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**Long-term N<sub>2</sub>O emission monitoring  
in biological wastewater treatment:  
methods, applications and relevance**

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## Summary

Nitrous oxide ( $\text{N}_2\text{O}$ ) emissions substantially contribute to global, environmental issues: climate change and stratospheric ozone depletion. Wastewater treatment plants (WWTPs) are potent point sources and significant contributors to anthropogenic  $\text{N}_2\text{O}$  emissions. However, emissions are currently underestimated in most greenhouse gas inventories due to overly simplified emission processes of WWTPs in the IPCC guidelines. Key limitations towards more realistic estimations are the low availability of representative monitoring campaigns and a limited understanding of  $\text{N}_2\text{O}$  formation mechanism during biological nitrogen removal in WWTPs. In particular,  $\text{N}_2\text{O}$  emission patterns assessed in long-term full-scale monitoring campaigns have shown strong seasonal variations, highlighting the requirement for long-term monitoring campaigns as basis for representative emission factor estimation. But underlying causes for the seasonal dynamics are unclear. Therefore, this thesis investigated (i) methods for long-term emission monitoring and the assessment of representative emission factors (EFs), (ii) methodologies for a more realistic estimation of countrywide  $\text{N}_2\text{O}$  emissions and (iii) causes of the seasonal emission pattern to ultimately propose mitigation measures.

Year-long  $\text{N}_2\text{O}$  monitoring campaigns were conducted on ten WWTPs using a newly developed version of the flux chamber approach, which allows high spatial and temporal resolution off-gas monitoring with a fully automated monitoring setup. The monitoring strategies identified differed between types of WWTPs. Monitoring one lane was found to be sufficient for a representative EF assessment, given comparable influent characteristics and process control over all lanes of a WWTP.

The yearly EFs assessed in this thesis ranged from 0.1-8% of the total nitrogen load in the inflow of the WWTP and strongly correlated with effluent nitrite relative to the incoming nitrogen load. An approach was suggested for the countrywide estimations of  $\text{N}_2\text{O}$  emissions based on the weighted average EFs of three nutrient removal categories (carbon removal, nitrification only, and full nitrogen removal). High uncertainties remain for carbon removal plants, given the wide range of expected EFs (0.1-8%). Applying the approach takes into account significant differences in EF for different processes and provides realistic estimates compared

to standard IPCC approaches. N<sub>2</sub>O emissions from WWTPs were estimated to contribute 0.3-1.4% of the total greenhouse gas emissions in the case of Switzerland. To identify key factors and patterns causing N<sub>2</sub>O formation, we applied extensive process monitoring, genomics, and isotopic technologies. Five highly relevant features for the seasonal pattern and N<sub>2</sub>O mitigation strategies could be identified. Firstly, nitrite accumulation was detected during high-emission phases in the seasonal pattern. Consequently, nitrite accumulation should be limited. Secondly, seasonal microbial dynamics exhibited a strong correlation with the seasonal N<sub>2</sub>O emission pattern and nitrite accumulation. A higher diversity and stability of the microbial community could be linked to lower emissions. Thirdly, WWTPs featuring year-round denitrification emitted significantly less N<sub>2</sub>O. The implementation of an anoxic phase lead to an immediate decrease of emissions. Fourthly, uncontrolled nitrification in carbon removal WWTPs can cause very high emissions. A stringent control of sludge age in carbon removing plants will lower emissions. Fifthly, reject water dosage was demonstrated to increase the daily emission variation significantly. Reject water should be optimally dosed during times with high organic substrate concentrations to maximize N<sub>2</sub>O reduction.

This thesis shows that N<sub>2</sub>O emission monitoring on WWTPs is required to quantify and mitigate N<sub>2</sub>O emissions until the dynamics and causes for the emission variation are better understood. Open key questions are the EF variation of different carbon removal WWTP and the causes for seasonal nitrite peaks. Solving these will require the joint efforts of different technologies, such as mathematical modeling, microbial tools and isotopic technologies as well as N<sub>2</sub>O emission monitoring.

## Zusammenfassung

Lachgasemissionen ( $\text{N}_2\text{O}$ ) tragen substantiell zu bedeutenden, globalen Umweltproblemen bei: dem Klimawandel und der stratosphärischen Zerstörung von Ozon. Kläranlagen (ARAs) sind relevante  $\text{N}_2\text{O}$  Punktquellen und verursachen einen relevanten Teil der anthropogenen Emissionen. Gegenwärtig werden die  $\text{N}_2\text{O}$  Emissionen aus ARAs in den meisten Treibhausgasinventaren jedoch unterschätzt, da die Emissionsprozesse in den üblicherweise verwendeten IPCC Richtlinien unzureichend abgebildet werden. Die Hauptgründe dafür sind eine limitierte Verfügbarkeit an repräsentativen Langzeitdaten und ein fehlendes Verständnis der  $\text{N}_2\text{O}$  Bildung während der biologischen Stickstoffelimination auf ARAs. Insbesondere die Ursachen für die saisonale Variabilität der Emission sind unbekannt. Die hohe Variabilität im Jahresverlauf unterstreicht die Notwendigkeit von Langzeitmess-kampagnen als Grundlage für eine repräsentative Schätzung der Emissionsfaktoren. Deshalb hatte die vorliegende Doktorarbeit folgende Zielsetzung: (i) Entwicklung und Anwendung einer Methode zur langfristigen Emissions-überwachung und zur Abschätzung repräsentativer Emissionsfaktoren (EFs), (ii) Erarbeitung einer Methode für eine realistischere Abschätzung der landesweiten  $\text{N}_2\text{O}$  Emissionen ARAs sowie (iii) Charakterisierung von ursächlichen Faktoren für die saisonale Variabilität, um schließlich Maßnahmen zur Emissionsminderung vorzuschlagen.

An zehn Kläranlagen wurden einjährige  $\text{N}_2\text{O}$  Messkampagnen durchgeführt, wobei eine neu entwickelte Methode verwendet wurde, die eine räumlich und zeitlich hoch aufgelöste Abluftbeprobung mit einem vollautomatisierten Messaufbau ermöglicht. Mithilfe der erhobenen Messdaten konnte gezeigt werden, dass die Langzeitmessung auf einer Abwasserstrasse, bei gleichen Zulaufmengen und -konzentrationen sowie identischer Steuerung auf allen Abwasserstrassen, ausreichend ist für die repräsentative Erhebung des Emissionsfaktors.

Die bestimmten EF auf den untersuchten ARAs lagen im Bereich zwischen 0.1-8.0% und korrelierten stark mit dem Nitritgehalt im Ablauf im Verhältnis zur zulaufenden Stickstofffracht. Für die landesweite Abschätzung der  $\text{N}_2\text{O}$  Emissionen wurde eine vereinfachte Methode vorgeschlagen, die auf den gewichteten durchschnittlichen EF



von drei Nährstoffentfernungskategorien basiert (Kohlenstoffelimination, nur Nitrifikation und vollständige Stickstoffelimination). Für Kohlenstoff eliminierende ARAs bestehen weiterhin grosse Unsicherheiten auf Grund des starken variierenden Erwartungsbereichs der EF (0.1-8.0% der Stickstofffracht im Zulauf der Kläranlagen). Insgesamt führt die Anwendung der Methode aber zu einer realistischeren Abschätzung der Emissionen im Vergleich zu den Standard IPCC Methoden. Für die Schweiz wurde der Anteil der N<sub>2</sub>O-Emissionen aus Kläranlagen an den gesamten Emissionen auf 0.3-1.4% abgeschätzt.

Um Schlüsselfaktoren und Betriebsstrategien gegen erhöhte Emissionen zu identifizieren, wurden verschiedene, komplementäre Methoden angewendet: eine umfangreiche Prozessüberwachung, Langzeitmessungen der N<sub>2</sub>O Emissionen, Genomik und Isotopentechnologien. Fünf Faktoren mit starkem Einfluss auf die N<sub>2</sub>O Bildung konnten festgestellt werden. (i) Eine saisonal auftretende Nitritakkumulation führte zu stark erhöhten Emissionen. Folglich sollte die Nitritakkumulation begrenzt werden (ii) Die saisonale mikrobielle Dynamik wies eine starke Korrelation mit N<sub>2</sub>O Emissionen und Nitritakkumulation auf. Eine höhere Diversität und Stabilität in der mikrobiellen Gemeinschaft korrelierte mit tiefen Emissionen. (iii) Kläranlagen mit ganzjähriger Denitrifikation emittierten deutlich weniger N<sub>2</sub>O. Die Implementierung einer anoxischen Phase führte zu einem sofortigen Rückgang der Emissionen. (iv) Eine unkontrollierte Nitrifikation in lediglich kohlenstoffeliminierenden Kläranlagen führte zu sehr hohen Emissionen. Eine strenge Kontrolle des Schlammalters auf diesen Anlagen würde die Emissionen senken. (v) Die Dosierung Faulwasser erhöhte die Emissionen deutlich bei tiefen organischen Substratgehalten, da die Reduktion von Lachgas verringert war.

Diese Arbeit zeigt, dass eine Überwachung der N<sub>2</sub>O Emissionen auf Kläranlagen erforderlich ist, um die N<sub>2</sub>O-Emissionen zu quantifizieren und zu reduzieren, bis die Dynamiken und Ursachen für die Emissionsschwankungen besser verstanden werden. Offene Schlüsselfragen sind die EF Variation verschiedener Kläranlagen zur Kohlenstoffentfernung und die Ursachen für saisonaler Nitritspitzen. Die Lösung dieser Fragen erfordert die Nutzung verschiedener Technologien, wie z. B. mathematische Modellierung, mikrobiellen Werkzeugen und Isotopentechnologie sowie der Langzeitmessung der N<sub>2</sub>O Emissionen.

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# **Chapter 1**

## **Introduction**



## **1.1 General introduction of the thesis**

### **1.1.1 History of climate change science – from science to policy**

Climate changes describes the long-term change of the earth's climate system with tremendous global impacts on different aspects, such as global warming (Figure 1.1 a), precipitation and wind patterns. This process has started at the beginning of the pre-industrial period and is ongoing since then (IPCC 2013). The temperature increase is induced, with a very high probability, by emissions of greenhouse gases (GHG) caused by human activities (Figure 1.1 b, c), as demonstrated by several researchers at the beginning of the 1980s (Hansen et al. 1981).

The physical basis, however, was laid more than one century earlier by three researchers and others. In 1827, Jean-Baptiste Joseph Fourier was the first to propose heat storage of interstellar radiation in Earth's atmosphere, later referred as greenhouse effect at a conceptual level (Fourier 1827). John Tyndall provided experimental evidence for Fourier's theory by measuring emissions and absorption of infrared radiation for visually transparent gases present in the atmosphere, such as water vapor, hydro carbons (e.g. methane ( $\text{CH}_4$ )), and carbon dioxide ( $\text{CO}_2$ ) (Tyndall 1861). Finally, Svante Arrhenius was the first to quantitatively estimate the temperature increase/decrease induced by an increase/decrease of atmospheric carbon dioxide concentration (Arrhenius 1896). Half a century later and after a general dispute on the global warming theory, new experimental methods and advances in modeling further provided evidence for and improved understanding of climate change. Quantitative projections of the global temperature increase induced by rising atmospheric  $\text{CO}_2$  concentrations were made using one of the first computer based climate models (Manabe and Wetherald 1967). Coupled with the projected increase of  $\text{CO}_2$  concentrations in the atmosphere based on  $^{14}\text{C}$  measurements (Revelle and Suess 1957, Suess 1955), concerns about the respective impacts on the climate were increasing in academia. In a long-term study from 1958 to 1976, a steady increase of atmospheric  $\text{CO}_2$  concentration was observed at the Mouna Loa Observatory in Hawaii (Keeling et al. 1976). Additionally, the importance of other GHG, such as  $\text{CH}_4$  and nitrous oxide ( $\text{N}_2\text{O}$ ) was demonstrated (Wang et al. 1976). In the mid-1980s, after a decade of increasing consensus on human-made climate

change, researchers started to speak out to the public about the causes and massive risks of climate change (Weart 2008). The rising political pressure led to the foundation of the international panel of climate change (IPCC) by World Meteorological Organization (WMO) and the United Nations Environment Program (UNEP) in 1988. The goal of the organization is to strengthen the international scientific collaboration on climate change research and provide a scientific basis for policy makers as well as public awareness. Until 2021, the IPCC has released five assessment reports (AR1-AR5), summarizing the most important scientific findings about climate change. These ARs are the standard references in international climate change research and policy.

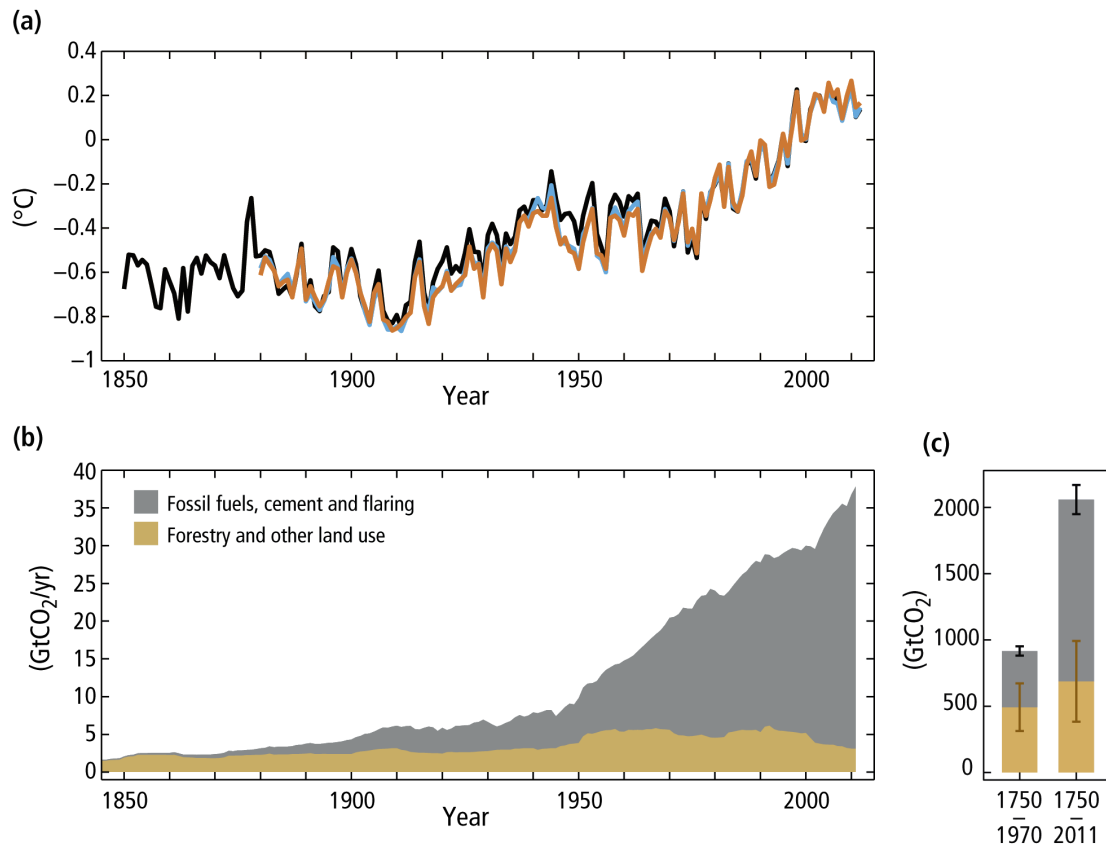


Figure 1.1: a) Annually and globally averaged combined land and ocean surface temperature anomalies relative to the average over the period 1986 to 2005. Colors denote different data sets: HadCRUT4 (version 4.1.1.0) (black), NASA GISS (blue), CDC MLOST (version 3.5.2) (orange). Origin: (IPCC 2013). b) Annual global anthropogenic carbon dioxide (CO<sub>2</sub>) emissions (gigatonne of CO<sub>2</sub>-equivalent per year, GtCO<sub>2</sub>/yr.) from fossil fuel combustion, cement production and flaring, and forestry and other land use (FOLU), 1750–2011. c) Cumulative emissions and their uncertainties are shown as bars and whiskers, respectively.

With the public alerts of scientist on the risks of rising GHG emissions and the successful adoption of the Montreal protocol for ozone mitigation (UNEP 1987), the stage was set for an international treaty covering global warming (Kuyper et al. 2018). The United Nations Framework Convention on Climate Change (UNFCCC) was founded at the 1992 Earth Summit in Rio de Janeiro to provide a panel for international climate governance (Oppenheimer and Petsonk 2005). An important element of the UNFCCC is the annually occurring conferences of parties (COP), where the two main treaties (Kyoto and Paris protocol) within the UNFCCC were negotiated (United Nations Climate Change 1997, 2015). In both agreements, ratified by 191 respectively 190 parties, legally binding emission reduction targets were set for Annex 1 parties, including industrialized countries and economies in transition (Held and Roger 2018). Consequently, an essential element of the treaty is the yearly reporting of country specific GHG emissions in national GHG inventories submitted to the UNFCCC (Hare et al. 2010).

#### **1.1.2 GHG reporting and N<sub>2</sub>O emission estimates**

The IPCC provides standardized guidelines for national GHG inventories, which are binding for Annex 1 parties in their mandatory yearly reporting (United Nations Climate Change 1997). In the Paris agreement, non-Annex 1 parties are obliged to conduct a bi-yearly GHG inventory (United Nations Climate Change 2015). The inventories are divided in four main reporting categories: (i) energy, (ii) industrial processes and construction, (iii) agriculture and (iv) waste (IPCC 2019a). For each category, several sub-categories exist. Conceptually, the reporting is organized in three Tiers, according to data availability. Tier 1 includes the estimation of GHG emissions with standard values defined in the IPCC guidelines. Tier 2 and Tier 3 approaches deviate by being country specific in terms of emission factors (EF) and activity data assessment (Tier 2) or the reporting method in general (Tier 3). Current emission timelines from countrywide GHG inventories based on the IPCC guidelines show that overall GHG emissions have been rising globally (Figure 1.1 b, c) compared to the reference year 1990 until recently (IEA 2020). The three most important GHG are CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O with respective shares of 76%, 16% and 6% of the globally emitted GHG (IPCC 2014).

For wastewater treatment research, N<sub>2</sub>O emissions are of high importance given its possibly dominant share in the GHG balance of wastewater treatment plants (WWTP) (Daelman et al. 2013b). GHG inventories based on the IPCC guidelines report that the largest share of N<sub>2</sub>O emissions (~50%) originates from agriculture while wastewater treatment contributes less than 4% to the total anthropogenic emissions (Tian et al. 2020). However, N<sub>2</sub>O emissions from wastewater treatment are likely an underestimated source of N<sub>2</sub>O emissions in the inventories, since (1) the Tier 1 method described in the IPCC guidelines is based on outdated literature and (2) the data basis to update the guidelines is insufficient (Table 1.1) (Daelman et al. 2015, Vasilaki et al. 2019): The applied N<sub>2</sub>O EF for wastewater treatment plants of 0.034%, as suggested in the 2006 IPCC guidelines for emission reporting originates from one of the first N<sub>2</sub>O monitoring studies on a single wastewater treatment plant and is based on short-term grab-sampling (Czepiel et al. 1995, IPCC 2006). Most of the GHG inventories are based on this value from the 2006 IPCC guidelines. N<sub>2</sub>O EFs from WWTP have been shown to exhibit a great variation (Chen et al. 2019). Hence, continuous long-term (at least year-long) monitoring is required for the assessment of representative EFs (Daelman et al. 2013a, Vasilaki et al. 2019). The recommended EF has been increased to 1.6% in the 2019 refinement of the IPCC guidelines, but current estimations still mostly rely on the short-term data (Table 1) and the updated EF is quite uncertain (IPCC 2019a, Vasilaki et al. 2019). Thus, more long-term monitoring campaigns are required in order to (i) cover a wider range of WWTP and (ii) to decrease uncertainties of emission estimations "(e.g. by appropriate categorization WWTPs according to their N<sub>2</sub>O emission).

Table 1.1 Overview on the existing N<sub>2</sub>O EF estimates and their data basis (monitoring campaigns, prior to this PhD).

Sourced of EFs	EF (%)	Type of study (duration, sampling)	Number of studies
2006 IPCC guidelines (IPCC 2006).	0.034	Short-term, grab sampling	1
2019 IPCC guidelines (IPCC 2019b).	1.6	Short-term, grab sampling	13
		Short-term, continuous sampling	14
		Long-term, grab sampling	2
		Long-term, continuous sampling	1
All monitoring campaigns in mainstream wastewater treatment according to Vasilaki et al. (2019).	0.87	Short-term, grab sampling	37
		Short-term, continuous sampling	17
		Long-term, grab sampling	5
		Long-term, continuous sampling	2
All existing continuous long-term monitoring campaigns according to Chen et al. (2019), Daelman et al. (2015), Kosonen et al. (2016).	1.9	Long-term, continuous sampling	3

### 1.1.3 N<sub>2</sub>O emissions and the nitrogen cycle

Atmospheric concentrations of N<sub>2</sub>O have risen drastically compared to preindustrial levels and exhibited a faster growth in the last five decades (Figure 1.2) - similarly to CO<sub>2</sub> and CH<sub>4</sub> (IPCC 2013, Thompson et al. 2019). Photolysis and oxidative reaction are the most important sinks of N<sub>2</sub>O in the atmosphere (Montzka et al. 2011). However, N<sub>2</sub>O strongly absorbs infrared radiation and its atmospheric removal is quite slow, implying a high GHG potential of 265 gCO<sub>2</sub>-eq/gN<sub>2</sub>O (IPCC 2013) and a long average atmospheric lifetime for N<sub>2</sub>O of 116 years (Prather et al. 2015). Additionally, N<sub>2</sub>O is the most important ozone depleting substance in the stratosphere since the Montreal protocol to limit ozone depleting substances has been ratified (Ravishankara et al. 2009).



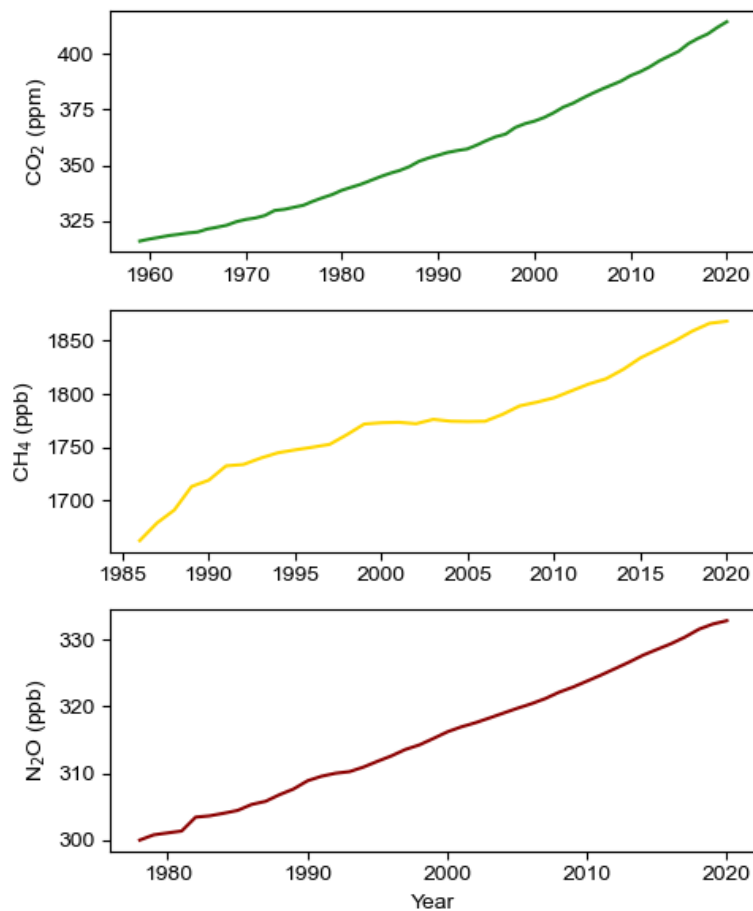


Figure 1.2: Atmospheric concentrations of the greenhouse gases carbon dioxide (CO<sub>2</sub>, green), methane (CH<sub>4</sub>, orange) and nitrous oxide (N<sub>2</sub>O, red) from direct atmospheric measurements. Origin: (Prinn et al. 2018, Tans and Keeling 2021)

Globally, most of the N<sub>2</sub>O is produced through two key microbial processes in the nitrogen cycle – nitrification and denitrification (Stein and Klotz 2016). The nitrogen cycle is driven by complex networks of microbes and the corresponding microbial processes (Figure 1.3). Nitrogen fixation of atmospheric molecular nitrogen and transformation of various reactive nitrogen species via nitrification, denitrification and anammox are the main transformation processes (Kuypers et al. 2018). Reactive nitrogen is transformed, recycled and stored in terrestrial and aquatic systems, where the most important inputs from the atmosphere are microbial nitrogen fixation and anthropogenic fertilizer production (Fowler et al. 2013). A minor part of the anthropogenic emissions originates from combustion processes (Hayhurst and Lawrence 1992, Tian et al. 2020). A substantial growth of the anthropogenic sources is to be expected, since fertilizer application in agricultural systems for crop

production is still increasing, given the expected global population growth (Canfield et al. 2010). A small part is transformed to  $N_2O$  and released to the atmosphere (Fowler et al. 2013). However,  $N_2O$  emissions have become a pressing topic in research, due to its substantial impact on atmospheric chemistry and radiation absorption. Currently, anthropogenic and natural systems contribute roughly equally to global  $N_2O$  emissions, but the anthropogenic share is expected to increase substantially, since  $N_2O$  emissions from agriculture and other sectors are growing (Tian et al. 2020). How wastewater treatment will contribute to the global  $N_2O$  emissions in the next decades has so far not been discussed. The formation pathways of  $N_2O$  during nitrification and denitrification are discussed in chapter 1.1.5.

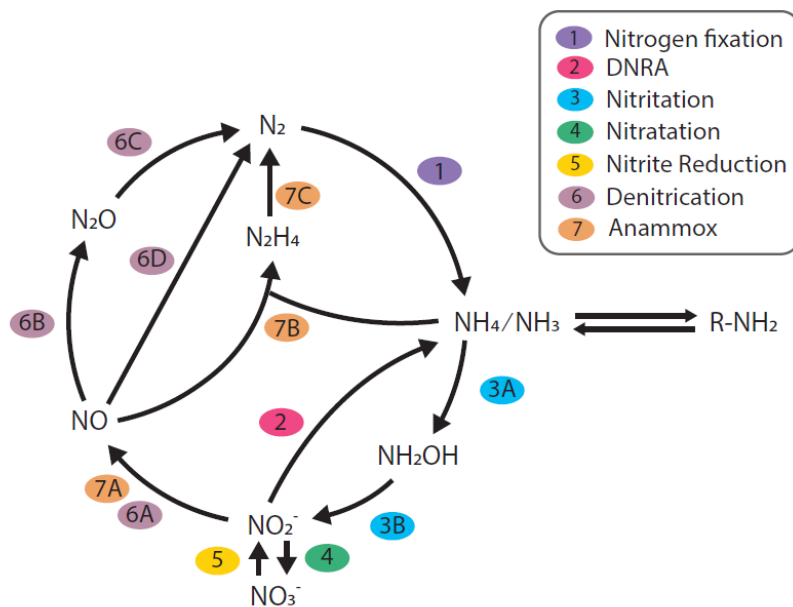


Figure 1.3: Major processes of the nitrogen cycle are represented by the numbered circles. Ammonification may be accomplished either by process 1, reduction of dinitrogen (nitrogen fixation), or by process 2, dissimilatory nitrite reduction to ammonium (DNRA). Nitrification is composed of process 3, oxidation of ammonia to nitrite (also referred to as ‘nitrification’), and process 4, oxidation of nitrite to nitrate. Process 5, reduction of nitrate to nitrite, can be coupled to processes 2, 6 or 7 in a mixed microbial population. Denitrification is shown as process 6, which is also referred to as ‘nitrogen-oxide gasification’. Anammox is shown as process 7. Process colors refer to Figure 1.5, where production pathways are shown. Origin: (Stein and Klotz 2016)

#### 1.1.4 $N_2O$ emissions from wastewater treatment plants

In wastewater treatment plants (WWTP),  $N_2O$  is produced during biological nitrogen removal (Kampschreur et al. 2009b). The main production processes of  $N_2O$  in WWTP are the biological treatment in the water line, the biological treatment for

reject water from the sludge line, and sludge incineration (Figure 1.4) (Joss et al. 2009, Law et al. 2012, Marias et al. 2015). While in the latter  $\text{N}_2\text{O}$  is a product of thermal oxidation of nitrogen in the sewage sludge,  $\text{N}_2\text{O}$  in biological systems is a by-product of nitrification and denitrification (Tallec et al. 2006, Von Schulthess and Gujer 1996). The produced  $\text{N}_2\text{O}$  can be emitted in follow up processes after the biological treatment, such as the secondary clarifier or in the effluent of the WWTP (Mikola et al. 2014). Additionally, nitrogen in the effluent of the WWTP in the form of nitrate ( $\text{NO}_3^-$ ) can be transformed biologically to  $\text{N}_2\text{O}$  in the receiving water and emitted to the atmosphere (Marescaux et al. 2018). The biological treatment of a WWTP is considered the most important source of GHG due to high  $\text{N}_2\text{O}$  emissions.  $\text{N}_2\text{O}$  emissions from the biological treatment are estimated to contribute roughly 80% to the GHG emissions from WWTP (Daelman et al. 2013b).

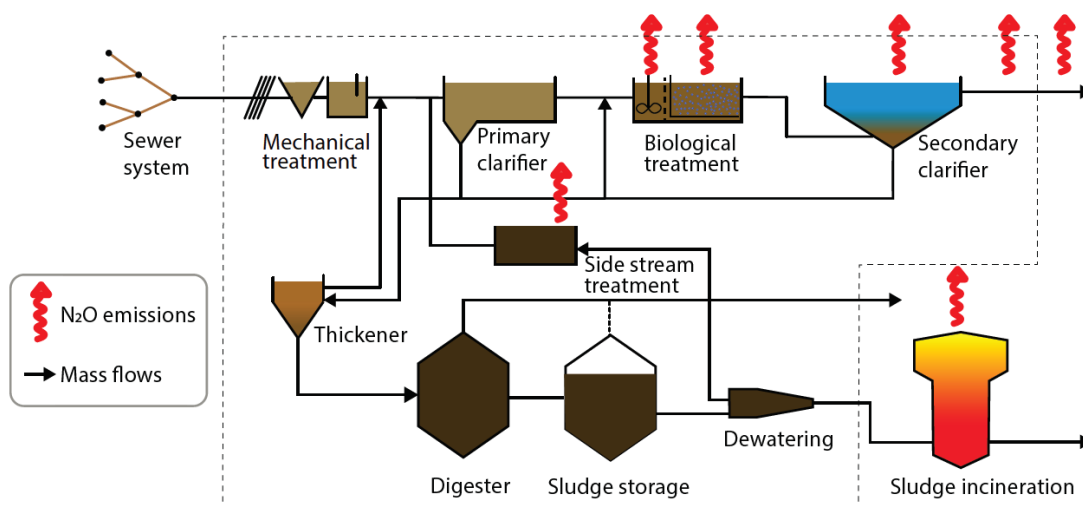


Figure 1.4:  $\text{N}_2\text{O}$  emissions in a standard wastewater treatment process. Dashed line indicates system boundaries of a WWTP.

$\text{N}_2\text{O}$  emissions from biological treatment have been monitored in numerous studies (Vasilaki et al. 2019). The resulting EFs showed a substantial variability, ranging from less than one per mill to several percent of the total nitrogen load in the influent of the WWTP (Daelman et al. 2015). Although the variability of the EFs can be partly linked to different process configurations (Yan et al. 2014), the type of monitoring strategy plays an important role for the wide range of EFs reported (Vasilaki et al. 2019). In terms of monitoring setup, three conceptually different approaches exist all based on temporally highly resolved online measurements, i.e. i) the flux chamber

based monitoring (Chandran et al. 2016) , ii) liquid sensor monitoring (Marques et al. 2016), iii) off-gas monitoring in the collected off-gas of covered WWTP (Daelman et al. 2015, Kosonen et al. 2016), and iv) plant-wide tracer dispersion (Yoshida et al. 2014), which is not further considered due to the limited applicability for long-term monitoring. Approach iii) is the most accurate, since the full spatial variability of the emissions on a WWTP can be represented. However, since most of the WWTP are uncovered, approaches i) and ii) have to be applied for monitoring. One of the key challenges using approaches i) and ii) is the high spatial variability of emissions from the biological treatment and even in single biological reactors (Pan et al. 2016). A further important source of uncertainty in the monitoring strategy is the duration of a monitoring campaign. Most of the reported campaigns are based on either short-term or non-continuous monitoring campaigns, although the assessment of representative EFs requires continuous, long-term monitoring campaigns (Daelman et al. 2013a). Guiding principles on how to perform spatially and temporally highly resolved, continuous, and long-term flux chamber based monitoring campaigns are missing.

Two of three existing, long-term monitoring campaigns report strong seasonal emission pattern, with an emission peak in the first half of the year (winter and spring) and lower emissions in the second half (summer and autumn) (Chen et al. 2019). The underlying reasons for the seasonal variability, however, remain uncovered and further monitoring campaigns are thus required for better understanding as well as for reducing uncertainties (Vasilaki et al. 2019). The complexity of N<sub>2</sub>O emissions from WWTP is probably linked to the complex formation, involving several microbial pathways (Wunderlin et al. 2012). In full-scale WWTP, this complexity has not been investigated in further detail.

#### **1.1.5 N<sub>2</sub>O formation in WWTP**

Multiple pathways for N<sub>2</sub>O formation in WWTP have been reported for ammonia (NH<sub>4</sub><sup>+</sup>) oxidizing bacteria (AOB) and heterotrophic denitrifying bacteria (HET) (Figure 1.5) (Ren et al. 2019). AOB can produce N<sub>2</sub>O as a by-product via at least two different pathways, such as i) nitrifier denitrification where nitrite (NO<sub>2</sub><sup>-</sup>) is reduced by the enzymes NirK and NorB to N<sub>2</sub>O and ii) hydroxylamine (NH<sub>2</sub>OH)

oxidation, where  $\text{NH}_2\text{OH}$  is oxidized to  $\text{N}_2\text{O}$  by the enzyme P460 (Stein 2018). Both pathways can interact with abiotic reactions, but are of minor importance in biological wastewater treatment under mainstream conditions (Soler-Jofra et al. 2020). Typical, AOB species found in wastewater treatment are *Nitrosomonas* and *Nitrosospira* (Cydzik-Kwiatkowska and Zielinska 2016). Besides AOB, other organisms able to oxidize  $\text{NH}_4^+$  have been found to play an important role in WWTP, such as  $\text{NH}_4^+$  oxidizing archaea (AOA) and *Comammox*, phylogenetically a NOB that can perform complete nitrification within one organism (Pan et al. 2018, Roots et al. 2019). Both clades have been linked to generally lower emissions than AOB, since they lack the genetic potential to perform nitrifier denitrification (Daims et al. 2015, Stein 2018). Heterotrophic denitrifying bacteria form  $\text{N}_2\text{O}$  as intermediate product during the reduction of  $\text{NO}_3^-$  to molecular nitrogen ( $\text{N}_2$ ), involving various genes (Figure 1.5). Denitrification involves many different clades (Lu et al. 2014). Although, multiple organisms exist that perform the complete denitrification within one organism, different strategists exist splitting denitrification and some organisms only reduce  $\text{N}_2\text{O}$  to  $\text{N}_2$ , making denitrification an important process to optimize for  $\text{N}_2\text{O}$  mitigation (Conthe et al. 2018b).

As a consequence of the presence of different pathways for  $\text{N}_2\text{O}$  formation in wastewater treatment systems, various process conditions have been discussed to enhance  $\text{N}_2\text{O}$  emissions, such as i) low DO,  $\text{NO}_2^-$  or free nitrous acid (FNA) accumulation and changes in the  $\text{NH}_4^+$  concentrations in nitrifying zones, ii) limitation of organic substrate and  $\text{NO}_2^-$  accumulation iii) alternation of anoxic and aerobic conditions, and iv) abrupt changes in the processes and system shocks (Vasilaki et al. 2019). An important factor for increased  $\text{N}_2\text{O}$  emissions in WWTP is  $\text{NO}_2^-$  accumulation, provoking increased  $\text{N}_2\text{O}$  formation by the pathways of heterotrophic denitrification and nitrifier denitrification (Castro-Barros et al. 2016). Hence, NOB are assumed to play an important role in  $\text{N}_2\text{O}$  formation in WWTPs, although they are not known to produce  $\text{N}_2\text{O}$ . While  $\text{NO}_2^-$  accumulation is crucial in partial nitrification-anammox and shortcut biological nitrogen removal systems (Duan et al. 2019, Hellinga et al. 1998), it is undesired in nitrification-denitrification nitrogen removal systems due to the toxicity of the protonated form of  $\text{NO}_2^-$ , FNA, and a reported mechanistic link to the growth of bulking organisms (Philips et al.

2002, Zhou et al. 2011).  $\text{NO}_2^-$  accumulation is caused by imbalances of AOB and NOB activities that can be caused by increased temperatures, low DO concentrations, and the presence of free ammonia (FA) or FNA (Ward et al. 2011). However, occasionally  $\text{NO}_2^-$  accumulation can be observed at low temperatures in full-scale WWTPs (Philips et al. 2002). Niche differentiation of different NOB strains may explain  $\text{NO}_2^-$  being dependent on varying conditions at WWTP, as several NOB genera have been detected, such as *Nitrospira*, *Nitrotoga*, and *Nitrobacter* (Daims et al. 2001, Lucker et al. 2015). While *Nitrospira* are reported to dominate under higher temperatures and have low affinities for their substrate  $\text{NO}_2^-$ , *Nitrotoga* are cold-climate NOB with higher affinities for  $\text{NO}_2^-$  (Wegen et al. 2019). Monitoring studies on full-scale WWTP combined with microbial assays are required in full-scale WWTP in order to (i) better understand the link between microbial community dynamics and  $\text{N}_2\text{O}$  emissions and (ii) explain the seasonal emission variations.

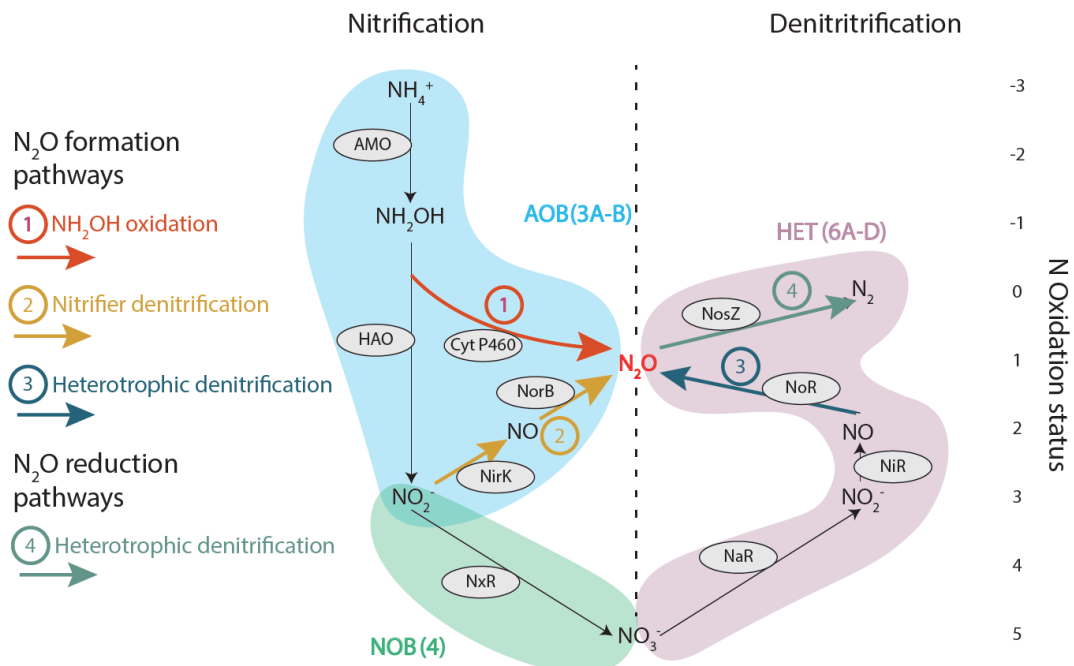


Figure 1.5:  $\text{N}_2\text{O}$  formation during nitrification and denitrification in biological wastewater treatment (Process 3A-B, 4, and 6A-D in Figure 1.3). Number in brackets next to the organisms indicate respective process in Figure 1.3. Grey oval boxes encode genes performing the reaction shown by the arrows.

### 1.1.6 Pathway detection in N<sub>2</sub>O formation

The understanding of the production pathways and their contributions to N<sub>2</sub>O production in the biological treatment is key for an effective mitigation of N<sub>2</sub>O emissions. Isotopic analysis of N<sub>2</sub>O isotopocules (constitutional isotopomers) abundances has been demonstrated to provide quantitative information on N<sub>2</sub>O formation pathways and their respective contributions (Toyoda and Yoshida 1999). From a methodological point, <sup>15</sup>N and <sup>18</sup>O isotopes in N<sub>2</sub>O are measured to assess the ratios of the four most abundant isotopocules (<sup>14</sup>N<sup>14</sup>N<sup>16</sup>O, <sup>15</sup>N<sup>14</sup>N<sup>16</sup>O, <sup>14</sup>N<sup>15</sup>N<sup>16</sup>O, <sup>14</sup>N<sup>14</sup>N<sup>18</sup>O). Abundances are reported relative to a standard in the  $\delta$ -notation in per mil (‰). Key measurers for pathway detection are i) the  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  abundance in N<sub>2</sub>O molecules as well as in the substrates of the expected N<sub>2</sub>O formation pathways (NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup>) and ii) the intramolecular distribution of  $\delta^{15}\text{N}$  at the central ( $\alpha$ ) and terminal ( $\beta$ ) positions in the asymmetric N<sub>2</sub>O molecule are valuable information to differentiate pathways (Sutka et al. 2006). Production pathways can be deduced by comparing the measured values in environmental samples with literature values from pure cultures and single process experiments. Isotopic analysis of N<sub>2</sub>O has been successfully applied for pathway detection in wastewater systems (Wunderlin 2013). However, recent review studies clearly emphasize considerable uncertainties linked to N<sub>2</sub>O isotope analysis and the need to couple experiments with complementary methods (Duan et al. 2017, Ostrom and Ostrom 2017, Yu et al. 2020). For pathway identification in wastewater systems, three types of methods for validation are suggested, such as <sup>15</sup>N tracer experiments (Ma et al. 2017), mathematical modeling of biochemical processes (Domingo-Félez and Smets 2016), and molecular methods from microbiology, such as qPCR, metatranscriptomics, or metaproteomics (Duan et al. 2017). Furthermore, isotope technologies has not been applied to full-scale WWTPs but potentially is valuable to investigate pathways and N<sub>2</sub>O reduction strategies.

## **1.2 Overall objectives of this thesis**

The present thesis quantifies N<sub>2</sub>O emissions from full-scale WWTP, discusses emission monitoring strategies and analyses production pathways in full-scale WWTP. The main goal is to advance our understanding for N<sub>2</sub>O production and dynamics in full-scale WWTP in order to develop N<sub>2</sub>O mitigation strategies. This challenge is tackled conducting extensive, continuous, long-term off-gas monitoring campaigns on WWTP combined with methods from (i) process engineering applied to wastewater treatment, (ii) molecular microbiology, and (iii) isotopic signature measurements. The following research questions were posed:

- i) What are essential components and important strategies for continuous, long-term off-gas monitoring campaigns on full-scale WWTP based on the flux-chamber approach?
- ii) What is the contribution of N<sub>2</sub>O emissions from WWTP to the total GHG emissions in a country and how are these emissions optimally estimated?
- iii) What are causes for the pronounced N<sub>2</sub>O emission dynamics over days and over seasons and how do they link to N<sub>2</sub>O mitigation strategies?



### 1.3 Outline of the thesis

In Chapter 2, the current methods to estimate N<sub>2</sub>O emissions from the biological treatment of a WWTP are reviewed. Based on the results, methods are developed to conduct long-term monitoring campaigns to ultimately reduce uncertainties for emissions factors estimations in full-scale WWTP (Chapters 3 and 4). A novel approach to estimate country-wide N<sub>2</sub>O emissions from WWTP is presented in Chapter 5. Potential causes for the high variability of N<sub>2</sub>O emissions are discussed using genomics and N<sub>2</sub>O isotope analysis in Chapter 6 and 7. N<sub>2</sub>O emissions are compared with other GHG emissions from WWTP in Chapter 8. The thesis is concluded and an outlook for future research is presented in Chapter 9. In the following, I present a short overview of chapters 2 to 7:

In Chapter 2, the current method applied by the Federal Office for the Environment (FOEN) of Switzerland to estimate countrywide emissions of N<sub>2</sub>O is reviewed. In a second step, a method to estimate the GHG emissions from WWTP based on current literature is suggested and open research questions are identified, which are then tackled in chapter 3-5.

In Chapter 3, the monitoring setup and methods are described with a high level of details to allow other research groups or practitioners to copy the whole or parts of the off-gas monitoring system. The description of the system consists of multiple elements, such as PI&D, wiring schemes, material list, programs, and user manuals, which are provided.

First monitoring results are presented, conducted with the first version of the presented setup (Chapter 3) and emission dynamics are discussed on different time scales in Chapter 4. The results are then used to propose a long-term off-gas monitoring strategy for various processes in full-scale WWTP using the floating flux chamber approach.

Using the monitoring setup described in chapter 3 and applied as well as improved in terms of the monitoring strategy in chapter 4, seven further monitoring campaigns were conducted. In Chapter 5, we discuss the dynamics and potential emission drivers using an extensive data-set of 14 at least year-long, continuous monitoring campaigns. Based on a correlation analysis we discuss important drivers for N<sub>2</sub>O

production and mitigation strategies. Ultimately, a novel method to estimate N<sub>2</sub>O emissions from WWTP for a whole country is suggested and compared with the current estimation methods from the IPCC guidelines. The uncertainties are estimated for each method.

Microbial community dynamics are discussed as potential drivers for the seasonality of N<sub>2</sub>O emission patterns in a full-scale SBR wastewater treatment plant in Chapter 6. 16s rRNA amplicon sequencing is used to assess changes in the microbial community of the activated sludge in several SBR reactors and correlated these changes with N<sub>2</sub>O emissions and variations in WWTP performance.

Stable isotope techniques, analyzing <sup>15</sup>N and <sup>18</sup>O content in N<sub>2</sub>O, NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup> and NO<sub>2</sub><sup>-</sup> are used to investigate N<sub>2</sub>O formation on a full-scale WWTP under different operational modes. Chapter 7 explores the applicability of stable isotope methods in full-scale WWTP to study N<sub>2</sub>O formation mechanisms and optimize operation of a WWTP.

Finally, the importance of N<sub>2</sub>O emissions is compared with other GHG emissions from WWTP based on scientific literature and methane measurements (Chapter 8). Additionally, the relevance of N<sub>2</sub>O emissions from the biological treatment of a WWTP is discussed in relation to other production processes of N<sub>2</sub>O in a WWTP. This chapter enables the comparison of various GHG optimization strategies and sets the monitoring data assessed in a larger context.

## 1.4 References

- Arrhenius, S. (1896) On the Influence of Carbonic Acid in the Air upon the Temperature of the Ground. *Philosophical Magazine and Journal of Science* 41, 237-276.
- Canfield, D.E., Glazer, A.N. and Falkowski, P.G. (2010) The evolution and future of Earth's nitrogen cycle. *Science* 330(6001), 192-196.
- Castro-Barros, C.M., Rodríguez-Caballero, A., Volcke, E.I.P. and Pijuan, M. (2016) Effect of nitrite on the  $\text{N}_2\text{O}$  and NO production on the nitrification of low-strength ammonium wastewater. *Chemical Engineering Journal* 287, 269-276.
- Chandran, K., Volcke, E.I. and Van Loosdrecht, M.C.M. (2016) *Experimental Methods in Wastewater Treatment*. Van Loosdrecht, M.C.M., Nielen, P.H., López Vázquez, C.M. and Brdjanovic, D. (eds), IWA, London.
- Chen, X., Mielczarek, A.T., Habicht, K., Andersen, M.H., Thornberg, D. and Sin, G. (2019) Assessment of Full-Scale  $\text{N}_2\text{O}$  Emission Characteristics and Testing of Control Concepts in an Activated Sludge Wastewater Treatment Plant with Alternating Aerobic and Anoxic Phases. *Environmental Science & Technology* 53(21), 12485-12494.
- Conthe, M., Wittorf, L., Kuenen, J.G., Kleerebezem, R., van Loosdrecht, M.C.M. and Hallin, S. (2018) Life on  $\text{N}_2\text{O}$ : deciphering the ecophysiology of  $\text{N}_2\text{O}$  respiring bacterial communities in a continuous culture. *ISME Journal* 12(4), 1142-1153.
- Cydzik-Kwiatkowska, A. and Zielinska, M. (2016) Bacterial communities in full-scale wastewater treatment systems. *World Journal of Microbiology and Biotechnology* 32(4), 66.
- Czepiel, P., Crill, P. and Harriss, R. (1995) Nitrous oxide emissions from municipal wastewater treatment. *Environmental Science & Technology*, 2352-2356.
- Daelman, M.R., De Baets, B., van Loosdrecht, M.C. and Volcke, E.I. (2013a) Influence of sampling strategies on the estimated nitrous oxide emission from wastewater treatment plants. *Water Research* 47(9), 3120-3130.
- Daelman, M.R., van Voorthuizen, E.M., van Dongen, L.G., Volcke, E.I. and van Loosdrecht, M.C. (2013b) Methane and nitrous oxide emissions from municipal wastewater treatment - results from a long-term study. *Water Science and Technology* 67(10), 2350-2355.
- Daelman, M.R., van Voorthuizen, E.M., van Dongen, U.G., Volcke, E.I. and van Loosdrecht, M.C. (2015) Seasonal and diurnal variability of  $\text{N}_2\text{O}$  emissions from a full-scale municipal wastewater treatment plant. *Science of The Total Environment* 536, 1-11.
- Daims, H., Lebedeva, E.V., Pjevac, P., Han, P., Herbold, C., Albertsen, M., Jehmlich, N., Palatinszky, M., Vierheilig, J., Bulaev, A., Kirkegaard, R.H., von Bergen, M., Rattei, T., Bendinger, B., Nielsen, P.H. and Wagner, M. (2015) Complete nitrification by *Nitrospira* bacteria. *Nature* 528(7583), 504-509.

- Daims, H., Nielsen, J.L., Nielsen, P.H., Schleifer, K.H. and Wagner, M. (2001) In situ characterization of Nitrospira-like nitrite-oxidizing bacteria active in wastewater treatment plants. *Applied Environmental Microbiology* 67(11), 5273-5284.
- Domingo-Félez, C. and Smets, B.F. (2016) A consilience model to describe N<sub>2</sub>O production during biological N removal. *Environmental Science: Water Research & Technology* 2(6), 923-930.
- Duan, H., Ye, L., Erler, D., Ni, B.J. and Yuan, Z. (2017) Quantifying nitrous oxide production pathways in wastewater treatment systems using isotope technology - A critical review. *Water Research* 122, 96-113.
- Duan, H., Ye, L., Lu, X. and Yuan, Z. (2019) Overcoming Nitrite Oxidizing Bacteria Adaptation through Alternating Sludge Treatment with Free Nitrous Acid and Free Ammonia. *Environmental Science & Technology* 53(4), 1937-1946.
- Fourier, J.J. (1827) On the Temperatures of the Terrestrial Sphere and Interplanetary Space. *Mémoires de l'Académie Royale des Sciences* 7, 569-604.
- Fowler, D., Coyle, M., Skiba, U., Sutton, M.A., Cape, J.N., Reis, S., Sheppard, L.J., Jenkins, A., Grizzetti, B., Galloway, J.N., Vitousek, P., Leach, A., Bouwman, A.F., Butterbach-Bahl, K., Dentener, F., Stevenson, D., Amann, M. and Voss, M. (2013) The global nitrogen cycle in the twenty-first century. *Philos Trans R Soc Lond B Biol Sci* 368(1621), 20130164.
- Hansen, J., Johnson, D., Lacis, A., Lebedeff, S., Lee, P., Rind, D. and Russell, G. (1981) Climate impact of increasing atmospheric carbon dioxide. *Science* 213(4511), 957-966.
- Hare, W., Stockwell, C., Flachslund, C. and Oberthür, S. (2010) The architecture of the global climate regime: a top-down perspective. *Climate Policy* 10(6), 600-614.
- Hayhurst, A.N. and Lawrence, A.D. (1992) Emissions of nitrous oxide from combustion sources. *Progress in Energy and Combustion Science* 18(6), 529-552.
- Held, D. and Rogger, C. (2018) Three Models of Global Climate Governance: From Kyoto to Paris and Beyond. *Global Policy* 9(4), 527-537.
- Hellinga, C., Schellen, A.A.J.C., Mulder, J.W., van Loosdrecht, M.C.M. and Heijnen, J.J. (1998) The SHARON process: An innovative method for nitrogen removal from ammonium-rich waste water. *Water Science and Technology* 37(9), 135-142.
- IEA (2020) Global Energy Review 2020, Paris
- IPCC (2006) Chapter 5.6 Wastewater treatment and discharge. 2006 IPCC Guidelines for National Greenhouse Gas Inventories, <http://www.ipcc-nggip.iges.or.jp/public/2006gl/index.html>.
- IPCC (2013) Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

- IPCC (2014) Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, p. 151, IPCC, Geneva, Switzerland.
- IPCC (2019a) 2019 Refinement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories. Buendia, E.C., Guendehou, S., Limmeechokchai, B., Pipatti, R., Rojas, Y., Sturgiss, R., Tanabe, K., Wirth, T., Romano, D., Witi, J., Garg, A., Weitz, M.M., Cai, B., Ottinger, D.A., Dong, H., MacDonald, J.D., Ogle, S.M., Rocha, M.T., Sanchez, M.J.S., Bartram, D. and Towprayoon, S. (eds).
- IPCC (2019b) Chapter 5.6 Wastewater treatment and discharge. 2019 Refinement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories, <https://www.ipcc-nggip.iges.or.jp/public/2019rf/index.html>.
- Joss, A., Salzgeber, D., Eugster, J., König, R., Rottermann, K., Burger, S., Fabijan, P., Leumann, S., Mohn, J. and Siegrist, H. (2009) Full-Scale Nitrogen Removal from Digester Liquid with Partial Nitrification and Anammox in One SBR. *Environmental Science & Technology* 43(14), 5301-5306.
- Kampschreur, M.J., Temmink, H., Kleerebezem, R., Jetten, M.S. and van Loosdrecht, M.C. (2009) Nitrous oxide emission during wastewater treatment. *Water Research* 43(17), 4093-4103.
- Keeling, C.D., Bacastow, R.B., Bainbridge, A.E., Ekdahl, C.A., Guenther, P.R. and Waterman, L.S. (1976) Atmospheric carbon dioxide variations at Mauna Loa Observatory, Hawaii. *Tellus* 28(6), 538-551.
- Kosonen, H., Heinonen, M., Mikola, A., Haimi, H., Mulas, M., Corona, F. and Vahala, R. (2016) Nitrous Oxide Production at a Fully Covered Wastewater Treatment Plant: Results of a Long-Term Online Monitoring Campaign. *Environmental Science & Technology* 50(11), 5547-5554.
- Kuyper, J., Schroeder, H. and Linnér, B.-O. (2018) The Evolution of the UNFCCC. *Annual Review of Environment and Resources* 43(1), 343-368.
- Kuypers, M.M.M., Marchant, H.K. and Kartal, B. (2018) The microbial nitrogen-cycling network. *Nature Reviews Microbiology* 16(5), 263-276.
- Law, Y., Ye, L., Pan, Y. and Yuan, Z. (2012) Nitrous oxide emissions from wastewater treatment processes. *Philos Trans R Soc Lond B Biol Sci* 367(1593), 1265-1277.
- Lu, H., Chandran, K. and Stensel, D. (2014) Microbial ecology of denitrification in biological wastewater treatment. *Water Research* 64, 237-254.
- Lucker, S., Schwarz, J., Gruber-Dorninger, C., Spieck, E., Wagner, M. and Daims, H. (2015) Nitrotoga-like bacteria are previously unrecognized key nitrite oxidizers in full-scale wastewater treatment plants. *ISME Journal* 9(3), 708-720.
- Ma, C., Jensen, M.M., Smets, B.F. and Thamdrup, B. (2017) Pathways and Controls of N<sub>2</sub>O Production in Nitrification-Anammox Biomass. *Environmental Science & Technology*.

- Manabe, S. and Wetherald, R.T. (1967) Thermal Equilibrium of the Atmosphere with a Given Distribution of Relative Humidity. *Journal of Atmospheric Science* 24, 241-258.
- Marescaux, A., Thieu, V. and Garnier, J. (2018) Carbon dioxide, methane and nitrous oxide emissions from the human-impacted Seine watershed in France. *Science of The Total Environment* 643, 247-259.
- Marias, F., Benzaoui, A., Vaxelaire, J., Gelix, F. and Nicol, F. (2015) Fate of Nitrogen during Fluidized Incineration of Sewage Sludge. Estimation of NO and N<sub>2</sub>O Content in the Exhaust Gas. *Energy and Fuels* 29(7), 4534-4548.
- Marques, R., Rodriguez-Caballero, A., Oehmen, A. and Pijuan, M. (2016) Assessment of online monitoring strategies for measuring N<sub>2</sub>O emissions from full-scale wastewater treatment systems. *Water Research*.
- Mikola, A., Heinonen, M., Kosonen, H., Leppanen, M., Rantanen, P. and Vahala, R. (2014) N<sub>2</sub>O emissions from secondary clarifiers and their contribution to the total emissions of the WWTP. *Water Science and Technology* 70(4), 720-728.
- Montzka, S.A., Dlugokencky, E.J. and Butler, J.H. (2011) Non-CO<sub>2</sub> greenhouse gases and climate change. *Nature* 476(7358), 43-50.
- Oppenheimer, M. and Petsonk, A. (2005) Article 2 of the UNFCCC: Historical Origins, Recent Interpretations. *Climatic Change* 73(3), 195-226.
- Ostrom, N.E. and Ostrom, P.H. (2017) Mining the isotopic complexity of nitrous oxide: a review of challenges and opportunities. *Biogeochemistry* 132(3), 359-372.
- Pan, K.L., Gao, J.F., Fan, X.Y., Li, D.C. and Dai, H.H. (2018) The more important role of archaea than bacteria in nitrification of wastewater treatment plants in cold season despite their numerical relationships. *Water Research* 145, 552-561.
- Pan, Y., van den Akker, B., Ye, L., Ni, B.J., Watts, S., Reid, K. and Yuan, Z. (2016) Unravelling the spatial variation of nitrous oxide emissions from a step-feed plug-flow full scale wastewater treatment plant. *Scientific Reports* 6, 20792.
- Philips, S., Laanbroek, H.J. and Verstraete, W. (2002) Origin, causes and effects of increased nitrite concentrations in aquatic environments. *Reviews in Environmental Science & Bio/Technology* 1(2), 115-141.
- Prather, M.J., Hsu, J., DeLuca, N.M., Jackman, C.H., Oman, L.D., Douglass, A.R., Fleming, E.L., Strahan, S.E., Steenrod, S.D., Sovde, O.A., Isaksen, I.S., Froidevaux, L. and Funke, B. (2015) Measuring and modeling the lifetime of nitrous oxide including its variability. *Journal of Geophysical Research-Atmospheres* 120(11), 5693-5705.
- Prinn, R., Weiss, R., Arduini, J., Arnold, T., DeWitt, H., Fraser, P., Ganesan, A., Gasore, J., Harth, C., Hermansen, O., Kim, J., Krummel, P., Li, S., Loh, Z., Lunder, C., Maione, M., Manning, A., Miller, B., Mitrevski, B., Muhle, J., O'Doherty, S., Park, S., Reimann, S., Rigby, M., Saito, T., Salameh, P., Schmidt, R., Simmonds, P., Steele, L., Vollmer, M., Wang, H.J., Yao, B., Yokouchi, Y., Young, D. and Zhou, L. (2018) History of chemically and radiatively important atmospheric gases from the Advanced Global Atmospheric Gases Experiment (AGAGE). *Carbon Dioxide*

- Information Analysis Center (CDIAC), Oak Ridge National Laboratory (ORNL), Oak Ridge, TN (United States). AGAGE (ed).
- Ravishankara, A.R., Daniel, J.S. and Portmann, R.W. (2009) Nitrous Oxide (N<sub>2</sub>O): The Dominant Ozone-Depleting Substance Emitted in the 21st Century. *Science* 326(5949), 123-125.
- Ren, Y., Ngo, H.H., Guo, W., Ni, B.-J. and Liu, Y. (2019) Linking the nitrous oxide production and mitigation with the microbial community in wastewater treatment: A review. *Bioresource Technology Reports* 7.
- Revelle, R. and Suess, H.E. (1957) Carbon Dioxide Exchange Between Atmosphere and Ocean and the Question of an Increase of Atmospheric CO<sub>2</sub> during the Past Decades *Tellus* 9(1), 18-27.
- Roots, P., Wang, Y., Rosenthal, A.F., Griffin, J.S., Sabba, F., Petrovich, M., Yang, F., Kozak, J.A., Zhang, H. and Wells, G.F. (2019) Comammox Nitrospira are the dominant ammonia oxidizers in a mainstream low dissolved oxygen nitrification reactor. *Water Research* 157, 396-405.
- Soler-Jofra, A., Perez, J. and van Loosdrecht, M.C.M. (2020) Hydroxylamine and the nitrogen cycle: A review. *Water Research* 190, 116723.
- Stein, L.Y. (2018) Insights into the physiology of ammonia-oxidizing microorganisms. *Current Opinion in Chemical Biology* 49, 9-15.
- Stein, L.Y. and Klotz, M.G. (2016) The nitrogen cycle. *Current Biology* 26(3), R94-98.
- Suess, H.E. (1955) Radiocarbon Concentration in Modern Wood. *Science* 122, 415-417.
- Sutka, R.L., Ostrom, N.E., Ostrom, P.H., Breznak, J.A., Gandhi, H., Pitt, A.J. and Li, F. (2006) Distinguishing nitrous oxide production from nitrification and denitrification on the basis of isotopomer abundances. *Applied Environmental Microbiology* 72(1), 638-644.
- Tallec, G., Garnier, J., Billen, G. and Gousailles, M. (2006) Nitrous oxide emissions from secondary activated sludge in nitrifying conditions of urban wastewater treatment plants: effect of oxygenation level. *Water Research* 40(15), 2972-2980.
- Tans, P. and Keeling, R. (2021) Trends in Atmospheric Carbon Dioxide. NOAA/GML (ed).
- Thompson, R.L., Lassaletta, L., Patra, P.K., Wilson, C., Wells, K.C., Gressent, A., Koffi, E.N., Chipperfield, M.P., Winiwarter, W., Davidson, E.A., Tian, H. and Canadell, J.G. (2019) Acceleration of global N<sub>2</sub>O emissions seen from two decades of atmospheric inversion. *Nature Climate Change* 9(12), 993-998.
- Tian, H., Xu, R., Canadell, J.G., Thompson, R.L., Winiwarter, W., Suntharalingam, P., Davidson, E.A., Ciais, P., Jackson, R.B., Janssens-Maenhout, G., Prather, M.J., Regnier, P., Pan, N., Pan, S., Peters, G.P., Shi, H., Tubiello, F.N., Zaehle, S., Zhou, F., Arneeth, A., Battaglia, G., Berthet, S., Bopp, L., Bouwman, A.F., Buitenhuis, E.T., Chang, J., Chipperfield, M.P., Dangal, S.R.S., Dlugokencky, E., Elkins, J.W., Eyre, B.D., Fu, B., Hall, B., Ito, A., Joos, F., Krummel, P.B., Landolfi, A., Laruelle, G.G., Lauerwald, R., Li, W., Lienert, S., Maavara, T., MacLeod, M., Millet, D.B., Olin,

- S., Patra, P.K., Prinn, R.G., Raymond, P.A., Ruiz, D.J., van der Werf, G.R., Vuichard, N., Wang, J., Weiss, R.F., Wells, K.C., Wilson, C., Yang, J. and Yao, Y. (2020) A comprehensive quantification of global nitrous oxide sources and sinks. *Nature* 586(7828), 248-256.
- Toyoda, S. and Yoshida, N. (1999) Determination of nitrogen isotopomers of nitrous oxide on a modified isotope ratio mass spectrometer. *Analytical Chemistry* 71(20), 4711-4718.
- Tyndall, J. (1861) XXIII. On the absorption and radiation of heat by gases and vapours, and on the physical connexion of radiation, absorption, and conduction.—The bakerian lecture. *The London, Edinburgh, and Dublin Philosophical Magazine and Journal of Science* 22(146), 169-194.
- UNEP (1987) Montreal Protocol on Substances that Deplete the Ozone Layer.
- United Nations Climate Change (1997) Kyoto Protocol to the United Nations Framework Convention on Climate Change. Change, U.N.C. (ed).
- United Nations Climate Change (2015) Paris Protocol to the United Nations Framework Convention on Climate Change. Change, U.N.C. (ed).
- Vasilaki, V., Massara, T.M., Stanchev, P., Fatone, F. and Katsou, E. (2019) A decade of nitrous oxide (N<sub>2</sub>O) monitoring in full-scale wastewater treatment processes: A critical review. *Water Research* 161, 392-412.
- Von Schulthess, R. and Gujer, W. (1996) Release of nitrous oxide (N<sub>2</sub>O) from denitrifying activated sludge: verification and application of a mathematical model. *Water Research* 30(3), 521-530.
- Wang, W.C., Yung, Y.L., Lacis, A.A., Mo, T. and Hansen, J.E. (1976) Greenhouse Effects Due to Man-Made Perturbations of Trace Gases. *Science* 194(4266), 685-690.
- Ward, B.B., Arp, D.J. and Klotz, M.G. (2011) Nitrification, American Society of Microbiology.
- Weart, S.R. (2008) The Discovery of Global Warming  
Revised and Expanded Edition, Harvard University Press.
- Wegen, S., Nowka, B. and Spieck, E. (2019) Low Temperature and Neutral pH Define "Candidatus Nitrotoga sp." as a Competitive Nitrite Oxidizer in Coculture with *Nitrospira defluvii*. *Applied Environmental Microbiology* 85(9).
- Wunderlin, P. (2013) Mechanisms Of N<sub>2</sub>O Production In Biological Wastewater Treatment From Pathway Identification To Process Control. Cumulative thesis, ETH Zurich, Zurich.
- Wunderlin, P., Mohn, J., Joss, A., Emmenegger, L. and Siegrist, H. (2012) Mechanisms of N<sub>2</sub>O production in biological wastewater treatment under nitrifying and denitrifying conditions. *Water Research* 46(4), 1027-1037.



- Yan, X., Li, L. and Liu, J. (2014) Characteristics of greenhouse gas emission in three full-scale wastewater treatment processes. *Journal of Environmental Sciences* 26(2), 256-263.
- Yoshida, H., Monster, J. and Scheutz, C. (2014) Plant-integrated measurement of greenhouse gas emissions from a municipal wastewater treatment plant. *Water Research* 61, 108-118.
- Yu, L., Harris, E., Lewicka-Szczebak, D., Barthel, M., Blomberg, M.R.A., Harris, S.J., Johnson, M.S., Lehmann, M.F., Liisberg, J., Muller, C., Ostrom, N.E., Six, J., Toyoda, S., Yoshida, N. and Mohn, J. (2020) What can we learn from N<sub>2</sub>O isotope data? - Analytics, processes and modelling. *Rapid Communications in Mass Spectrometry* 34(20), e8858.
- Zhou, Y., Oehmen, A., Lim, M., Vadivelu, V. and Ng, W.J. (2011) The role of nitrite and free nitrous acid (FNA) in wastewater treatment plants. *Water Research* 45(15), 4672-4682.

# **Chapter 2**

## **Review of "Source category 5D – Wastewater treatment and discharge" in Switzerland**

This chapter has been accepted as expert review by the FOEN of Switzerland:

Luck, M., Gruber, W., Joss, A. (2018) Review of “Source category 5D – Wastewater treatment and discharge” in Switzerland

## Abstract

This review examines the application of the ‘2006 IPCC Guidelines Sector 5 – Chapter 6 - Wastewater treatment and discharge’ for Switzerland. The calculations of nitrous oxide (N<sub>2</sub>O) emissions are carried out correctly as stated in the IPCC Guidelines and no methodological issues were detected. A detailed assessment of the activity data showed that the nitrogen content in the protein consumption is a good indicator of nitrogen loads, but additional factors for unconsumed and industrial nitrogen lead to an overestimation for Switzerland. Furthermore, the sludge amount has not been adjusted in recent years. It is recommended that this amount be calculated via the per capita production. The emission factors used agree with the default values of the IPCC Guidelines and *best practice*. The review of the methane (CH<sub>4</sub>) emissions from wastewater treatment showed methodological differences from the IPCC Guidelines. The biogas usage from wastewater treatment plants (WWTPs) is allocated to the waste sector, unlike in the guidelines. It is stated there that biogas usage in combination with energy recovery should be allocated to Sector 1: Energy and not Sector 5: Waste (IPCC Guidelines 2006). The allocation applied by FOEN is not explained in detail. Furthermore, the activity data of the biogas is incomplete, i.e., the total amount of biogas production is not assessed and described correctly. It is assumed that the percentages for leakages and losses refer to the total production of sewage gas, but the calculations are carried out differently. The emission factors agree with the default values from the IPCC Guidelines and *best practice*. Even though the N<sub>2</sub>O IPCC methodology is applied correctly, the assessment does not represent the situation in advanced and well-managed WWTPs in Switzerland. It is recommended that a country-specific methodology should be used for Switzerland. In this report, a method is proposed which is based on the same activity data but includes nitrogen removal from WWTP and country-specific emissions factors. CH<sub>4</sub> emissions from WWTP, as currently calculated, are not in a plausible numerical range but are attributed to the wrong treatment steps for Switzerland’s advanced and well-maintained plants. Therefore, a different method for CH<sub>4</sub> emissions is proposed in which CH<sub>4</sub> production from the sewers and losses from sewage sludge treatment are implemented.

## 2.1 Introduction

In Switzerland, wastewater is commonly collected in closed sewers. Most of the population are connected to sewer systems (97% in 2016) and their wastewater is treated in centralized plants (FOEN Indicator WS076 2018). Primary (physical) and secondary (biological) treatment is applied at all advanced centralized plants in Switzerland. Tertiary treatment (removal of nutrients such as nitrogen and phosphorus and purification from micropollutants) is widely used and is implemented step-by-step at additional plants (Strähl et al. 2013). During wastewater treatment, the greenhouse gases CO<sub>2</sub>, N<sub>2</sub>O and CH<sub>4</sub> are emitted. These form a part of a country's impact on global climate and climate change. CO<sub>2</sub> emissions are not assessed, as these are of biogenic origin and not included in the inventories (IPCC 2006).

In a WWTP, N<sub>2</sub>O is produced by nitrification and denitrification (Wunderlin 2013). The IPCC GL does not specify any production mechanisms. The discharge of nitrogen to the effluent can lead to the production of N<sub>2</sub>O in natural water bodies and its subsequent emission to the atmosphere. The degradation of organic material under anaerobic conditions leads to the production and emissions of CH<sub>4</sub>. In Switzerland, anaerobic conditions arise mostly in the sewer system, but are rarely found in the water treatment of centralized plants. During sewage sludge treatment, anaerobic processes take place and CH<sub>4</sub> is produced. The biogas (mostly CH<sub>4</sub> and carbon dioxide) can leak and be emitted to the atmosphere in uncovered processes or is captured and used in energy recovery systems. Both scenarios result in CH<sub>4</sub> leakage, which should be considered.

The aim of this report is to review the application of Sector 5 – Chapter 6 - Wastewater treatment and discharge for Switzerland of the 2006 IPCC Guidelines. The National Inventory Report and the calculation files are reviewed and assessed in terms of their completeness, consistency, accuracy, transparency and potential for improvement. This task is further described in the document “Experten-Review: Anforderungen und Anregungen, 05.02.2018” (Bock 2018) and the methodology is described in the IPCC GL (IPCC Guidelines 2006).

The following files were received from FOEN and were assessed in this review:

- EMIS Kommentar: 5 D 1\_5 D 2\_Kläranlagen  
THG\_20170412.pdf
- Guidelines:  
<http://www.ipcc-nggip.iges.or.jp/public/2006gl/vol5.html>
- 5\_Kläranalagen\_\_EMIS-Bericht-ohne-Mesap-Formeln-  
20180308.xls
- NIR\_5\_Waste\_EMIS-Bericht-Ohne-Mesap-  
Formlen\_20180308.xls
- NIR\_CHE\_2018\_Master\_Kap7-5.pdf
- CHE\_2018\_2016\_01032018\_122642\_started.xls

## **2.2 Methodology on the basis of the IPCC guidelines**

The current methodologies for accounting N<sub>2</sub>O and CH<sub>4</sub> emissions from wastewater treatment, as described in the 2006 IPCC Guidelines, are summarized below (IPCC Guidelines 2006).

### **2.2.1 Methodology for N<sub>2</sub>O emissions**

The IPCC accounts for direct emissions from advanced centralized wastewater treatment plants – originating from nitrification and denitrification processes – and emissions from effluent containing nitrogen. It is stated that the emissions from WWTP are minor while N<sub>2</sub>O emissions from wastewater treatment effluent are typically more substantial. A scheme of the perceived emission locations and nitrogen flows is shown in Figure 2.1.

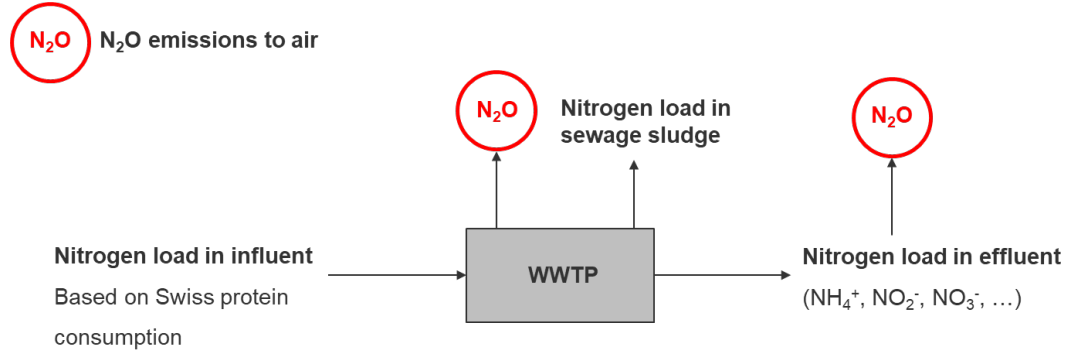


Figure 2.1: Simplified model of nitrogen flows for the assessment of N<sub>2</sub>O emissions according to the IPCC 2006 Guidelines.

Direct emissions from WWTP are assessed on the basis of the number of persons, an additional factor for industry loads and a per capita emission factor (Eq. (2.1)). The emission factor is based on field testing by Czepiel et al. (1995). The amount of nitrogen directly emitted as N<sub>2</sub>O must be considered in the calculation of the effluent nitrogen load.

$$N_2O_{PLANTS} = P * T_{PLANTS} * F_{IND-COM} * EF_{PLANTS} \quad (2.1)$$

Where N<sub>2</sub>O<sub>PLANTS</sub> are the N<sub>2</sub>O emissions from WWTPs (kg N<sub>2</sub>O-N / year), P is the population (# person), T<sub>PLANTS</sub> is the connection rate to WWTP (%), F<sub>IND-COM</sub> Factor for industrial and commercial protein (-), EF<sub>PLANTS</sub> Emission factor for N<sub>2</sub>O from WWTP (kg N<sub>2</sub>O-N/kgN)

The influent nitrogen load is based on the per capita protein consumption multiplied by factors accounting for non-consumed protein and co-discharged industrial protein. It is *good practice* not to account for nitrogen in the sludge removal. However, if the data is available it can be incorporated in the calculation of the effluent nitrogen load (Eq. (2.2)).

$$N_{EFFLUENTS} = (P * Protein * F_{NPR} * F_{NON-CON} * F_{IND-COM}) - N_{SLUDGE} - N_{PLANTS} \quad (2.2)$$

Where P is Population (# person), Protein is per capita protein consumption (kg protein / person / year),  $F_{NPR}$  is the nitrogen in protein (%),  $F_{NON-CON}$  is the factor for non-consumed protein (-),  $F_{IND-COM}$  is the,  $N_{SLUDGE}$  is the nitrogen in sewage sludge (kgN/year), and  $N_{PLANTS}$  is the nitrogen in  $N_2O$  emissions from WWTPs (kg $N_2O$ -N/year)

$N_2O$  emissions from the effluent are estimated from the nitrogen load in the effluent and an EF based on the literature (Eq. (2.3)).

$$N_2O_{EFFLUENTS} = N_{EFFLUENTS} * EF_{EFFLUENTS} \quad (2.3)$$

Where  $N_2O_{EFFLUENTS}$  are the  $N_2O$  emissions from WWTP effluents (kg  $N_2O$ -N / year),  $N_{EFFLUENTS}$  is the nitrogen load in WWTP effluents (kgN/year), and  $EF_{EFFLUENTS}$  is the emission factor for  $N_2O$  from effluents (kg $N_2O$ -N/kgN)

### 2.2.2 Methodology for $CH_4$ emissions

The default method is described in the 2006 IPCC Guidelines and accounts for emissions from wastewater treatment (IPCC Guidelines 2006). Emissions from sludge treatment are neglected in most countries.

The methodology categorizes three methods (Tiers 1 to 3), according to the data available and the methods applied. Tier 1 corresponds to the application of the default AD and EF for countries with limited data. Tier 2 follows the same method but allows country-specific data. The Tier 3 method applies country-specific methods and values for activity parameters and the EF.

In the default method, as shown in Figure 2.2,  $CH_4$  emissions are assessed with a daily BOD (organically degradable matter) load per capita (Eq. (2.4)).

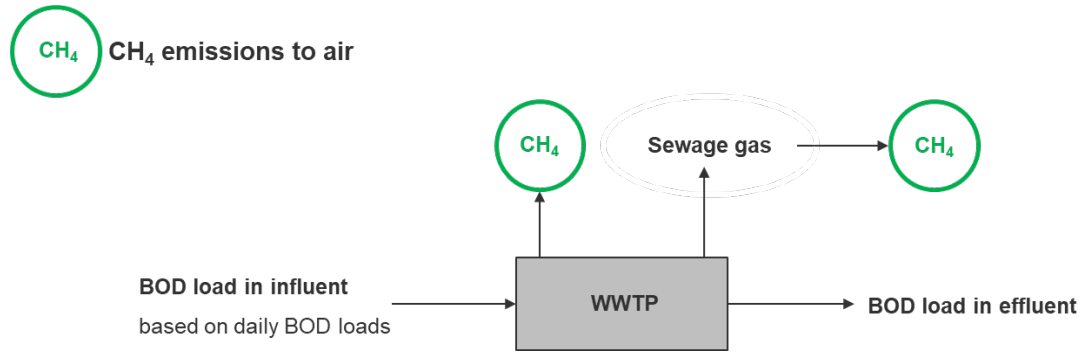


Figure 2.2: Simplified model for assessing  $\text{N}_2\text{O}$  emissions according to the IPCC 2006 Guidelines.

$$TOW = P * BOD * 0.001 * I * 365 \quad (2.4)$$

Where TOW are the total organics in wastewater (kg BOD / year), P is the Population size (# person), BOD is the per capita load of organically degradable material (g BOD / pers. / day), and I is the Correction factor for additional industrial BOD (-).

Different emission factors are assessed depending on the collection and treatment scenario. It is *good practice* to account for three categories: the rural population, the urban high-income population and the urban low-income population, applying different treatment systems to each category (Eq. (2.5) and (2.6)). It is *good practice* to neglect emissions from sewage sludge treatment, as only few countries have sludge-removal and  $\text{CH}_4$  recovery data. If sludge treatment is considered, it is recommended to distinguish between flaring and  $\text{CH}_4$  recovery for energy generation.

$$EF_{PLANTS,j} = B_{PLANTS} * MCF_{PLANTS} \quad (2.5)$$

Where  $EF_{PLANTS,j}$  is the emission factor for  $\text{CH}_4$  emissions from WWTP (kg  $\text{CH}_4$  / kg BOD),  $B_{PLANTS}$  is the maximum  $\text{CH}_4$  production capacity (kg  $\text{CH}_4$  / kg BOD), and  $MCF_{PLANTS}$  is the  $\text{CH}_4$  correction factor (depending on treatment pathway) (-).



$$CH_{4, PLANTS} = \sum (U_i * T_{i,j} * EF_j) * TOW \quad (2.6)$$

Where  $CH_{4, PLANTS}$  are the  $CH_4$  emissions from wastewater treatment (kg  $CH_4$  / year),  $U_i$  is the Fraction of population in group  $i$  in income year (%),  $T_{i,j}$  is the degree of utilisation of treatment pathway or system  $j$  for each income group  $I$  (-), and  $EF_j$  is the emission factor for treatment pathway  $j$  (kg  $CH_4$  / kg BOD).

## 2.3 Application of Guidelines in Switzerland's NIR

In the following section, a check is made to see if the application for Switzerland's National Inventory Report is in line with the Guidelines or whether any different assumptions or methods correspond to the current state of knowledge. It is in line with the Guidelines to implement country-specific methods or data if these are stated and documented correctly.

### 2.3.1 Application of Guidelines for $N_2O$ emissions

The IPCC GL were correctly applied. The protein consumption is based on national statistics and is adjusted yearly. The application of additional factors is explained and is reproducible.  $F_{NON-CON}$  is set to 1.1, as waste is not allowed to be discharged into wastewater, and the default value of 1.25 is used for  $F_{IND-CON}$ . Data on the amount of sewage sludge and its nitrogen content was available for the earlier years (Külling 2002) and was extrapolated from statistics for subsequent years (FOEN 2016)

Summary: The assessment of  $N_2O$  emissions is in line with the IPCC GL.

### 2.3.2 Application of Guidelines for $CH_4$ emissions

Switzerland's NIR applies a Tier 3 country-specific method. Only the pathway of collecting wastewater in covered sewers and treatment in advanced and well-managed WWTPs is considered. All collected wastewater is treated in advanced WWTPs independently of income group. The emissions from uncollected

wastewater are neglected, since the CH<sub>4</sub> emissions are very small due to the low mean temperatures in the uncollected areas and the high connection rate to the wastewater treatment system (FOEN Indicator WS076 2018). The emission factor is based on the maximum CH<sub>4</sub> production capacity and the CH<sub>4</sub> correction factor for well-managed plants. AD and EF are multiplied as in Eq. (2.6).

In Switzerland, biogas handling and usage is also assessed. Different pathways for biogas were implemented and assessed (Eq. (2.7)). The AD of the energy flows from biogas usage is based on the national statistics on renewable energies and expert judgments given to FOEN and provided by them. These emission factors are also based on expert judgements.

$$CH_{4, \text{ BIOGAS}} = \sum P_{j, \text{ BIOGAS}} * EF_{j, \text{ BIOGAS}} \quad (2.7)$$

Where CH<sub>4, BIOGAS</sub> are the CH<sub>4</sub> emissions from biogas usage (kg CH<sub>4</sub> / year), P<sub>j, BIOGAS</sub> is the activity data for biogas usage (TJ / year), and EF<sub>j, BIOGAS</sub> is CH<sub>4</sub> emission factor for biogas usage (kg CH<sub>4</sub> / TJ).

The methodology for CH<sub>4</sub> emissions from wastewater is correctly applied and the underlying assumptions are reasonable and justified. The focus on two pathways for wastewater treatment is explained. Omitting the emissions from uncollected wastewater is reasonable and in line with the IPCC Guidelines.

According to a Tier 3 method, it is reasonable to assess emissions from biogas treatment and usage. However, the Guidelines advise making a distinction between flaring and energy recovery systems (IPCC Guidelines 2006). The applied country-specific methodology proposes five categories for sewage gas: Furnaces, Combined Heat and Power (CHP) installations, torches, leakages and upgrading. In the NIR, emissions from all categories are allocated to Sector 5: Waste (Chapter 6 Wastewater). However, it is clearly stated in the IPCC guidelines that the emissions from biogas usage in furnaces and CHP installations should be allocated to Sector 1: Energy.

Summary: The Calculation of CH<sub>4</sub> emissions from wastewater on WWTP is carried out correctly according to the IPCC Guidelines with country-specific parameters.

Proposed changes: The biogas classifications should be edited and allocated to their respective sectors. Furnace and CHP installations are not part of Sector 5: Waste, and any emissions correspond to the energy production in Sector 1.

## 2.4 Activity data

The activity data provides the basis for calculating emissions. The IPCC Guidelines report default activity data, which can be adapted to a specific country.

### 2.4.1 Activity data of N<sub>2</sub>O emissions

#### *Protein consumption*

The protein consumption is the main indicator of nitrogen loads. Therefore, the estimated protein consumption, according to the IPCC Guidelines, was cross-compared with three independent data sources for nitrogen loads to Swiss WWTP:

- Nitrogen loads in Switzerland in the year 2005 – published by FOEN (FOEN 2010)
- Estimation of nitrogen loads in Switzerland in 2020 – published by FOEN (FOEN 2013)
- Nitrogen loads based on operational data from Swiss WWTP operators, collected in datasets by SWA and FOEN and evaluated by Eawag (Strähl et al. 2013)

The SWA dataset comprises the loads and sizes of a representative selection (around 70% of the person equivalents connected) of Switzerland's WWTPs. The FOEN dataset includes all WWTPs in Switzerland and enables the extrapolation of the SWA dataset to a country-wide level. A total influent load of 46,490 t N/year in 2010 resulted from the analysis. Together with the two reports and considering the different references years, it was concluded that the current application of the IPCC

GL results in significantly higher nitrogen loads in the NIR. The overestimation of nitrogen loads to the WWTPs leads to an overestimation of N<sub>2</sub>O emissions.

In Eq. (2.2), two additional factors ( $F_{\text{IND-COM}}$  and  $F_{\text{NON-CON}}$ ) account for industrial protein while additional loads from garbage account for other nitrogen sources. According to the load estimates noted above, it seems appropriate for industrial protein to be already included in the statistics. Furthermore, it is not allowed to dispose garbage in wastewater in Switzerland. Both factors therefore seem too large for the Swiss situation. It is therefore proposed that both factors  $F_{\text{IND-COM}}$  and  $F_{\text{NON-CON}}$  be set to 1.0, which is in the range provided by the IPCC GL. The application of the adjusted factors is shown in Table 2.1 and results in comparable nitrogen loads.

Table 2.1: Comparison of nitrogen accounting.

<b>Nitrogen load to WWTP</b>	<b>N<sub>INFLUENT</sub> load (t/year)</b>	<b>N<sub>EFFLUENT</sub> load (t/year)</b>
Nload in 2005 from report (FOEN 2010)	43,200	26,000
Nload in 2020 from report (FOEN 2013)	47,900	25,390
Nload in 2010 from datasets (Strähl et al. 2013)	46,490	23,830
Nload in 2015, Eq. (2.2) calculation according to IPCC GL	65,780	47,550
Nload in 2015, Eq. (2.2) calculation with $F_{\text{IND-COM}}$ and $F_{\text{NON-CON}}$ set to 1.0	47,840	-

### *Sewage sludge*

The AD on sewage sludge is based on a study of Külling (2002) for the years 1990 to 1999 and subsequently on the official waste statistics of FOEN, which indicate the total production and per capita values (FOEN 2016). Since 2006, the amount of sewage sludge was not updated in the waste statistics and was therefore not adjusted in the calculations for the NIR. 2011 was the last year in which a per capita value was stated. The nitrogen content of the sewage sludge was examined in the same study of Külling (2002). There is no updated information on this topic in the literature.

Summary: There is good data coverage on protein consumption, population size, connection rate and sewage sludge (in the years 1990 – 1999). However, the data has

not been cross-checked with independent sources, which suggests an adaption of the default values.

Proposed changes: The additional factors  $F_{\text{IND-COM}}$  and  $F_{\text{NON-CON}}$  should be set to 1.0, as the current values overestimate the situation in Switzerland. The proposed changes affect the assessment of direct emissions from WWTP and indirect emissions from the discharges. However, if these changes are implemented, it is necessary to state in the NIR that industrial wastewaters are included in the protein consumption and not via additional factors.

Even though no recent studies or literature on the sludge amount and nitrogen content are available, it is clearer for the sludge production to be assessed with a per capita production. The inclusion of population change allows a more realistic assessment of sludge production in the NIR. A per capita sludge production of 26 kg per person and year based on the 2011 waste statistics should be applied from 2010 onwards (FOEN 2016). If the sludge amount is adapted in the assessment, it must also be corrected in the other categories of Sector 5: Waste and Sector 1: Energy.

#### **2.4.2 Activity data of CH<sub>4</sub> emissions**

##### *Organics load in wastewater (TOW)*

The calculation of TOW loads via daily BOD production per person is reasonable and correctly executed. The values match an expert judgment and the literature values.

It is correctly explained that the uncollected wastewaters are treated in alternative systems. Only remote and sparsely populated regions are permitted to treat their wastewaters in such systems (FOEN 2018). The high connection rate, 97% in 2013, to the sewer system also shows this (FOEN Indicator WS076 2018). The mean temperatures in these regions only rarely exceed 15°C so that CH<sub>4</sub> production is limited by the low temperatures. CH<sub>4</sub> emissions from these systems can be neglected.

##### *Biogas production and usage*

The AD of biogas is divided into several categories: Furnace, CHP installations, upgrading, torches and leakages. The first three categories are available from national statistics of the Federal Office for Energy (FOE 2017). The other two

categories (torches and leakages) are not assessed in the statistics. These are extrapolated by expert judgements, which are provided by FOEN, for the total biogas production. The total gas production includes five categories: Furnace, CHP installations, upgrading, torches and leakages

However, the execution of the calculation currently differs from the stated methodology. As seen in Table 2.2, the categories (4) torches and (5) leakages are calculated from only a few categories, rather than from the total biogas production. In this review, the conversion of the gas production, reported in the statistics, to the total gas production is revised and corrected according to Table 2.2.

Table 2.2: Mass balance for biogas in 2016: The middle column indicates the status quo, while the right column shows the proposed corrections.

Category number	Description	Current mass balance sewage gas	Review: Mass balance sewage gas
-	<i>In Statistics:</i>		
(1)	Furnace (TJ/y)	370.8	370.8
(2)	CHP installations (TJ/y)	1,278.0	1,278.0
(3)	Upgrading (TJ/y)	526.36	526.36
-	Biogas in statistics (TJ/y)	(1) + (2) + (3) = 2,175.2	(1) + (2) + (3) = 2,175.2
-	Biogas prod. In energy statistics (%)	100%	100% - 2% - 0.75% = 97.25%
-	Total production (TJ/y)	2,175.2	2,175.2 / 97.25% = 2,236.7
(4)	Torches (TJ/y)	(1) + (2) * 2% = 33.0	(2,236.7) * 2% = 44.7
(5)	Leakages (TJ/y)	(1) + (2) * 0.75% = 12.4	(2,236.7) * 0.75% = 16.8
-	Total biogas in assessment (TJ/y)	(1) + (2) + (3) + (4) + (5) = 2,220.5	(1) + (2) + (3) + (4) + (5) = 2,236.7

Summary: The TOW loads match the default value from the IPCC Guidelines and a cross-check with the literature. However, the biogas is not accounted correctly. The missing categories are based on only part of the biogas production.

Proposed changes: The correct application is to refer the two categories of torches and leakages to the total biogas production (all five categories.).

## 2.5 Emission factors

The multiplication of the AD and EF results in emissions. The given EF mostly refer to a general case and are therefore often adapted to a country's specific application. In the following section, the emission factors given by the IPCC Guidelines and country-specific factors for Switzerland are reviewed.

### 2.5.1 N<sub>2</sub>O emission factors

Switzerland applies the default emission factors  $EF_{\text{PLANTS}}$  and  $EF_{\text{EFFLUENT}}$  (IPCC Guidelines 2006). These are *best practice*. A literature review and the data evaluation from the running measurement campaign performed by Eawag showed that the current value of  $EF_{\text{PLANTS}}$  proposed by the IPCC GL is too low and underestimates the emissions significantly, as seen in Daelman et al. and Kosonen et al. (Daelman et al. 2015, Kosonen et al. 2016) .

The default emission factors are also applied in Germany (FEA Germany 2017). However, Austria's Environmental Agency requested measurement campaigns on different WWTP over varying time spans (Parravicini et al. 2015). The result was a much higher emission factor of 43 g N<sub>2</sub>O / person / a (an increase by a factor of 13 compared to IPCC GL). This shows how essential monitoring campaigns and an assessment of the  $EF_{\text{N}_2\text{O}}$  are. In future, the necessary input for the revision of the emission factors will be generated from a measurement campaign run by Eawag in collaboration with FOEN. The preliminary results are stated in Chapter 8.1.2 and suggest a higher EF. The final results are expected in 2020. The default EF for N<sub>2</sub>O emissions from the wastewater effluent was reviewed by the expert judgement of M.

Lehmann. He stated that the varying conditions made a generalisation difficult but he would nevertheless estimate an EF in the same range (Lehmann and Gruber 2018).

Summary: As only a preliminary emission factor for Switzerland can currently be proposed, it is *best practice* to use the default IPCC GL value.

### **2.5.2 CH<sub>4</sub> emission factors**

The CH<sub>4</sub> Emission factor (EF<sub>CH<sub>4</sub></sub>) is based on the maximum CH<sub>4</sub> production capacity and the CH<sub>4</sub> correction factor. It is *good practice* to use the default value. The CH<sub>4</sub> correction factor depends on the treatment system and its management. The Swiss treatment systems contain (mostly) aerobic processes and are well managed. Therefore, a default value of 0.05 (-) is applied and explained in detail.

The EF values for biogas usage are based on an expert judgment, correspond to *best practice* and are reasonable.

Summary: The emission factors are reproducible and understandable.



## 2.6 Emissions

The emissions according to Switzerland's NIR and with the proposed changes are shown in the following section and the comparison with other countries is discussed.

### 2.6.1 N<sub>2</sub>O emissions

The emissions from Switzerland's NIR and those including the recommended changes for the year 2016 are shown in Figure 2.3. The changes neglect the additional factors for industry nitrogen and unconsumed protein in the influent nitrogen and couple the sludge amount to the population size. It is proposed to change the emission factors according to the conclusions of the ongoing measurement campaigns by Eawag and these are therefore not implemented in this comparison. The monitoring campaigns and their necessity are described in Chapters 5.1 and 8.1.2.

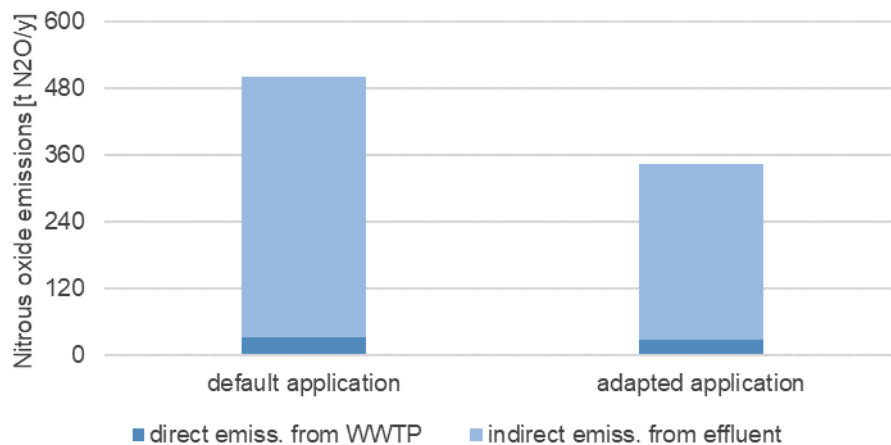


Figure 2.3: Assessment of N<sub>2</sub>O emissions in the year 2016 - The data from Switzerland's NIR are displayed on the left and the implementation of the proposed changes on the right.

The IPCC GL assumes that indirect emissions from the effluent are much higher than direct emissions, so that only indirect emissions are of interest for advanced centralized wastewater treatment plants. However, the comparison with Austria's NIR showed a flipped ratio if higher EF values for direct emissions are applied (Umweltbundesamt Austria 2017). The same trend of a flipped ratio is expected for Switzerland, which again points to the fact that direct emissions are greatly underestimated.

### 2.6.2 CH<sub>4</sub> emissions

CH<sub>4</sub> emissions from the WWTP are dominant compared to biogas handling and treatment, as can be seen in Figure 2.4. The proposed changes adapt the mass balance of biogas production and usage. They do not alter the results significantly but ensure consistent calculations.

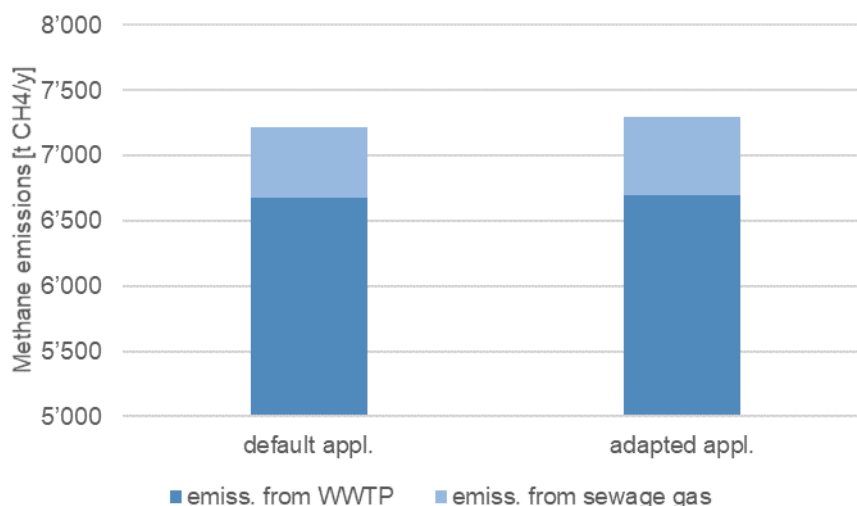


Figure 2.4: Assessment of CH<sub>4</sub> emissions in the year 2016 - The data from Switzerland's NIR are displayed on the left and the implementation of the proposed changes on the right.

Different country-specific methodologies can be found in the Austrian and German NIRs (FEA Germany 2017, Umweltbundesamt Austria 2017). The main difference between these and Switzerland's assessment is that CH<sub>4</sub> emissions from advanced WWTP are neglected. However, Germany and Austria also include the wastewater not treated at centralized advanced plants in the assessment and calculate the emissions accordingly. This share of wastewater is treated under anaerobic conditions. Both differences to Switzerland lead to the reporting of significantly lower CH<sub>4</sub> emissions.

However, it is correctly stated in Switzerland's NIR that the wastewater not connected to centralized WWTP is treated at low temperatures, so that CH<sub>4</sub> production is minimal and can be neglected (FOEN 2017, 2018). It is therefore proposed to retain the current approach for Switzerland.

## 2.7 Documentation

This review of the documentation examines whether the relevant data and information provided by FOEN are accessible and the assumptions are explained.

### 2.7.1 N<sub>2</sub>O documentation

The documentation is complete and allows the calculations to be reproduced.

If the proposed changes are implemented, the update of the additional factors ( $F_{\text{NON-CON}}$  and  $F_{\text{IND-CON}}$ ) must be explained and mentioned as it corresponds to a deviation from the default value. Furthermore, it must be stated explicitly that industrial wastewater is included in the protein consumption and not accounted for by an external factor ( $F_{\text{IND-CON}}$ ).

Summary: The documentation is detailed and reproducible. Further changes to the default methodology must be stated clearly.

### 2.7.2 CH<sub>4</sub> documentation

The documentation in the “EMIS-Kommentar” is very specific and accurate for the assessment of CH<sub>4</sub> emissions from WWTP (FOEN 2018).

The source of AD for biogas is explained in the documentation. However, the calculations of the total biogas production and its separation into the categories of furnace, CHP installations, torches, leakages and upgrading are not stated in the commentary report (FOEN 2018). The calculation files (5\_Kläranalagen\_\_EMIS-Bericht-ohne-Mesap-Formeln-20180308.xls) are more specific about the AD and EF of the biogas and allow the reproduction and disclosure of errors. In the “EMIS-Kommentar”, an equation with the EF of biogas and a normalized population is stated but never applied in the calculation files.

Summary: The documentation of AD and EF of CH<sub>4</sub> from wastewater treatment is accurate and complete.

Proposed changes: The assumptions and calculations of the biogas emissions should be included in the “EMIS-Kommentar” and explained properly.

## 2.8 Proposal of revised methodology

In this section, additional methods are proposed which describe the production and emission pathways of GHG more accurately. The aim was to develop methods based on the IPCC GL but refined for Switzerland. These are therefore labelled as *country-specific* according to the guidelines. Nevertheless, one goal was to design methodologies which are transferrable to other countries. The methods are based on literature reviews, expert judgements and running monitoring campaigns, so the finalisation of the proposals is pending until these campaigns have been concluded.

### 2.8.1 Country-specific N<sub>2</sub>O methodology

The proposed methodology for N<sub>2</sub>O assessment is based on the protein consumption as the main indicator of nitrogen flows. These are the same indicator and activity data as in the IPCC GL. However, the mass balance of nitrogen on a WWTP is constructed differently in order to include the nitrogen removal via nitrification and denitrification.

#### *Activity data on nitrogen loads and mass balance*

The calculation of the nitrogen load in the wastewater treatment is similar to the IPCC GL. The additional factors for industrial and non-consumed protein are omitted, as these are not representative for the case of Switzerland, shown in Section 2.4.1. The calculation is shown in Eq. (2.8).

$$N_{INFLUENT} = T_{PLANTS} * Protein * F_{NPR} \quad (2.8)$$

Where  $N_{INFLUENT}$  is the influent nitrogen Nload (kg N / year),  $T_{PLANT}$  is the connection rate to WWTP (-), Protein is the Protein consumption (kg protein / year), and  $F_{NPR}$  is the nitrogen in protein (%).

It is proposed that no differentiation be made between plants with or without nitrification or denitrification. N<sub>2</sub>O emissions are only expected from plants with nitrification and denitrification treatment steps. However, as carbon-only removal

plants could be nitrifying in the summer, no simple assessment of the plants could be made and it is most practical not to distinguish between the types of plants.

The generalized mass balance for nitrogen on a WWTP includes nitrogen removal as a pathway which is not integrated in the IPCC GL approach. A simplified but correct way is to determine this value via the nitrogen removal rate based on data from the operators. The effluent load is assessed without specifying sludge amounts and nitrogen content in sludge: it is shown in Figure 2.5 and Eq. (2.9).

$$N_{\text{EFFLUENT}} = N_{\text{INFLUENT}} * (1 - r_{\text{NITROGEN REMOVAL RATE}}) \quad (2.9)$$

Where  $N_{\text{EFFLUENT}}$  is the Effluent nitrogen load (kg N / year),  $N_{\text{INFLUENT}}$  is the influent nitrogen Nload (kg N / year), and  $r_{\text{NITROGEN REMOVAL RATE}}$  is the average nitrogen removal rate - data series is displayed above (-)

The proposed nitrogen removal rate (Eq. (2.9)) is based on a 1993 report on WWTP in CH (Meyer et al. 1996) and on two datasets from SWA and FOEN (Strähl et al. 2013). The assessment of the WWTP dataset by SWA, which contains the influent and effluent quality of about two thirds of the plants for the year 2010, resulted in an average nitrogen removal rate for Switzerland. The available influent and effluent data were evaluated and resulted in an average nitrogen removal rate of 48.7% over all plants in 2010 (Strähl et al. 2013).

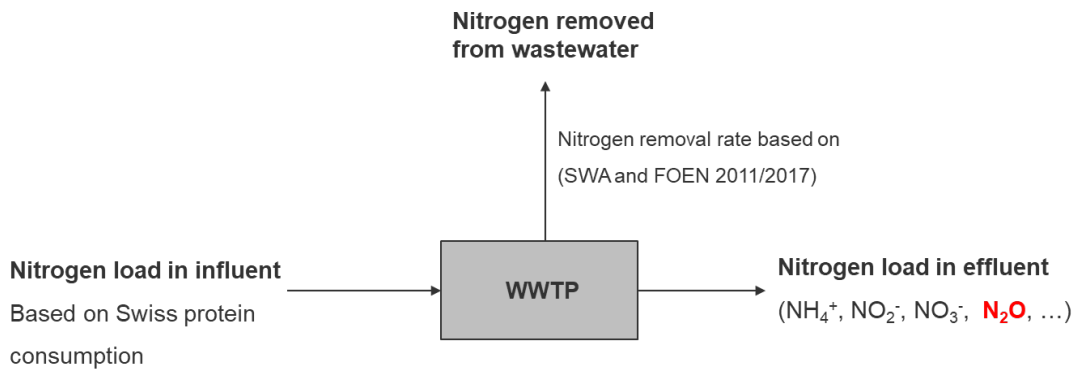


Figure 2.5: Simplified nitrogen balance on WWTP for climate reporting.

The literature values for 1993 and the dataset for 2010 for the nitrogen removal rate were used to inter- and extrapolate a time series (Table 2.3) (Meyer et al. 1996, Strähl et al. 2013). The expert stated that the relevant processes are gradually being implemented at plants and no change in legislation had taken place. Both factors result in a slow change of the removal rate and a linear function can be applied according to the expert. The full data series can be found in the file “Expert Review - additional data” under number 1. Additional information can be found in the notes from the expert discussion with Hansruedi Siegrist (Siegrist and Luck 2018).

Table 2.3: Data series on the nitrogen removal rate for Switzerland: (1993\* and 2010\* are set points – the other years are linearly extrapolated).

1990	1993*	2000	2005	2010*	2015	2020	2025	2030
25.3%	28.9%	37%	42.9%	48.7%	54.6%	60.4%	66.3%	72.1%

#### *Emission factor for $N_2O$ in the wastewater sector*

Three pathways for  $N_2O$  emissions from wastewater treatment are considered, as shown in Figure 2.6. The  $N_2O$  produced onsite is either emitted through stripping into the air or remains in the wastewater stream and can subsequently be emitted to the air from the effluent. The third pathway is the production of  $N_2O$  outside the plant from nitrogen in the effluent. The calculation of the emissions is shown in Eq. (2.10), (2.11), and (2.12); the subscripts firstly indicate the production and secondly the emission location.

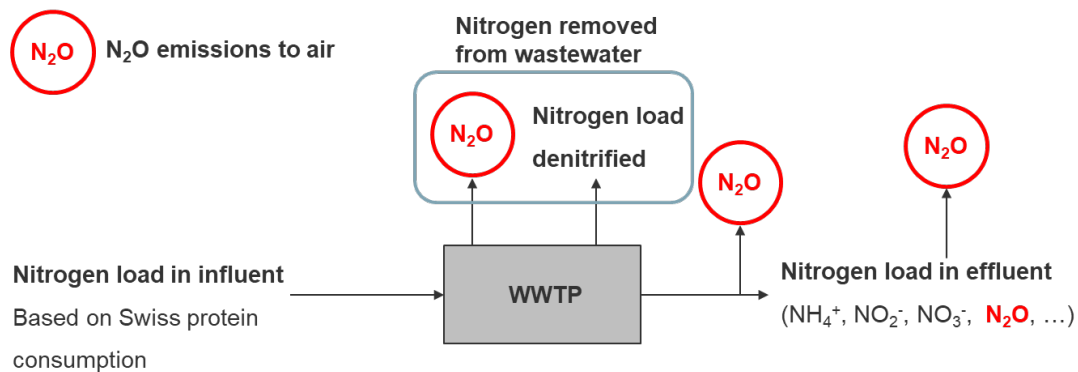


Figure 2.6:  $N_2O$  emission pathways in the proposed methodology:  $N_2O$  emissions from wastewater treatment, emissions from  $N_2O$  in the effluent,  $N_2O$  production and emissions in the effluent.

The  $EF_{N_2O, PLANT}$  for  $N_2O$  from WWTP in the IPCC GL (IPCC Guidelines 2006) is based on a paper from the United States (Czepiel et al. 1995) in which field measurements at a plant with only nitrification processes are evaluated. A cross-check with the literature showed that the default value is significantly lower than recent long-term studies suggest (Daelman et al. 2015, Kosonen et al. 2016). The limited availability of the literature data and methodical differences in the strategies do not allow the revision of emission factors based on the available literature.

Long-term monitoring campaigns by Eawag are consequently planned and in operation. The goal is to identify an EF ( $kg\ N_2O-N / kg\ N_{INFLUENT}$ ) based on monitoring campaigns extending over at least ten years. The selection of WWTP should be representative of the WWTP landscape in Switzerland and allow the extrapolation to a national EF. The preliminary results, as shown in Table 2.4, indicate much higher emission factors than the default values in the guidelines. Each EF constitutes the aggregation of the monthly means to annual emission factors (containing each month once).

Table 2.4: Compilation of measurement campaigns used to identify a preliminary emission factor (the EF identified with a star \* are preliminary values from running monitoring campaigns).

WWTP	Literature	Timespan	EF (%)
<i>Campaigns by Eawag/ETHZ</i>			
Altenrhein SG	-	Dec. 2015 – Mar. 2017	1.4
Emmen, REAL LU	-	Mar. 2014 – Sep. 2015	0.8
Bazenheid SG	-	Jan. 2018 – Jul. 2018	2.5 *
Werdhölzli ZH	-	Aug. 2016 – Jul. 2018	0.5 *
<i>Published campaigns:</i>			
Kralingseveer WWTP	Daelmann, et al., 2013	Oct. 2010 – Dec. 2011	2.8
Viikinmäki WWTP	Kosonen et al., 2016	Jul. 2012 – Jun. 2013	1.9
<i>IPCC guidelines:</i>			
Durnham WWTP	Czepiel et al. (1995)	Apr. 1993 – Aug. 1993	0.032

The  $EF_{N_2O, PLANT-EFFLUENT}$  is assessed from the equilibrium of dissolved to stripped  $N_2O$  and refers to the total influent nitrogen load. The emission factor is based on the current measurement campaign at WWTP Bazenheid and data from February 2018 to July 2018. The result is a concentration of  $0.03\ g\ N_2O-N / m^3$  in the effluent and an emission factor  $EF_{N_2O, PLANT-EFFLUENT}$  of  $0.0008\ kg\ N_2O-N\ per\ kg\ N_{EFFLUENT}$ . This

emission factor is based on one measurement campaign by Eawag and could be validated by a second campaign at the end of the project, although no significant changes are expected. It is assumed that the EF is constant and can also be applied in the future. More detailed explanations and the full data series can be found in the additional data under Number 2 (Luck 2018).

$$N_2O_{PLANT} = N_{INFLUENT} * EF_{N2O, PLANT} \quad (2.10)$$

Where  $N_2O_{PLANT}$  are the  $N_2O$  emissions from WWTP (kg  $N_2O$ -N / year),  $N_{INFLUENT}$  is the influent nitrogen Nload (kg N / year),  $EF_{N2O; PLANT}$  is the emission factor for onsite production and emissions (kg  $N_2O$ -N / kg N)

$$N_2O_{PLANT-EFFLUENT} = N_{INFLUENT} * EF_{N2O, PLANT-EFFLUENT} \quad (2.11)$$

Where  $N_2O_{PLANT-EFFLUENT}$  are the  $N_2O$  emissions to the effluent and subsequently to air (kg  $N_2O$ -N / year),  $N_{INFLUENT}$  is the influent nitrogen Nload (kg N / year), and  $EF_{N2O; PLANT-EFFLUENT}$  is the emission factor for onsite production and emissions to effluent (kg  $N_2O$ -N / kg N).

$$N_2O_{EFFLUENT-WATERBODY} = N_{EFFLUENT} * EF_{N2O, EFFLUENT-WATERBODY} \quad (2.12)$$

Where  $N_2O_{EFFLUENT-WATERBODY}$  are the  $N_2O$  emissions from effluent (kg  $N_2O$ -N/year),  $N_{EFFLUENT}$  is the Effluent nitrogen load (kg N / year), and  $EF_{N2O; EFFLUENT-WATERBODY}$  is the emission factor for production and emissions in effluent (kg  $N_2O$ -N / kg N).

No updated information on the emission factor from the discharge ( $EF_{N2O, EFFLUENT-WATERBODY} = 0.005$  kg  $N_2O$ -N /kg  $N_{EFFLUENT}$ ) is available. However, it is *best practice* to use the default value of the IPCC GL and this is therefore also proposed here (IPCC Guidelines 2006). The multiplication of AD and EF is displayed in Eq. (2.10), (2.11), and (2.12).



### 2.8.2 Country-specific CH<sub>4</sub> methodology

The wastewater collected in Switzerland is treated under aerobic conditions in well-managed plants. No significant CH<sub>4</sub> emissions from the biological treatment steps are therefore expected. This assumption has been validated by the ongoing monitoring campaign run by Eawag at WWTP Uster, where only about 0.001 kg CH<sub>4</sub> per kg COD<sub>INFLEUNT</sub> is emitted.

However, CH<sub>4</sub> is produced and possibly emitted in the covered sewer system at the WWTP in the grit chamber, where the wastewater is aerated for the first time. In addition, the emission of CH<sub>4</sub> from sludge treatment & storage and from biogas handling and usage also have to be considered. The revised methodology for CH<sub>4</sub> emissions therefore includes CH<sub>4</sub> production in the sewer as well as emissions from sewage sludge treatment and usage, as shown in Figure 2.7.

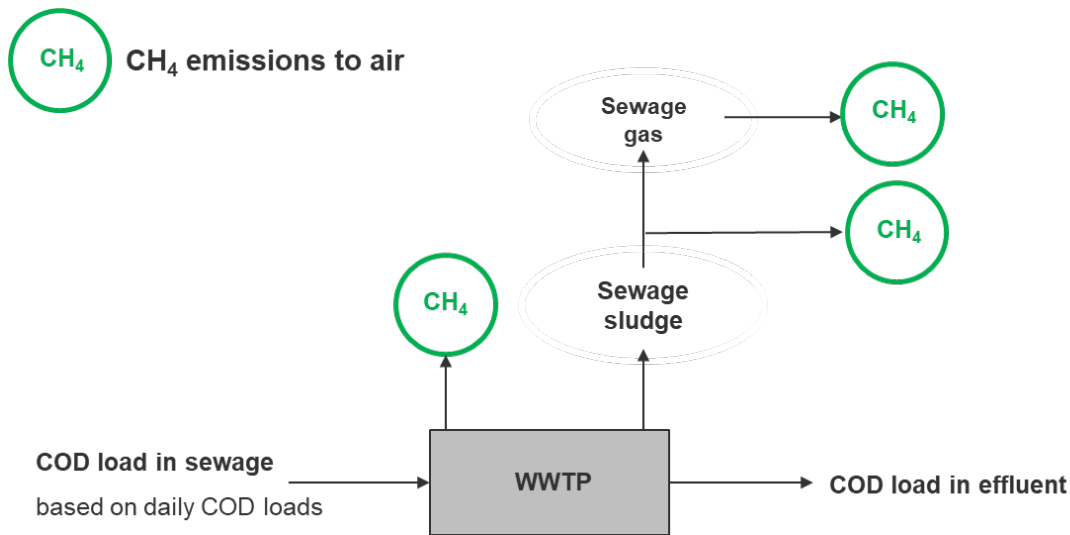


Figure 2.7: CH<sub>4</sub> emission pathways in the proposed methodology: CH<sub>4</sub> emissions from the sewer system, CH<sub>4</sub> emissions from sludge treatment and biogas usage.

#### *Activity data of organic load in sewer and sewage sludge treatment*

The main difference is that this method considers the organically degradable material (TOW) per person in the sewer and not in the WWTP because the production of CH<sub>4</sub> takes place in the sewer and not in the WWTP. It is assumed that the load of organic material in the sewer is similar to the influent of the WWTP and the same indicators as in the IPCC GL can be used, as shown in Eq. (2.13).

$$TOW_{SEWER} = P * T_{SEWER} * COD * 0.001 * I * 365 \quad (2.13)$$

Where TOW are the total organics in wastewater (kg COD / year), P is the Population size (# person),  $T_{SEWER}$  is the Connection rate to sewer system equal to the connection rate to WWTP (-), COD is the per capita load of organically degradable material (g COD / pers. / day), and I is the Correction factor for additional industrial COD (-).

The organically degradable material is indicated in kg BOD in the currently applied methodology, whereas it is reported in kg COD in the proposed methodology - Eq. (2.13). The units were changed, as the influent and effluent loads in Switzerland are commonly indicated in COD. The two different units of organically degradable material can be converted on the basis of 2.0 kg COD / kg BOD.

The leakages from the treatment and storage of sewage sludge are assessed via the loss of sewage gas. An expert judgement allowed a time series to be developed in which the change from open to covered sludge storage is considered (Siegrist and Luck 2018). The expert stated that open sludge storage could result in emissions of up to 10% of the gas production. The study of Cunningham et al. (2015) showed similar emissions and confirms the expert statement.

A data series was developed together with an assumption on the number of open and covered plants. It shows the decrease of the total EF as a result of the decrease in the number of WWTP with open sludge storage (Table 2.5). The data can be extrapolated to the future, but should be reviewed in the period 2025 – 2030. The full data series is displayed in the additional file under Number 3 (Luck 2018).

Table 2.5 Data series on losses from sludge treatment and storage: (1990 and 2015 are set points based on expert judgements – other years were linearly extrapolated; the time series ends in 2030)

1990	1995	2000	2005	2010	2015	2020	2025	2030
10.0%	9.0%	8.0%	7.0%	6.0%	5.0%	4.0%	3.0%	2.0%

The activity data from biogas production is similar to the approach currently in use. The main difference is that the “leakages” category refers not only to leakages from

captured biogas but includes those from sludge treatment, as discussed in the paragraph above.

The biogas usage in the Federal statistics (FOE 2017) only reports part of the total production. The total production is calculated by dividing the reported biogas production by the reporting percentage (2.15). This percentage is obtained from the assumptions on “leakages & torches” and is listed in Table 2.6. The full dataset is included in the additional data under Number 3 (Luck 2018). An example of this calculation was discussed and executed in Section 2.4.2 and shown in Table 2.2.

$$P_{TOT, SEWAGE GAS} = \frac{\sum P_{j, SEWAGE GAS}}{T_{Rep}} \quad (2.14)$$

Where  $P_j$  Reported usages in Federal statistics (TJ / year), and  $T_{REP}$  is the percentage of biogas reported (%)

Table 2.6 Data series on percentage of biogas reported in national statistics (FOE 2017): (Leakages from sludge treatment & stocking and burned biogas in torches are not included in the statistics, which are therefore divided by this factor to calculate the full biogas production)

1990	1995	2000	2005	2010	2015	2020	2025	2030
88.0%	89.0%	90.0%	91.0%	92.0%	93.0%	94.0%	95.0%	96.0%

#### *Emission factor for CH<sub>4</sub> in wastewater sector*

Studies of the CH<sub>4</sub> production in sewers are rare and not transferable to Switzerland due to different climatic and geographical conditions. The EF for CH<sub>4</sub> production in the sewers is therefore based on laboratory studies (Liu et al. 2015a). A mean concentration of CH<sub>4</sub> was assumed on the basis of this compilation of CH<sub>4</sub> concentrations in the raw wastewater. To determine the emission factor, the mean concentration was related to the COD concentration in the sewer, which is listed in textbooks (Gujer 2007).

The resulting emission factor of 0.015 kg CH<sub>4</sub> per kg COD was reviewed with the expert judgement of Hansruedi Siegrist (Siegrist and Luck 2018). He stated that he would expect 5% of COD in the raw wastewater to be transformed to CH<sub>4</sub>, which

results in a similar EF of 0.0125 kg CH<sub>4</sub> per kg COD. CH<sub>4</sub> emissions from wastewater treatment and sewers were estimated according to Eq. (2.15).

$$CH_{4, \text{ WASTEWATER}} = TOW_{\text{SEWER}} * EF_{CH_4, \text{ WASTEWATER}} \quad (2.15)$$

Where CH<sub>4, WASTEWATER</sub> are the total CH<sub>4</sub> emissions from sewer system/WWTP (kg CH<sub>4</sub> / year), TOW are the total organics in wastewater (kg COD / year),

The emission factors of different biogas usage are the default values provided by FOEN. They are based on an expert judgement, adapted to Switzerland and applied as in Eq. (2.16) (FOEN 2018).

$$CH_{4, \text{ BIOGAS}} = \sum P_{j, \text{ BIOGAS}} * EF_{CH_4, \text{ BIOGAS}, j} \quad (2.16)$$

Where CH<sub>4, BIOGAS</sub> are the CH<sub>4</sub> emissions from biogas usages / leakages (kg CH<sub>4</sub> / year), P<sub>j, BIOGAS</sub> is the biogas usage (TJ / year), and EF<sub>CH<sub>4</sub>, BIOGAS</sub> is the emission factor for CH<sub>4</sub> from biogas usage (kg CH<sub>4</sub> / TJ)

### 2.8.3 Discussion of proposed methodologies

The new methodology proposed in this review assesses higher N<sub>2</sub>O emissions than the currently applied IPCC GL. The preliminary results from the monitoring campaigns in particular indicate higher N<sub>2</sub>O emissions from nitrification and denitrification. Emissions from dissolved N<sub>2</sub>O in the effluent and later emitted to the air are only assessed in the proposed methodology and therefore increase the difference.

The emissions from N<sub>EFFLUENT</sub> have decreased as the load of nitrogen in the effluent was reduced due to the nitrogen removal step, which removes more nitrogen than in the currently applied assessment.

The assessment of methane emissions according to the new proposed methodology results in more CH<sub>4</sub> being released to the atmosphere. Coincidentally the change in the activity data from BOD to COD and in the emission factor from EF<sub>CH<sub>4</sub>, WWTP</sub> to EF<sub>CH<sub>4</sub>, SEWER</sub> offset each other, so the emissions are the same in both methods.

However, the calculation of leakages from sludge treatment and storage increases the total CH<sub>4</sub> emissions. In 2016, the total emissions increased by a factor of 1.27 in the proposed method compared to the application of IPCC GL, shown in Figure 2.8. The difference between the two approaches decreased from 1990 to 2016, as the biogas usage increased and the leakages decreased.

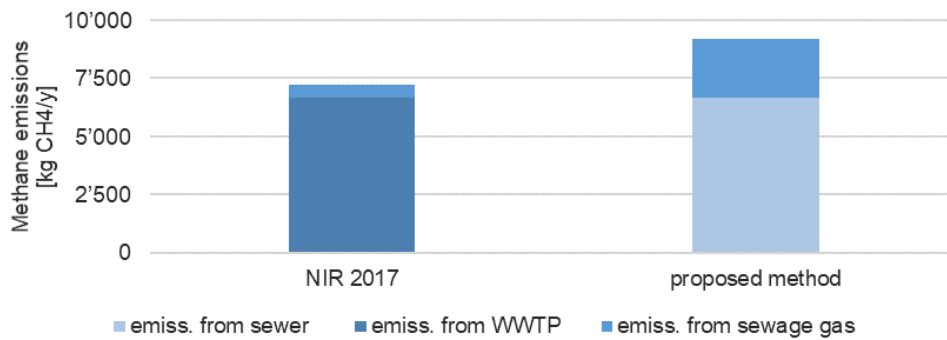


Figure 2.8: Impact of proposed methodology: Methane emissions based on IPCC GL and the new methodology of 2016.

## 2.9 References

- Bock, M. (2018) Nationale Expertenreview - Anforderungen und Anregungen, Federal Office for the Environment, Bern.
- Cunningham, M., Fink, A. and Baier, U. (2015) Methanemissionen Auf Kläranlagen. Aqua & Gas 3/2015.
- Czepiel, P., Crill, P. and Harriss, R. (1995) Nitrous oxide emissions from municipal wastewater treatment. Environmental Science & Technology, 2352-2356.
- Daelman, M.R., van Voorthuizen, E.M., van Dongen, U.G., Volcke, E.I. and van Loosdrecht, M.C. (2015) Seasonal and diurnal variability of N<sub>2</sub>O emissions from a full-scale municipal wastewater treatment plant. Science of The Total Environment 536, 1-11.
- FEA Germany, F.E.A. (2017) National Inventory Report for the German Greenhouse Gas Inventory 1990 – 2015.
- FOE (2017) Schweizerische Statistik der erneuerbaren Energien. Federal Office for Energy FOE (ed), Bern.
- FOEN (2010) Stickstoffflüsse in der Schweiz 2005. Federal Office for the Environment FOEN (ed), Bern.
- FOEN (2013) Stickstoffflüsse in der Schweiz 2020. Federal Office for the Environment FOEN (ed), Bern.
- FOEN (2016) Annual statistics on waste generation in Switzerland. Federal Office for the Environment FOEN (ed), Bern.
- FOEN (2017) Switzerland's National Inventory Report 2017. Federal Office for the Environment FOEN (ed), Bern.
- FOEN (2018) EMIS-Kommentar zu Sektor 5D. Federal Office for the Environment FOEN (ed), Bern.
- FOEN Indicator WS076 (2018) WS076 - Anschlussgrad an Abwasserreinigungsanlage. Federal Office for the Environment, F. (ed), <https://www.bafu.admin.ch/bafu/de/home/themen/thema-wasser/wasser--daten--indikatoren-und-karten/wasser--indikatoren/indikator-wasser.pt.html>.
- Gujer, W. (2007) Siedlungswasserwirtschaft, Springer, Berlin.
- IPCC (2006) Chapter 5.6 Wastewater treatment and discharge. 2006 IPCC Guidelines for National Greenhouse Gas Inventories, <http://www.ipcc-nggip.iges.or.jp/public/2006gl/index.html>.
- IPCC Guidelines (2006) Chapter 6 - Wastewater treatment and discharge.
- Kosonen, H., Heinonen, M., Mikola, A., Haimi, H., Mulas, M., Corona, F. and Vahala, R. (2016) Nitrous Oxide Production at a Fully Covered Wastewater Treatment Plant:

- Results of a Long-Term Online Monitoring Campaign. *Environmental Science & Technology* 50(11), 5547-5554.
- Külling, D. (2002) Nährstoffe und Schwermetalle im Klärschlamm 1975 - 1999. *AGRARForschung* 9.
- Lehmann, M. and Gruber, W. (2018) Personal communication with Moritz Lehmann. Gruber, W. (ed), eawag Dübendorf.
- Liu, Y., Ni, B.-J., Ganigué, R., Werner, U., Sharma, K.R. and Yuan, Z. (2015) Sulfide and methane production in sewer sediments. *Water Research* 70, 350-359.
- Luck, M. (2018) Expert Review - Additional data. eawag (ed).
- Meyer, J., Sommer, M. and Von Schulthess, R. (1996) Stickstoff-Frachten aus Abwasserreinigungsanlagen. Federal Office for the Environment, F. (ed), Bern.
- Parravicini, V., Valkova, T., Haslinger, J., Saracevic, E., Winkelbauer, A., Tauber, J., Svardal, K., Hohenblum, P., Clara, M., Windhofer, G., Pazdernik, K. and Lampert, C. (2015) Reduktionspotential bei den Lachgasemissionen aus Kläranlagen durch Optimierung des Betriebes Bundesministerium Landwirtschaft, Regionen und Tourismus, Wien.
- Siegrist, H. and Luck, M. (2018) Personal communication with Hansruedi Siegrist. Luck, M. (ed), eawag Dübendorf.
- Strähl, S., Ort, C., Siegrist, H., Thomann, M., Obrecht, J. and Kurz, E. (2013) Stickstoffelimination in Schweizer ARA. *Aqua & Gas* 5/2013, 74-84.
- Umweltbundesamt Austria (2017) Austria's National Inventory Report 2017, Wien.
- Wunderlin, P. (2013) Mechanisms Of N<sub>2</sub>O Production In Biological Wastewater Treatment From Pathway Identification To Process Control. Cumulative thesis, ETH Zurich, Zurich.

## **Chapter 3**

# **notos: multi-channel apparatus for monitoring off-gas in WWTPs**

This chapter has been published as a part of a methods description:

Joss, A., Gruber, W., Bühner, T., Feller, K., Biolley, L., von Känel, L., Braun, D. (2021)  
notos: multi-channel apparatus for monitoring off-gas in WWTPs



## Abstract

Long-term monitoring of  $\text{N}_2\text{O}$  emissions on full-scale WWTPs is required to assess representative emission factors. Emissions have been shown to substantially vary over time, from hours to years, and in space. Hence, a monitoring setup should guarantee high resolution measurements (<15 minutes, multiple measurement channels) in the long-term. Here, we present an apparatus to monitor off-gas concentrations in WWTPs, which can be applied on both roofed and unroofed WWTPs. Most often the setup was used in the combination with flux chambers on unroofed biological treatment steps. Off-gas fluxes are assessed via blower speed or flow measurements in the blower system. The system allows to measure multiple off-gas compounds, such as  $\text{CH}_4$ ,  $\text{CO}_2$ ,  $\text{O}_2$  or  $\text{NO}$ . Full automation reduces operational efforts. A focus is laid on the protection of the system against water leakage, which can lead to failure of the system. This description is meant to all rebuilding and installing the system and is currently being tested by two water boards (Emscher-Genossenschaft – Lippeverband, GER; VASYD, SE).

### 3.1 Scope

Purpose of the described apparatus is to monitor the off-gas in wastewater treatment plants (WWTPs) with high resolution in space and time: up to 14 sampling points can be sampled every 15 minutes and analysed for different gas components. The apparatus was developed to serve the following scopes:

- Quantify direct greenhouse gas emissions ( $\text{N}_2\text{O}$ ,  $\text{CH}_4$ ) in short-term or long-term monitoring campaigns.
- Online measurement of the oxygen uptake for monitoring or process control.
- Assess the state of the aeration equipment to evaluate the need for its refurbishing.
- The apparatus is suitable for both open as well as roofed reactors.

### 3.2 Documentation

The description of the monitoring system is available from the Eawag Research Data Institutional Collection (Eric) at <https://doi.org/10.25678/0003WD>

The description of the measuring apparatus consists of the following documents:

- This document describes the general set-up and commissioning of the unit.
- The P&ID scheme is part of this description and provides an overview of the individual elements.
- The technical drawings are available for the flux chamber for sampling open reactors.
- Technical drawing of the chamber.
- Technical drawing of the exhaust pipe, composed of different modules.
- The automation of the control system is described by the following documents:

- i) Wiring diagram of the electric cabinet: currently being corrected for consistency with other documents.
- ii) Description of the software for the programmable logic controller (PLC)
- iii) Copy of the PLC software in CoDeSys Version 2.3
- iv) PDF printout of the PLC software
- v) Documents describing the single components: the file names begin with the ID of the component according to the P&ID scheme
- vi) Instruction for the data evaluation

### **3.3 The equipment**

The apparatus consists in the following elements:

- Flux chambers for collecting representative off-gas samples on the surface of aerated basins.
- Pipes conveying the gas samples from the sampling point to the analytic devices.
- Set of valves for sequentially measuring different sampling points.
- Pumping and pre-treatment of the gas sample.
- Analytical devices for measuring several gas components.
- Automated control and supervision for long term monitoring.
- Installation in a container (outdoors) or on a handcart (indoors).

#### **3.3.1 Flux chambers**

Each flux chamber collects the off-gas from of one square meter basin surface (Figure 3.3). At the basis of the exhaust pipe a sample is diverted in direction of the centralized analytical device. Roofed reactors with exhaust collection system can be sampled directly without chambers if suitable sampling points are available.

The chambers are made of polyethylene and weight 16 kg. These float thanks to their frame made of a sealed 2-inch pipe. The chambers are tied with ropes on all four

corners to the guardrail of the basin. The chambers are designed for minimizing the enclosed gas volume (faster measurement), to drain off rain and splashes, and to allow stacking for storage and transport.

The collected air is conveyed away via a vertical exhaust pipe consisting in the following elements (Figure 3.4):

- Coupling to the chamber with 2-inch screw connector.
- Lateral connector for the hose of the gas sample (1/8-inch thread).
- Ending: an open 180 ° bend facing downwards to prevent rain in the exhaust air pipe.
- Optional: module with check valve consisting of a ping-pong ball with rubber seal; the valve prevents ambient air from flowing back into the chamber. In the case of weakly aerated or non-aerated reactors, however, the valve can cause the chamber to sink because it is evacuated. For such case installing a ceramic aerator under the chamber is recommended (see next point).
- Optional: for measurements in reactors that are not, weakly or intermittently aerated, a ceramic aerator can be attached below the chamber, which ensures minimal gas exposure. The ceramic aerator must be supplied with air.
- Optional: selected chambers can be equipped with gas flowmeter, each consisting of three thermal sensors. In order to prevent condensation, the exhaust air must be heated before the flow measurement (heating powered with 48 VDC). Contact the group of Daniel Braun (ETH, Switzerland) concerning this module.

### **3.3.2 Tubing connecting sampling point and analytical device**

The gas sample is conveyed from the sampling point to the central measurement using pneumatic tubing:

- Distances up to 150 m: polyurethane pneumatic tubing, UV resistant, 4 mm inner diameter.
- Distances longer than 150 m: polyurethane pneumatic tubing, UV resistant, 6 mm inner diameter.

- The coupling of tubing sections is done using commercially available plug-in couplings pneumatic tubing.

At locations where frost is expected, pipe clogging due to freezing condensate may represent an issue. As a counter measure installing a flushing pump is recommended (M004 and M002 in P&ID schemes in Figure 3.1 and Figure 3.2, respectively), which ensures a constant air flow in all pipes during the measurement intervals. Pausing of the gas flow would lead to the accumulation of condensate in some spots and then to freezing. Heating the tubing is not recommended because a) costly and laborious to install, and b) it is hardly possible to install a heating system along the entire tubing avoiding thermal bridges. Even a thermal bridge of a few centimeter is sufficient for condensation and freezing.

### 3.3.3 Valves and calibration gases

Typically a single measurement takes around one minute: 30 seconds of flushing and 30 seconds measurement (if flushing pumps are implemented). Thus a set of valves (Y001 to Y014) allows to sequentially sample every point several times per hour. Additional valves (Y015 to Y020) allow to periodically perform reference measurements with calibration gases: ambient air for O<sub>2</sub>, bottled gases for N<sub>2</sub>O, NO, CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub> as blank.

Another valve (Y021) is required for switching between sampling points and calibration gases, i.e. to avoid flooding the entire gas pre-treatment system with calibration gas.

All calibration gas bottles must be equipped with a pressure reducer. A T-junction with a > 2 m hose (open at the end to the ambient atmosphere) is required in order to provide all calibration gas at ambient pressure and thus enable an accurate calibration measurement.

Commercially available valves for pressurized air are used, which can be controlled either with 24VDC or 230VAC.

### 3.3.4 Gas pumps and pre-treatment

For the following reasons, the analytical devices are preceded by a multistage pre-treatment:

- To protect the analytic equipment from liquids and dirt: in addition to condensate, foam can also be expected on aeration tanks. Further it may rarely occur that flux chambers sink and therefore liquid is aspirated.
- To prevent condensation forming in the analytic equipment.
- To allow a measurement every minute, even with lines > 150 m.
- In order provide the sample gas at an appropriate flow for long and short distances.

The gas pre-treatment consists of the following elements:

- Gas pumps (M002, M004, M006, M008)
- Condensate separator (E001, E002, E003) with the corresponding condensate pumps (M001, M003, M005)
- Gas drying (E004) with its condensate pump (M007)
- Fine filter (E005), protection filter (E007)
- Pressure relief valve (E006, E009)
- Mass flow controller (E008)
- Water sensor (B001).

The gas is pumped using the following pumps:

- M008: The sample gas pump provides the analytical device with a flow of 1 L/min.
- M002: The flushing pump ensures that all lines are flushed also during the measurement intervals, in order to a) constantly renew the gas sample allowing for faster measurement and b) to reduce icing problems in winter, since condensate is not accumulating at specific points of the gas tubing.
- M004: A second flushing pump is required for installations with distances of > 150 m because the pressure drop in the long and short lines differs too much.
- M006: A second sample gas pump is also required in installations with distances of > 150 to ensure that for all lines a gas flow of at least 1 L / min is delivered, regardless of the pressure drop in the lines. To avoid

overpressure, a T-branch with pressure relief valve (E009) ensures that excess sample gas is diverted.

Liquid separators:

- Each gas pump is preceded by a gas/liquid separator (E001, E002, E003) with its corresponding condensate pump (M001, M003, M005) to protect the gas pumps from contamination by water and particles.
- The gas cooler (E004 with its condensate pump M007) ensures that the relative humidity in the gas is  $< 100\%$ , so that no condensation occurs in the analysers.

The following safety elements are also part of the gas pre-treatment:

- A fine filter (E005) holds back any dust and particles.
- A water sensor (B001) monitors that no liquid is detected after the gas drying. If water is detected the sample gas pump (M008) is switched off.
- A 3-way valve (Y021) switches between sample gas from the chambers and the calibration gas.
- A gas-permeable microfilter (E007) is used as a final barrier for liquids: In case of exposure to water the filter increases its flow resistance significantly. An upstream pressure relief valve (E006) ensures that the protection filter is not exposed to a too high a pressure, thus avoiding water breakthrough.
- A mass flow controller (E008) monitors and controls the flow of the sample gas (1 L/min).

### 3.3.5 Analytical devices

Up to 5 different components can be monitored simultaneously:

- Oxygen ( $O_2$ ; B002): Is of interest because a) aeration is one of the most important electricity consumers in wastewater treatment and b) because the oxygen consumption provides information about the current activity in the reactor (e.g. to recognize the end of nitrification). Typical measuring range: 10% to 25%.
- Nitrous oxide ( $N_2O$ ; B003) is a relevant greenhouse gas. In waste water treatment emitted typically in the range of 0 to 3000 ppm.
- Nitric oxide ( $NO$ ; B004): this measurement could be interesting. According to the first measurements, the concentrations in the exhaust air appears to be low and in the range of the limit of quantification. Also,  $NO$  has no great greenhouse gas potential because it reacts relatively quickly in the atmosphere. Typical measuring range: 0 to 100 ppm.
- Carbon dioxide ( $CO_2$ ; B005): This signal is mostly redundant to the  $O_2$  signal, but is strongly influenced by pH fluctuations in the reactor due to the carbonate equilibrium. Typical measuring range: 0% - 10%.
- Methane ( $CH_4$ ; B006): is not formed in the activated sludge process, but in the sewer and during sludge treatment. Typical measuring range: 0 to 1000 ppm in the exhaust air from the aerated reactors (stripping of dissolved  $CH_4$  input); in the exhaust air from sludge treatments: 0 to 2000 ppm.

### 3.3.6 Automation and supervision devices

Monitoring campaigns reliably sampling each point with intervals of <15 minutes require an automation, which is composed of the following elements:

- The programmable logic controller (PLC type Wago 750-8207) providing the following:
- Control of the valves (Y001 to Y021).
- Conversion of analogic signals to digital values (B002 to B006).



- Storing the measured values and the system parameterisation a micro SD card.
- Sending out alarms and perform emergency shutdown.
- A computer (E013, E014) serves as an interface for parameterization as well as for remote access. The PC should be set up to automatically restart and login after a power outage, in order to be available again for remote access as soon as possible.
- Optionally a virtual PC can be implemented (e.g. HyperV from Microsoft Windows or VMware): herewith a complete backup of the PC may be stored, allowing for a computer replacement within only a few hours. However, this requires an external USB interface (E012) because the PC-internal USB interfaces are not available in the virtual PC.
- A modem is used for sending data, for alarming as well as to remotely access the PC (e.g. using the software TeamViewer)
- An electric cabinet suitable for indoor use. All certificates for installation on WWTP must be available. It may be appropriate to leave 20% of free space in the electric cabinet to allow for future adjustments. The wiring diagram as a paper printout as well as in PDF format should be supplied. The PLC is usually part of the control cabinet.

### **3.3.7 Installation in a container or on a handcart**

The following elements are usually set up on a pallet or a mobile handcart:

- The valves
- The complete gas pre-treatment including gas pumps
- The analytic devices
- The electric cabinet including PLC

The following also belongs to the measurement setup:

- The PC complete with screen, keyboard and mouse
- A work table
- A rack with the secured gas bottles for the calibration gas.

Outdoor installation: When installing those elements besides or between wastewater treatment lanes, a small office container is worthwhile (e.g. 10 feet = 3 x 2.5 x 2.5 m). Indoor installation: When setting up the equipment in the interior, it is often advantageous to use a mobile handcart.

### **3.4 Control and operation**

#### **3.4.1 Positioning the chambers**

The chambers are positioned with ropes fixed loosely on the guardrails of the reactor, so that the flux chambers may not reach the edge of the reactor. Contact with the rough concrete walls leads to wear and can thus damage and sink the flux chamber. The tubing departing from the chamber must be tied loosely to one of the ropes positioning the chamber.

#### **3.4.2 Check the tightness and assignment of the chambers**

The tightness of the system can be checked in two different ways:

- Connect a gas bag (10 L) filled with calibration gas to the gas tubing, as close as possible to the flux chambers. The measured value should confirm the absence of leaks and the valve position at which the calibration gas values are found confirm the assignment of valves and chambers. If the measured value differs from the calibration gas, check whether a leak is present or whether the device must be recalibrated: to this, connect the gasbag directly to the sample gas pump (M008; i.e. without sample gas line) and repeat the measurement.
- Seal the tubing in the vicinity of the flux chamber and check the sample gas flow monitored by the mass flow controller (E008): it should falls towards zero. If this is not the case, outside air is sucked in through a leak in the system.

Due to the length of the lines, the assignment of the individual chambers to the respective valves must be checked after every installation.

### 3.4.3 Calibration of gas analysers

The calibration and reference measurements of the analytical devices are usually carried out manually. Periodic reference measurements can be carried out automatically as quality control. This requires, however, that the fittings of the gas bottles (pressure reducing valves and the valves Y016 to Y020) to be reliably gas tight: otherwise after the calibration gas bottles are found empty a few days only. If the reference measurements are carried out exclusively manually, the calibration gas bottles can be closed on the main tap and thus usually last for years.

### 3.4.4 Parametrizing the automated monitoring

The following parameters are normally set for the automated monitoring:

- Test and set the time for sample flushing, i.e. the delay between the switching of the valve to a new position and the recording of the signal outputs. This delay is identified by manually switching between two sampling points with clearly different gas compositions and noting the time required for the signal to reach a representative value. This is normally done with sampling points and not with calibration gases to include also the flushing of the gas pre-treatment. Typical value: 30 seconds.
- Decide the required total opening time of a valve for a single measurement (i.e. delay for sample flushing + measuring time). Typical value: 1 minute.
- Name and switch to Auto-mode all required valve position.
- Set sample gas flow to be controlled by mass flow controller (E008) and parametrize the minimally required value. Typical values: 1 and 0.5 L/min.

### 3.4.5 Supervision of monitoring campaigns

It is best to check the operation of the system roughly daily via remote access. Regular evaluation of the data (e.g. monthly calculating the expected key results) is also recommended so that any malfunctioning can be detected as soon as possible.

The breakdowns experienced so far include:

- Power outages: PLC and computer are to be set up to restart automatically after a power outage, included all required software.
- Foam: is normally handled by the solid liquid separators. Back-flushing of the tubing with pressurized air may be required to remove dirt.
- Gas pipes freezing up: Flushing pumps help avoiding freezing. Back-flushing of the tubing with pressurized air when thawing may help to keep frost-outages short. Some very cold days without measurement may be acceptable; if not: heating the entire tubing length is probably the only option.
- Drift of the measured signals: e.g. O<sub>2</sub> may require recalibration few times per month.
- Damage and sinking of the chambers due to wear on the concrete wall. Countermeasure: positioning of the chambers avoiding the chambers touching the concrete walls.

### **3.5 P&ID schemes and technical drawings**

P&ID schemes are provided for a measurement setup for systems with some lines longer than 150 m (Figure 3.1), and for a measurement setup for systems with all lines shorter than 150 m (Figure 3.2). A technical drawings of the floating flux chamber is displayed in Figure 3.3. The exhaust pipe of the flux chamber is shown Figure 3.4.

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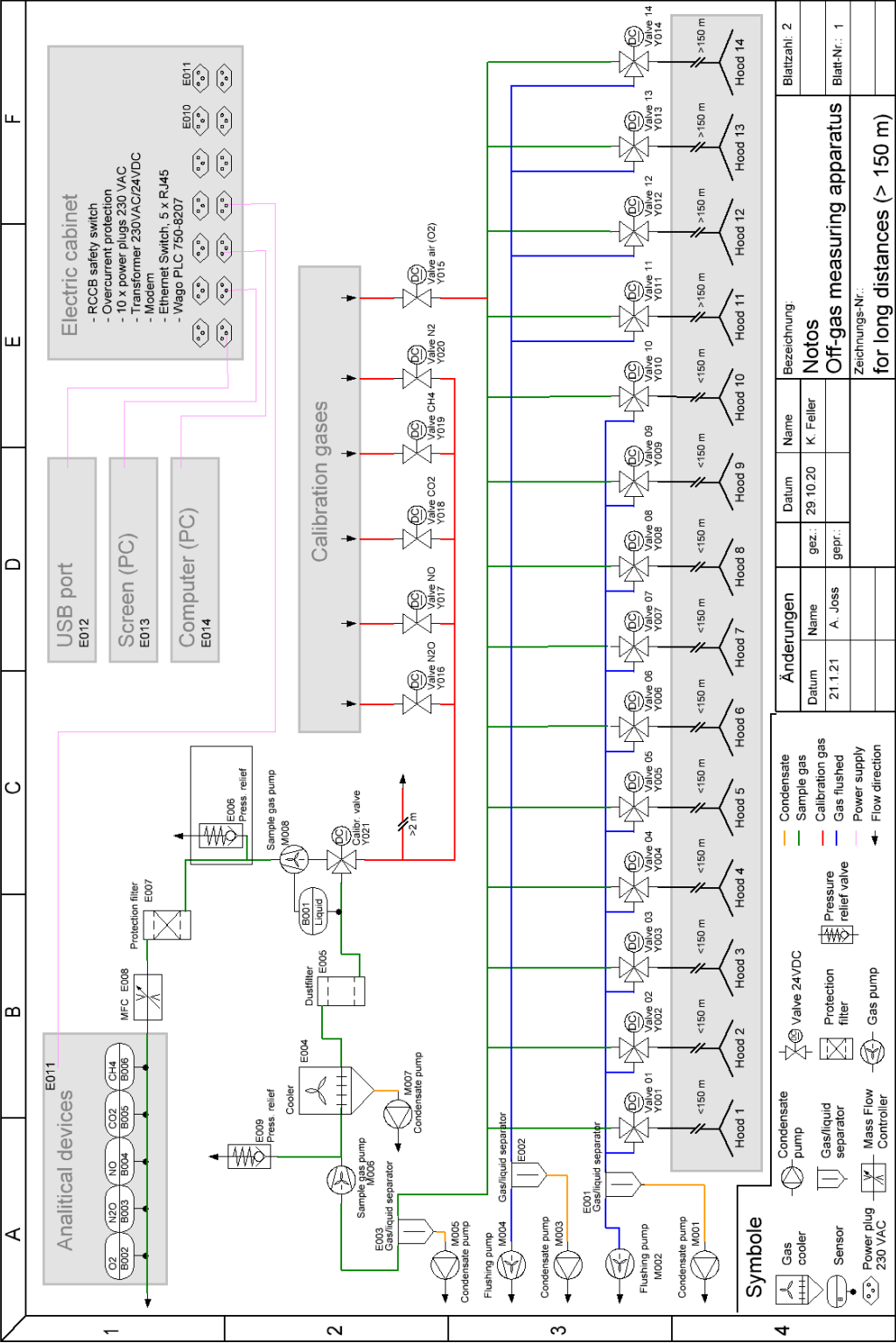


Figure 3.1 P&ID diagram of a standardized setup of the measurement system for systems with some lines longer than 150 m.

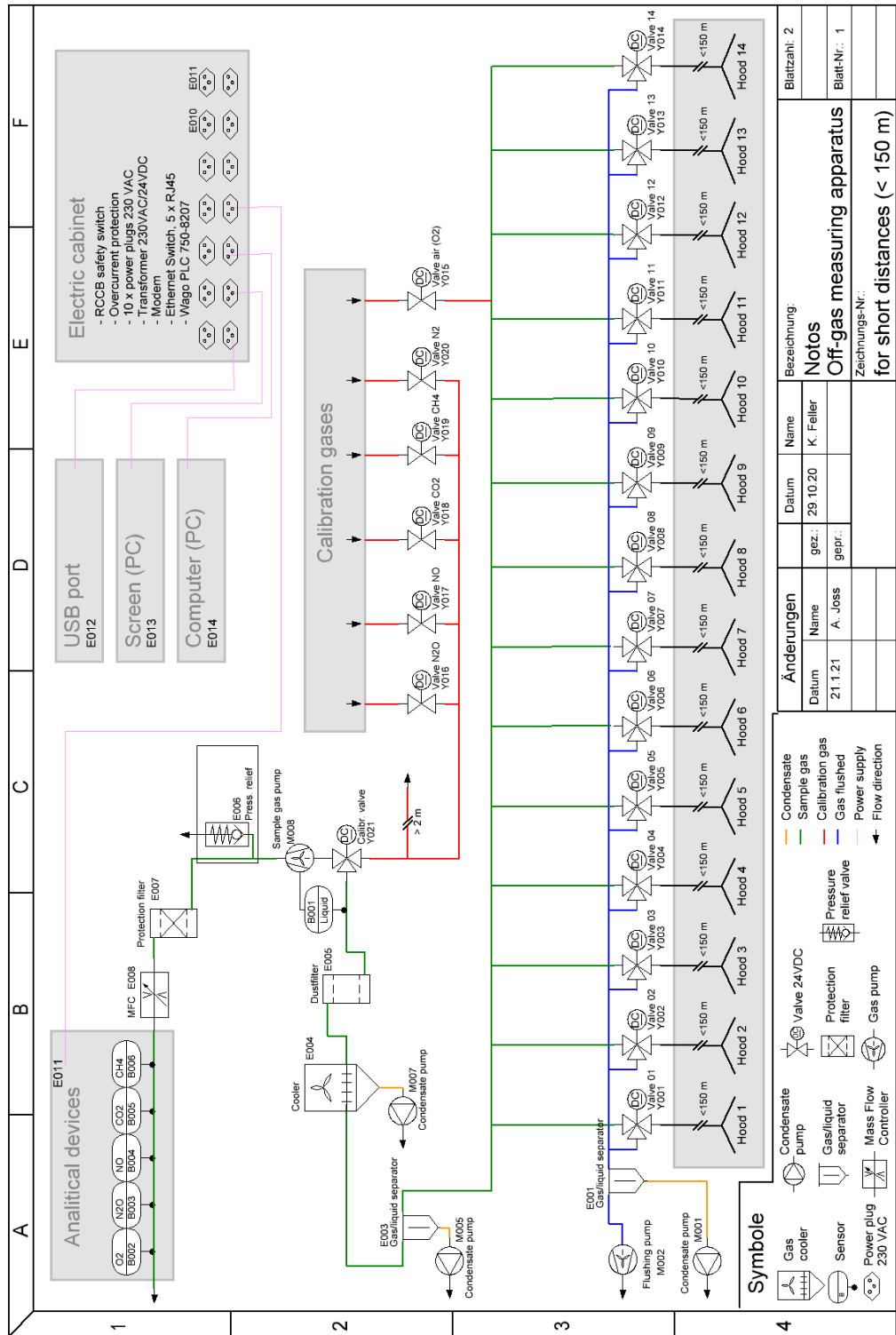


Figure 3.2 P&ID diagram of a standardized setup of the measurement system for systems with all lines shorter than 150 m.

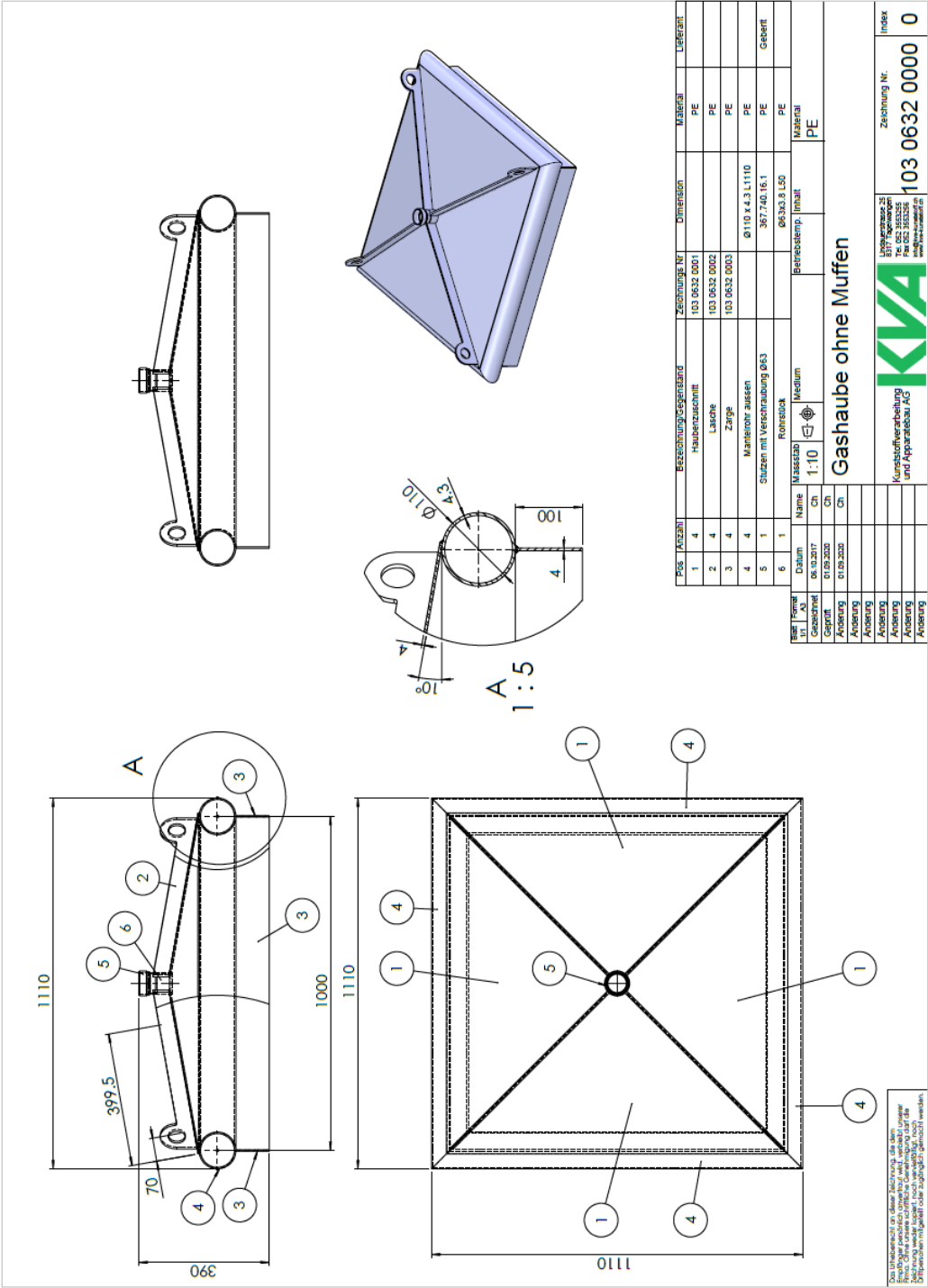


Figure 3.3 Drawing of the floating measuring chamber without exhaust pipe. Distances in mm/valve.

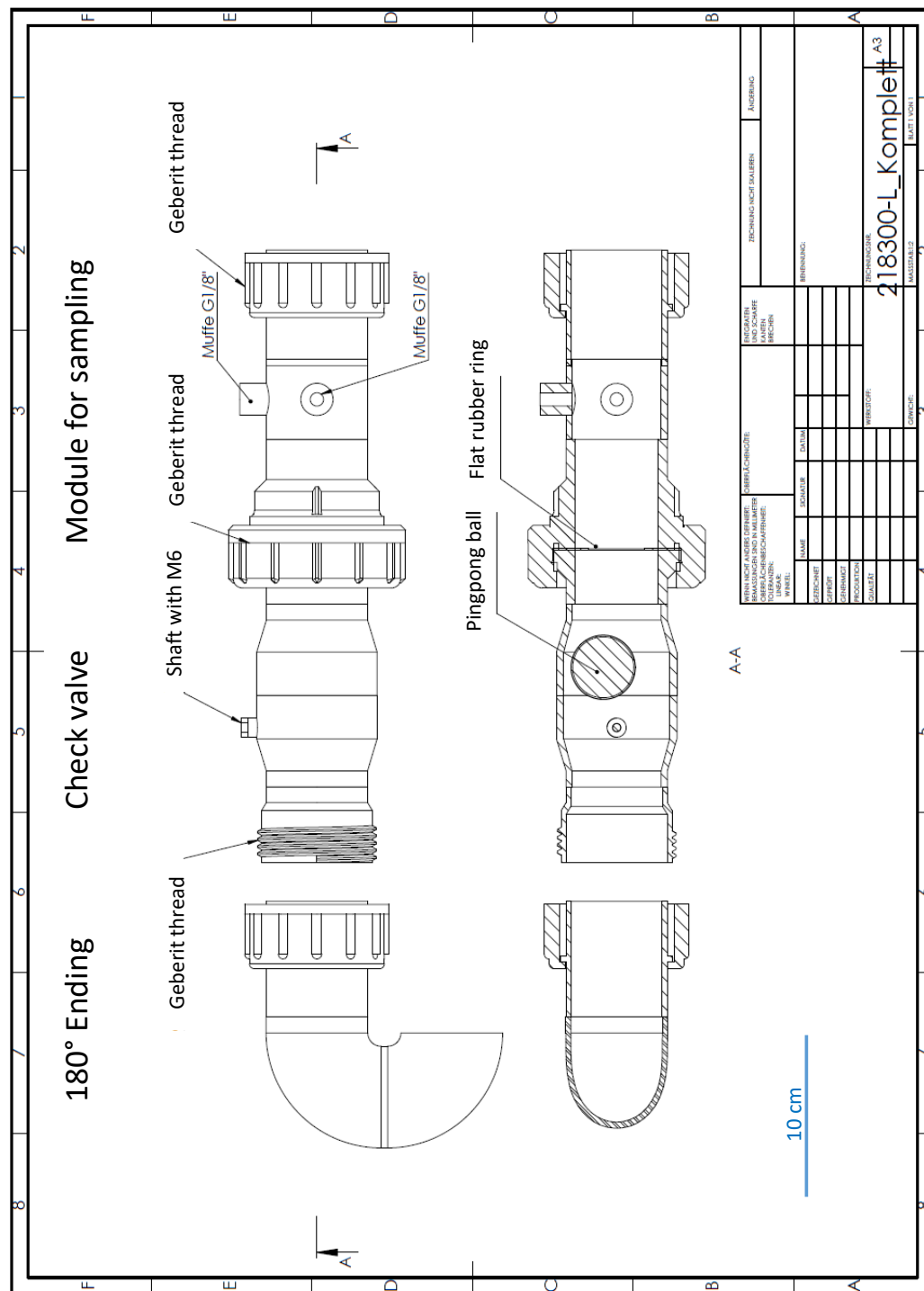


Figure 3.4 Drawing of the exhaust pipe for the flux chamber, with module for check valve.



# **Chapter 4**

## **N<sub>2</sub>O emission in full-scale wastewater treatment: Proposing a refined monitoring strategy**

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N<sub>2</sub>O emission in full-scale wastewater treatment: Proposing a refined monitoring strategy.  
Science of The Total Environment 699, 134157

## Abstract

Nitrous oxide (N<sub>2</sub>O) emissions from wastewater treatment contribute significantly to greenhouse gas emissions. They have been shown to exhibit a strong seasonal and daily profile in previously conducted monitoring campaigns. However, only two year-long online monitoring campaigns have been published to date. Based on three monitoring campaigns on three full-scale wastewater treatment plants (WWTPs) with different activated sludge configurations, each of which lasted at least one year, we propose a refined monitoring strategy for long-term emission monitoring with multiple flux chambers on open tanks. Our monitoring campaigns confirm that the N<sub>2</sub>O emissions exhibited a strong seasonal profile and were substantial on all three plants (1 - 2.4% of the total nitrogen load). These results confirm that N<sub>2</sub>O is the most important greenhouse gas emission from wastewater treatment. The temporal variation was more distinct than the spatial variation within aeration tanks. Nevertheless, multiple monitoring spots along a single lane are crucial to assess representative emission factors in flow-through systems. Sequencing batch reactor systems were shown to exhibit comparable emissions within one reactor but significant variation between parallel reactors. The results indicate that considerable emission differences between lanes are to be expected in cases of inhomogeneous loading and discontinuous feeding. For example, N<sub>2</sub>O emission could be shown to depend on the amount of treated reject water: lanes without emitted less than 1% of the influent load, while parallel lanes emitted around 3%. In case of inhomogeneous loading, monitoring of multiple lanes is required. Our study enables robust planning of monitoring campaigns on WWTPs with open tanks. Extensive full-scale emission monitoring campaigns are important as a basis for reliable decisions about reducing the climate impact of wastewater treatment. More specifically, such data sets help us to define general emission factors for wastewater treatment plants and to construct and critically evaluate N<sub>2</sub>O emission models.

## 4.1 Introduction

Nitrous oxide ( $\text{N}_2\text{O}$ ) emissions have a strong impact on the environment.  $\text{N}_2\text{O}$  has a global warming potential 300 times greater than that of carbon dioxide and is emitted in significant quantities across the globe (Stocker et al. 2013). Additionally, it is considered the most important ozone-depleting substance of anthropogenic origin (Ravishankara et al. 2009). Wastewater treatment (WWT), in particular biological nitrogen removal, has been shown to produce  $\text{N}_2\text{O}$  (Hanaki et al. 1992). Even if only small amounts of the influent nitrogen load are emitted as  $\text{N}_2\text{O}$ , it can represent the dominant greenhouse gas emission of a wastewater treatment plant (WWTP): Two year-long monitoring campaigns, at Kralingseveer in the Netherlands (Daelman et al. 2015) and Viikinmäki in Finland (Kosonen et al. 2016), found that  $\text{N}_2\text{O}$  constituted the majority (78% and 86%) of the total greenhouse gas emissions of the WWTP (Daelman et al. 2013b, Kosonen et al. 2016). In these studies, 2–3% of the influent nitrogen load was emitted as  $\text{N}_2\text{O}$ .

The IPCC guidelines, which were updated in 2019, suggest an emission factor of 1.6% of the total nitrogen load. The value is an average of current short-term and long-term monitoring campaigns (IPCC 2019b). Based on the new value it can be expected that  $\text{N}_2\text{O}$  from wastewater contributes significantly more to the total  $\text{N}_2\text{O}$  emissions than previously assumed 3.1% for Europe in 2017 (EEA 2017). For almost all of the countries, the calculations were based on the previously used, significantly lower emission factor of 0.035% (Czepiel et al. 1995, EEA 2017).

No quantitative model currently explains both long-term and short-term  $\text{N}_2\text{O}$  emission dynamics. Although long-term monitoring studies confirm significant emission variation on a yearly scale, no modeling study covering more than a few months has been published (Ni and Yuan 2015). Without significant advances in long-term emissions modeling, monitoring campaigns remain necessary to determine emission factors and provide a solid data basis for validating the current emission models (Vasilaki et al. 2019).

Emission factors have been reported ranging from 0.01% to 25% of the incoming nitrogen load (Ahn et al. 2010, Kampschreur et al. 2009b). Although emissions vary significantly between treatment plants and processes, the wide range of emission

factors assessed can at least partly be attributed to differences in monitoring strategies and methods (Kosonen et al. 2016). A consensus on a generally accepted emission factor has yet to be reached. Because emissions have been found to vary greatly over the year as well as over single days, a representative quantification of N<sub>2</sub>O emissions requires long-term online measurement, which is currently rarely applied (Kosonen et al. 2016). Preferably, measurement campaigns should cover the complete annual temperature range of the incoming wastewater at a time resolution of <30 minutes (Daelman et al. 2013a).

In both published long-term monitoring campaigns, fully covered WWTPs were monitored by measuring the N<sub>2</sub>O off-gas concentration in the centrally collected off-gas (Daelman et al. 2015, Kosonen et al. 2016). The application of this monitoring approach is only possible on fully covered WWTP. To date, no studies have been published of monitoring campaigns over a year or longer on WWTPs with open tanks. Hence, guiding principles for long-term monitoring on open tanks are not yet available. Since many WWTPs in industrialized countries are equipped with open tanks, long-term online monitoring campaigns on uncovered WWTPs are crucial to establish a representative N<sub>2</sub>O emission factor for greenhouse gas reporting (Pan et al. 2016).

N<sub>2</sub>O emissions from open tanks are typically collected with flux chambers (Chandran et al. 2016). Another possible approach to monitor the emissions are the application of a measurement for dissolved N<sub>2</sub>O via i) Clark-Type electrodes (Marques et al. 2014) or ii) stripping of N<sub>2</sub>O from a sludge sample (Mampaey et al. 2015) combined with an air stripping model (Marques et al. 2014). Due to the potentially high spatial heterogeneity of N<sub>2</sub>O emissions within a treatment process, measurements at various spots on a WWTP are required to arrive at a representative measurement of the N<sub>2</sub>O emission factor for an entire WWTP (Aboobakar et al. 2013, Pan et al. 2016). For this purpose, a flux chamber based monitoring approach is more appropriate than a dissolved N<sub>2</sub>O measurement based monitoring, due to i) significantly higher costs for additional point measurements and ii) that quantifying stripping efficiency with sufficient accuracy is not a simple task at full-scale. N<sub>2</sub>O emissions from activated sludge lanes with open reactors operated in parallel have not yet been compared, although this is an important step in assessing plant-wide emission factors.

The present study proposes a refined strategy to quantify N<sub>2</sub>O emission factors for biological treatment in open tanks, based on long-term high-resolution monitoring campaigns on three municipal WWTPs in Switzerland with three different activated sludge configurations. At each plant, multiple spots were monitored at high resolution (10-15 min) over more than one year. A decision tree is proposed for planning the number of sampling points required by the type of treatment plant and operational choices (e.g. feeding regimes). All monitoring data and operational data series are made available at high resolution in the supplementary data.

## **4.2 Method**

### **4.2.1 Field sites**

N<sub>2</sub>O emissions were monitored in three Swiss municipal WWTPs operated with different activated sludge process configurations: a conventional activated sludge process (CAS), an alternately fed and intermittently aerated activated sludge process (A/I), and a sequencing batch reactor activated sludge process (SBR). Key numbers and general information on the WWTPs are summarized in Table 4.1. The positioning of the hoods on the reactors are visualized in Figure 4.1, Figure 4.2, and Figure 4.3.

Table 4.1: Features and key numbers of the monitored WWTP. A/I: alternating / intermitting feed plant; CAS: conventional activated sludge; CH: Switzerland; SBR: sequencing batch reactor. PE: Person equivalent.

<b>Biological process</b>	<b>CAS</b>	<b>A/I</b>	<b>SBR</b>
Location	Altenrhein (CH)	Lucerne (CH)	Uster (CH)
Wastewater design load (m <sup>3</sup> /d)	30,000	90,000	15,000
Biological treatment design load (PE)	70,000	250,000	40,000
Pre-treatment	Screening Grit Primary settler	Screening Grit Primary settler	Screening Grit Primary settler
Lanes with separated sludges	3 (each divided into 2 lanes)	6 (each divided into 2 lanes)	6
Aerobic sludge age (days)	Winter: 12 Summer: 10	Winter: 14 Summer: 10	Winter: 12 Summer: 8
Dissolved oxygen concentration (mgO <sub>2</sub> /l)	2	2	2
Nitrogen removal rate (%)	65	75	40
Nitrification discharge limit (mgNH <sub>4</sub> <sup>+</sup> -N/l)	2	2	2

### *CAS plant*

The nitrogen load of 125,000 PE exceeds the COD load, since the WWTP treats the sludge containing reject water of other plants in the region. After pretreatment, most of the wastewater is treated with a conventional activated sludge process on three lanes. During the winter season, the anoxic zone is aerated completely, and denitrification is reduced. Additionally, eight fixed-bed biofilm reactors (Biostyr®) are operated in parallel to treat the rest of the wastewater. These are equipped with a lower anoxic zone and an aerobic zone above this. During low influent flow, only two regularly switched fixed biofilm reactors are operated. On average, the fixed-bed system treats 30% of the wastewater.

Reject water was bypassed around one activated sludge lane at Altenrhein WWTP over a period of two months (March 2016 to May 2016). The experiment was then repeated one year later for three months (December 2016 to March 2017). In the experiments, reject water was dosed after the primary clarifier instead of before it. The surplus reject water was shared in a ratio of two to one between the remaining activated sludge lanes and the fixed-bed reactor. The reject water supply to the fixed-bed and the activated sludge process was controlled and monitored separately by

flow measurement (Endress+Hauser, Promag P). Emissions were monitored on both lanes with and without reject water, with three floating gas hoods per lane.

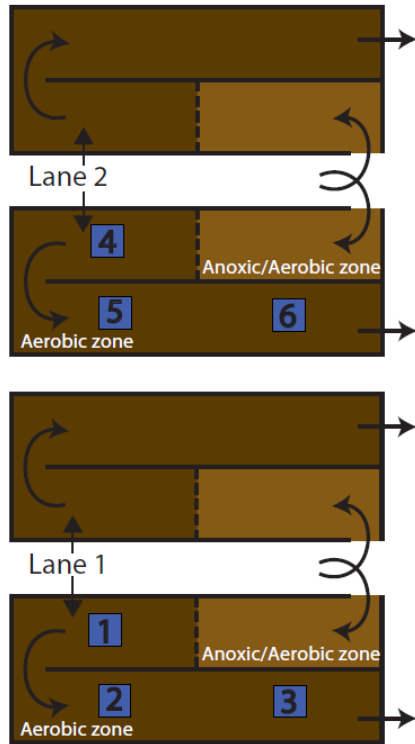


Figure 4.1: Monitoring setup of the Altenrhein WWTP monitoring campaign.

#### *AI plant*

The biological treatment comprises six lanes each consisting of two reactors of 5,500 m<sup>3</sup>. One of the reactors in each lane is always filling. In the middle of a cycle, the influent is directed to the other reactor. During the filling, the reactors are operated in plug-flow mode, starting with an anoxic phase followed by an aerobic treatment (main-aeration). After filling, the reactors are operated in batch mode. However, the reactors cannot be assumed to be fully mixed, since longitudinal mixing is limited due to the rectangular shape of the reactors. The cycle time is at least 1.5 hours in summer and 2 hours in winter and includes a 1 hour filling time and a variable aeration time, depending on the  $\text{NH}_4^+$  concentration. Subsequently, one post-aeration reactor per lane ( $6 \times 1,600 \text{ m}^3$ ) allows the nitrification discharge limit of  $2 \text{ mgNH}_4^+-\text{N/L}$  to be safely met. Anoxic cycles are shortened or canceled at low temperatures during the winter season to enable sufficient nitrification. During

anoxic periods, the activated sludge is mixed at intervals by pulses of coarse bubble aeration, and fine bubble aeration is used to aerate the mixed liquor.

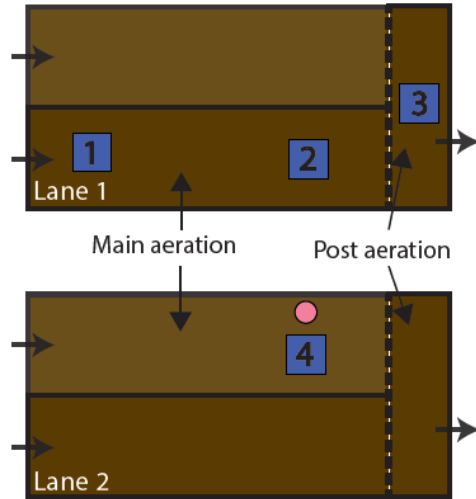


Figure 4.2: Monitoring setup of the Lucerne WWTP monitoring campaign.

#### *SBR plant*

A sequencing batch reactor system with activated sludge was monitored at Uster WWTP (Uster, Switzerland). Most SBR cycles last about 6 hours and exhibit a fixed sequence of operational phases: 30 minutes to 1 hour of feeding, about 3 hours of reaction phase (anoxic, aerobic), 1.5 hours settling phase, and 0.5 hours decanting. The reaction phase ends as soon as the target  $\text{NH}_4^+$  concentration is reached. During the winter season, an anoxic phase is skipped completely, and denitrification is reduced. In the summer season, the anoxic phase is set to a maximum of 25 minutes. Depending on the influent load, the anoxic phase can be skipped also in summer.



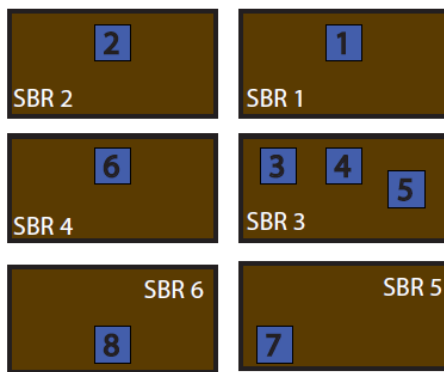


Figure 4.3: Monitoring setup of the Uster WWTP monitoring campaign.

#### 4.2.2 N<sub>2</sub>O measurement and monitoring

An adapted version of the flux chamber method was used for the assessment of the gaseous emissions from the open tanks (Chandran et al. 2016). Gas emissions were collected with four to nine flux floating chambers per sampling campaign, each covering an area of 1 m<sup>2</sup> (Chapter 3). In contrast to the standard method, an open outlet tube, with a diameter of 50 mm and length of 500 mm, was installed on top of the flux chambers as an off-gas pipe (Chandran et al. 2016). A small share of the off-gas (1 l/min) was diverted through a tube with a diameter of 5 mm from the off-gas pipe's base to the central measuring unit. The gas was dehumidified by cooling (JCP SL, JCT, Wiener Neustadt, AUT). The N<sub>2</sub>O concentrations were measured with a non-dispersive infra-red sensor (X-stream, Emerson, St. Louis MO, US). The device measures at a controlled, constant temperature of 60°C. Measurements were spatially resolved with an automated valve system preceding the dehumidifier and the analyzer. This enables the measurement of the off-gas composition sequentially at several spots within the WWTP, as in the procedure presented by Pan et al. (2016). The off-gas sampling location was changed every 4 to 6 minutes. The first 2 minutes after switching were ignored to account for purging of the connection hose, which had a maximum dead volume of 1.2 liters. The N<sub>2</sub>O measuring range spanned from 0 ppm to 1000 ppm. The detection limit was 1 ppm. The devices were serviced and calibrated according to the manufacturer's instructions.

The Altenrhein WWTP was monitored with six to seven flux chambers: two activated sludge lanes, with three flux chambers each according to the scheme in Figure 4.1 and one to three on biofilm fixed-bed reactors. Each hood was monitored

for 5 minutes, resulting in a measurement at each spot every 30 minutes. The off-gas was monitored for 16 months (from December 2015 to March 2017). Daily influent nitrogen loads were obtained with flow proportional composite samples for nitrogen (Hach, LCK338) measured by the plant operators twice a week.

The N<sub>2</sub>O emissions of two out of six A/I-reactors were monitored at Lucerne WWTP for 19 months between March 2014 and September 2015. Four flux chambers were installed according to the scheme in Figure 4.2: three on one lane and one on a separate lane. Each hood was monitored for 5 minutes, leading to a sample at each spot every 15 minutes. Daily influent nitrogen loads were obtained with flow proportional composite samples for nitrogen (Hach, LCK338) measured by the plant operators twice a week.

At the Uster WWTP, the emissions of all six SBR reactors were monitored between March 2018 and April 2019. On each reactor a flux chamber was installed Figure 4.3. On one reactor (SBR 3), three chambers were installed to resolve the spatial variation of the emissions. Each hood was monitored for 3 minutes, resulting in a measurement at each spot every 20 minutes. Daily influent nitrogen loads were obtained with flow proportional composite samples for nitrogen (Hach, LCK338) measured by the plant operators twice a week.

For each plant, a figure of the plant layout (Appendix A.1) and a data set with the monitoring data, operational data and standard wastewater indices (Appendix A.3) are provided in the SI. The respective resolution was chosen according to the frequency of the gas measurement.

#### **4.2.3 Calculation of N<sub>2</sub>O emissions**

The air flow supplied to the reactor was estimated via the blower speed recorded at intervals of 1 minute. Emissions were calculated in 1 minute intervals for the aerated phases only by linearly interpolating the measured N<sub>2</sub>O concentrations. The N<sub>2</sub>O net flux was calculated according to the formula provided by Aboobakar et al. (2013): the air flow supplied to each reactor section equipped with a flux chamber and the respective N<sub>2</sub>O concentration was calculated according to Eq. (4.1) (Aboobakar et al. 2013). Monthly and yearly average values of the nitrogen load, measured in 24 h composite samples, were used to calculate the N<sub>2</sub>O emissions relative to the influent

nitrogen load. For lanes with inhomogeneous influent distribution amongst lanes (Altenrhein WWTP, Uster WWTP), assumptions on the respective loads were made according to composite samples taken by the operators.

$$F = C * Q_{air} \cdot \left( \frac{AH}{AT} \right) \quad (4.1)$$

Where  $F$  is the net  $N_2O$  flux [ $kg\ N_2O-N/min$ ],  $C$  is the measured  $N_2O$  concentration in the off-gas [ $kg\ N_2O-N/m^3$ ],  $Q_{air}$  is airflow supplied by the blower [ $m^3/min$ ],  $AH$  is the number of air blowers attributed to a specific flux chamber [-], and  $AT$  is the total number of air blowers in the reactor [-].

## 4.3 Results and Discussion

### 4.3.1 Significant emission variation on a yearly scale

The measured  $N_2O$  emissions from the monitored activated sludge configurations all exhibit a similar strong seasonal pattern when plotted as a function of time (Figure 4.4). Emissions occurred mainly in one half-year (December–May), and they were very low in the other half-year (June–November). Daelman et al. (2015) also reported a seasonal emission pattern with similarly high differences on a year (Daelman et al. 2015). Emissions at Kralingseveer showed a very similar temperature variation, but their yearly peak shifted towards the temperature rise between March and June. Overall, the period of the emission peaks in this study were more distinct and shorter during the campaigns in this study than at the Kralingseveer WWTP. The seasonal emission patterns assessed by Kosonen et al. (2016) at the Viikinmäki WWTP were not as pronounced as in the other studies, which could be linked to the higher wastewater temperatures (20–40°C) (Kosonen et al. 2016). Additionally, the wastewater temperatures at Viikinmäki WWTP do not exhibit the seasonal profile typical of WWTPs connected to combined sewer systems in the temperate climate zone. The emission variation monitored in this study confirms the necessity of long-term ( $\geq 1$  year) monitoring for the assessment of emission factors (Daelman et al. 2013a).

A potential cause for the high emission variation could be failure of nitrification. In the Lucerne and the Uster campaign, nitrite concentrations peaked with N<sub>2</sub>O emissions, which could be expected according to literature (Colliver and Stephenson 2000). However, in the Lucerne campaign nitrite peaked also in summer while emissions were low. Another potential cause is the reduced denitrification capacity of the WWTP at cold temperatures (Conthe et al. 2018a). In the Uster campaign, the dramatic emission variation among the reactors clearly speaks against this. Linear and non-linear regression models show that the seasonal variation of the Altenrhein and the Lucerne campaign cannot be explained with the available operational data (Appendix A.2 (SI)). We conclude that at least one important driver governing the high emission variation in the yearly course has not yet been identified. The necessary information to solve this challenges may be extracted from newer data types, such as microbiological data (Stein 2018), inorganic carbon measurements (Peng et al. 2016), and concentration of isotopomers (Ostrom and Ostrom 2017). For example, the measurement of microbial diversity and gene, protein, and metabolites expression has been applied successfully to explain variations in lab-scale measurements of N<sub>2</sub>O emissions (Ge et al. 2018, Perez-Garcia et al. 2014).

The yearly average N<sub>2</sub>O emission factor from the reactors monitored at the three WWTPs are summarized in Table 4.2. The SBR system at the Uster WWTP exhibited the highest emissions with 2.4%. The emission values of each reactor are shown in the Appendix A.4 (SI). The A/I system at Lucerne WWTP was shown to have the lowest emissions with 1%. The average emission factor assessed in this study is 1.7% of the total incoming nitrogen load. The high standard deviations of the emissions reflect the strong emission variability (Table 4.2). In general, the emissions are in the same order of magnitude as the values assessed in the other two long-term studies published, where N<sub>2</sub>O emission factors of 2.8% were found by Daelman et al. (2015) (Kralingseveer WWTP) and 1.9% by Kosonen et al. (2016) (Viikinmäki WWTP).

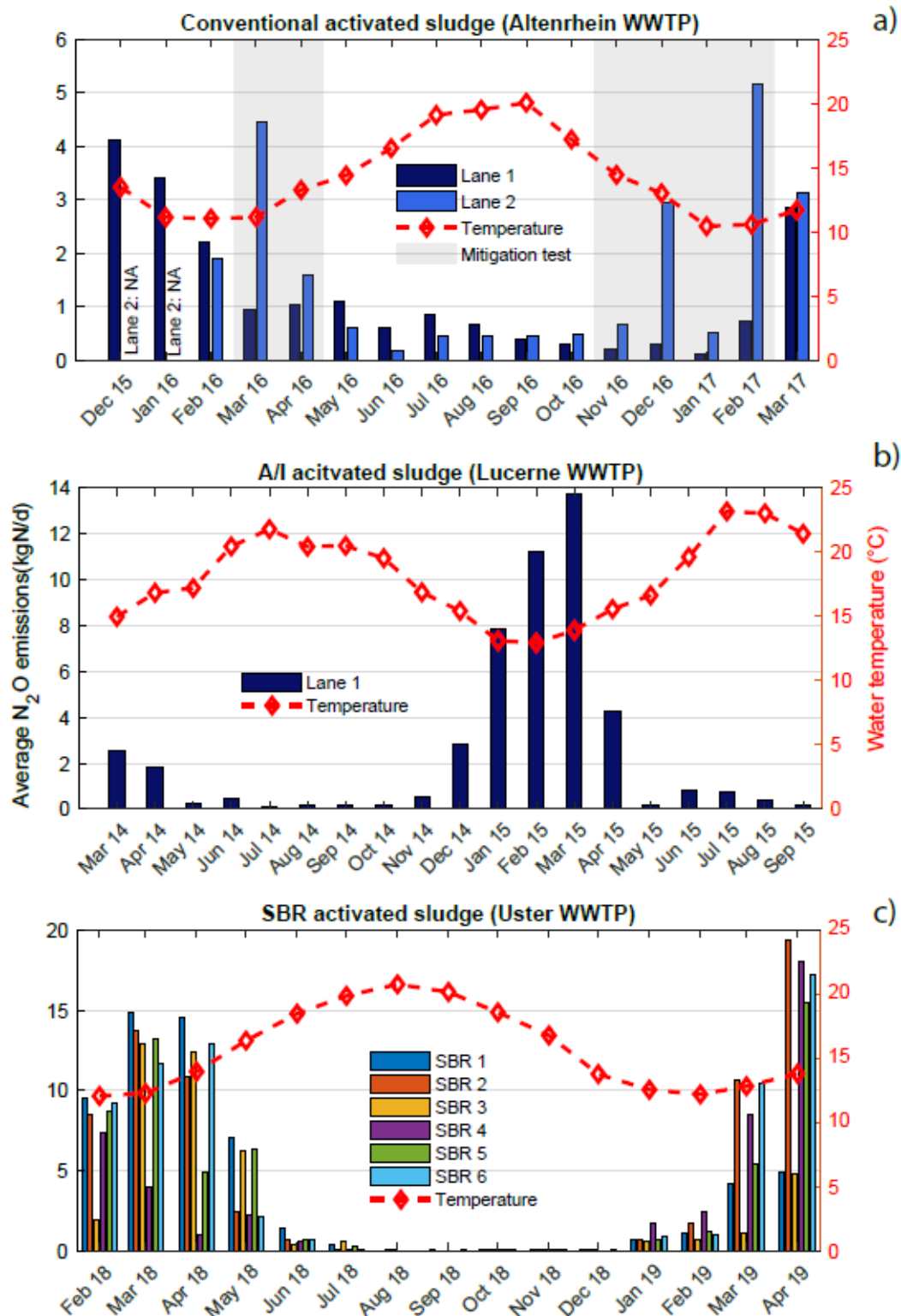


Figure 4.4: a) Monthly mean  $N_2O$  emissions of the activated sludge lane 1 and the fixed-bed reactor, and wastewater temperatures at Altenrhein WWTP. Grey background indicates times when reject water was omitted from the feed to lane. The activated sludge lanes 2 and 3 and the fixed bed reactors treated all the reject water during this period. In Dec 2015 and Jan 2016 lane 2 was not monitored. b) Monthly mean  $N_2O$  emissions and wastewater temperatures at Lucerne WWTP. c) Monthly mean  $N_2O$  emissions and wastewater temperatures at Uster WWTP.

Table 4.2: Yearly average emissions and standard deviations assessed in the monitoring campaigns; L: lane number. CAS: conventional activated sludge plant of Altenrhein; A/I: alternating / intermitting plant of Lucerne; SBR: sequencing batch reactor of Uster, all six lanes monitored separately.

WWTP	Yearly average emissions		Standard deviation
	kgN <sub>2</sub> O-N/year	% of total nitrogen load	kgN <sub>2</sub> O-N/year
CAS, L1	537	1.6	701
CAS, L2	749	2.0	795
A/I, L1	1204	1.0	1737
SBR, L1 to L6	447-1172		824-1059
SBR, average	805	2.4	976

Considering all published long-term monitoring campaigns, the average emission factor is 1.9%, which is in the same range as the value suggested by the IPCC guidelines (1.6%). However, more long-term campaigns are required given the site-specific variation of observed emission factors as well as the current lack of understanding differences amongst plant types (IPCC 2019b). Assuming the long-term monitoring campaign's emission factor, the contribution of wastewater treatment to the total N<sub>2</sub>O emissions would increase from 4% to 19% for Switzerland. The emission factors monitored in this study confirm the relevance of N<sub>2</sub>O to the greenhouse gas balance of WWTP: it is quantitatively more important than the indirect emissions caused by WWTPs' electricity consumption. An N<sub>2</sub>O emission factor of 2% causes 40% higher emissions than a WWTP powered exclusively by coal (Larsen 2015, Wunderlin et al. 2013b). Hence, the N<sub>2</sub>O emission factors assessed in our study confirm that N<sub>2</sub>O is the most important GHG source from WWTP.

#### 4.3.2 Low spatial emission variation

At all plants, abrupt changes from low emissions to high emissions were observed to occur over a few days. For example, at Lucerne WWTP, a 7 month low emission period was terminated by an emission rise from <0.2% to 4% within 4 days at the end of December 2014 (Figure 4.5a). Similarly, dramatic changes in N<sub>2</sub>O emissions were observed on a daily basis at Uster WWTP, as shown in Figure 4.5b. The rapid dynamic highlights confirm the previously discussed importance of online

monitoring approaches with sufficient temporal resolution (Daelman et al. 2013a). Typical daily  $\text{N}_2\text{O}$  emission profiles are shown in Appendix A.4 (SI).

In the same periods, the spatial emission distribution remained relatively stable. In the monitoring campaigns of this study, the spatial emission variation was generally not as pronounced as the temporal variation within one lane. However, systematic differences were also seen in this study in the A/I and the CAS reactors at Lucerne (Figure 4.5a) and Altenrhein WWTP. In contrast, all three sampling points in one SBR reactor at Uster WWTP exhibited comparable  $\text{N}_2\text{O}$  concentrations (Figure 4.5b).

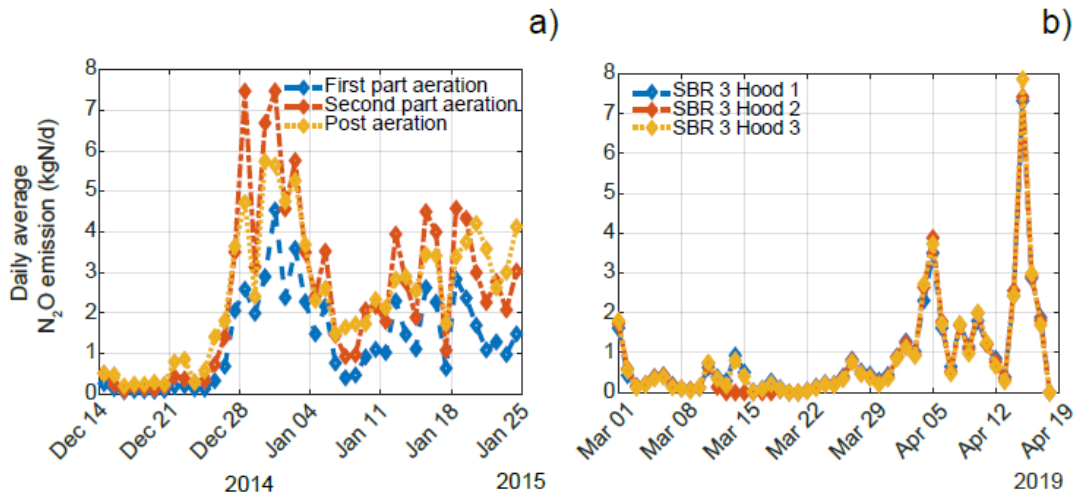


Figure 4.5: Spatial distribution of  $\text{N}_2\text{O}$  emissions within one lane as daily average at a) CAS plant (Lucerne WWTP) and b) SBR plant (Uster WWTP).

Aboobakar et al. (2013) found high spatial variation (0.01-0.1%) in a CAS process; however, this was paired with a low overall emission factor of 0.1% and a short-term emission period (Aboobakar et al. 2013). For a two-step activated sludge process, the spatial distribution was shown to be substantial (0.6-3.5%), with significantly higher emissions in the second step (Pan et al. 2016). This is to be expected, since nitrification occurs predominantly in the second step. It is hypothesized that multiple measuring points are required if substrate gradients or different aeration strategies are applied along a lane. This is typically the case for lanes featuring plug-flow characteristics. Multiple measurement points are not required in a single SBR reactor.

#### 4.3.3 How many lanes to monitor?

The emissions from parallel lanes in both plants with continuous feeding (CAS, A/I) exhibited strong variations from day to day, but these were reproducible across lanes even though these were operated with completely separated activated sludges (Figure 4.6a). In cases of uneven substrate load distribution between lanes, emissions can be significantly different. Two mitigation test phases between March 16 and April 16 and between November 16 and March 17 at Altenrhein WWTP provide an extreme example: During these phases, lane 2 treated part of the reject water from the sludge treatment, but lane 1 did not. During the second period, N<sub>2</sub>O emissions from lane 2 increased dramatically to values around 6 kg N<sub>2</sub>O-N/d. Emissions from lane 1 remained low at roughly 1 kg N<sub>2</sub>O-N/d. The resulting emissions relative to the influent load for lane 1 and lane 2 were 0.7% and 2.7% during the first experiment and 0.5% and 3.1% during the second experiment. The increase in N<sub>2</sub>O emissions of 80% was clearly higher than the increase in nitrogen load of 30%.

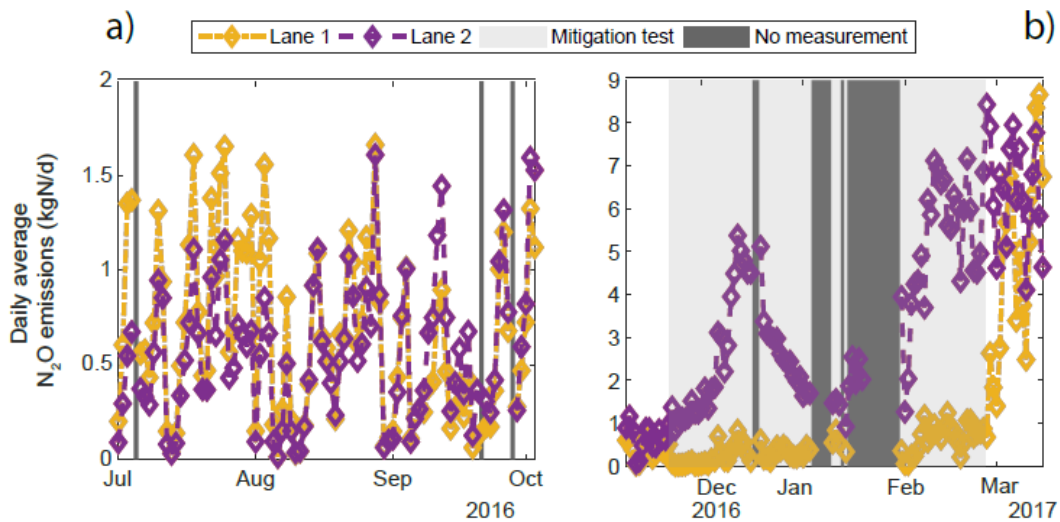


Figure 4.6: N<sub>2</sub>O emissions of two parallel activated sludge lanes operated with separated sludge lanes at Altenrhein WWTP during a) the same influent composition and b) different influent composition: activated sludge lane 1 was operated without reject water in the feed; activated sludge lane 2 was fed with wastewater containing reject water.

It is expected that inhomogeneous influent load can be caused not only by WWTP-internal recycles but also other factors, such as incomplete mixing in the sewer system or uneven preferential flows in the influent distribution. Key performance indicators of the WWTP, such as oxygen requirement and effluent concentration, are expected to be indicative of inhomogeneous influent loads.



Emission from discontinuously fed reactors may exhibit different emission profiles, as was seen in the monitored SBR system (Figure 4.4c). The monthly average emissions of all reactors differed substantially during the whole monitoring campaign. Based on the finding from parallel CAS lanes (Figure 4.6a), it is expected that parallel-fed SBR reactors also exhibit parallel N<sub>2</sub>O emissions. It still needs to be confirmed that monitoring a restricted number of reactors is sufficiently representative in this case.

#### 4.3.4 Refined monitoring strategy

The monitoring campaigns described above allow a refined monitoring strategy to be derived for assessing N<sub>2</sub>O emission factors and for generating data for identification and validation of full-scale GHG emission models.

Both emission factors and models should be based on data series of at least a 1 year duration, since seasonal emission patterns can be very conspicuous (Figure 4.4; (Daelman et al. 2015, Kosonen et al. 2016). Given the short-term variability, a sampling frequency higher or equal to twice per hour for each point of measurement is required (Daelman et al. 2013a). For a flux-chamber-based monitoring study of open reactors, the choice of the number and the positioning of flux chambers is crucial to assessing a representative emission factor. A decision tree for such a representative monitoring campaign is provided in Figure 4.7.

To design a sampling campaign, the following criteria are considered crucial:

- a) Homogenous influent and continuous feeding of all lanes: All lanes fed simultaneously and performing comparably (i.e. supplied with comparable amounts of air and exhibiting similar soluble O<sub>2</sub> concentration and effluent concentrations) can be assumed to exhibit comparable N<sub>2</sub>O emission dynamics (Figure 4.6a). Inhomogeneous influent may result from WWTP-internal recycles (e.g. supernatant from digesters; Figure 4.6b) or from incomplete mixing of the influent sewage. At most full-scale sites, it is easier to identify feed heterogeneity by comparing key performance indicators among lanes than by sampling the influent of different lanes separately. Also, in cases of intermittent feeding not occurring simultaneously for different

- lanes, separate monitoring is required due to variations in the distribution of the daily incoming load.
- b) Mixing conditions: reactors that are completely stirred during aeration do not require multiple off-gas measuring points (Figure 4.5b); this is mostly the case for SBR reactors.
  - c) Cascaded lanes: Lanes featuring spatial concentration gradients require an adequate number of measuring points. This is also the case if the density of aeration units varies along a lane: each section requires one measuring point. A minimum of three measuring points (e.g. after 1/6 of the reactor length, in the middle and at 5/6) is suitable when a lane exhibits no subdivision into cascades and aerators are evenly distributed (Figure 4.5a).

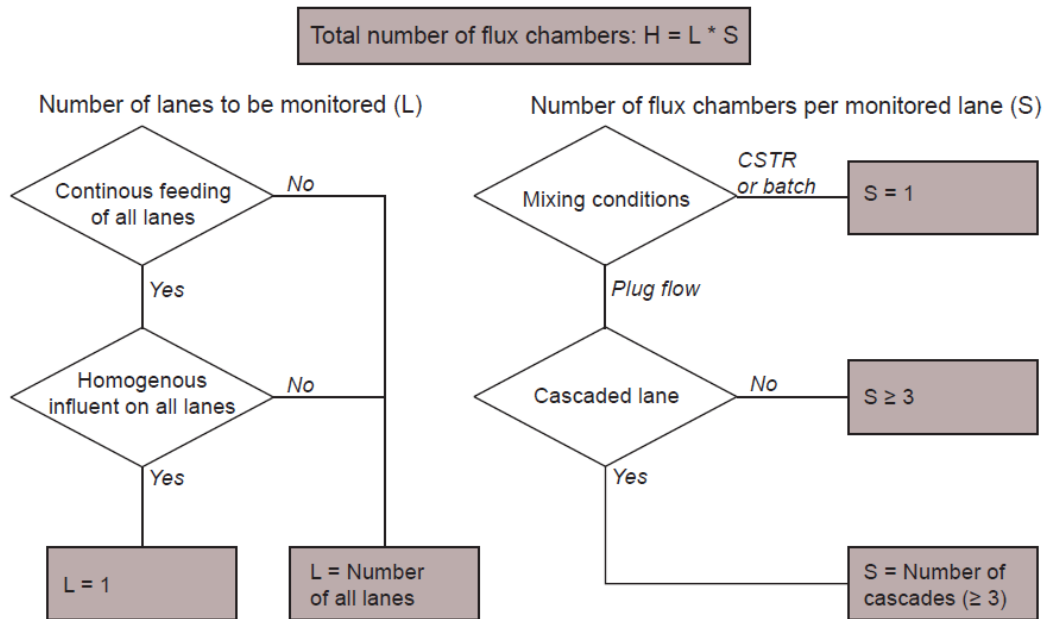


Figure 4.7: Decision tree for the selection of the number of flux chambers and the placement on a particular lane. H = total number of flux chambers, L = number of lanes to be monitored, S = number of flux chambers per monitored lane.

#### 4.3.5 Outlook

Given a) that short and long-term N<sub>2</sub>O emission variations are conspicuous, and b) that no model is available that can describe and quantify all sources of N<sub>2</sub>O emissions, it is advisable to plan monitoring campaigns with some redundancy, such as in the number of measuring points. Nevertheless, it must be acknowledged that the uncertainty of a monitoring campaign is not only dependent on the number and positioning of the measuring points but also on such other factors as the long-term accuracy of the measuring device and emission changes from year to year (Figure 4.4b).

Reliable monitoring strategies applicable to a range of treatment schemes are deemed of great importance to improving understanding of the mechanisms relevant to N<sub>2</sub>O emissions in WWTPs (Vasilaki et al. 2019). Since the majority of WWTPs are composed of open reactors, a flux-chamber-based measurement approach is required. The present paper therefore contributes to a critical evaluation of this off-gas sampling method. A significant simplification of the required monitoring effort is expected only once all the important parameters influencing N<sub>2</sub>O emission have been identified and a reliable model is available. At present, we consider the number of publicly available data sets describing long-term variations in GHG emissions too low to achieve this. Until then, model identification and validation are expected to be feasible only with reliable and representative long-term monitoring data. Consequently, further long-term monitoring campaigns are necessary to establish a broadly supported emission factor for N<sub>2</sub>O from wastewater treatment (Vasilaki et al. 2019).

The separate treatment of reject water was shown to be an efficient measure for reducing N<sub>2</sub>O emissions (Figure 4.6b). In the Altenrhein WWTP, about 80% of the emissions could be reduced, so the emission increase during winter could be avoided almost completely. The fact that this resulted from reducing the influent nitrogen load by only 30% shows that quantitative modeling cannot be based on simple linear correlation with standard parameters. This promising result also confirms the practical value of a better quantitative understanding of these emissions, since solutions for significantly reducing them are feasible in at least some cases.

## 4.4 Conclusions

- This study confirms the high N<sub>2</sub>O emission factors suggested in recent literature for three different activated sludge process configurations: N<sub>2</sub>O is the most important GHG caused by wastewater treatment.
- The temporal emission variation (short- and long-term) was shown to be significant for all process configurations, while the spatial emissions variation along a single lane is only significant in flow through systems with plug-flow characteristics. Here, multiple monitoring spots are required per lane.
- On WWTPs with inhomogeneous loading or discontinuous feeding of different lanes, monitoring of all or at least representative lanes is required to calculate emission factors reliably.
- A separate reject water treatment is a promising strategy to mitigate N<sub>2</sub>O emissions from biological treatment.

## 4.5 Acknowledgements

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## 4.6 References

- Aboobakar, A., Cartmell, E., Stephenson, T., Jones, M., Vale, P. and Dotro, G. (2013) Nitrous oxide emissions and dissolved oxygen profiling in a full-scale nitrifying activated sludge treatment plant. *Water Research* 47(2), 524-534.
- Ahn, J.H., Kim, S.W., Park, H., Rahm, B., Pagilla, K. and Chandran, K. (2010) N<sub>2</sub>O Emissions from activated sludge Processes, 2008-2009: Results of a national monitoring survey in the United States. *Environmental Science & Technology* 44(12), 4505-4511.
- Chandran, K., Volcke, E.I. and Van Loosdrecht, M.C.M. (2016) *Experimental Methods in Wastewater Treatment*. Van Loosdrecht, M.C.M., Nielen, P.H., López Vázquez, C.M. and Brdjanovic, D. (eds), IWA, London.
- Colliver, B.B. and Stephenson, T. (2000) Production of nitrogen oxide and dinitrogen oxide by autotrophic nitrifiers. *Biotechnology Advances* 18, 219-232.
- Conthe, M., Lycus, P., Arntzen, M.O., Ramos da Silva, A., Frostegard, A., Bakken, L.R., Kleerebezem, R. and van Loosdrecht, M.C.M. (2018) Denitrification as an N<sub>2</sub>O sink. *Water Research* 151, 381-387.
- Czepiel, P., Crill, P. and Harriss, R. (1995) Nitrous oxide emissions from municipal wastewater treatment. *Environmental Science & Technology*, 2352-2356.
- Daelman, M.R., De Baets, B., van Loosdrecht, M.C. and Volcke, E.I. (2013a) Influence of sampling strategies on the estimated nitrous oxide emission from wastewater treatment plants. *Water Research* 47(9), 3120-3130.
- Daelman, M.R., van Voorthuizen, E.M., van Dongen, L.G., Volcke, E.I. and van Loosdrecht, M.C. (2013b) Methane and nitrous oxide emissions from municipal wastewater treatment - results from a long-term study. *Water Science and Technology* 67(10), 2350-2355.
- Daelman, M.R., van Voorthuizen, E.M., van Dongen, U.G., Volcke, E.I. and van Loosdrecht, M.C. (2015) Seasonal and diurnal variability of N<sub>2</sub>O emissions from a full-scale municipal wastewater treatment plant. *Science of The Total Environment* 536, 1-11.
- EEA (2017) *Annual European Union greenhouse gas inventory 1990-2015 and inventory report 2017.*, European Environment Agency, Copenhagen, Denmark.
- Ge, J., Huang, G., Li, J., Sun, X. and Han, L. (2018) Multivariate and Multiscale Approaches for Interpreting the Mechanisms of Nitrous Oxide Emission during Pig Manure-Wheat Straw Aerobic Composting. *Environmental Science & Technology* 52(15), 8408-8418.
- Hanaki, K., Hong, Z. and Matsuo, T. (1992) Production of nitrous oxide gas during denitrification of wastewater. *Water Science and Technology* 26(5-6), 1027-1036.

- IPCC (2019) Chapter 5.6 Wastewater treatment and discharge. 2019 Refinement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories, <https://www.ipcc-nggip.iges.or.jp/public/2019rf/index.html>.
- Kampschreur, M.J., Temmink, H., Kleerebezem, R., Jetten, M.S. and van Loosdrecht, M.C. (2009) Nitrous oxide emission during wastewater treatment. *Water Research* 43(17), 4093-4103.
- Kosonen, H., Heinonen, M., Mikola, A., Haimi, H., Mulas, M., Corona, F. and Vahala, R. (2016) Nitrous Oxide Production at a Fully Covered Wastewater Treatment Plant: Results of a Long-Term Online Monitoring Campaign. *Environmental Science & Technology* 50(11), 5547-5554.
- Larsen, T.A. (2015) CO<sub>2</sub>-neutral wastewater treatment plants or robust, climate-friendly wastewater management? A systems perspective. *Water Research* 87, 513-521.
- Mampaey, K.E., van Dongen, U.G., van Loosdrecht, M.C. and Volcke, E.I. (2015) Novel method for online monitoring of dissolved N<sub>2</sub>O concentrations through a gas stripping device. *Environmental Science & Technology* 36(13-16), 1680-1690.
- Marques, R., Oehmen, A. and Pijuan, M. (2014) Novel microelectrode-based online system for monitoring N<sub>2</sub>O gas emissions during wastewater treatment. *Environmental Science & Technology* 48(21), 12816-12823.
- Ni, B.J. and Yuan, Z. (2015) Recent advances in mathematical modeling of nitrous oxides emissions from wastewater treatment processes. *Water Research* 87, 336-346.
- Ostrom, N.E. and Ostrom, P.H. (2017) Mining the isotopic complexity of nitrous oxide: a review of challenges and opportunities. *Biogeochemistry* 132(3), 359-372.
- Pan, Y., van den Akker, B., Ye, L., Ni, B.J., Watts, S., Reid, K. and Yuan, Z. (2016) Unravelling the spatial variation of nitrous oxide emissions from a step-feed plug-flow full scale wastewater treatment plant. *Scientific Reports* 6, 20792.
- Peng, L., Ni, B.-J., Law, Y. and Yuan, Z. (2016) Modeling N<sub>2</sub>O production by ammonia oxidizing bacteria at varying inorganic carbon concentrations by coupling the catabolic and anabolic processes. *Chemical Engineering Science* 144, 386-394.
- Perez-Garcia, O., Villas-Boas, S.G., Swift, S., Chandran, K. and Singhal, N. (2014) Clarifying the regulation of NO/N<sub>2</sub>O production in *Nitrosomonas europaea* during anoxic-oxic transition via flux balance analysis of a metabolic network model. *Water Research* 60, 267-277.
- Ravishankara, A.R., Daniel, J.S. and Portmann, R.W. (2009) Nitrous Oxide (N<sub>2</sub>O): The Dominant Ozone-Depleting Substance Emitted in the 21st Century. *Science* 326(5949), 123-125.
- Stein, L.Y. (2018) Insights into the physiology of ammonia-oxidizing microorganisms. *Current Opinion in Chemical Biology* 49, 9-15.
- Stocker, T.F., Qin, D., Plattner, G.K., Tignor, M.M.B., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V. and Midgley, P.M. (2013) Climate change 2013 the physical

science basis: Working Group I contribution to the fifth assessment report of the intergovernmental panel on climate change.

Vasilaki, V., Massara, T.M., Stanchev, P., Fatone, F. and Katsou, E. (2019) A decade of nitrous oxide (N<sub>2</sub>O) monitoring in full-scale wastewater treatment processes: A critical review. *Water Research* 161, 392-412.

Wunderlin, P., Siegrist, H. and Joss, A. (2013) Online N<sub>2</sub>O measurement: the next standard for controlling biological ammonia oxidation? *Environmental Science & Technology* 47(17), 9567-9568.



# **Chapter 5**

## **Estimation of countrywide N<sub>2</sub>O emissions from wastewater treatment in Switzerland using long-term monitoring data**

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## Abstract

Nitrous oxides (N<sub>2</sub>O) emissions contribute to climate change and stratospheric ozone depletion. Wastewater treatment is an important, yet likely underestimated, source of N<sub>2</sub>O emissions, as recent, long-term monitoring campaigns have demonstrated. However, the available data are insufficient to representatively estimate countrywide emission due to the brevity of most monitoring campaigns. This study showed that the emission estimates can be significantly improved using an advanced approach based on multiple continuous, long-term monitoring campaigns. In monitoring studies on 14 full-scale wastewater treatment plants (WWTPs), we found a strong variability in the yearly emission factors (EFs) (0.1 to 8% of the incoming nitrogen load) which exhibited a good correlation with effluent nitrite. But countrywide data on nitrite effluent concentrations is very limited and unavailable for emission estimation in many countries. Hence, we propose a countrywide emission factor calculated from the weighted EFs of three WWTP categories (carbon removal, EF: 0.1–8%, nitrification only: 1.8%, and full nitrogen removal: 0.9%). However, EF of carbon removal WWTPs are still highly uncertain given the expected variability in performance.

The newly developed approach allows representative, country-specific estimations of the N<sub>2</sub>O emissions from WWTP. Applied to Switzerland, the estimations result in an average EF of 0.9 to 3.6% and total emissions of 410 to 1690 tN<sub>2</sub>O-N/year, which corresponds to 0.3–1.4% of the total greenhouse gas emissions in Switzerland. Our results demonstrate that better data availability and an improved understanding of long-term monitoring campaigns is crucial to improve current emission estimations. Finally, our results confirm several measures to mitigate N<sub>2</sub>O emissions from wastewater treatment: year-round denitrification, limiting nitrite accumulation, and stringent control of sludge age in carbon removal plants.

## 5.1 Introduction

Nitrous oxide ( $\text{N}_2\text{O}$ ) emissions contribute substantially to climate change (IPCC 2014) and stratospheric ozone depletion (Ravishankara et al. 2009). The atmospheric  $\text{N}_2\text{O}$  concentration is expected to rise until the middle of the 21st century (Tian et al. 2020). Wastewater treatment plants (WWTPs) and their  $\text{N}_2\text{O}$  production are currently often underestimated emission processes in national greenhouse gas (GHG) inventories. Estimations of countrywide  $\text{N}_2\text{O}$  emissions are based on assumed emission factors (EFs). EFs in the standard reporting guidelines are much lower (0.03% to 0.14%; (Daelman et al. 2015, IPCC 2006)) than reported long-term full-scale measurements. With the refinement of the IPCC methodology in 2019, increased EFs of 0.01% to 2.9% appeared in the reporting guidelines (IPCC 2019b). In wastewater treatment,  $\text{N}_2\text{O}$  formation is primarily caused by biological nitrogen removal through nitrification and denitrification (Kampschreur et al. 2009b). The main production processes are the biological stage in the water lines of WWTPs (Kosonen et al. 2016) and the side-stream treatment for reject water from the sludge lines (Joss et al. 2009, Kampschreur et al. 2009a). Emissions of  $\text{N}_2\text{O}$  produced on WWTPs can occur either on site or in receiving waters if dissolved  $\text{N}_2\text{O}$  is discharged (Marescaux et al. 2018, Mikola et al. 2014). However, roughly 80% of the  $\text{N}_2\text{O}$  emissions from WWTPs are released in the aerated zone of biological treatment (Baeten et al. 2020, Chen et al. 2019).

Quantifying representative EFs at the biological stage of WWTPs requires continuous long-term monitoring campaigns due to the substantial daily variation and seasonality of emissions (Daelman et al. 2013a). A strong seasonal emission pattern with high emission in spring and low emissions in autumn was previously shown in several long-term monitoring campaigns (Gruber et al. 2020). However, only six continuous monitoring campaigns of at least one year's duration have been reported to our knowledge (Chen et al. 2019, Daelman et al. 2015, Gruber et al. 2020, Kosonen et al. 2016). Five of these studies were conducted in temperate climates, with a monthly mean temperature of over 10 °C during 4–7 months (Belda et al. 2014). The EF assessed (0.8–2.9% of the total nitrogen loads) were shown to be substantially higher than those found by many short-term campaigns (Chen et al. 2019, Daelman et al. 2015, Gruber et al. 2020, Kosonen et al. 2016, Vasilaki et al.

2019). The causes of the wide range of EFs assessed are still unknown (Vasilaki et al. 2019). Hence, we conclude that additional online long-term monitoring campaigns are much needed to better characterize the variability from N<sub>2</sub>O emissions in full-scale WWTPs. Ultimately, a broad data basis is crucial for countrywide assessment of the N<sub>2</sub>O EFs from wastewater treatment.

Various approaches have been suggested for the extrapolation of N<sub>2</sub>O emissions from WWTPs at countrywide level (Ramirez-Melgarejo et al. 2020). The standard approach is described by the IPCC guidelines (IPCC 2006), which are designed to be calculated with generally accessible variables. Consequently, the approach leads to high uncertainties of the total emission estimates for two reasons. Firstly, in a top-down approach, the nitrogen load to WWTPs is estimated from a country's protein consumption, resulting in substantial differences compared to a bottom-up approach, using measured nitrogen influent loads to WWTPs (Ramirez-Melgarejo et al. 2020). Secondly, the IPCC guidelines suggest a uniform EF independent of the type of WWTP (IPCC 2006). The assumption of a uniform EF leads to high uncertainties, due to the wide range of EFs reported for different types of WWTPs (Cadwallader and Van Briesen 2017) and poorly described key factors characterizing N<sub>2</sub>O EFs (Vasilaki et al. 2019). Several methods have been suggested to overcome these shortcomings arising from short-term monitoring campaigns (Ahn et al. 2010, Valkova et al. 2020). However, the integration of continuous long-term monitoring campaigns on different process types is needed to obtain the most accurate estimates for countrywide N<sub>2</sub>O EFs.

Here, we propose a refined approach to estimating N<sub>2</sub>O emissions from WWTPs based on the EFs of a dozen long-term monitoring campaigns on full-scale WWTPs in Switzerland. To acquire a broad data basis, we conducted seven monitoring campaigns of at least one year's duration on full-scale WWTPs using an adaptation of the flux chamber method (Gruber et al. 2020). Additionally, we extracted the same results from seven long-term monitoring campaigns reported in literature (Chen et al. 2019, Gruber et al. 2020, Kosonen et al. 2016). We used Spearman's correlation analysis to characterize key variables of a WWTP to predict N<sub>2</sub>O EFs. We use our results to link the variables to patterns detected in our monitoring campaigns to provide experimental evidence for the correlations found, and we conducted full-

scale tests on one WWTP to prove concepts from the correlation analysis. Finally, we propose a method for calculating N<sub>2</sub>O emissions for Switzerland and compare it with the methods described in the IPCC guidelines (IPCC 2006, 2019b) regarding total emissions and corresponding uncertainties.

## 5.2 Material & Methods

### 5.2.1 Monitoring approach

N<sub>2</sub>O emissions were assessed using an adapted version of the flux-chamber method as described in Gruber et al. (2020) (Figure 5.1). In total, five different setups based on the same general concept were applied to monitor the emission on seven WWTPs (see diagrams in the Supporting Information: SI). In short, all discontinuously fed reactors were equipped with a flux chamber. On WWTPs with continuously fed lanes, one or more lanes were monitored, each with three or more flux chambers per lane placed on the aerated compartments.

In contrast to the standard method (Chandran et al. 2016), a chamber (surface: 1 m<sup>2</sup>) with an open outlet was used, and therefore sample gas was not recirculated to the flux chamber after measurement. A sample of the gas flowing through the chamber was diverted at the outlet through a tube with a length of up to a few hundred meters to the central monitoring station, where the off-gas was measured in a non-dispersive infrared analyser (X-stream, Emerson, St. Louis MO, USA). Prior to the measurement, the off-gas was dehumidified by cooling to 4 °C (JCP SL, JCT, Wiener Neustadt, AUT or TC-Standard (PKE 521), Bühler, Ratingen, GER). To monitor the entire biological treatment at multiple sampling points and on various lanes, up to 14 floating hoods can be connected to our system over three-way valves (Parker Lucifer type 7131KBG2JV00, Cleveland, USA). While one of the valves is open to the measurement line, the other channels are purged with a pump (ME 2 NT, Vaccubrand, Wertheim, GER) to ensure a constant flow through all the tubes. The constant flow is considered important for two reasons: (i) to reduce system response

time and thus allow fast switching between valves (1 min per measurement) and ii) to avoid freezing of sampling tubes due to humidity in tubing and cold temperatures.

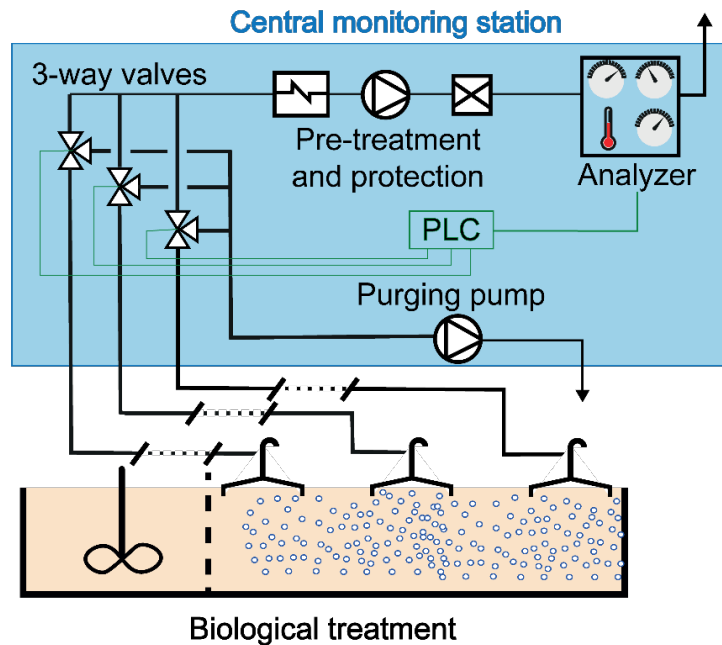


Figure 5.1: Schematic overview of the monitoring setups applied. Blue rectangle indicates all elements within central monitoring station. PLC: programmable logical controller.

The system is controlled with a programmable logical controller (PLC type WAGO 750–881) which provides two key functions: (i) switching between the valves, and (ii) analogue–digital signal conversion and data storage. Key control variables of the system are a flowmeter (MEMS flow sensor D6F-10A6–000, Omron, Kyoto, JP) in the sample gas duct, a paramagnetic oxygen sensor in the gas analyser (Xstream, Emerson, St. Louis MO, USA), and a humidity sensor (KW1, JCT, Wiener Neustadt, AUT) after the pre-treatment. The flowmeter allows detection of malfunctioning across the whole system or in specific channels when the measured flow drops below a set threshold. The oxygen sensor signals substantial leaks in the channel when oxygen concentration is close to atmospheric concentrations and does not show variation. Finally, the humidity sensor is used as a binary sensor. In case of humidity after the pre-treatment, the measurement is switched off to prevent the analyser malfunctioning. As a second barrier against water entering the analyser, a protection filter is installed directly after the pre-treatment. A field computer is used to parametrize the PLC. Additionally, the field computer establishes data transmission

to a data server via the Secure File Transfer Protocol (SFTP) and a 3 G modem (IMON-U300, Insys Icom, Regensburg, GER). On the data server, the monitoring data is processed and synchronized with the operational data of the WWTPs and visualized on demand. The operators of the WWTPs assessed lab data on concentrations of total nitrogen, nitrogen species, and COD sampled from influent, effluent primary clarifier, effluent biological treatment, and effluent WWTP at various intervals. Operational data on influent flow, air supply, wastewater temperature, dissolved oxygen concentrations was acquired over the WWTP's supervisory control and data acquisition (SCADA) system. A detailed description of the monitoring setup can be found in Appendix C (SI).

### **5.2.2 WWTP selection and monitoring campaigns**

The seven WWTPs monitored were selected to represent the range of nutrient removal goals set and common types of processes installed in WWTPs in Switzerland (Table 5.1). Common removal goals are i) carbon removal ii) nitrification and iii) denitrification. Common processes include various types of activated sludge (AS) systems, such as plug-flow (conventional activated sludge (CAS), anoxic-aerobic (AO), anaerobic-anoxic-aerobic (A2O)), alternately fed intermittently aerated (A/I) and, sequencing batch reactors (SBR)) and biofilm systems (hybrid fixed bed and activated sludge (IFAS), fixed bed (FB)). We sought to represent various types of WWTP size classes (Table 5.1). Monitoring campaigns were conducted over at least 1 year. Flux chambers were placed on lanes in accordance with the monitoring strategy proposed in Gruber et al. (2020) (see section 5.2.1 and SI). The monitoring campaigns reported in Gruber et al. (2020) were included in the selection. Additionally, data from three long-term monitoring campaigns of previous studies was included for the statistical analysis of the data (Chen et al. 2019, Daelman et al. 2015, Kosonen et al. 2016). These studies include data on one AO process and two carrousel (CARR) activated sludge processes. Detailed information on the WWTPs monitored and the results of the newly conducted monitoring campaigns can be found in the SI.

Table 5.1: Characteristics of monitored WWTPs and origin of monitoring campaigns used in this study.

WWTP (country)	Design size (PE)	Process	Removal goal	Origin
Altenrhein (CH)	80,000	CAS	Nitrification	(Gruber et al. 2020)
Altenrhein (CH)	40,000	FB	Nitrification	(Gruber et al. 2020)
Avdore (DK)	350,000	CARR	Denitrification	(Chen et al. 2019)
Bazenheid (CH)	50,000	IFAS	Denitrification	this study
Birs (CH)	150,000	SBR	Denitrification	this study
Giubiasco (CH)	100,000	CAS	Carbon removal	this study
Hofen (CH)	50,000	AO	Denitrification	this study
Kralingseveer (NL)	360,000	CARR	Denitrification	(Daelman et al. 2015)
Luzern (CH)	280,000	A/I	Denitrification	(Gruber et al. 2020)
Moossee (CH)	50,000	AO	Denitrification	this study
Schönau (CH)	180,000	A2O	Denitrification	this study
Uster (CH)	50,000	SBR	Nitrification	(Gruber et al. 2020)
Vikinmäkki (FI)	840,000	CAS	Denitrification	(Kosonen et al. 2016)
Werdhölzli (CH)	670,000	A/I	Denitrification	this study

### 5.2.3 N<sub>2</sub>O emission and EF calculation

The net N<sub>2</sub>O flux for a specific lane was computed with the approach presented by Aboobakar et al. (2013) from the airflow supplied to each reactor section equipped with a flux chamber and the respective N<sub>2</sub>O concentration. When gaps between two consecutive data points did not exceed 30 min, N<sub>2</sub>O concentrations were linearly interpolated; data gaps longer than 30 min were excluded (see Table B.1: Duration of monitoring campaigns and number of days evaluated (i.e. availability of the monitoring device)). The air flow supplied to the reactor was estimated with three different methods depending on the WWTP: i) from the blower speed and the blower specification provided by the plant operators, ii) by measurement of the total air flow provided to a treatment lane, or iii) by measurement of the air flow provided to a compartment of the treatment lane. The airflow was recorded at intervals of 1 min, and emissions were calculated in 1 min intervals only for the aerated phases. Emissions from the whole treatment plant were extrapolated according to suggestions made in Gruber et al. (2020): when wastewater loadings were expected to differ or operation strategies to change, all lanes were monitored, but lanes with comparable process performance and operation were assumed to emit comparable quantities of N<sub>2</sub>O.



N<sub>2</sub>O EFs were calculated with Eq. (1) for the whole duration of the monitoring campaigns (Aboobakar et al. 2013, Gruber et al. 2020). For the monitoring campaigns that were substantially longer than 1 year (+ 3 months), we evaluated the EFs for all possible data sets of 1 year and calculated their average and standard deviation. In the newly conducted monitoring campaigns of more than 15 months, the yearly average EFs did not exhibit substantial variation. EFs always refer to the yearly average influent nitrogen load of the WWTPs calculated based on 24-hour composite samples. The samples were taken and analysed by the operators of the WWTPs every 5 to 14 days. Where nitrogen influent measurements were not available, we estimated the influent nitrogen load by evaluating a nitrogen mass balance over the primary clarifier based on typical values for mass flow coefficients from standard textbooks (Gujer 2007, Tchobanoglous et al. 2014), because at least effluent loads of the primary clarifier were available in every case (Figure S3, SI). All monitoring data and the values for the nitrogen loads can be found in the SI. (Gujer 2007), since at least effluent loads of the primary clarifier were available in every case (Figure B.3 (SI)). All monitoring data and the values for the nitrogen loads can be found in the Appendix B (SI).

$$EF_{N_2O} = \frac{\sum_{d=1}^{365} \sum_{m=1}^{1440} (C_{N_2O,m,d} * Q_{air,m,d})}{365 * Nload_{daily}} \quad (5.1)$$

Where  $EF_{N_2O}$  is the N<sub>2</sub>O EF [kg N<sub>2</sub>O-N/d],  $C_{N_2O,m,d}$  is the measured N<sub>2</sub>O concentration in the off-gas during minute  $m$  at day  $d$  [kg N<sub>2</sub>O-N/m<sup>3</sup>],  $Q_{air,m,d}$  is airflow supplied by the blower of the aeration system of the WWTP to reactor surface area representative for a sampling point during minute  $m$  at day  $d$  [m<sup>3</sup>/d], and  $Nload_{daily}$  is the yearly average nitrogen load per day (kgN/d).

#### 5.2.4 Meta data and correlation analysis

The correlation analysis included key figures and performance indicators that were collected for each WWTP. Spearman rank correlation was used to study the relationships between variables. The following variables on the design and operation of the WWTPs were included: design load, process for biological treatment, type of

biomass, biological reactor volume, aerated volume in biological treatment, non-aerated volume in biological treatment, volume of secondary clarifier (if present), nutrient removal goal, aerobic solids retention time (aerobic SRT), total solids retention time (SRT) and information on post, side-stream and sludge treatment (fast sand filtration, supernatant treatment type, co-digestion). The corresponding data for all WWTPs is available in Table B.2 (SI). If possible, we collected and calculated values for the total nitrogen and COD loads at the influent and the effluent of the biological treatment and of the WWTPs. Additionally, we collected values on nitrite (NO<sub>2</sub><sup>-</sup>) concentration in the effluent of the biological treatment. The input variables for the correlation analysis are summarized in Table B.3 (SI). All statistical calculations were performed using Python programming language (version 3.8.3) (Van Rossum and Drake 2009) and the Pandas (McKinney 2010), Numpy (Harris et al. 2020a), and Scipy packages (Virtanen et al. 2020).

#### **5.2.5 Full scale tests at the Hofen WWTP**

We conducted full-scale experiments to compare the effects on N<sub>2</sub>O emissions of a pre-denitrification zone with those of a fully oxic process at the Hofen WWTP (see SI for further details). The plant has an AO activated sludge process consisting of six lanes where two lanes use the same clarifier and sludge recycle (Appendix B). Every lane consists of three zones with an equal size, of which the first zone is typically anoxic. Four of the six lanes were monitored: lane 1–1, lane 2–1, lane 2–2, and lane 3–2. Whereas lane 2–1 and lane 2–2 share the same secondary clarifier, lane 1–1 and lane 3–2 are independent of each other. Between February and April 2020, the first of three zones on two lanes (lanes 2–1 and 3–2) were fully aerated. The exact dates and durations for each experiment are given in Table 5.2.

Table 5.2: Start date, end date, duration, and experimental lanes during experimental with one lane fully aerated at the Hofen WWTP.

Start date	End date	Days	Lane fully aerated
4.2.2020	14.4.2020	70	2-1
14.4.2020	27.4.2020	13	3-2
17.6.2020	24.6.2020	10	2-1
9.9.2020	23.9.2020	13	2-1

### 5.2.6 Extrapolation to the whole Switzerland and uncertainty estimation

Countrywide N<sub>2</sub>O emissions were estimated by multiplying the total nitrogen load to the WWTP and different estimates of the EFs from WWTPs for Switzerland. We used four approaches for the estimation of EFs: a) the 2006 IPCC guidelines (IPCC 2006), b) the 2019 refinement of the IPCC guidelines (IPCC 2019b), c) the average EF of all monitoring campaigns analysed in this study with a bottom-up approach for activity data estimation, based on extrapolation from data on 70% of the Swiss wastewater load treated in WWTPs (Strähl et al. 2013), and d) a method developed in this study that used the same nitrogen loads as in c). Emissions outside of the biological treatment of a WWTPs were not considered. All equations refer to the standard IPCC terminology.

#### *Approach a*

The 2006 version of the IPCC guidelines (IPCC 2006), the current standard method, were applied according to Eq. (5.2) of this study. The values for the estimates were chosen according to the Swiss implementation of the 2006 IPCC guidelines. The EF applied was 0.032%, as suggested by the guidelines. A standard deviation could not be calculated for the resulting countrywide emission, because the 2006 EF is based on a single monitoring campaign. Numerical values for other variables are given in Table 5.3.

$$N_2O_{PLANTS} = Protein * F_{NPR} * T_{PLANTS} * F_{IND-COM} * EF_{PLANTS} \quad (5.2)$$

Where N<sub>2</sub>O<sub>PLANTS</sub> are the total, direct N<sub>2</sub>O emissions from WWTPs in Switzerland per year (kgN<sub>2</sub>O-N/year), Protein is the Swiss Protein consumption (kg Protein/year), F<sub>NPR</sub> is the fraction of nitrogen in protein (-), T<sub>PLANTS</sub> is the connection

rate to WWTPs (-),  $F_{IND-COM}$  is the factor for industrial and commercial protein (-),  $EF_{PLANTS}$  is the EF for N<sub>2</sub>O from WWTPs (kg N<sub>2</sub>O-N / kg N).

### *Approach b*

The 2019 refinement of the 2006 IPCC guidelines (IPCC 2019b) were applied according to Eq. (5.3) of this study. The values for the estimates were chosen from the Swiss implementation of the 2006 IPCC guidelines, and if not available, according to the default values in the 2019 refinement. The EF applied was  $1.6 \pm 0.5\%$ , which was calculated as the mean and the standard error of the monitoring campaigns used for the guideline (Table B.5 (SI)) (IPCC 2019b). Numeric values of further variables are given in Table 5.3.

$$N_2O_{PLANTS} = Protein * F_{NPR} * T_{PLANTS} * N_{HH} * F_{NON-COM} * F_{IND-COM} * EF_{PLANTS} \quad (5.3)$$

Where  $N_2O_{PLANTS}$  are the total, direct N<sub>2</sub>O emissions from WWTPs in Switzerland per year (kgN<sub>2</sub>O-N/year), Protein is the Swiss Protein consumption (kg Protein/year),  $T_{PLANT}$  is the connection rate to WWTPs (-),  $F_{NPR}$  is the fraction of nitrogen in protein (-),  $N_{HH}$  is the additional nitrogen from household products added to the wastewater (-),  $F_{IND-COM}$  is the factor for nitrogen in non-consumed protein disposed in sewer system (-),  $F_{IND-COM}$  is the factor for industrial and commercial protein (-),  $EF_{PLANTS}$  is the EF for N<sub>2</sub>O from WWTPs (kg N<sub>2</sub>O-N / kg N).

Table 5.3: Selected values for the estimation of the activity data (nitrogen load) in Switzerland for 2011 based on the IPCC methods (IPCC 2006, 2019b).

Variable	2006 IPCC guidelines	2019 refinement
P: Swiss protein consumption (t/year)	306,000	306,000
F <sub>NPR</sub> : Nitrogen content of protein (default value) (kgN/kg protein)	0.16	0.16
T <sub>PLANT</sub> : Connection rate to WWTP (%)	97%	97%
F <sub>IND-COM</sub> : Factor for industrial and commercial protein (default value) (-)	1.25	1.25
F <sub>NON-COM</sub> : Factor for industrial and commercial protein (default value) (-)	-	1.09
N <sub>HH</sub> : Additional nitrogen from household products added to the wastewater (default value) (-)	-	1.08
Calculated total nitrogen load to WWTP (kg N/year)	60,000	70,000

#### *Approaches c, d*

For the bottom-up approach, we used the data set described in (Strähl et al. 2013) to estimate the nitrogen load in the wastewater treated of all Swiss WWTPs. This dataset contains information on influent loads, treatment type and treatment performance for WWTPs covering ~70% of the wastewater treated in CH in 2011 (Strähl et al. 2013). We assumed the same fractions of WWTP types and extrapolated the nitrogen loads found in the dataset to the WWTPs treating the remaining ~30% of the wastewater in Switzerland. An overview of the data set is provided in Appendix B.7 (SI). Countrywide emissions in approaches c and d were calculated according to Eq. (5.4). Approach (d) was calculated for two scenarios: d1 incorporates a high emission factor for the carbon removal category and d2 a low emission factor.

$$N_2O_{PLANTS} = EF_{PLANTS} * N_{PLANTS} \quad (5.4)$$

Where  $N_2O_{PLANTS}$  are the total, direct  $N_2O$  emissions from WWTPs in Switzerland per year (kg $N_2O$ -N/year),  $EF_{PLANTS}$  is the estimated average EF of Switzerland (kg

N<sub>2</sub>O-N / kg N) and N<sub>PLANTS</sub> is the total nitrogen load treated in wastewater treatment plant (kgN/year).

The average EF was assessed by calculating the arithmetic mean of all monitoring campaigns reported in this study (approach c) or a weighted sum of emission factors estimated separately for the three categories of WWTPs (carbon removal, nitrification only, and nitrogen removal) in Switzerland (approaches d1 and d2) (Eq. (5.5)).

$$EF_{PLANTS} = \sum_{i=1}^3 f_i * EF_i \quad (5.5)$$

Where EF<sub>PLANTS</sub> is the estimated average EF of Switzerland in scenarios (d1) or (d2) of approach (d) (kg N<sub>2</sub>O-N / kg N), f<sub>i</sub> is the fraction of nitrogen loading in Switzerland treated in WWTPs belonging to category i, and EF<sub>i</sub> is the estimated EF for category i.

#### *Estimation of uncertainties*

We estimated the uncertainties (standard deviation) of the estimated average EFs (EF<sub>PLANTS</sub>) using linear error propagation. The standard error (SE) of the countrywide EF estimates for approaches (b), (c), (d1) and (d2) were calculated by Eq. (5.6). It was assumed that the average EFs for each category in scenarios (d1) and (d2) are uncorrelated with each other. Approach (a) is based on a single fixed EF, so uncertainty cannot be quantified.

$$SE = \sqrt{\sum_{i=1}^m \left( \frac{f_i * \sigma_i}{\sqrt{n_i}} \right)^2} \quad (5.6)$$

W where SD<sub>EF</sub> is the standard deviation of the estimated average EF, m is the number of categories of WWTPs (b,c: 1, d1, d2: 3), n<sub>i</sub> is the number of samples in category i, f<sub>i</sub> is the fraction of nitrogen loading in Switzerland treated in WWTPs belonging

to category  $i$ , and  $\sigma_i$  is the standard deviation of the EFs in category  $i$ . The fraction  $\frac{\sigma_i}{\sqrt{n_i}}$  represents the standard deviation of EF <sub>$i$</sub> .

## 5.3 Results and discussions

### 5.3.1 N<sub>2</sub>O emission factors from long-term monitoring campaigns

The monitoring campaigns studied showed a yearly N<sub>2</sub>O EF ranging from 0.1% to 8% of the total influent nitrogen load (Figure 5.2). The range of EF is significantly wider than has been reported from previous long-term monitoring campaigns (1.1 to 2.9%), mainly due to the high EF of the Giubiasco WWTP, whose treatment goal is carbon removal. The average value of 1.6% for all studies is comparable to the value proposed in the updated IPCC guidelines (IPCC 2019b). However, the high standard deviation of the average EF (2% equivalent to 125% of the mean value) shows clearly that using average EFs for countrywide extrapolations leads to very high uncertainties, which is in line with previous research (Valkova et al. 2020). Characterizing the N<sub>2</sub>O EF of a WWTP depending from key indicators is therefore essential for the robust calculation of countrywide EFs.

None of the explanatory factors displayed in Figure 5.2 exhibits a strong relation with the N<sub>2</sub>O EF. While nitrogen removal efficiency displays a weak and insignificant correlation ( $r = 0.51$ ,  $p = 0.06$ , Figure 5.3), treatment size does not correlate at all with N<sub>2</sub>O EF ( $r = 0.002$ ,  $p = 0.99$ , Figure 5.3). The low relevance of the reactor configuration and the treatment process can be only discussed qualitatively due to the low number of monitoring campaigns for different processes: Firstly, flow through activated sludge systems exhibited a wide range of EFs (0.08% - 8%;  $n = 6$ ). The systems monitored with our setup with an anoxic zone during the whole year (processes: AO and A2O,  $n = 3$ ) did not exceed an EF of 0.3%. The very low EFs are not in line with the value (1.9%) for the Viikinmäki WWTP, which uses with an AO process. This dissimilarity may be explained by the difference between its influent conditions: Viikinmäki is served by a separated sewer system and the climate in Finland differs from that of central Europe (Kosonen et al. 2016). Moreover, the nitrogen removal efficiency of the biological treatment is 60%: quite

low compared to the other AO and A2O systems (Figure 5.2). Secondly, the EFs of two activated sludge plants with SBR configuration differed substantially (0.3% vs. 2.3%) as their nutrient removal goal differed: nitrification vs. denitrification. Previously, SBR systems were reported to cause generally higher N<sub>2</sub>O emissions (Vasilaki et al. 2019), but our results indicate that low EFs can be reached in SBR systems too. Thirdly, the two A/I activated sludge systems varied substantially (0.2% vs 0.8%) even though they shared the same removal goal, denitrification. Finally, both biofilm systems monitored had a high EF (1.4%), which is closely in line with previously reported values (Bollon et al. 2016). The systems monitored by Daelman et al. (2015) at Kralingseveer WWTP and Chen et al. (2019) at Avedøre WWTP have carrousel reactors for the biological treatment and exhibited high EFs (1.1% and 2.9% respectively).

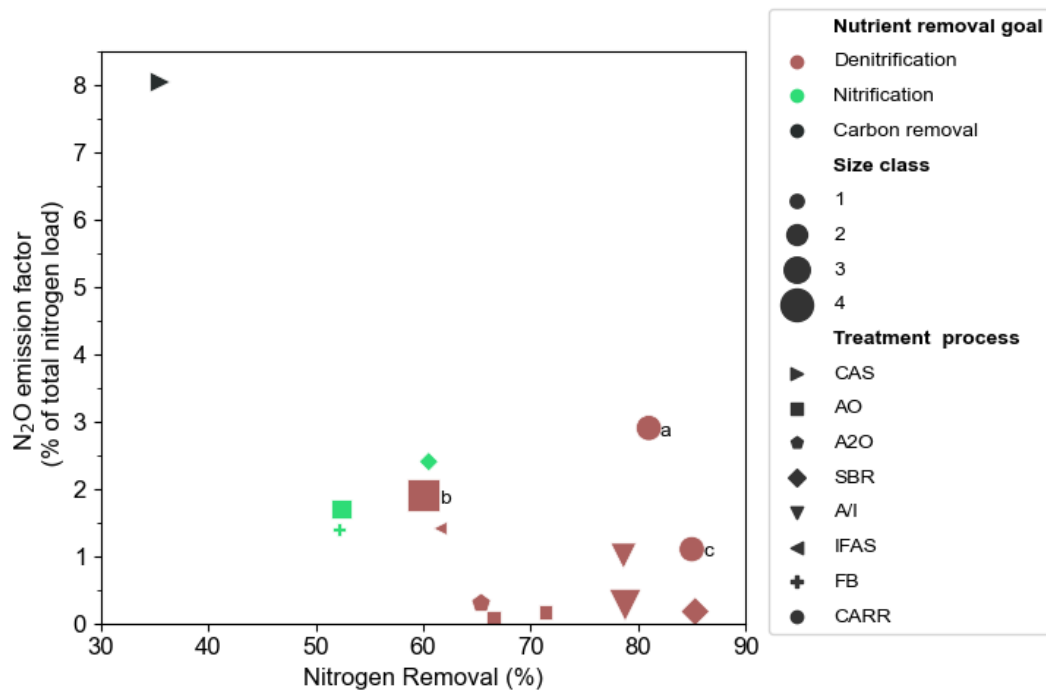


Figure 5.2: N<sub>2</sub>O EF calculated for all available monitoring campaigns as a function of the yearly average nitrogen removal efficiency. Colors describe nutrient removal goal. Small letters highlight campaigns assessed with a different monitoring method than the flux chamber method (a: (Daelman et al. 2015), b: (Kosonen et al. 2016), c: (Chen et al. 2019)). Size of data points describes the size class of the WWTPs (1: < 50,000 PE, 2: 50,000 – 200,000 PE, 3: 200,000 – 500,000 PE, 4: > 500,000 PE). Shape of data points describe treatment process.



The EFs assessed by other research groups are higher on average. This might be linked to the monitoring approach. In contrast to our flux chamber based monitoring approach, the other monitoring campaigns were conducted using i) measurement of dissolved  $\text{N}_2\text{O}$  in the biological treatment combined with a stripping model and ii) the measurement of the collected off-gas of covered WWTP. The study by Chen et al. (2019) used approach i) and resulted in an EF similar to that found in this study. The studies by Daelman et al. (2015) and Kosonen et al. (2016) used approach ii) and reported very high EFs despite high nitrogen removal in the biological treatment. Unlike the flux chamber approach, emissions from unaerated zones in the biological treatment and secondary clarification can be assessed using approaches i) and ii). However, the relative contribution of these emissions has been found to be of minor importance (Chen et al. 2019, Mikola et al. 2014). Three main sources of uncertainty arise when using the flux chamber method for monitoring  $\text{N}_2\text{O}$  emissions from the aerated zone in the biological treatment. Firstly, inhomogeneous air supply within the biological treatment due to preferential flows of air and aging of the membrane aerators can locally affect airflow rates and calculated emissions substantially. To reduce those uncertainties, we used a multi-flux-chamber-approach with at least three flux chambers per lane to cover the full range of off-gas concentrations within a treatment lane. Secondly, inhomogeneity in nutrient and sewage loading of parallel lanes in a WWTP may contribute to variation in the emissions between lanes, which remains unobserved if not all lanes are monitored (Gruber et al. 2020). Thirdly, the quality of air flow and blower data differs highly between plants, ranging from blower frequency data of single blowers supplying several lanes to installed air flow meters on each zone of a treatment lane. To quantify the relevance of the uncertainties and compare the off-gas monitoring approaches, a study comparing all monitoring approaches applied on one WWTP would be needed.

### 5.3.2 Variables relevant for yearly $\text{N}_2\text{O}$ EF

Figure 5.3 shows that the EF displays the highest and only statistically significant correlation with the effluent  $\text{NO}_2^-$  load expressed as a fraction of the incoming nitrogen load. Similarly, concentration peaks coincided with  $\text{N}_2\text{O}$  emissions peaks at WWTP with high EF, where  $\text{NO}_2^-$  effluent concentrations were measured

(Giubiasco, Lucerne, Kralingseveer). NO<sub>2</sub><sup>-</sup> accumulation has previously been linked to high N<sub>2</sub>O emissions in nitrification-denitrification systems (Kampschreur et al. 2008, Peng et al. 2015). A negative correlation with nitrogen removal efficiency was expected, because denitrifying communities have a high capacity to scavenge N<sub>2</sub>O produced during both anoxic and aerobic conditions, which has been demonstrated in lab- and full-scale WWTP studies (Conthe et al. 2018a, Rodriguez-Caballero et al. 2015). However, a few WWTPs (Lucerne, Avedøre and, most strikingly, Kralingseveer) had a high EF factor ( $\geq 1\%$ ) despite high nitrogen removal efficiency, most likely due to nitrite accumulation. Excluding the Kralingseveer WWTP data from the correlation analysis results in a strong and substantial relation between the EF and nitrogen removal efficiencies ( $r = 0.73$ ,  $p = 0.005$ ). Other variables that could potentially be linked to N<sub>2</sub>O did not show significant correlation: nitrogen loading, specific reactor volume, C to N ratio at inflow, aerobic SRT, and WWTP utilization (average/design load). However, yearly average values are only partly useful for such correlation analysis, because seasonal peak phases are not well-represented (Vasilaki et al. 2018). Nevertheless, we conclude from the relations found that countrywide estimations can be improved by considering three factors governing the N<sub>2</sub>O EF of a WWTP: i) seasonal emission pattern and NO<sub>2</sub><sup>-</sup> accumulation, ii) all-year denitrification, and iii) unstable nitrification. These factors are discussed in the following sections.

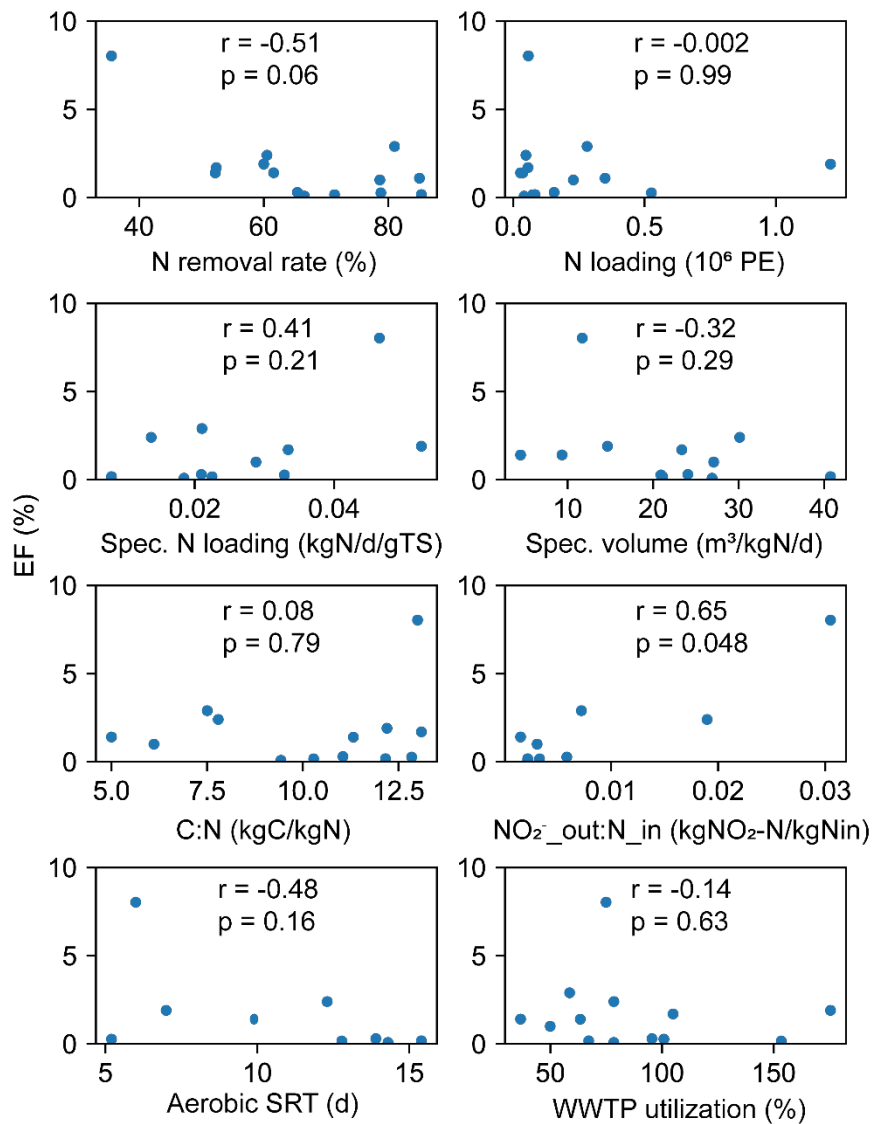


Figure 5.3: Correlation coefficients (spearman's rank,  $r$ ) and significance levels ( $p$ ) of selected variables with  $\text{N}_2\text{O}$  EFs. Abbreviations: C:N = ratio in the influent;  $\text{N}_{\text{in}}$  = nitrogen inflow;  $r$  = correlation coefficient; Spec. = specific; SRT = sludge retention time.

### 5.3.3 Seasonal emission pattern and $\text{NO}_2^-$ accumulation

A strong and reproducible emission pattern has been found in our own  $\text{N}_2\text{O}$  emission monitoring campaign and in all previous studies except for the Viikinmäki stud (Figure 5.4). Emissions typically peaked in March, April or May and dropped over several months to a minimum in September or October. Hence, we analysed the seasonal emission pattern of the long-term monitoring campaigns for all biological nutrient removal (BNR) activated sludge processes in temperate climates, since this category represents the majority ( $n = 10$ ) of the WWTPs monitored. The analysis of

seasonality is considered representative, because for some WWTPs more than 1 year of data were available (Uster, Lucerne, Altenrhein) and the observations represent independent measurements of comparable processes during different years.

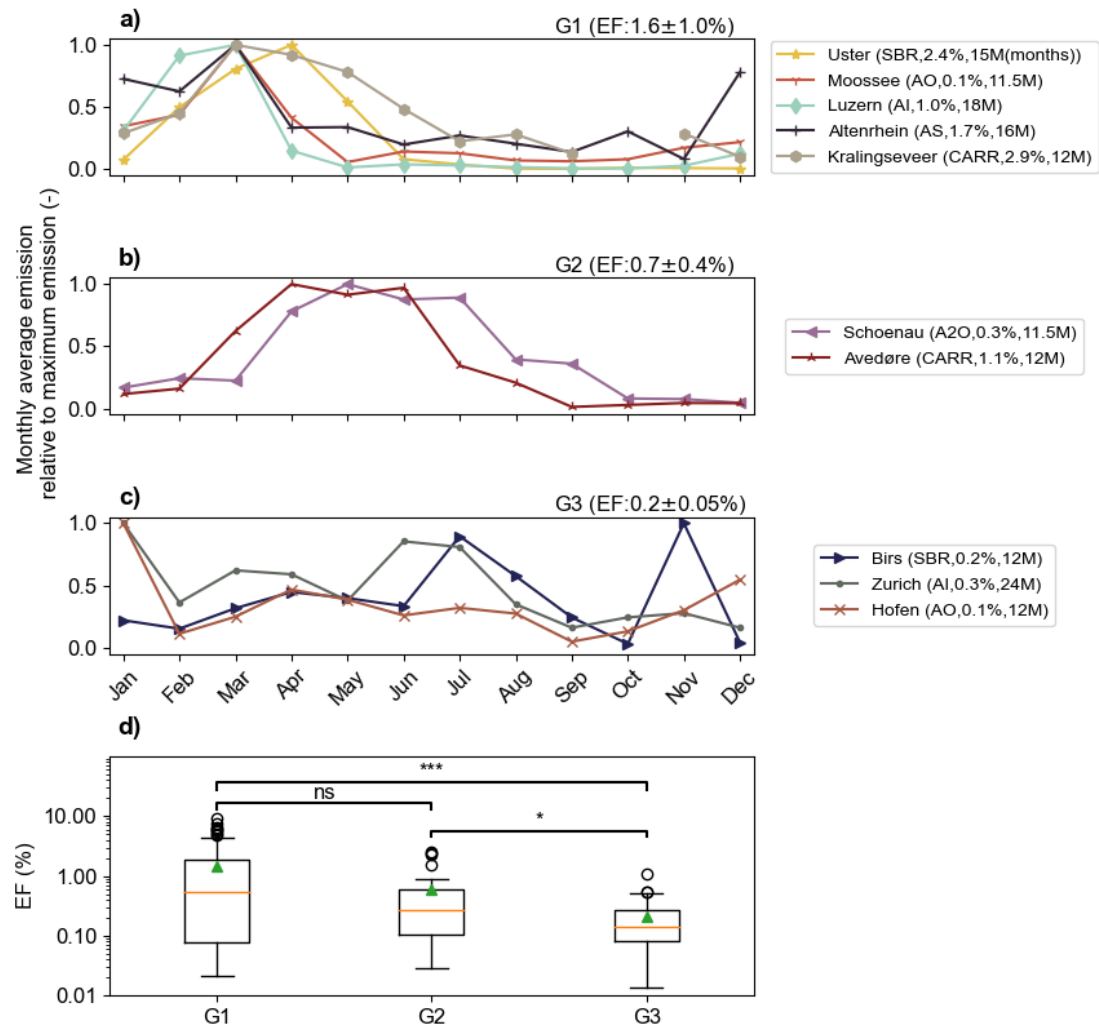


Figure 5.4: Monthly average N<sub>2</sub>O emission as a fraction of the monthly maximum emission for all monitoring campaigns analysed (a-c). The monitoring campaigns are grouped by the presence and shape of a seasonal emission pattern (G1: peak month in Feb/March, G2: peak months Apr/May-Jun/Jul, G3: no clear emission peak phase). EFs displayed above panels represent average EFs and standard deviation for each group. Boxplots (d) displaying the EFs in different groups (G1–3). First quartiles, medians (red line), and third quartiles are displayed in the box. Triangles indicate mean values and circles represent outliers. Significance levels (Mann-Whitney-Wilcoxon) indicates whether groups (G1 or G2) are significantly higher than pairing groups (G2 or G3) (not significant (ns) > 0.05, \* < 0.05, \*\* < 0.01, \*\*\* < 0.001).

The seasonal emission pattern with a high emission peak in March or April was only apparent in five of the monitoring campaigns (Figure 5.4a). Two WWTPs exhibited a shifted pattern with extended peaks between April and July (group G2, panel b),

and three WWTPs featured a rather uniform pattern with only occasional peaks (group G3, panel c). The average EF was significantly higher for the WWTPs exhibiting the seasonal EF than those without (Mann-Whitney- Wilcoxon:  $U = 3095$ ,  $p = 6 \times 10^{-4}$ ; (Figure 5.4d). Only one of the WWTPs with an emission peak in March or April had an EF clearly below 1% (Moossee WWTP). WWTPs with a seasonal emission pattern emitted mostly between February and May. The high contribution of the emission peak phase to the total emissions highlights the strong need to better understand the causes of the seasonal emission pattern (Chen et al. 2019). Additionally, it signifies that emission reduction strategies should be based and tested on long-term monitoring campaigns covering at least 1 year (Duan et al. 2020). The reproducibility of the emission pattern on WWTPs with high EF (panel a, b) and between different lanes in a WWTP suggests that monitoring  $N_2O$  emissions over 1 year is sufficient.

On several WWTPs (Lucerne, Uster, Kralingseveer), the pattern could be partly linked to  $NO_2^-$  accumulation in biological treatment during the spring season. We hypothesize that reduced  $NO_2^-$  oxidizing bacteria (NOB) performance could be the cause of seasonal  $NO_2^-$  accumulation (Gruber et al. 2021, Vieira et al. 2018) and lead to  $N_2O$  peak emissions induced by enhanced nitrifier denitrification or incomplete heterotrophic denitrification. The emission pattern and the emission peaks in spring help to prioritize efforts to mitigate  $N_2O$  emissions, but the high impact on the yearly EF is problematic for a countrywide extrapolation based on only a few monitoring campaigns, since the frequency of WWTPs with seasonal peaks is unknown. Additionally,  $NO_2^-$  effluent concentrations do not fully represent the  $NO_2^-$  concentrations in the reactors, because  $NO_2^-$  can accumulate locally in a WWTP or even within sludge flocs (Chen et al. 2018). As a consequence, monitoring of  $N_2O$  emission patterns at WWTPs with plug-flow characteristics or multiple biological treatment steps, such as the Kralingseveer WWTP, may be less accurate if effluent concentrations are used or if concentrations are sampled only at one location. For such cases, extensive monitoring of nitrogen species at a range of locations may be advantageous. In order to avoid  $NO_2^-$  accumulation, all-year denitrification appears to be an efficient strategy. WWTPs without seasonal emission patterns (Birs, Zurich and Hofen) or with low EFs (Moossee) had a very low proportion of  $NO_2^-$  in the

effluent of the biological treatment (0.2–0.5% relative to the yearly nitrogen influent load). All of these WWTPs practise all-year denitrification.

#### **5.3.4 Year-round denitrification and N<sub>2</sub>O reduction**

To test the seasonally varying influence of an anoxic zone and denitrification on N<sub>2</sub>O emissions, full-scale tests were performed at the Hofen WWTP. Some WWTPs in Switzerland are operated for nitrogen removal year-round whereas other treatment plants are fully aerated in winter, and thus only perform denitrification in summer. The responses of N<sub>2</sub>O emissions to switching aeration on or off in the first compartment of the biological treatment train are shown in Figure 5.5. The N<sub>2</sub>O emissions were substantially increased on the fully aerated lanes (lanes 2–1 and 3–2). Amongst the monitored lanes sharing the same secondary clarifier, the one with an anoxic zone (2–2) had lower emissions than the fully aerated one (2–1) but still had higher emissions than the lane with anoxic zones on both lanes sharing the same secondary clarifier (lane 3–2). When the conditions were swapped and lane 3–2 was fully aerated, the emission pattern reacted immediately and switched completely. The anoxic zone, however, does not always have such a substantial impact on the emissions. During the first phase of the first experiment (beginning of February to mid-March), emissions on lanes 2–1 and 2–2 only slightly increased. Additionally, the emissions rose to a lower level in June and only marginally in September. The importance of denitrification to reaching low emissions highlights the need to consider nitrogen removal rates in countrywide estimations of N<sub>2</sub>O emissions.

The varying impact of an anoxic zone underpin our conclusion that the emission peaks in spring are caused by imbalanced nitrification and NO<sub>2</sub><sup>-</sup> accumulation. An anoxic zone prevents NO<sub>2</sub><sup>-</sup> and N<sub>2</sub>O accumulation probably via the return sludge, since both intermediates can be reduced by denitrification as long as sufficient organic substrate is available (Pan et al. 2013b). The seasonal dependence of denitrification on the emissions might be linked to the yearly variation of the microbial community and nitrifiers in a WWTP and relates to the previous discussion about yearly NOB variation (Griffin and Wells 2017, Gruber et al. 2021, Ju et al. 2014). Experimental support for the relevance of seasonal nitrifier variation can be

found in the monitoring campaign at the Giubiasco WWTP, which represents an extreme case of unstable nitrification and highlights its impact on  $\text{N}_2\text{O}$  emissions.

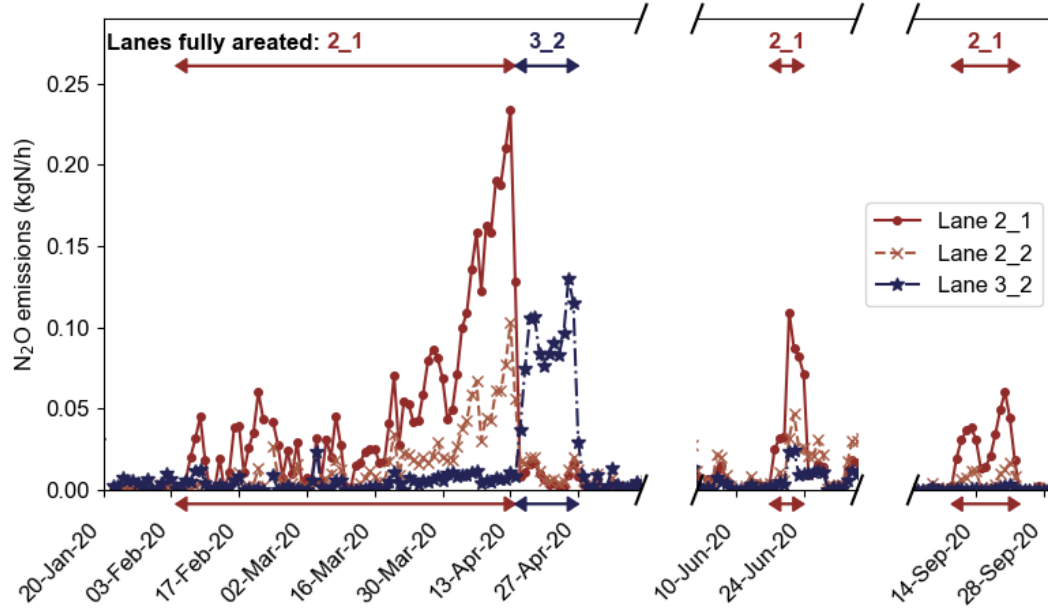


Figure 5.5:  $\text{N}_2\text{O}$  emission at Hofen WWTP between January 15 and May 20 during. Arrows indicate the lanes where three out of three compartments were fully aerated.

### 5.3.5 Unstable nitrification and $\text{N}_2\text{O}$ emission pattern

To characterize the risk of high  $\text{N}_2\text{O}$  emissions in a process with unstable nitrification, we analysed the Giubiasco WWTP in more detail. The Giubiasco WWTP is a CAS system with carbon removal as a treatment goal. The  $\text{N}_2\text{O}$  emission pattern exhibited a strong seasonal variation (Figure 5.6a). As a consequence of higher temperatures, nitrifiers can actually proliferate during the summer months and a seasonal occurrence of nitrification is the result at Giubiasco WWTP (Figure 5.6b). The yearly emission pattern exhibits two emission peak phases (January – March & June - July): these coincide with a dramatic change in nitrification performance. Nitrification did not occur during winter, at low temperatures. Therefore, the ammonium effluent concentration of the WWTP exhibits a pattern that is opposite of the wastewater temperature curve. During the transition from a nitrifying to a non-nitrifying process, the  $\text{NO}_2^-$  concentrations in the WWTP effluent increased and massive amounts of  $\text{N}_2\text{O}$  were emitted.

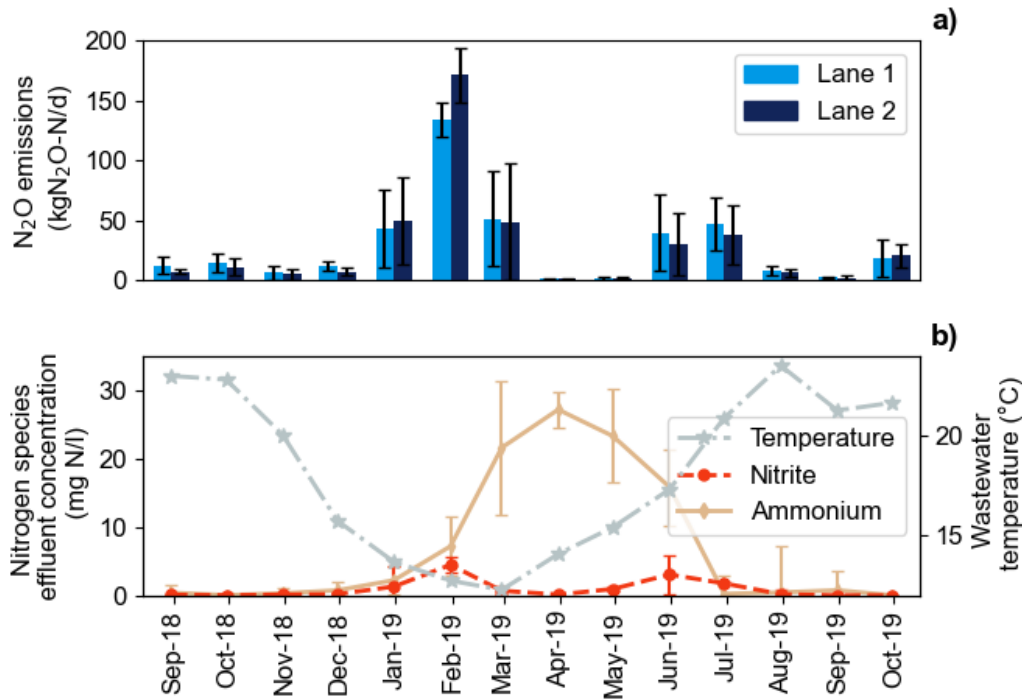


Figure 5.6: Monthly average N<sub>2</sub>O emissions measured for both lanes at the Giubiasco WWTP (panel a) and wastewater temperature, monthly average NH<sub>4</sub><sup>+</sup>-N, and NO<sub>2</sub><sup>-</sup>-N effluent concentrations (panel b). Error bars indicate standard deviation.

The high EF (8%) for a non-BNR system contrasts strongly with previous studies and the IPCC guidelines, which suggests that a high EF is linked to uncontrolled and incomplete nitrification. In most previous studies, non-BNR systems were found to have low emissions (Vasilaki et al. 2019). Our study demonstrates that long-term monitoring is required to assess EFs from non-BNR systems, unless nitrification can be excluded all year. Most of the previously reported monitoring campaigns were conducted over short periods, but a few discontinuous long-term monitoring campaigns have been conducted for non-BNR system. A study on a Brazil WWTP over 1 year resulted in a very low EF of 0.12% (Brotto et al. 2015). The nitrogen removal efficiency and nitrification exhibited a strong seasonal variation, but NO<sub>2</sub><sup>-</sup> was comparatively low (0–0.8 mg NO<sub>2</sub><sup>-</sup>-N/L) in all effluent samples. A possible explanation for the differences from the Giubiasco WWTP is the tropical climate, which led to very high wastewater temperature (24 °C at minimum). High emissions for WWTPs with variable nitrogen removal efficiencies were reported from three monitoring campaigns using a discontinuous monitoring approach over a year (Yan



et al. 2014). In that study, a WWTP with similarly low nitrogen removal efficiencies as the Giubiasco WWTP had a very high average EF of 3.6%. The yearly temperature patterns were very similar to the WWTPs monitored in our study.

Further continuous, long-term monitoring campaigns on carbon removal WWTPs are essential to provide better estimates of EF variability. We expect negligible EFs from carbon removal systems that completely avoid BNR, partly explaining the low EF of previous studies. For emission mitigation, limiting the SRT to prevent nitrifier growth could be a feasible strategy for carbon removing plants. However, the strategy leads to impaired removal of organic compounds in the biological treatment (Falas et al. 2016) and, thus, to a trade-off decision between effluent quality and the carbon footprint of a WWTP.

### **5.3.6 Countrywide extrapolation of N<sub>2</sub>O emissions and uncertainties**

The dependencies of the N<sub>2</sub>O EF discussed above and the corresponding understanding of the mechanistic processes derived from the 14 long-term monitoring campaigns on eight types of WWTP processes led us to propose a refined approach to estimating on-site N<sub>2</sub>O emissions of WWTPs in Switzerland. We suggest calculating an EF for the three nutrient removal categories of carbon removal, nitrification only, and year-round nitrogen removal with the overall assumption that a lower nitrogen removal results in higher emissions and a higher probability of NO<sub>2</sub><sup>-</sup> accumulation unless nitrification can be excluded completely. We calculated an average EF for Switzerland by multiplying the average EF for each category (Figure 5.7a) with the share of nitrogen load treated by each category (Figure 5.7b), (Table 5.4). The total emissions were calculated for two scenarios with our approach (d1 and d2), because data availability for carbon removal plant was not sufficient and emissions were expected to differ substantially between such WWTPs. We did not propose a linear regression model for EF extrapolation, since data on effluent NO<sub>2</sub><sup>-</sup> the best predictor for EFs (Figure 5.3), was limited on a countrywide level in Switzerland, and is in most other countries.

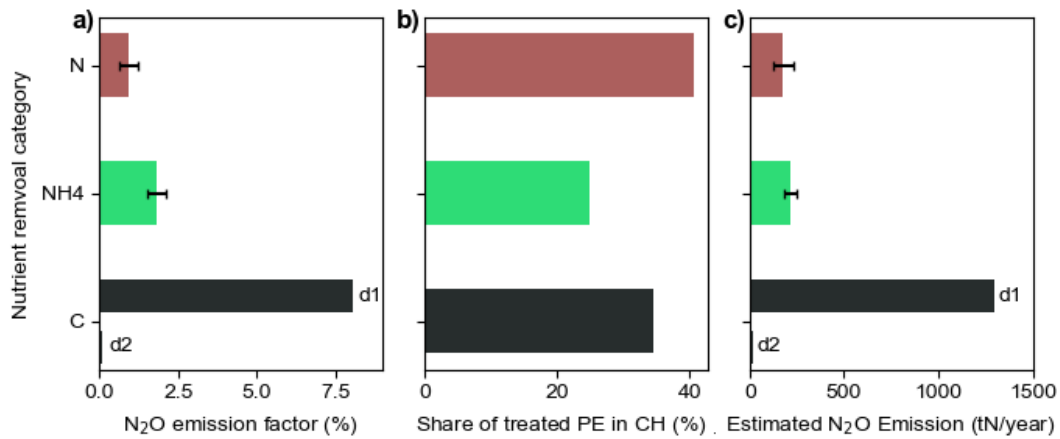


Figure 5.7: Estimation of the countrywide EF for Switzerland (CH). N = anoxic zone for year-round denitrification; NH<sub>4</sub> = nitrifying plants denitrifying only during the warm season; C = plants not required to nitrify. (a) Estimated EFs per nutrient removal category. C (d1) and (d2) denote EF for the two scenarios tested. Error bars indicate the standard errors. (b) Treated nitrogen load per nutrient removal categories in CH in 2011. (c) Estimate emissions for each category assuming activity data in Table 5.4.

The results of the new methods (approaches d1 and d2) are compared with the current IPCC approaches (approaches a and b) and an average EF of all 14 EFs (approach c) discussed in this study (Table 5.4). approach (c, d) is lower than that calculated with the IPCC approaches (a, b). Nevertheless, the emission estimates calculated with the 2006 guidelines are drastically lower than the other estimates due to the very low EF applied (Table 5.4). N<sub>2</sub>O emissions calculated with our approaches (c, d1, d2) vary substantially and depend primarily on the estimated EF for carbon removal plants (Figure 5.7c). The 2019 refinements are in the range of the total emissions estimated with our approaches and data. In summary, all the estimates show that direct N<sub>2</sub>O emissions from WWTPs are important and sum up to 0.3–1.4% of total Swiss GHG emissions (~50 MtCO<sub>2,e</sub>/year in 2011) (FOEN 2020). Notably, these estimations do not include N<sub>2</sub>O emissions originating from the effluent of the WWTP after discharge to the environment and the total GHG emission in Switzerland in 2011 were around 10% higher than in 2018. Accordingly, we expect an increasing importance of N<sub>2</sub>O emissions from WWTPs to the total Swiss GHG emissions, especially assuming the projected, continued decline of total GHG emissions.

Table 5.4: Estimation of direct N<sub>2</sub>O emissions from wastewater treatment plants in Switzerland using different calculation methods.

<b>Estimation approach</b>	<b>Activity data (nitrogen load) (tN/year)</b>	<b>EF<sub>PLANTS</sub> (Estimated average EF) (%)</b>	<b>N<sub>2</sub>O<sub>PLANTS</sub> (Total emissions and uncertainties) (tN<sub>2</sub>O-N/year)</b>
a (2006 guidelines)	60,000	0.032	190
b (2019 refinement)	70,000	1.6±0.4	1,120±280
c (Swiss specific activity data, average EF)	47,000	1.6±0.5	744±230
d1 (Swiss specific activity data, weighted EF, Carbon removal: high EF)	47,000	3.6±0.2	1,690±90
d2 (Swiss specific activity data, weighted EF, Carbon removal: low EF)	47,000	0.9±0.2	410±90

The discrepancies between the activity data from the IPCC approaches and our data confirm that a country-specific bottom-up approach should be prioritized for estimation if sufficient data is available (Ramirez-Melgarejo et al. 2020). The low emission estimates yielded from the 2006 guidelines highlight that an update of the estimation methods was clearly required for more accurate representation of wastewater treatment in GHG inventories. However, our results show that the 2019 refinement is not optimal for calculating country specific EFs for two reasons: (i) The selection of monitoring campaigns in the guidelines used to calculate the average EF is arbitrary and not necessarily representative for a country. (ii) The EFs applied (Appendix B.6 (SI)) originate mostly from short-term grab sampling, which does not provide representative EFs (Daelman et al. 2013a). Methods building on a country-specific representation of wastewater treatment, such as ours, not only yield more accurate estimations but are better suited to resolve changes in wastewater treatment efficiencies over time (Figure B.2, Figure B.4 (SI)). An increase of the nitrogen removal efficiencies in wastewater treatment plants over the last decades, as reported in most developed countries due to increased effluent requirements, could be implemented with our approach to obtain representative emission time series (Van Drecht et al. 2009). The expected change in emissions over time could thus be quantified. As required by the Kyoto and Paris protocols (United Nations Climate Change 1997, 2015), the reporting of GHG emissions to the UNFCCC (United

Nations Climate Change 1992) always refers back to 1990 and representative emission time series are therefore considered important.

We conclude that identifying robust predictors for EFs from WWTPs is of high importance to making reliable predictions for the total N<sub>2</sub>O emission from wastewater treatment. Previously, linear regression modeling was proposed by Valkova et al. (2020) as a versatile and precise predictor for countrywide N<sub>2</sub>O emissions from WWTPs. Their study was based on short-term monitoring campaigns over a few weeks, which does not provide representative EF estimation, and reported substantially lower EFs (0–1.5%) than the values reported in our study and other long-term monitoring campaigns (Chen et al. 2019). Moreover, a linear relation between nitrogen removal efficiency and EF cannot be assumed (Figure 5.2 and potentially low EFs for carbon removal WWTPs). Our approach is based on long-term monitoring data and on grouping of EFs according to meaningful characteristics of WWTPs, which additionally reduces the uncertainties of EFs (Table 5.4). However, a clear need to better characterize emissions from carbon removal WWTPs has been identified. Estimates of the proportion and EFs of carbon removal WWTPs have to be made by expert judgements until more monitoring data is available. A fourth nutrient removal category could then be implemented in our method to differentiate between the two types of carbon removal WWTPs and combine scenarios (d1) and (d2). Ultimately, we believe that our approach can be used for N<sub>2</sub>O emission estimations in other countries with temperate climates and significantly improve the representativity of emission estimation over the IPCC approach. For the extrapolation to countries in other climate zones, continuous, long-term monitoring campaigns in those climate zones are absolutely necessary.

## **5.4 Conclusions**

N<sub>2</sub>O EFs from WWTPs exhibit a strong relation with the effluent NO<sub>2</sub><sup>-</sup> (compared to the influent nitrogen load) and nitrogen removal efficiency. Since data availability for NO<sub>2</sub><sup>-</sup> effluent loads on a countrywide level is usually limited, we suggest calculating a countrywide EF from the weighted, average EFs of three nutrient

removal categories carbon removal, (EF: 0.1–8%), nitrification only: (1.8%), and full nitrogen removal (0.9%). The overall assumption of the approach is that categories with higher nitrogen removal result in lower emissions unless nitrification is not active at all. The approach allows representative, country-specific estimations of the N<sub>2</sub>O emissions from WWTPs. Applied to Switzerland, the estimations result in an average EF of 0.9–3.6% and total emissions of 410–1690 tN<sub>2</sub>O-N/year, which corresponds to 0.3–1.4% of total Swiss GHG emissions.

Uncontrolled nitrification in WWTPs with carbon removal as a treatment goal can cause very high emissions coupled with NO<sub>2</sub><sup>-</sup> accumulation during wash-out and re-growth of nitrifiers. Partial nitrification should be avoided by stringent SRT control, because increased N<sub>2</sub>O emissions can be excluded, as long as nitrification is not present in a WWTP. Consequently, uncertainties linked to the high variability of carbon removal WWTPs (estimated EFs: 0.1–8%) have to be considered in countrywide emission estimations. Further continuous, long-term monitoring campaigns on carbon removal WWTPs are required to reduce the uncertainties of EF estimates.

Yearlong continuous monitoring campaigns are essential to assess representative EFs given the high temporal variability encountered in 14 long-term monitoring campaigns. Yearly N<sub>2</sub>O emissions patterns for activated sludge based WWTPs with high EFs (>0.5%) are very dynamic and comparable among different WWTPs and lanes of the same WWTP with separated sludges. Hence, assuming yearly reproducibility of EFs is justified for a particular WWTP. The emission peak phase often coincides with NO<sub>2</sub><sup>-</sup> accumulation in the biological treatment. We expect that limiting NO<sub>2</sub><sup>-</sup> accumulation is the key factor reducing N<sub>2</sub>O emissions in WWTPs.

WWTPs with year-round denitrification often exhibit low EFs and rather uniform emission pattern. Introducing anoxic conditions at the beginning of the biological treatment immediately reduces N<sub>2</sub>O emissions over the whole biological treatment, including the aerobic zones. However, fully aerobic conditions do not always result in high N<sub>2</sub>O emissions.

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## 5.6 Author contributions

W.G., L.v.K., L.B., D.B., E.M. and A.J. designed the study. All authors provided helpful feedback and suggestions throughout work on the study. L.v.K., L.B. and W.G. were responsible for data collection of process performance data and monitoring data. L.V., L.v.K., W.G. and R.L. programmed the routines to evaluate the monitoring data. L.B., D.B., L.v.K., W.G., A.M., M.K., L.V., N.K., and A.J. planned and conducted the monitoring campaigns. K.F., L.v.K., M.L., R.L., and W.G. analyzed the data. The manuscript was written by W.G with helpful reviews from A.J., L.v.K., D.B., and E.M.

## 5.7 References

- Aboobakar, A., Cartmell, E., Stephenson, T., Jones, M., Vale, P. and Dotro, G. (2013) Nitrous oxide emissions and dissolved oxygen profiling in a full-scale nitrifying activated sludge treatment plant. *Water Research* 47(2), 524-534.
- Ahn, J.H., Kim, S.W., Park, H., Rahm, B., Pagilla, K. and Chandran, K. (2010) N<sub>2</sub>O Emissions from activated sludge Processes, 2008-2009: Results of a national monitoring survey in the United States. *Environmental Science & Technology* 44(12), 4505-4511.
- Baeten, J.E., van Loosdrecht, M.C.M. and Volcke, E.I.P. (2020) When and why do gradients of the gas phase composition and pressure affect liquid-gas transfer? *Water Research* 178.
- Belda, Belda, M., Holtanová, E., Halenka, T. and Kalvová, J.M. (2014) Climate classification revisited: from Köppen to Trewartha. *Climate Research* 59(1), 1-13.
- Bollon, J., Filali, A., Fayolle, Y., Guerin, S., Rocher, V. and Gillot, S. (2016) N<sub>2</sub>O emissions from full-scale nitrifying biofilters. *Water Research*.
- Brotto, A.C., Kligerman, D.C., Andrade, S.A., Ribeiro, R.P., Oliveira, J.L., Chandran, K. and de Mello, W.Z. (2015) Factors controlling nitrous oxide emissions from a full-scale activated sludge system in the tropics. *Environmental Science and Pollution Research* 22(15), 11840-11849.
- Cadwallader, A. and Van Briesen, J.M. (2017) Incorporating Uncertainty into Future Estimates of Nitrous Oxide Emissions from Wastewater Treatment. *Journal of Environmental Engineering* 143(8).
- Chandran, K., Volcke, E.I. and Van Loosdrecht, M.C.M. (2016) *Experimental Methods in Wastewater Treatment*. Van Loosdrecht, M.C.M., Nielen, P.H., López Vázquez, C.M. and Brdjanovic, D. (eds), IWA, London.
- Chen, X., Mielczarek, A.T., Habicht, K., Andersen, M.H., Thornberg, D. and Sin, G. (2019) Assessment of Full-Scale N<sub>2</sub>O Emission Characteristics and Testing of Control Concepts in an Activated Sludge Wastewater Treatment Plant with Alternating Aerobic and Anoxic Phases. *Environmental Science & Technology* 53(21), 12485-12494.
- Chen, X., Yuan, Z. and Ni, B.J. (2018) Nitrite accumulation inside sludge flocs significantly influencing nitrous oxide production by ammonium-oxidizing bacteria. *Water Research* 143, 99-108.
- Conthe, M., Lycus, P., Arntzen, M.O., Ramos da Silva, A., Frostegard, A., Bakken, L.R., Kleerebezem, R. and van Loosdrecht, M.C.M. (2018) Denitrification as an N<sub>2</sub>O sink. *Water Research* 151, 381-387.
- Daelman, M.R., De Baets, B., van Loosdrecht, M.C. and Volcke, E.I. (2013) Influence of sampling strategies on the estimated nitrous oxide emission from wastewater treatment plants. *Water Research* 47(9), 3120-3130.

- Daelman, M.R., van Voorthuizen, E.M., van Dongen, U.G., Volcke, E.I. and van Loosdrecht, M.C. (2015) Seasonal and diurnal variability of N<sub>2</sub>O emissions from a full-scale municipal wastewater treatment plant. *Science of The Total Environment* 536, 1-11.
- Duan, H., van den Akker, B., Thwaites, B.J., Peng, L., Herman, C., Pan, Y., Ni, B.J., Watt, S., Yuan, Z. and Ye, L. (2020) Mitigating nitrous oxide emissions at a full-scale wastewater treatment plant. *Water Research* 185, 116196.
- Falas, P., Wick, A., Castronovo, S., Habermacher, J., Ternes, T.A. and Joss, A. (2016) Tracing the limits of organic micropollutant removal in biological wastewater treatment. *Water Research* 95, 240-249.
- FOEN (2020) Kenngrößen zur Entwicklung der Treibhausgasemissionen in der Schweiz 1990–2018. Federal Office for the Environment, F., Abteilung Klima (ed), Bern.
- Griffin, J.S. and Wells, G.F. (2017) Regional synchrony in full-scale activated sludge bioreactors due to deterministic microbial community assembly. *ISME Journal* 11(2), 500-511.
- Gruber, W., Niederdorfer, R., Ringwald, J., Morgenroth, E., Bürgmann, H. and Joss, A. (2021) Linking seasonal N<sub>2</sub>O emissions and nitrification failures to microbial dynamics in a SBR wastewater treatment plant. *Water Research X* 11, 100098.
- Gruber, W., Villez, K., Kipf, M., Wunderlin, P., Siegrist, H., Vogt, L. and Joss, A. (2020) N<sub>2</sub>O emission in full-scale wastewater treatment: Proposing a refined monitoring strategy. *Science of The Total Environment* 699, 134157.
- Gujer, W. (2007) *Siedlungswasserwirtschaft*, Springer, Berlin.
- Harris, C.R., Millman, K.J., van der Walt, S.J., Gommers, R., Virtanen, P., Cournapeau, D., Wieser, E., Taylor, J., Berg, S., Smith, N.J., Kern, R., Picus, M., Hoyer, S., van Kerkwijk, M.H., Brett, M., Haldane, A., del Río, J.F., Wiebe, M., Peterson, P., Gérard-Marchant, P., Sheppard, K., Reddy, T., Weckesser, W., Abbasi, H., Gohlke, C. and Oliphant, T.E. (2020) Array programming with NumPy. *Nature* 585(7825), 357-362.
- IPCC (2006) Chapter 5.6 Wastewater treatment and discharge. 2006 IPCC Guidelines for National Greenhouse Gas Inventories, <http://www.ipcc-nggip.iges.or.jp/public/2006gl/index.html>.
- IPCC (2014) Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, p. 151, IPCC, Geneva, Switzerland.
- IPCC (2019) Chapter 5.6 Wastewater treatment and discharge. 2019 Refinement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories, <https://www.ipcc-nggip.iges.or.jp/public/2019rf/index.html>.
- Joss, A., Salzgeber, D., Eugster, J., König, R., Rottermann, K., Burger, S., Fabijan, P., Leumann, S., Mohn, J. and Siegrist, H. (2009) Full-Scale Nitrogen Removal from Digester Liquid with Partial Nitrification and Anammox in One SBR. *Environmental Science & Technology* 43(14), 5301-5306.



- Ju, F., Guo, F., Ye, L., Xia, Y. and Zhang, T. (2014) Metagenomic analysis on seasonal microbial variations of activated sludge from a full-scale wastewater treatment plant over 4 years. *Environmental Microbiology Reports* 6(1), 80-89.
- Kampschreur, M.J., Poldermans, R., Kleerebezem, R., van der Star, W.R., Haarhuis, R., Abma, W.R., Jetten, M.S., Jetten, M.S. and van Loosdrecht, M.C. (2009a) Emission of nitrous oxide and nitric oxide from a full-scale single-stage nitrification-anammox reactor. *Water Science and Technology* 60(12), 3211-3217.
- Kampschreur, M.J., Tan, N.C.G., Kleerebezem, R., Picoreneau, C., Jetten, M.S. and Van Loosdrecht, M.C. (2008) Effect of dynamic process conditions on nitrogen oxides emission from a nitrifying culture. *Environmental Science & Technology* 42(2), 429-435.
- Kampschreur, M.J., Temmink, H., Kleerebezem, R., Jetten, M.S. and van Loosdrecht, M.C. (2009b) Nitrous oxide emission during wastewater treatment. *Water Research* 43(17), 4093-4103.
- Kosonen, H., Heinonen, M., Mikola, A., Haimi, H., Mulas, M., Corona, F. and Vahala, R. (2016) Nitrous Oxide Production at a Fully Covered Wastewater Treatment Plant: Results of a Long-Term Online Monitoring Campaign. *Environmental Science & Technology* 50(11), 5547-5554.
- Marescaux, A., Thieu, V. and Garnier, J. (2018) Carbon dioxide, methane and nitrous oxide emissions from the human-impacted Seine watershed in France. *Science of The Total Environment* 643, 247-259.
- McKinney, W. (2010) Data Structures for Statistical Computing in Python, pp. 51-56.
- Mikola, A., Heinonen, M., Kosonen, H., Leppanen, M., Rantanen, P. and Vahala, R. (2014) N<sub>2</sub>O emissions from secondary clarifiers and their contribution to the total emissions of the WWTP. *Water Science and Technology* 70(4), 720-728.
- Pan, Y., Ni, B.J. and Yuan, Z. (2013) Modeling electron competition among nitrogen oxides reduction and N<sub>2</sub>O accumulation in denitrification. *Environmental Science & Technology* 47(19), 11083-11091.
- Peng, L., Ni, B.J., Ye, L. and Yuan, Z. (2015) The combined effect of dissolved oxygen and nitrite on N<sub>2</sub>O production by ammonia oxidizing bacteria in an enriched nitrifying sludge. *Water Research* 73, 29-36.
- Ramirez-Melgarejo, M., Reyes-Figueroa, A.D., Gasso-Domingo, S. and Guereca, L.P. (2020) Analysis of empirical methods for the quantification of N<sub>2</sub>O emissions in wastewater treatment plants: Comparison of emission results obtained from the IPCC Tier 1 methodology and the methodologies that integrate operational data. *Science of The Total Environment* 747, 141288.
- Ravishankara, A.R., Daniel, J.S. and Portmann, R.W. (2009) Nitrous Oxide (N<sub>2</sub>O): The Dominant Ozone-Depleting Substance Emitted in the 21st Century. *Science* 326(5949), 123-125.

- Rodriguez-Caballero, A., Aymerich, I., Marques, R., Poch, M. and Pijuan, M. (2015) Minimizing N<sub>2</sub>O emissions and carbon footprint on a full-scale activated sludge sequencing batch reactor. *Water Research* 71, 1-10.
- Strähl, S., Ort, C., Siegrist, H., Thomann, M., Obrecht, J. and Kurz, E. (2013) Stickstoffelimination in Schweizer ARA. *Aqua & Gas* 5/2013, 74-84.
- Tchobanoglous, G., Stensel, D.H., Tsuchihashi, R. and Burton, F. (2014) *Wastewater Engineering: Treatment and Resource Recovery* McGrawHill, New York.
- Tian, H., Xu, R., Canadell, J.G., Thompson, R.L., Winiwarter, W., Suntharalingam, P., Davidson, E.A., Ciais, P., Jackson, R.B., Janssens-Maenhout, G., Prather, M.J., Regnier, P., Pan, N., Pan, S., Peters, G.P., Shi, H., Tubiello, F.N., Zaehle, S., Zhou, F., Arneth, A., Battaglia, G., Berthet, S., Bopp, L., Bouwman, A.F., Buitenhuis, E.T., Chang, J., Chipperfield, M.P., Dangal, S.R.S., Dlugokencky, E., Elkins, J.W., Eyre, B.D., Fu, B., Hall, B., Ito, A., Joos, F., Krummel, P.B., Landolfi, A., Laruelle, G.G., Lauerwald, R., Li, W., Lienert, S., Maavara, T., MacLeod, M., Millet, D.B., Olin, S., Patra, P.K., Prinn, R.G., Raymond, P.A., Ruiz, D.J., van der Werf, G.R., Vuichard, N., Wang, J., Weiss, R.F., Wells, K.C., Wilson, C., Yang, J. and Yao, Y. (2020) A comprehensive quantification of global nitrous oxide sources and sinks. *Nature* 586(7828), 248-256.
- United Nations Climate Change (1992) United Nations Framework Convention on Climate Change. Change, U.N.C. (ed).
- United Nations Climate Change (1997) Kyoto Protocol to the United Nations Framework Convention on Climate Change. Change, U.N.C. (ed).
- United Nations Climate Change (2015) Paris Protocol to the United Nations Framework Convention on Climate Change. Change, U.N.C. (ed).
- Valkova, T., Parravicini, V., Saracevic, E., Tauber, J., Svardal, K. and Krampe, J. (2020) A method to estimate the direct nitrous oxide emissions of municipal wastewater treatment plants based on the degree of nitrogen removal. *Journal of Environmental Management*, 111563.
- Van Drecht, G., Bouwman, A.F., Harrison, J. and Knoop, J.M. (2009) Global nitrogen and phosphate in urban wastewater for the period 1970 to 2050. *Global Biogeochemical Cycles* 23(4), n/a-n/a.
- Van Rossum, G. and Drake, F.L. (2009) *Python 3 Reference Manual*, CreateSpace, Scotts Valley, CA.
- Vasilaki, V., Massara, T.M., Stanchev, P., Fatone, F. and Katsou, E. (2019) A decade of nitrous oxide (N<sub>2</sub>O) monitoring in full-scale wastewater treatment processes: A critical review. *Water Research* 161, 392-412.
- Vasilaki, V., Volcke, E.I.P., Nandi, A.K., van Loosdrecht, M.C.M. and Katsou, E. (2018) Relating N<sub>2</sub>O emissions during biological nitrogen removal with operating conditions using multivariate statistical techniques. *Water Research* 140, 387-402.

- Vieira, A., Galinha, C.F., Oehmen, A. and Carvalho, G. (2018) The link between nitrous oxide emissions, microbial community profile and function from three full-scale WWTPs. *Science of The Total Environment* 651(Pt 2), 2460-2472.
- Virtanen, P., Gommers, R., Oliphant, T.E., Haberland, M., Reddy, T., Cournapeau, D., Burovski, E., Peterson, P., Weckesser, W., Bright, J., van der Walt, S.J., Brett, M., Wilson, J., Millman, K.J., Mayorov, N., Nelson, A.R.J., Jones, E., Kern, R., Larson, E., Carey, C.J., Polat, İ., Feng, Y., Moore, E.W., VanderPlas, J., Laxalde, D., Perktold, J., Cimrman, R., Henriksen, I., Quintero, E.A., Harris, C.R., Archibald, A.M., Ribeiro, A.H., Pedregosa, F., van Mulbregt, P., Vijaykumar, A., Bardelli, A.P., Rothberg, A., Hilboll, A., Kloeckner, A., Scopatz, A., Lee, A., Rokem, A., Woods, C.N., Fulton, C., Masson, C., Häggström, C., Fitzgerald, C., Nicholson, D.A., Hagen, D.R., Pasechnik, D.V., Olivetti, E., Martin, E., Wieser, E., Silva, F., Lenders, F., Wilhelm, F., Young, G., Price, G.A., Ingold, G.-L., Allen, G.E., Lee, G.R., Audren, H., Probst, I., Dietrich, J.P., Silterra, J., Webber, J.T., Slavič, J., Nothman, J., Buchner, J., Kulick, J., Schönberger, J.L., de Miranda Cardoso, J.V., Reimer, J., Harrington, J., Rodríguez, J.L.C., Nunez-Iglesias, J., Kuczynski, J., Tritz, K., Thoma, M., Newville, M., Kümmerer, M., Bolingbroke, M., Tartre, M., Pak, M., Smith, N.J., Nowaczyk, N., Shebanov, N., Pavlyk, O., Brodtkorb, P.A., Lee, P., McGibbon, R.T., Feldbauer, R., Lewis, S., Tygier, S., Sievert, S., Vigna, S., Peterson, S., More, S., Pudlik, T., Oshima, T., Pingel, T.J., Robitaille, T.P., Spura, T., Jones, T.R., Cera, T., Leslie, T., Zito, T., Krauss, T., Upadhyay, U., Halchenko, Y.O., Vázquez-Baeza, Y. and SciPy, C. (2020) SciPy 1.0: fundamental algorithms for scientific computing in Python. *Nature Methods* 17(3), 261-272.
- Yan, X., Li, L. and Liu, J. (2014) Characteristics of greenhouse gas emission in three full-scale wastewater treatment processes. *Journal of Environmental Sciences* 26(2), 256-263.

# **Chapter 6**

## **Linking seasonal N<sub>2</sub>O emissions and nitrification failures to microbial dynamics in a SBR wastewater treatment plant**

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## Abstract

Nitrous oxide ( $\text{N}_2\text{O}$ ) is a strong greenhouse gas and causal for stratospheric ozone depletion. During biological nitrogen removal in wastewater treatment plants (WWTP), high  $\text{N}_2\text{O}$  fluxes to the atmosphere can occur, typically exhibiting a seasonal emission pattern. Attempts to explain the peak emission phases in winter and spring using physico-chemical process data from WWTP were so far unsuccessful and new approaches are required. The complex and diverse microbial community of activated sludge used in biological treatment systems also exhibit substantial seasonal patterns. However, a potentially causal link between the seasonal patterns of microbial diversity and  $\text{N}_2\text{O}$  emissions has not yet been investigated. Here we show that in a full-scale WWTP nitrification failure and  $\text{N}_2\text{O}$  peak emissions, bad settleability of the activated sludge and a turbid effluent strongly correlate with a significant reduction in the microbial community diversity and shifts in community composition. During episodes of impaired performance, we observed a significant reduction in abundance for filamentous and nitrite oxidizing bacteria in all affected reactors. In some reactors that did not exhibit nitrification and settling failures, we observed a stable microbial community and no drastic loss of species. Standard engineering approaches to stabilize nitrification, such as increasing the aerobic sludge age and oxygen availability failed to improve the plant performance on this particular WWTP and replacing the activated sludge was the only measure applied by the operators to recover treatment performance in affected reactors. Our results demonstrate that disturbances of the sludge microbiome affect key structural and functional microbial groups, which lead to seasonal  $\text{N}_2\text{O}$  emission patterns. To reduce  $\text{N}_2\text{O}$  emissions from WWTP, it is therefore crucial to understand the drivers that lead to the microbial population dynamics in the activated sludge.

## 6.1 Introduction

Nitrous oxide (N<sub>2</sub>O) is the third most important greenhouse gas (GHG), contributing roughly 8% to the globally emitted GHG potential of anthropogenic origin (IPCC 2013). Additionally, it is considered the dominant ozone depleting substance in the stratosphere (Ravishankara et al. 2009). Biological nitrogen removal during wastewater treatment can cause high N<sub>2</sub>O fluxes to the atmosphere with a significant contribution to global N<sub>2</sub>O emissions (Vasilaki et al. 2019). In wastewater treatment plants (WWTP), emissions ranging from very low amounts up to a few percent of the total nitrogen load were shown to exhibit a strong seasonal pattern (Gruber et al. 2020). Typically, emissions exhibited a seasonal emission pattern with high emissions between March and June, and low emissions between July and November (Chen et al. 2019).

N<sub>2</sub>O in wastewater treatment systems can be produced by ammonia-oxidizing bacteria (AOB) and heterotrophic denitrifying bacteria (DNB) (Schreiber et al. 2012). AOB can produce N<sub>2</sub>O through hydroxylamine oxidation and nitrifier denitrification (Caranto and Lancaster 2017, Wrage-Mönnig et al. 2018). DNB produce N<sub>2</sub>O as an intermediate during denitrification (Von Schulthess and Gujer 1996). Chemical oxidation of hydroxylamine to N<sub>2</sub>O is the only known abiotic source and mostly occurs in systems with high ammonia (NH<sub>4</sub><sup>+</sup>) concentrations (> 100 mg NH<sub>4</sub><sup>+</sup>-N\*L<sup>-1</sup>) and high or low pH (≥ 8, ≤ 5), such as in side stream treatment for reject water from sludge treatment (Soler-Jofra et al. 2020). In general, the abiotic reactions are of minor importance in biological nitrogen removal systems (Su et al. 2019).

In activated sludge systems, high biological production and emissions of N<sub>2</sub>O have been linked to several patterns, such as i) ammonia or toxicity shocks and quickly changing process conditions, ii) low dissolved oxygen concentrations and increased concentrations of nitrite (NO<sub>2</sub><sup>-</sup>), iii) transient zones with alternating aerobic/anoxic conditions, and iv) limitation of organic substrate (Vasilaki et al. 2019). However, these factors are not exclusive and could only partly explain emission patterns assessed in long-term monitoring campaigns (Vasilaki et al. 2019). Statistical regression algorithms and mechanistic process modeling based on the activated

sludge modeling framework have been applied with limited success to model N<sub>2</sub>O emissions from WWTP (Ni and Yuan 2015, Song et al. 2020, Vasilaki et al. 2018). Thus, to better understand the N<sub>2</sub>O emissions from WWTP and identify relevant mechanisms, new aspects may have to be taken into account. Among other factors, microbial community dynamics has been proposed in previous studies as a potential driver of long-term N<sub>2</sub>O dynamics (Daelman et al. 2015).

The activated sludge in a WWTP is a unique engineered ecosystem consisting of a complex microbial community that orchestrates the biological removal of pollutants in the wastewater (Wu et al. 2019). However, as with all complex ecosystems, minor environmental changes may trigger internal dynamics in activated sludge that result in substantial impacts on the microbial community and its performance (Bürgmann et al. 2011, Griffin and Wells 2017, Johnston and Behrens 2020, Johnston et al. 2019, Shade et al. 2012). Previous studies have reported a reproducible, seasonally driven pattern for the bacterial alpha diversity at multiple WWTP (Griffin and Wells 2017, Johnston et al. 2019). Microbial diversity in temperate climates dropped dramatically at the beginning of the winter season (November and December), started to increase at the end of spring (April/May) and peaked at the end of autumn (October). Furthermore, these seasonal patterns appear to have a significant impact on the performance of valuable members involved in the nitrification but also other pollutant removal processes (de Celis et al. 2020, Ju et al. 2014).

Biological nitrogen removal through nitrification and denitrification in WWTP includes multiple species and can exhibit seasonal variation (Ju et al. 2014). While denitrification can be performed by a large number of organisms and there is therefore a high degree of functional redundancy in most cases (Lu et al. 2014), nitrification activity is linked to only a few specialized organisms (Siripong and Rittmann 2007). In conventional wastewater treatment with activated sludge, nitrification is typically a two-step process, with AOB oxidizing ammonium to NO<sub>2</sub><sup>-</sup> and nitrite oxidizing bacteria (NOB) oxidizing nitrite to nitrate. In biofilm systems and activated sludge with high solid retention times (SRT), complete nitrification performed by a single organism (*Comammox*) can be important (Cotto et al. 2020), but is expected to be a minor contributor to N<sub>2</sub>O emissions (Han et al. 2021). Several factors such as insufficient solids retention times (SRT), low oxygen concentrations,

low temperatures, elevated pH values and increased free ammonia concentrations have been linked to the loss of certain NOB species in activated sludge and NO<sub>2</sub><sup>-</sup> accumulation (Huang et al. 2010, Ren et al. 2019, Vuono et al. 2015). Similarly, yearlong community assembly studies in WWTP have reported lower abundances for nitrifiers during winter, especially for NOB from the Phylum *Nitrospira* (Griffin and Wells 2017). However, functional redundancy and niche differentiation for the NO<sub>2</sub><sup>-</sup> oxidation process in the activated sludge microbiome is theoretically possible given different NOB species present, such as *Nitrospira*, *Nitrobacter* and *Ca. Nitrotoga* (Huang et al. 2010, Lucker et al. 2015). Factors inducing a seasonal change in the NOB community of a full-scale WWTP and how such changes affect NO<sub>2</sub><sup>-</sup> accumulation as well as N<sub>2</sub>O production have not yet been studied.

Here, we test the hypothesis that seasonal NO<sub>2</sub><sup>-</sup> accumulation and N<sub>2</sub>O emission episodes can be linked directly or indirectly to shifts in the activated sludge microbiome. Of interest for full-scale operation are changes in the nitrogen converting populations resulting in reduced nitrification performance and potentially causing increased N<sub>2</sub>O production. To address our research questions, we combined an extensive N<sub>2</sub>O measurement campaign over 1.5 years and 16S rRNA sequencing for microbial community analysis during two seasonal N<sub>2</sub>O emission episodes. Using the floating flux chamber method, as described in Gruber et al. (2020), N<sub>2</sub>O emissions were assessed on six parallel SBR reactors in a Swiss WWTP. Using operational data and multivariate- and ecological-statistics, activated sludge composition analysis allowed us to uncover microbial dynamics that followed the trajectory of nitrification failures and N<sub>2</sub>O emission episodes. To the best of our knowledge, this is the first study to discuss shifts in microbial community composition as a potential cause for seasonal N<sub>2</sub>O emission pattern and nitrite accumulation based on long-term data of a full scale WWTP.



## 6.2 Material and Methods

### 6.2.1 Field site

The study was performed at the municipal WWTP of Uster (Switzerland, 47°21'02.8" N 8°41'34.0" E). On average, the plant treats 16,000 m<sup>3</sup> wastewater per day and is designed for a nutrient load of 45,000 person equivalents (PE) with average loading of 35,000 PE. Detailed information on the influent characteristics can be found in Table C.1 (SI). After mechanical treatment by screening, grit chambers, sand and fat traps, and primary clarification, the wastewater enters the biological stage. The biological treatment step consists of six sequencing batch reactors (SBR) with a volume of 3,000 m<sup>3</sup> each. On average, total solids retention time (SRT) was 34 days and aerobic SRT 10 days. Operating conditions of the SBRs are described in Table C.2 (SI). The SBRs were operated with dynamic cycle times depending on the same rules for all reactors (Table C.3 (SI)). A yearly average SBR cycle includes a fixed sequence of process steps (total time = 3.5 hours): 45 minutes feeding, 90 minutes reaction phase (30 minutes anoxic, 60 minutes aerobic), and 75 minutes settling and decanting. The total cycle length as well as the length of each step vary substantially over a year. The operation of the reaction and settling phases are adapted seasonally. During the warmer months and if sufficient nitrification capacity is available, a pre-anoxic phase is implemented. When nitrification performance is limiting, the reaction phase is fully aerated. The settling phase is adapted depending on the actual settling velocity. Following the biological treatment, the wastewater is polished in a rapid sand filtration and discharged into the environment.

The SBRs are controlled and monitored with several online sensors and 24 hours composite samples taken at multiple treatment steps of the WWTP (after primary clarifier, after biological treatment, and after filter). Except for the O<sub>2</sub>-probe, the online liquid sensors are situated in the analytics room of the WWTP where mixed liquor from the reactors is pumped to two identical monitoring trains equipped with multiple sensors (Figure 6.1). Each monitoring train receives mixed liquor from three reactors (R1, R3, R5 or R2, R4, R6). Each reactor is sampled for 5 minutes, consisting of a flushing period of the monitoring train to remove the mixed liquor from the previous reactor and a measurement phase. For the present study, the

following online signals were used for further analysis: NH<sub>4</sub><sup>+</sup> concentration, NO<sub>3</sub><sup>-</sup> concentration, O<sub>2</sub> concentration, pH and TS concentration (Table C.4 (SI)). Furthermore, data on wastewater flow, excess sludge flow, air flow, wastewater temperature, dosage of precipitant and sludge settling velocity were used to analyze process performance. To compare AOB and NOB activity among reactors, activities were estimated for each SBR cycle by subtracting the concentrations of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> measured at the beginning and the end of an aeration phase and dividing by the duration of the aeration phase. During the second campaign, NO<sub>2</sub><sup>-</sup> concentration was tracked online with UV/VIS sensors in both monitoring trains. The following operational data was used as input data for a Pearson's correlation analysis: oxygen concentration, total and aerobic SRT, anoxic cycle time, settling velocity, precipitant dosage, N<sub>2</sub>O emissions, estimated AOB and NOB activity and temperature. From weekly lab measurements, we extracted the following variables in the effluent of the biological treatment and after the sand filter: NO<sub>2</sub><sup>-</sup> effluent concentration, NH<sub>4</sub><sup>+</sup> effluent concentration, transparency determined with the Snellen method subsequently referred as transparency, and sludge volume index (SVI) (Table C.4 (SI)).

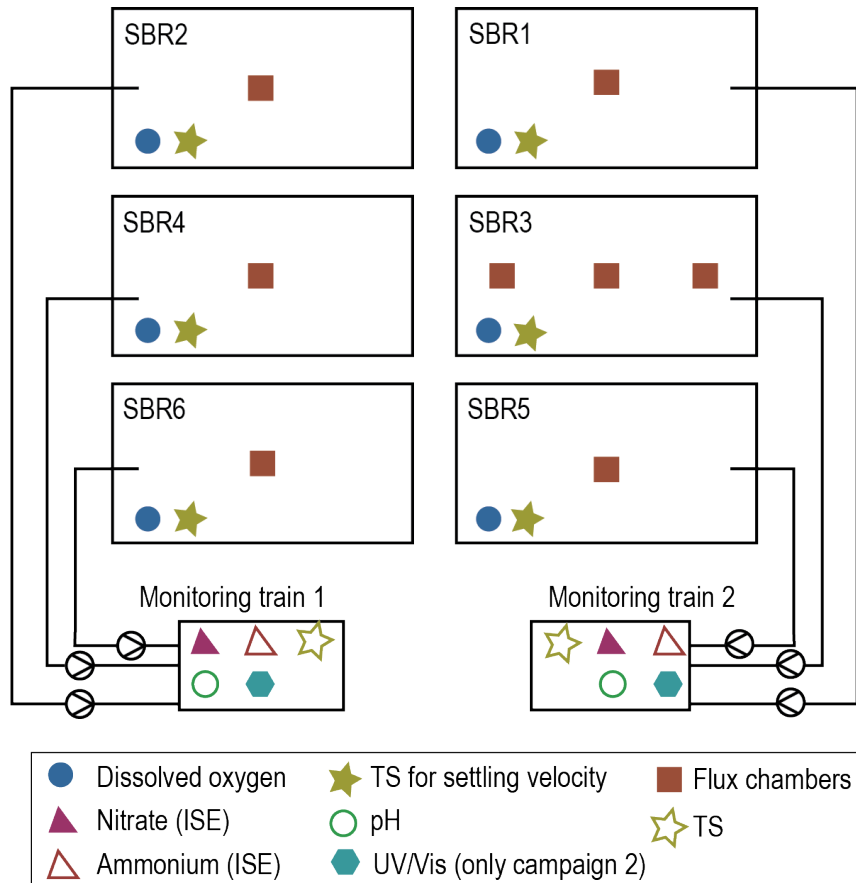


Figure 6.1: Sensor and flux chamber placement in the biological treatment at Uster WWTP. ISE: ion selective electrode, UV/Vis: online optical spectrophotometer, Flux chamber: Off gas monitoring.

### 6.2.2 N<sub>2</sub>O measurement and monitoring campaigns

The N<sub>2</sub>O monitoring campaign was conducted at the Uster WWTP from February 2018 to July 2019. The emissions were assessed using an adapted version of the flux chamber for off-gas monitoring on WWTP. At least one flux chamber was installed on every reactor (Figure 6.1). A detailed description of the monitoring setup can be found in Gruber et al. (2020). The emissions at Uster WWTP exhibited a strong seasonal pattern with two extended emission peaks (February 2018 to May 2018; March 2019 to May 2019) and low emissions between the two peaks. The study focuses on the processes around the two peaks subsequently called campaign 1 and campaign 2.

As stated above, the operation of Uster WWTP is adapted depending on wastewater flow and plant performance, changing significantly over a year. During campaign 1

and campaign 2, extended periods of process failure on the majority of reactors were observed with high NO<sub>2</sub>- effluent concentrations and bad settling qualities of the activated sludge. An overview of the WWTP operational changes and mitigation strategies is provided in Table 6.1. Table 6.2 gives detailed information on sludge exchange for each event.

Table 6.1: Mitigation strategies applied by the operator to reduce nitrification failure during campaign 1 & 2. Each type of operational change is indexed with a number.

	Start	End	Mitigation strategy
Campaign 1	10.02.2018	31.03.2018	i) Aerobic SRT increased by ~20% (10 to 12 days) (increase of total SRT & extension of aerobic phase)
	10.02.2018	31.03.2018	ii) Target oxygen concentration during aeration increased from 2 (default value) to 3 mgO <sub>2</sub> /l
	22.02.2018 27.04.2018	05.03.2018 07.05.2018	iii) No anoxic cycle phases before aeration
	26.04.2018	07.07.2018	iv) Exchange of activated sludge in selected reactors (Table 6.2)
Campaign 2	05.02.2019	11.05.2019	iii) No anoxic cycle phases before aeration
	09.06.2019	28.06.2019	iv) Exchange of activated sludge in selected reactors (Table 6.2)

Table 6.2: Sludge transfer from donating reactors to a receiving reactor (R).

Date	Receiving reactor	Donating reactor
26.04.2018	R2	R4, R5
18.05.2018	R1	R4, R6
07.07.2018	R6	R2, R3, R5
09.09.2018	R4	R1, R3
09.06.2019	R4	R1, R3
14.06.2019	R2	R1, R3, R4
24.06.2019	R5	R1, R2, R3, R4
28.06.2019	R6	R1, R2, R3, R4

### 6.2.3 Activated sludge sampling and DNA extraction

The activated sludge sampling was performed on a weekly basis for selected reactors during the sampling campaigns. To reduce the number of samples, R4 was

completely excluded from the sampling for the first campaign given the high similar behavior of all reactors. During the second campaign, samples were collected from all reactors. Overall, we sequenced 53 sludge samples from campaign 1 and 47 samples from campaign 2. For each sample, a 50 ml tube of mixed liquor was collected when the reactors were fully mixed during the aeration phase or the anoxic mixing phase and immediately transported to the lab. In the lab, 2 ml tubes were filled with the mixed liquor and centrifuged at 6,000 rcf and 4 °C for two minutes. The supernatant was withdrawn, and the procedure was repeated twice. Three aliquots of each sample were stored at -20 °C for further processing.

Nucleic acids from the 1st campaign were extracted with the MoBio power soil kit (Qiagen, Germany) following the standard operating procedure of the extraction kit. Nucleic acids from the 2nd campaign were extracted based on a method modified from Griffiths et al. (2002). Activated sludge samples from every time point were transferred to 1.5 ml Matrix E lysis tubes (MPbio) and 0.5 ml of both hexadecyltrimethylammonium bromide buffer and phenol:chloroform:isoamylalcohol (25:24:1, pH 6.8) was added. The activated sludge was lysed in a FastPrep machine (MPbio), followed by nucleic acid precipitation with PEG 6000 on ice. Nucleic acids were washed three times with ethanol (70%) and dissolved in 50 µl DEPC treated RNase free water. For all samples, DNA quality and quantity were assessed by using agarose gel electrophoresis and a Nanodrop ND-2000c (Thermo Fisher Scientific, USA).

#### **6.2.4 Sequencing**

16S rRNA gene amplicon sequencing from the 1st campaign was performed at the University of Basel (Switzerland) on an Illumina MiSeq platform, based on the pair-end algorithm (300bp, V3-V4) and the primer pair 341f and 806r resulting in an average number of  $92,200 \pm 34,700$  sequences. Due to the Covid-19 outbreak and entailed constraints, we were not able to perform sequencing of the samples from the second campaign at the same sequencing service provider. Samples from the 2nd campaign were sequenced at DNASense ApS (Aalborg, Denmark, [www.dnasense.com](http://www.dnasense.com)), using the same algorithm and primers, resulting in an average number of  $30,800 \pm 5,600$  sequences. Although using the same PCR chemistry

(2x300bp, V3/V4 region) and Illumina sequencer, the outcome from the sequence providers differed significantly in the number and quality of sequences, which made it particularly challenging to analyze both sequence sets together. Therefore, and due to the different DNA extraction protocols used, the microbial data from both campaigns were analyzed as separate datasets although they were observed in the same WWTP.

#### **6.2.5 Sequence analysis and microbial community analysis**

Raw sequences from both sequence runs were analyzed within the QIIME2 framework (Caporaso et al. 2010). Amplicon sequence variants (ASVs) were produced with the DADA2 (Callahan et al. 2016) pipeline and taxonomically annotated based on the Microbial Database for Activated Sludge (MiDAS3, (Nierychlo et al. 2020)). All subsequent biostatistics analysis were performed individually on the sequence tables, derived from this analysis pipeline. A link to the sequence tables are provided at the end of the manuscript. After normalization based on the variance stabilization algorithm within DESEQ2 (Love et al. 2014), we performed a non-metric multidimensional scaling (nMDS) analysis based on the Bray–Curtis dissimilarity using vegan and R software (Oksanen et al. 2007, R-Core-Team 2020). A hierarchic clustering approach (vegdist function, vegan, R) was applied on the dissimilarities in community composition, to statistically divide the samples from all reactors into different clusters within each campaign. While community dissimilarities in campaign 1 were statistically most robust when explained by 5 clusters (A, B, C, D, E), campaign 2 could be divided into 4 Clusters (X, Y<sup>α</sup>, Y<sup>β</sup>, Z). We assigned, if possible, ASVs to their putative functional role in the wastewater treatment plant based on the Global Database of Microbes in Wastewater Treatment Systems and Anaerobic Digesters (MIDAS) (Nierychlo et al. 2020). More information on sequence analysis and subsequent ecostatistics can be found in Appendix C.2.

## 6.3 Results

### 6.3.1 N<sub>2</sub>O emission, plant performance and incomplete nitrification

During our N<sub>2</sub>O monitoring campaign at Uster WWTP, the biological treatment went through two extended periods of severe nitrification and settling failure leading to high NO<sub>2</sub><sup>-</sup> concentrations and turbidity in the effluent. A detailed overview of the performance and operation of the biological treatment during both periods is shown in Figure 6.2. Starting in March 2018 and April 2019, increased N<sub>2</sub>O emissions, very low nitrification performance (NO<sub>2</sub><sup>-</sup> in effluent), bad settleability of the activated sludge (SVI) and a turbid effluent (low transparency value) were the most important process failure characteristics observed over a period of two to three months (Figure 6.2). After an extended transition phase of roughly one month, the reactors reverted to a satisfying treatment performance (as before the process failure period) and emitted very low amounts of N<sub>2</sub>O during both campaigns. Interestingly, the transition between phases was not synchronized between the different reactors. This asynchrony of the recovery is highlighted by the high standard deviations for the N<sub>2</sub>O emissions, estimated NOB and AOB activity in mid-April 2018 to mid-May 2018 and May 2019 (Figure 6.2a; for individual reactor data see Figure C.1 (SI), Figure C.2 (SI), Figure C.3 (SI)).

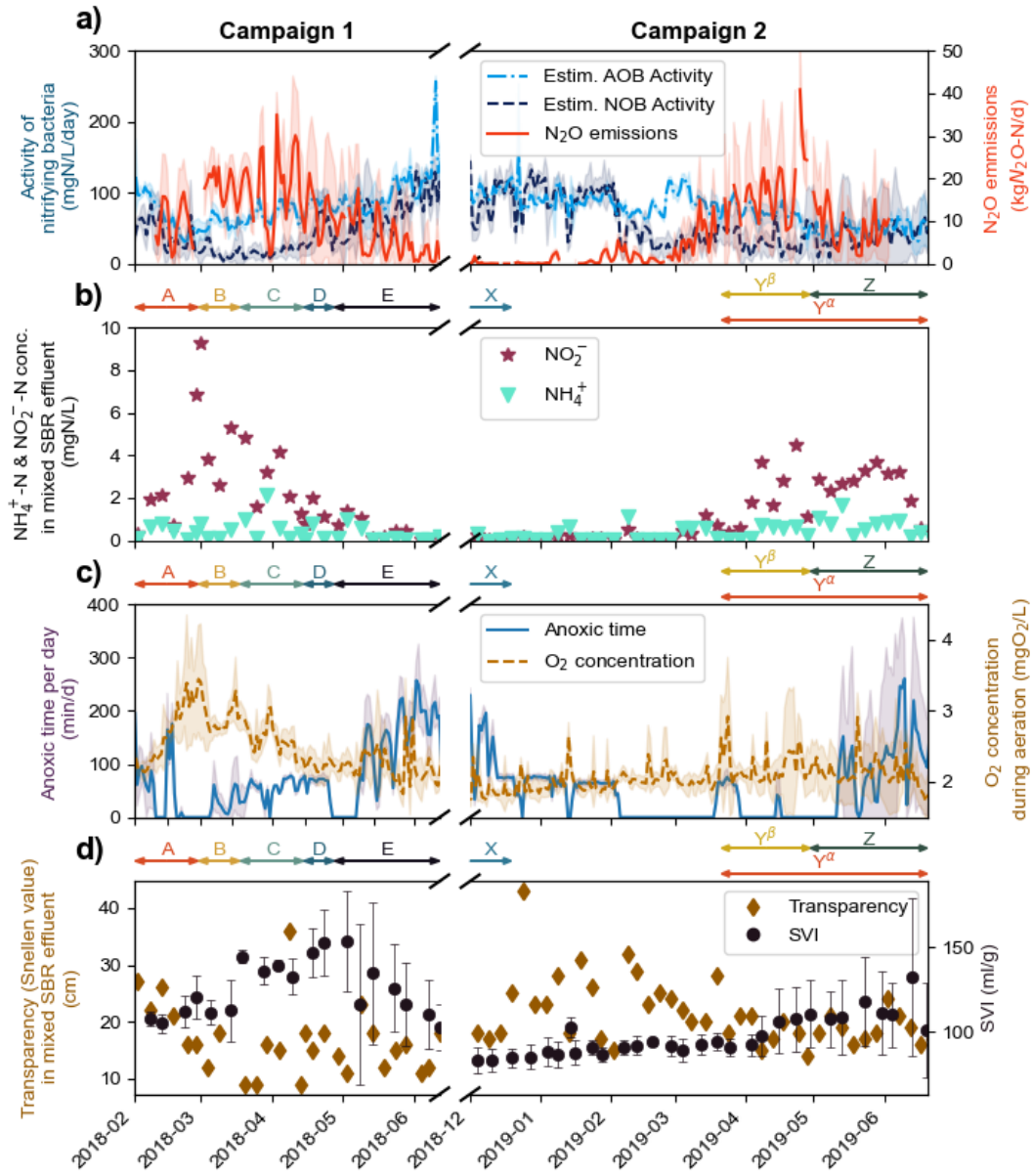


Figure 6.2: Treatment performance and operational conditions at Uster WWTP; AOB and NOB activities as well as N<sub>2</sub>O emissions as average values of all reactors incl. standard deviation as shaded area (a), ammonium and nitrite effluent concentrations in collected effluent of all SBRs (b), and operational parameters of all reactors incl. standard deviation as shaded area (c), sludge settling properties as average of all reactors (d). Arrows and letters between panels indicate time periods of samples aggregated to clusters identified by nMDS-cluster analysis (Figure 6.4).

During both campaigns, NO<sub>2</sub><sup>-</sup> concentrations in the mixed effluent of all reactors reached very high values of up to 9.3 mgNO<sub>2</sub><sup>-</sup>-N/l during campaign 1 and 4.9 mgNO<sub>2</sub><sup>-</sup>-N/l during campaign 2, as shown in Figure 6.2b. While NO<sub>2</sub><sup>-</sup> concentrations increased within a month from satisfying to peak concentrations, the return to normal concentrations took two to three months. Although the rapid sand filtration for



effluent polishing could reduce some of the produced  $\text{NO}_2^-$ , the effluent concentrations were still dramatically higher than the target value of  $0.3 \text{ mgNO}_2^- \text{-N/l}$  of the Swiss water protection law. The  $\text{NO}_2^-$  concentrations correlated negatively with the observed average NOB activity ( $r = -0.61$ ,  $p < 0.001$ ,  $n = 81$ ). While the NOB activity dropped by up to 100% to levels around  $20 \text{ mgN/l/d}$ , AOB activity decreased only slightly (campaign 2) or remained stable and increased later (campaign 1, cluster E). Therefore,  $\text{NH}_4^+$  effluent concentrations increased slightly but remained clearly below the discharge limits of  $2 \text{ mgNH}_4^+ \text{-N/L}$  after the filter. The transparency of the effluent dropped parallel to the decreasing NOB activity (Figure 6.2b, Figure C.4 (SI), Figure C.5 (SI), Figure C.6 (SI)).

The sludge settling characteristics changed dramatically leading to high SVI values and low sludge settling velocities (Figure 6.2, Figure C.5 (SI), Figure C.6 (SI)). Both properties showed a medium negative correlation ( $r = -0.51$ ,  $p < 0.001$ ,  $n = 332$ ) and were heavily affected during both process failure phases. The WWTP emitted significant amounts of  $\text{N}_2\text{O}$  during both campaigns. During peak days, up to 30% of the influent nitrogen load was emitted as  $\text{N}_2\text{O}$ , resulting in a massive impact on the greenhouse gas balance of the WWTP.  $\text{N}_2\text{O}$  emissions showed a close and highly significant positive correlation with  $\text{NO}_2^-$  concentrations in the effluent of the biological treatment ( $r = 0.81$ ,  $p < 0.001$ ,  $n = 60$ ). Generally, the emission pattern was highly variable. Under wet weather conditions e.g., at the beginning of April 2018,  $\text{N}_2\text{O}$  emissions dropped to very low levels and then peaked only a few days later when the influent wastewater amount returned to dry weather conditions.

Effluent  $\text{NO}_2^-$  concentrations and transparency values from the biological treatment indicate that similar events of incomplete nitrification were observed in the spring seasons of preceding years (Figure C.3 (SI)). Despite the evident periodicity of the nitrification failure episodes, the two campaigns indicate a different progression of process performance in different years. In campaign 1,  $\text{NO}_2^-$  rose and peaked rapidly, and the estimated NOB activity dropped accordingly to levels close to zero at the beginning of March. The effluent transparency mirrored the pattern of the  $\text{NO}_2^-$  concentrations. In campaign 2, the decline of NOB activity and the increase of  $\text{NO}_2^-$  effluent concentration happened more gradually with a peak in March while the effluent transparency value reached its minimum one month before the  $\text{NO}_2^-$

concentrations. Interestingly, the process failure phenomenology was overall less dramatic in campaign 2 compared to campaign 1 (Figure 6.2, Figure 6.3, Figure C.9 (SI)).

While all reactors performed similarly and exhibited a partial failure of nitrification and settling during campaign 1, R1 and R3 did not exhibit episodes of dramatic process instabilities during campaign 2. This fortuitous development allowed a comparative analysis of the characteristics of failing and functioning tanks in campaign 2. Elevated NO<sub>2</sub><sup>-</sup> concentrations ( $\geq 1$  mg NO<sub>2</sub><sup>-</sup>-N/L) during aeration can to some extent be observed in all reactors (Figure C.7 (SI)). However, R1 and R3 during campaign 2 had enough nitrite oxidation and denitrification capacity to avoid a drastic long-term NO<sub>2</sub><sup>-</sup> accumulation (Figure C.10 (SI)). Additionally, the N<sub>2</sub>O emissions of R1 and R3 were clearly lower compared to the other reactors (Figure 6.3c). The estimated AOB activity, however, was comparable in all reactors (Figure 6.3a). After the transient loss of nitrification and settling performance, overall process performance returned to the previous levels. After sludge exchange in the low performing reactors, settling and nitrite oxidation performance increased significantly.

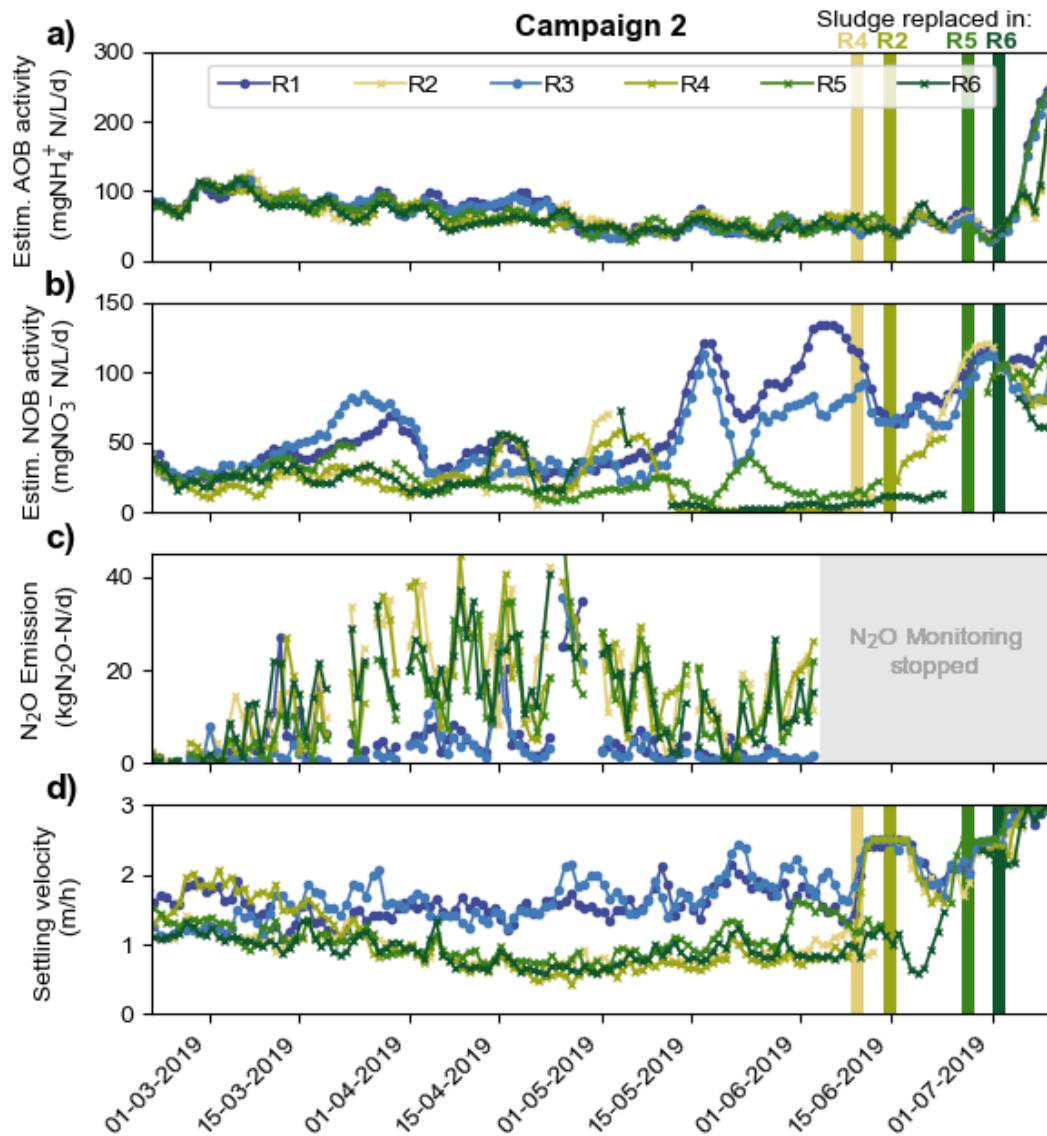


Figure 6.3: Comparison of reactor performance and  $\text{N}_2\text{O}$  emissions between the well running reactors (R1, R3) and the reactors exhibiting nitrification failure and settling problems (R2, R4, R5, R6) during campaign 2. Colored bars indicate dates of sludge exchange in respective reactors (Table 6.2). Data was smoothed with a moving average of 6 days in panels a), b), and c).

### 6.3.2 Mitigation measures applied by the operators and correlation analysis

In order to reduce the duration of the process failure phases in campaign 1 compared to previous years, the operators changed operation parameters according to the following four operational strategies (Table 6.1): i) increase of aerobic SRT to retain more nitrifiers (Figure C.8 (SI)), ii) increase the oxygen concentration during aeration to increase aerobic activity (see Figure 6.2c), iii) reduce or skip the anoxic reaction phase to allow lengthening the aeration phase (Figure 6.2c), and iv)

replacement of the activated sludge with sludge from a well running system (see Figure 6.3, Table 6.2). In the second campaign, dissolved oxygen and aerobic SRT were only slightly increased, since the strategies were not successful during campaign 1 (Figure 6.2c). Aerobic reaction phases were extended by reducing or skipping the anoxic reaction phase in both campaigns (Figure 6.2c). Overall, the strategies i), ii) and iii) were found insufficient, as they did not accelerate the recovery of nitrification performance (Figure 6.2c, Figure S11: DO, aerobic SRT, anoxic time). The complete exchange of activated sludge (strategy iv) appeared to be the only successful strategy to recover treatment performance (Figure 6.3).

In order to investigate potential causes for the seasonal process failure, Pearson correlation analysis was performed with standard operational parameters, performance indicators and influent indices (Figure C.11 (SI)). Although correlation analysis has been applied in previous N<sub>2</sub>O monitoring studies with limited success, WWTP operators often rely on strategies based on empirical correlations to address unexpected performance issues like incomplete nitrification. NO<sub>2</sub><sup>-</sup> ( $r = 0.8$ ,  $p < 0.001$ ,  $n = 59$ ) and COD ( $r = 0.71$ ,  $p < 0.001$ ,  $n = 59$ ) effluent concentrations showed the highest correlations with N<sub>2</sub>O emissions. N<sub>2</sub>O emissions showed a moderate negative correlation with temperature ( $r = -0.48$ ,  $p < 0.001$ ), and NOB activity ( $r = -0.5$ ,  $p < 0.001$ ), as well as a weak negative correlation with anoxic cycle time ( $r = -0.32$ ,  $p < 0.001$ ). While temperature only correlated on a daily average and is thus assumed to influence the emissions only indirectly, the latter two appear to be highly relevant variables for NO<sub>2</sub><sup>-</sup> accumulation and N<sub>2</sub>O emissions. No other significant correlations with operational parameters were found. Overall, the correlation analysis does not yield any strategies to optimize plant performance, since all process optimization strategies applied were shown to be ineffective and therefore exhibited correlations with N<sub>2</sub>O contrary to the intended effect.

### **6.3.3 Microbial community dynamics as a driver of N<sub>2</sub>O emissions and NO<sub>2</sub><sup>-</sup> accumulation**

As we were not able to explain the observed N<sub>2</sub>O dynamics and concomitant nitrification failures based on WWTP operational parameters, we decided to investigate the role of microbial community dynamics as a potential driver. We used 16S rRNA gene sequencing analyses to obtain time-series data of the microbial

community composition, with the goal of correlating the process performance with changes in the activated sludge microbiome. To identify distinct phases in the microbial community composition over time, we applied a hierarchical clustering approach to the ASV abundance table (amplicon sequence variants reflecting microbial “species”) of all samples from the different reactors within the consecutive sampling campaigns. Dissimilarities of microbial community composition and resulting clusters are visualized in Figure 6.4.

The resulting distinct clusters, based on the dissimilarities in microbial community composition, followed the temporal progression, and in campaign 2 additionally reflected the split between reactors with and without process failure. We therefore used these clusters to divide the campaigns into a sequence of distinct phases for subsequent analyses of microbial data. Within the 1st campaign we observed a significant (PERMANOVA;  $p < 0.05$ ) change in the microbial community composition from cluster A to E, which was comparable for all reactors. In the second campaign, a similar temporal dynamic could also be observed for the communities in reactors experiencing process failure (R2, R4, R5 and R6) in clusters X, Y<sup>β</sup>, Z. However, the microbial community structure in reactor R1 and R3 from campaign 2 remained nearly unchanged after the initial transition from cluster X to Y<sup>α</sup> and did not change thereafter, in line with the stable nitrification performance (Figure 6.3). Interestingly, while they displayed lower N<sub>2</sub>O emissions and no process failures during the second campaign, these two reactors were characterized by impaired nitrification and particularly high N<sub>2</sub>O peaks during the first campaign. Notably, these reactors were operated identically to the others over the period of both campaigns, as long as nitrification worked sufficiently. The failing reactors (R2, R4, R5 and R6), however, shared a common clustering pattern, as already observed during the first year, ending with a significantly distinct community structure in summer (cluster Z) compared to the initial state in late fall (cluster X) or the stable reactors (Y<sup>α</sup>).

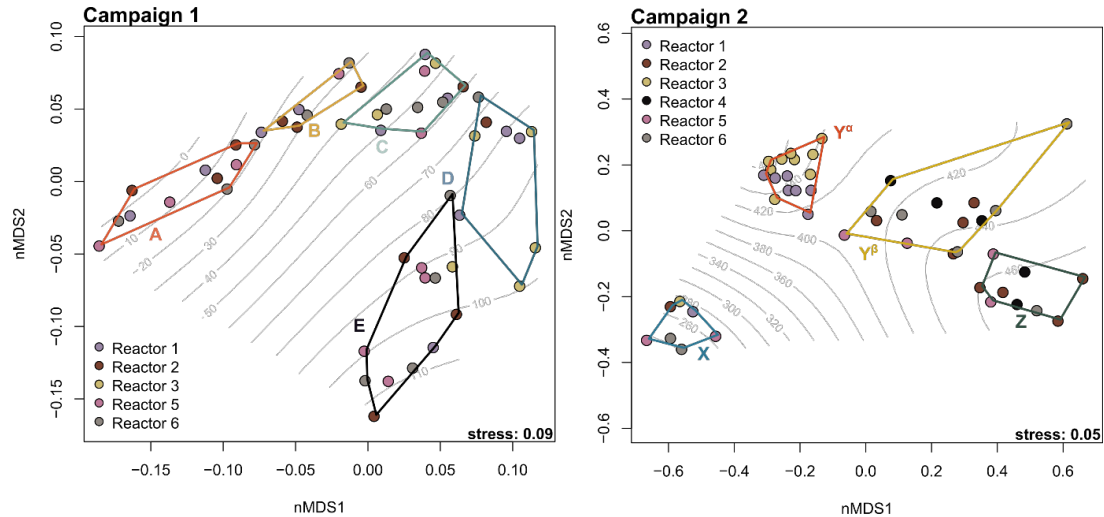


Figure 6.4: Non-metric multidimensional scaling analysis (nMDS) based on the Bray-Curtis dissimilarity between the different sampling time-points of both campaigns (letters A - E and X - Z and colored hull polygons indicate the naturally identified clusters in chronological sequence). Symbol color denotes the reactor source. A GAM model (grey lines) depicts the best fit for day of sampling (grey numbers in contour lines) to the data. Both stress levels indicate a good fit for the ordination.

The alpha diversity index (Shannon), average N<sub>2</sub>O concentrations and the SVI all varied considerably between the temporal clusters (Figure 6.5). We found that species diversity significantly decreased in all reactors during process failure episodes, i.e., from cluster A to C in campaign 1 and from X to Y<sup>β</sup> to Z (Figure 6.5a). While diversity was decreasing, N<sub>2</sub>O emissions and SVI tended to increase in both campaigns (Figure 6.5b, c). As with diversity, we did not observe a substantial change for these parameters between cluster X and cluster Y<sup>α</sup> in campaign 2. The diversity of the activated sludge increased again from cluster D to cluster E (campaign 1), accompanied by decreasing N<sub>2</sub>O emissions and SVI. The observed increase in diversity at the end of campaign 1 could not be observed in campaign 2, since the recovery phase was not sampled. The strong link between microbial diversity and performance indicators for settling and nitrification is confirmed by correlation analysis (Figure C.12 (SI)). The Shannon diversity and two other indices (Simpson diversity and species evenness) were found to be significantly negatively correlated with N<sub>2</sub>O emissions, SVI values, and NO<sub>2</sub><sup>-</sup> concentrations in effluent of the biological treatment during both campaigns. A weak positive correlation was found with effluent transparency during campaign 1 (Figure C.12 (SI)).

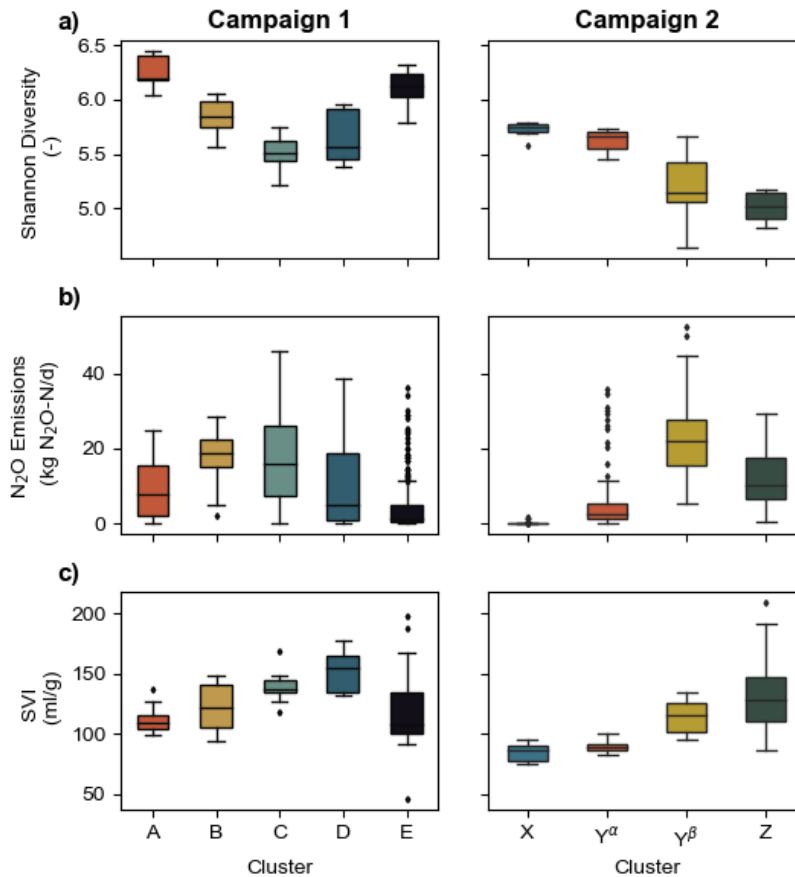


Figure 6.5: Boxplots displaying the changes in Shannon diversity (panel a), N<sub>2</sub>O (panel b), and SVI (panel c) over the different clusters in both campaigns. Colors denote the different clusters as shown in Fig. 4. Boxplot whiskers show 1.5 times the interquartile range. First quartiles, medians (line), third quartiles are displayed in the box. Diamonds represent outliers.

In order to identify which functional groups of the microbial community displayed the significant changes in abundance, we assigned all ASVs, based on their assigned genus and using the Global Database of Microbes in Wastewater Treatment Systems and Anaerobic Digesters (Nierychlo et al. 2020), either to the morphological group of filamentous bacteria or to a putative functional role in WWTP. Given the crucial importance of filamentous bacteria in WWTP (Nierychlo et al. 2019, Speirs et al. 2019), we decided to include this category into our assignment. Therefore, in case filamentous ASVs could be assigned in addition to other putative functions (aerobic heterotrophs or fermenters), we used the morphological feature rather than the putative function. To quantify which ASVs substantially contributed to observed fluctuations in relative abundance and diversity changes, we performed a differential abundance analysis and expressed the magnitude of change between consecutive clusters as  $\log_2\text{foldchange}$  (Figure C.13 (SI)). A positive  $\log_2\text{foldchange}$  indicates a

decrease in abundance over time while a negative log<sub>2</sub>foldchange means increasing counts.

The assignment to high-level functional roles allows for comparison between the two campaigns. We found that the transitions from clusters A → B → C (campaign 1) and X → Y<sup>β</sup> → Z displayed the highest numbers in ASVs that significantly ( $p < 0.05$ , Wald test) decreased in abundance (Figure C.13 (SI); number of bubbles). The transitions from D → E (campaign 1) and X → Y<sup>α</sup> (campaign 2) were characterized by an increase in abundance of ASVs, which decreased in the earlier clusters. During the early transition from cluster A → B and X → Y<sup>β</sup> that corresponds to the initial development toward process failure in both campaigns, we observed an increase in abundance of aerobic heterotrophs and fermenting bacteria while filamentous bacteria decreased in abundance (Figure 6.6, Figure C.13 (SI)). The declining abundance of filamentous bacteria continued during the transition from cluster Y<sup>β</sup> to Z during campaign 2. Fermenting bacteria, mostly affiliated to the genera *Arcobacter* and *Bacteroides*, tended to increase from A → B and X → Y<sup>β</sup> in both campaigns. Interestingly, they decreased during phases with elevated NO<sub>2</sub><sup>-</sup> concentrations and N<sub>2</sub>O emissions (i.e., campaign 1: B → C and C → D; campaign 2: Y<sup>β</sup> → Z), respectively. This dynamic was accompanied by an increase in aerobic heterotrophs and a decrease in denitrifying bacteria (DNB). We also found that NOB were low in abundance during cluster C → D (campaign 1) and Y<sup>β</sup> → Z (campaign 2). Associated with a recovery of the process performance, the transition from cluster D → E in campaign 1 was characterized by a re-increase in abundance of filamentous bacteria, DNBs and NOBs, while aerobic heterotrophs substantially decreased in abundance (Figure 6.6, Figure C.13 (SI)). We also observed a stabilization of the community for all reactors in cluster Z of campaign 2. In stark contrast to these dynamic cluster transitions, the shift from cluster X to Y<sup>α</sup> (stable reactors of campaign 2) entailed merely an increase in abundance for filamentous bacteria and AOB. Focusing on the temporal development of the microbial communities in reactor 1 and 3 (cluster Y<sup>α</sup>, Figure 6.6, Figure C.13 (SI)), we observed a surprisingly stable community with a significant increase (linear regression analyses,  $p < 0.05$ ) in abundance of filamentous bacteria in comparison to the starting condition (cluster X), in contrast to the decreasing trend for this group in the other reactors.



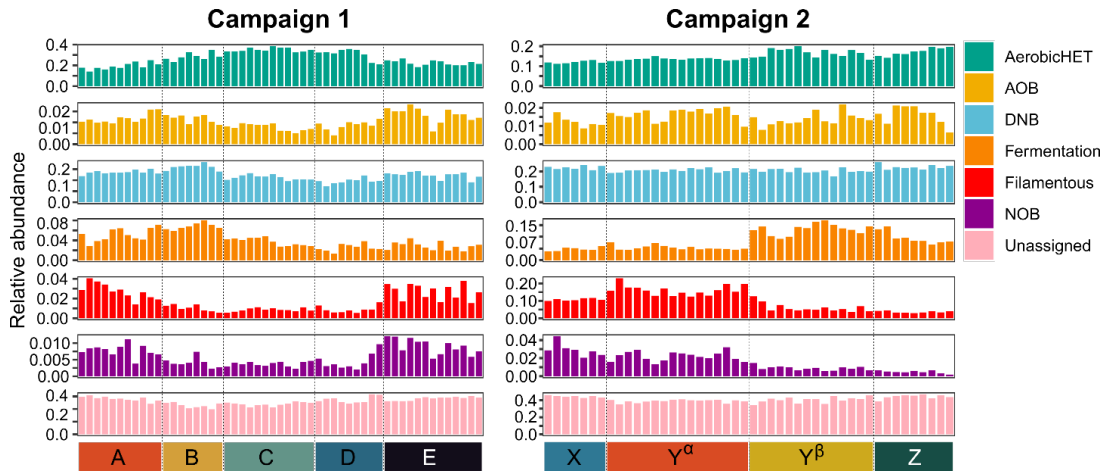


Figure 6.6: Cumulative relative abundances of ASVs assigned to their putative functional role in the WWTP. Colors denote the putative function. The X-axis displays all samples from different reactors organized into the different clusters as in Figure 6.2 and Figure 6.4 (53 samples in campaign 1, 47 samples for campaign 2). Within clusters individual samples are organized by increasing sampling date (except 2 samples from reactor 3 campaign 1) and by reactor.

Given the crucial importance of nitrifying bacteria in municipal wastewater treatment, we dissected the microbial communities from both campaigns to elucidate the individual dynamics of AOB and NOB affiliated bacteria (Figure 6.7). During both campaigns, *Nitrosomonas* was the only detected bacterial genus affiliated with aerobic ammonium oxidation and its abundance did not change dramatically over the course of the sampling campaigns despite process disturbances. However, bacteria affiliated with  $\text{NO}_2^-$  oxidation displayed surprising dynamics in abundance. During both campaigns, the abundance of the dominant NOB (*Nitrospira*) significantly decreased during the periods with a low nitrification performance (campaign 1: cluster B, C, D; campaign 2:  $Y^\beta$ , Z). During campaign 1, ASVs assigned to a different bacterium affiliated with  $\text{NO}_2^-$  oxidation (*Candidatus Nitrotoga*) started to emerge in cluster D and became the dominant NOB fraction of the community in cluster E. Interestingly, *Candidatus Nitrotoga* was not present in the prior clusters of campaign 1, nor could it be detected during campaign 2. The recovery phases of R2, R4, R5, and R6 were not sampled during campaign 2 and it is therefore not clear if the species may have emerged later. However, it is likely that *Nitrotoga* did not appear in the second campaign, since the operators started to replace the activated sludge of the unsatisfyingly performing reactor one week after the last sludge samples were taken (Figure 6.3).

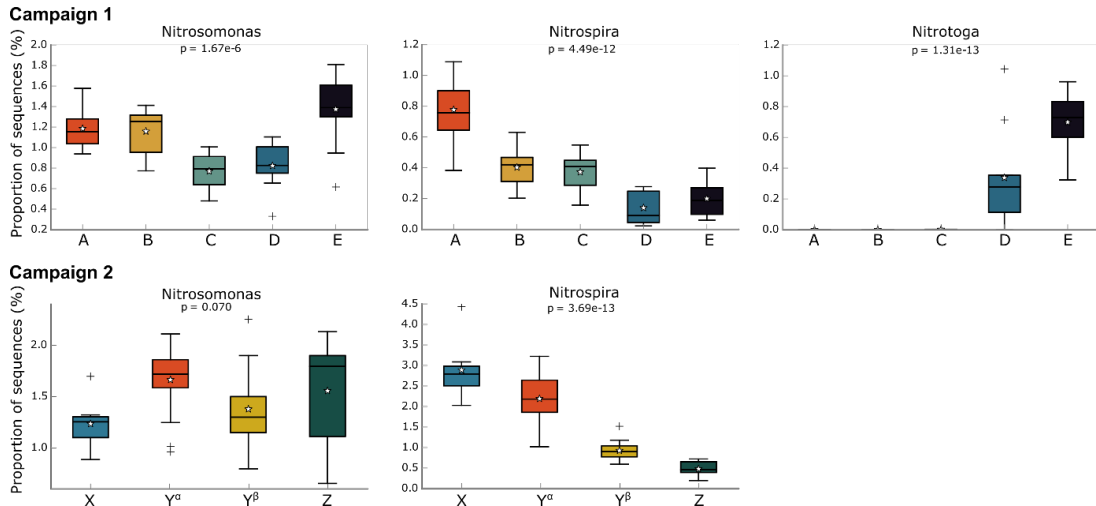


Figure 6.7: Boxplots displaying the proportion of sequences (%) from all reactors within respective clusters annotated as bacterial nitrifiers in the different clusters. Colors also denote the respective clusters. Boxplot whiskers show 1.5 times the interquartile range. First quartiles, medians (line), third quartiles are displayed in the box. Pluses represent outliers. The corrected (Benjamini-Hochberg FDR) p-value values are based on an ANOVA and Tukey-Kramer post-hoc test.

In order to identify potential process parameters or environmental factors, which could have initiated these drastic changes in community structure, we performed a correlation-based analysis. Here, we used all ASVs that were present in at least 25% of the samples and sorted them into their putative functional groups. We determined the correlation of these groups with the same, averaged process parameters, as used for the process correlation analysis described above, for each sampling point of the treatment plant for each campaign (Figure C.14 (SI)). However, we were not able to find a large number of significant correlations after the p-value adjustment, which would allow us to make assumptions on what might have caused the initiation of the community change. Further, diverging results between the two consecutive campaigns, which can perhaps be attributed to differences in operation strategy of the reactors and different periods of the clusters (Figure C.14 (SI)), ultimately do not allow to identify drivers.

## 6.4 Discussion

The yearly  $\text{N}_2\text{O}$  emissions at Uster WWTP are an example for a broadly observed pattern of seasonally driven  $\text{N}_2\text{O}$  emission from WWTP. Most previous  $\text{N}_2\text{O}$  monitoring campaigns at WWTP observed an emission pattern peaking in spring and reaching its minimum in autumn, such as the Kralingseveer WWTP (Daelman et al. 2015), Avedøre WWTP (Chen et al. 2019), Lucerne WWTP and Altenrhein WWTP (Gruber et al. 2020). Hence, these monitoring campaigns might represent observations of the same phenomenon. Given the reported correlation of  $\text{N}_2\text{O}$  emissions of  $\text{NO}_2^-$  concentration in two studies (Daelman et al. 2015, Gruber et al. 2020), we hypothesize that seasonally increased  $\text{NO}_2^-$  concentrations in the biological reactors of these treatment plants are directly and functionally linked to the  $\text{N}_2\text{O}$  emissions patterns. During both campaigns at Uster WWTP high  $\text{N}_2\text{O}$  emissions were observed after substantially diminished NOB activity resulting in  $\text{NO}_2^-$  accumulation in the effluent, which suggests a high contribution of denitrification (nitrifier or heterotrophic) to  $\text{N}_2\text{O}$  production (Domingo-Felez et al. 2016, Wunderlin et al. 2013a). Although the extent of nitrite accumulation in our monitoring campaign is extreme (Figure 6.2), seasonal nitrite accumulation has been previously reported for full-scale WWTPs and shown to be related to  $\text{N}_2\text{O}$  emissions (Castro-Barros et al. 2016, Philips et al. 2002, Randall and Buth 1984). At the Vikinmäkki WWTP, a very similar case with substantial NOB failure could be observed in a continuously fed activated sludge process with denitrification and nitrification (Kuokkanen et al. 2020).

The Uster WWTP is designed following the standard guidelines (Appendix C.1 (SI)). The strategies applied by the operator in campaign 1 to counter incomplete nitrification were shown to be unsuccessful (Figure 6.2; i.e., increasing aerobic SRT and oxygen setpoints). They target typical key operation parameters aiming to support nitrifying bacteria (Stenstrom and Poduska 1980). Other reported causes for NOB loss and nitrite accumulation, such as high temperatures, elevated pH values and increased free ammonia concentrations (Ren et al. 2019) can be clearly excluded for the case reported (Figure 6.2, Figure C.11 (SI)). Hence, the yearly recurring episodes (Figure C.4 (SI)) of substantial nitrite accumulation followed by  $\text{N}_2\text{O}$

emissions cannot be solved and explained using standard engineering approaches. In strong agreement with the microbial analysis, we find that the NOB loss correlates with important changes of the entire microbial community and thus the primary cause likely does not reside in the nitrifiers themselves. The clustering of the changing microbial community structure correlated surprisingly well with the changing nitrification performance and sludge characteristics in both campaigns (Figure 6.2, Figure 6.5). Our analysis of the microbial communities clearly revealed a progressive and quite well synchronized change of the community composition in all independent reactors (Figure 6.4) and that the respective species diversity negatively correlated with nitrite accumulation, changing sludge settleability and N<sub>2</sub>O emissions (Figure 6.5, Figure C.5 (SI)). With the exceptions of R1 and R3 during campaign 2, where the microbial community was very stable (Figure 6.4), the six reactors exhibited synchronized microbial communities and reproducible impaired treatment performances. The high similarity of the activated sludge microbiome within different independent reactors of the same WWTP or even in the same region has been observed in previous studies (Griffin and Wells 2017).

The microbial community analysis of the two campaigns revealed significant differences between the pre- and post-process-failure community compositions (Figure 6.4). Despite the differences in community structure, all reactors re-emerged to satisfying performances in N-removal (Figure 6.2) and displayed comparable diversity measures again at the end of campaign 1 and at the beginning of campaign 2 (Figure 6.5). We hypothesize that the destabilization of the activated sludge microbiome was initiated by the loss of certain key functional groups that maintain the sludge structure; this in turn triggered a cascading decline of other valuable members, including NOB, of the community (Van den Abbeele et al. 2011). Our observations on decreasing diversity and evenness (Figure 6.5, Figure C.13 (SI)) as well as the pronounced loss of specific microbial consortia during clusters A → C and X → Y<sup>β</sup> → Z, support this notion. Specifically, the observed decline in filamentous bacteria (mainly *Chloroflexi*) after cluster A and X appears likely to have initiated the cascading effect on the community in both campaigns as it provides a credible explanation for the reported changes in sludge settling (Figure 6.3 and Figure 6.5). The visible change in transparency and settling velocity further supports

the notion of the lost sludge integrity (Figure 6.2, Figure 6.3, Figure C.12 (SI)). Filamentous members of the phylum *Chloroflexi* are known to support the structural integrity of activated sludge. Their ability to degrade complex polymeric organic compounds to low molecular weight substrates is very beneficial for other members of the community (Kragelund et al. 2007, Nierychlo et al. 2019, Speirs et al. 2019). Burger et al. (2017) found a direct correlation between the abundance of filamentous bacteria and the strength of the floc, which further supports our findings. However, the mechanisms that lead to the decline of filamentous bacteria and NOB, while AOB are significantly less affected remain unclear. Both loss of structural integrity (e.g., pin-point floc formation and washout) and loss of mutualistic interactions (e.g., substrate transfer) could potentially play a role (Burger et al. 2017, Lau et al. 1984, Örmeci and Vesilind 2000, Sezgin et al. 1978).

Disturbance- or changing-condition-induced species loss can open up new niches within the sludge community that are prone to colonization by other bacterial consortia with ecological advantages under the given conditions (Vuono et al. 2016). We observed this phenomenon during campaign 1. While the NOB species *Nitrospira* declined substantially in abundance, another NOB species, *Nitrotoga*, emerged and took over as the dominant NOB in these reactors (Figure 6.7). During the transition phase between these two NOB species, we observed the highest N<sub>2</sub>O emissions (Figure 6.2). To our surprise, no sequences from the 2<sup>nd</sup> campaign could be annotated to the genus *Nitrotoga*. However, *Nitrotoga* was also not found during the first three clusters of campaign 1. We believe that, as fast as the cold affine *Nitrotoga* (Lucker et al. 2015, Wegen et al. 2019) was emerging, it was soon again replaced by *Nitrospira* as the dominating NOB species during the warm summer months preceding campaign 2. In stark contrast to the NOB community, the AOB fraction (*Nitrosomonas*) remained comparably stable in abundance over the course of both campaigns. We speculate that the changing sludge morphology, initiated by the loss of filamentous bacteria, could also affect the observed abundance dynamics within the nitrifying community. Given the increased effluent turbidity after biological treatment due to diminished sludge integrity in the affected reactors (Figure 6.2), we speculate that the NOB fraction could be preferentially washed out

in pin-point flocs. The washout of NOBs in turn leads to NO<sub>2</sub><sup>-</sup> accumulation as observed during campaigns after cluster A and X, respectively.

As our results indicate, the exchange of activated sludge can work as a mitigation strategy, but it should be only applied in emergency cases for two reasons. Firstly, the transfer of significant amounts of sludge leads to lower treatment performance in the source reactor. Secondly, the replacement of sludge speeds up the system recovery but does not prevent system failure later during a season or in the following year. The results from campaign 2 and the well performing reactors R1 and R3 show that probably only small changes are needed to stabilize the microbiome, since the same operational strategies were applied in the disturbed and the satisfying reactors. Although the initial causes for impaired plant performance remain unknown, strategies to reduce process failure should aim for a stabilization of activated sludge microbiome already well before the problem becomes acute. As reported in previous studies, several strategies could be applied, such as (i) increase of oxygen concentration (Huang 2010), (ii) increase SRT (Kim et al. 2011, Vuono et al. 2015) or (iii) maintaining a stable process operation strategy (Dytczak et al. 2008). Since strategies (i) and (ii) have been unsuccessfully applied during campaign 1 when the microbiome was already substantially disturbed, we hypothesize that the changes in operation should be implemented a few months before the expected phase of nitrification failure. Integrating a proactive management of the activated sludge microbiome in the operational strategy of a WWTP could be an asset for the mitigation of seasonally occurring nitrification failure and insufficient sludge settleability.

Our study highlights the need for further detailed sampling campaigns and experimental work to uncover the chain of events that leads to community disturbance and ultimately to significant peaks in N<sub>2</sub>O emissions and NO<sub>2</sub><sup>-</sup> accumulation. A better understanding of seasonal patterns of microbial population dynamics will be central to this objective. To investigate microbial dynamics as a potential cause or mediator of such patterns, further studies are required in three directions, i.e. (1) 16s rRNA amplicon sequencing with a higher resolution (weekly sampling over a whole year), (2) seasonal assessment of microbial activity with metagenomics or multi-omics approaches, and (3) systematic assessment of the

microbial community during tests of mitigation strategies and comparison with a reference system. In particular, multi-omics approaches could help to characterize the initial causes for strong dynamics in microbial communities. For seasonal studies, independent of the methods applied, it seems crucial to include not only species involved in the nitrogen cycle, but the whole activated sludge microbiome. Furthermore, future studies should always be coupled with spatially and temporally highly resolved long-term  $\text{N}_2\text{O}$  and  $\text{NO}_2^-$  monitoring and extended process monitoring as at Uster WWTP. Ultimately, suitable targets (organisms, genes or community traits) that can be measured reliably and cost-effectively would have to be characterized that are reliably linked to subsequent process failures – merely collecting microbial data does not automatically advance the operation of a WWTP. Our study clearly shows that extended discussions and a close collaboration between operators, engineers and microbiologists are required to take advantage of the full potential of microbial assays, to analyze the data appropriately and to suggest mitigation strategies.

## 6.5 Conclusions

- NO<sub>2</sub><sup>-</sup> accumulation correlates strongly with and is very likely the cause for the observed seasonal N<sub>2</sub>O emission peaks on a full-scale activated sludge SBR plant. While the AOB abundance and performance remained relatively stable throughout the campaigns, the NOB population disappeared and needed to re-establish.
- The phases of impaired nitrification and high N<sub>2</sub>O emissions correlated with the process of a drastic change in the microbial community affecting multiple process relevant species. The communities of reactors with high emissions differed significantly before and after the peak emission phases. On the contrary, reactors with a stable microbial community over the whole period did not exhibit increased N<sub>2</sub>O emissions.
- The NO<sub>2</sub><sup>-</sup> oxidation on the SBR plant repeatedly underperformed even though (i) the important operating parameters (aeration and aerobic SRT) were set according to standard guidelines and (ii) common factors known to cause NO<sub>2</sub><sup>-</sup> oxidation failure were not present. These results counter the notion that the accumulation of NO<sub>2</sub><sup>-</sup> and the seasonal N<sub>2</sub>O emission pattern are issues uniquely related to growth conditions of nitrifiers.
- Loss and re-establishment of NOB activity seems to coincide with loss and re-establishment of filamentous bacteria and entailed bad sludge settling properties (impaired settleability and a turbid effluent). This has considerable practical implications since measures to maintain complete nitrification might need to target floc structure rather than AOB and NOB growth conditions only.
- Regular, long-term microbial and physico-chemical monitoring of the activated sludge and a better understanding of its microbial community likely is important for understanding seasonal N<sub>2</sub>O emission patterns, while current standard engineering approaches could not explain the



process failure. Appropriate operational strategies to avoid large community shifts still need to be identified.

## 6.6 Acknowledgements

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## 6.7 Author contributions

W.G, A.J and E.M designed the study. All authors provided helpful feedback and suggestions throughout work on the study. J.R was responsible for data collection of process performance data. W.G performed the sludge sampling. R.N and W.G performed the laboratory work, sequencing and data analysis. R.N and W.G wrote the first draft of the manuscript. The manuscript was written by W.G, and R.N with critical and helpful reviews from H.B, A.J and E.M.

## **6.8 Data Availability**

Raw 16S sequences can be found on the NCBI sequence read archive under the repository number: PRJNA691692.

All other data (species abundance tables as comma-separated tables, physico-chemical data sheets and R codes) are available from the Eawag Research Data Institutional Collection (Eric) at [URL to be supplied after acceptance of manuscript].

## 6.9 References

- Burger, W., Krysiak-Baltyn, K., Scales, P.J., Martin, G.J.O., Stickland, A.D. and Gras, S.L. (2017) The influence of protruding filamentous bacteria on floc stability and solid-liquid separation in the activated sludge process. *Water Research* 123, 578-585.
- Bürgmann, H., Jenni, S., Vazquez, F. and Udert, K.M. (2011) Regime shift and microbial dynamics in a sequencing batch reactor for nitrification and anammox treatment of urine. *Applied and Environmental Microbiology* 77(17), 5897-5907.
- Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunencko, T., Zaneveld, J. and Knight, R. (2010) QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* 7(5), 335-336.
- Caranto, J.D. and Lancaster, K.M. (2017) Nitric oxide is an obligate bacterial nitrification intermediate produced by hydroxylamine oxidoreductase. *PNAS* 114(31), 8217-8222.
- Castro-Barros, C.M., Rodríguez-Caballero, A., Volcke, E.I.P. and Pijuan, M. (2016) Effect of nitrite on the  $N_2O$  and NO production on the nitrification of low-strength ammonium wastewater. *Chemical Engineering Journal* 287, 269-276.
- Chen, X., Mielczarek, A.T., Habicht, K., Andersen, M.H., Thornberg, D. and Sin, G. (2019) Assessment of Full-Scale  $N_2O$  Emission Characteristics and Testing of Control Concepts in an Activated Sludge Wastewater Treatment Plant with Alternating Aerobic and Anoxic Phases. *Environmental Science & Technology* 53(21), 12485-12494.
- Cotto, I., Dai, Z., Huo, L., Anderson, C.L., Vilardi, K.J., Ijaz, U., Khunjar, W., Wilson, C., De Clippeleir, H., Gilmore, K., Bailey, E. and Pinto, A.J. (2020) Long solids retention times and attached growth phase favor prevalence of comammox bacteria in nitrogen removal systems. *Water Research* 169, 115268.
- Daelman, M.R., van Voorthuizen, E.M., van Dongen, U.G., Volcke, E.I. and van Loosdrecht, M.C. (2015) Seasonal and diurnal variability of  $N_2O$  emissions from a full-scale municipal wastewater treatment plant. *Science of The Total Environment* 536, 1-11.
- de Celis, M., Belda, I., Ortiz-Alvarez, R., Arregui, L., Marquina, D., Serrano, S. and Santos, A. (2020) Tuning up microbiome analysis to monitor WWTPs' biological reactors functioning. *Scientific Reports* 10(1), 4079.
- Domingo-Felez, C., Pellicer-Nacher, C., Petersen, M.S., Jensen, M.M., Plosz, B.G. and Smets, B.F. (2016) Heterotrophs are key contributors to nitrous oxide production in activated sludge under low C-to-N ratios during nitrification-Batch experiments and modeling. *Biotechnology and Bioengineering* 114(1), 132-140.

- Griffin, J.S. and Wells, G.F. (2017) Regional synchrony in full-scale activated sludge bioreactors due to deterministic microbial community assembly. *ISME Journal* 11(2), 500-511.
- Gruber, W., Villez, K., Kipf, M., Wunderlin, P., Siegrist, H., Vogt, L. and Joss, A. (2020) N<sub>2</sub>O emission in full-scale wastewater treatment: Proposing a refined monitoring strategy. *Science of The Total Environment* 699, 134157.
- Han, P., Wu, D., Sun, D., Zhao, M., Wang, M., Wen, T., Zhang, J., Hou, L., Liu, M., Klumper, U., Zheng, Y., Dong, H.P., Liang, X. and Yin, G. (2021) N<sub>2</sub>O and NO<sub>y</sub> production by the comammox bacterium *Nitrospira inopinata* in comparison with canonical ammonia oxidizers. *Water Research* 190, 116728.
- Huang, Z., Gedalanga, P.B., Asvapathanagul, P. and Olson, B.H. (2010) Influence of physicochemical and operational parameters on *Nitrobacter* and *Nitrospira* communities in an aerobic activated sludge bioreactor. *Water Research* 44(15), 4351-4358.
- IPCC (2013) *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*, Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
- Johnston, J. and Behrens, S. (2020) Seasonal Dynamics of the Activated Sludge Microbiome in Sequencing Batch Reactors, Assessed Using 16S rRNA Transcript Amplicon Sequencing. *Applied and Environmental Microbiology* 86(19), e00597-00520.
- Johnston, J., LaPara, T. and Behrens, S. (2019) Composition and Dynamics of the Activated Sludge Microbiome during Seasonal Nitrification Failure. *Scientific Reports* 9(1), 4565.
- Ju, F., Guo, F., Ye, L., Xia, Y. and Zhang, T. (2014) Metagenomic analysis on seasonal microbial variations of activated sludge from a full-scale wastewater treatment plant over 4 years. *Environmental Microbiology Reports* 6(1), 80-89.
- Kuokkanen, A., Blomberg, K., Heinonen, M. and Mikola, A. (2020) Mainstream Shortcut Nitrification-denitrification Causing Massive N<sub>2</sub>O Emissions In A Continuous Activated Sludge Process, IWA, Helsinki.
- Lau, A.O., Strom, P.F. and Jenkins, D. (1984) Growth Kinetics of *Sphaerotilus natans* and a Floc Former in Pure and Dual Continuous Culture. *Journal (Water Pollution Control Federation)* 56(1), 41-51.
- Lu, H., Chandran, K. and Stensel, D. (2014) Microbial ecology of denitrification in biological wastewater treatment. *Water Research* 64, 237-254.
- Lucker, S., Schwarz, J., Gruber-Dorninger, C., Spieck, E., Wagner, M. and Daims, H. (2015) Nitrotoga-like bacteria are previously unrecognized key nitrite oxidizers in full-scale wastewater treatment plants. *ISME Journal* 9(3), 708-720.
- Ni, B.J. and Yuan, Z. (2015) Recent advances in mathematical modeling of nitrous oxides emissions from wastewater treatment processes. *Water Research* 87, 336-346.

- Nierychlo, M., Milobedzka, A., Petriglieri, F., McIlroy, B., Nielsen, P.H. and McIlroy, S.J. (2019) The morphology and metabolic potential of the Chloroflexi in full-scale activated sludge wastewater treatment plants. *FEMS Microbiology Ecology* 95(2), fiy228.
- Örmeci, B. and Vesilind, P.A. (2000) Development of an improved synthetic sludge: a possible surrogate for studying activated sludge dewatering characteristics. *Water Research* 34(4), 1069-1078.
- Philips, S., Laanbroek, H.J. and Verstraete, W. (2002) Origin, causes and effects of increased nitrite concentrations in aquatic environments. *Reviews in Environmental Science & Bio/Technology* 1(2), 115-141.
- Randall, C.W. and Buth, D. (1984) Nitrite Build-Up in Activated Sludge Resulting from Temperature Effects. *Journal (Water Pollution Control Federation)* 56(9), 1039-1044.
- Ravishankara, A.R., Daniel, J.S. and Portmann, R.W. (2009) Nitrous Oxide (N<sub>2</sub>O): The Dominant Ozone-Depleting Substance Emitted in the 21st Century. *Science* 326(5949), 123-125.
- Ren, Y., Ngo, H.H., Guo, W., Ni, B.-J. and Liu, Y. (2019) Linking the nitrous oxide production and mitigation with the microbial community in wastewater treatment: A review. *Bioresource Technology Reports* 7.
- Schreiber, F., Wunderlin, P., Udert, K.M. and Wells, G.F. (2012) Nitric oxide and nitrous oxide turnover in natural and engineered microbial communities: biological pathways, chemical reactions, and novel technologies. *Frontiers in Microbiology* 3, 372.
- Sezgin, M., Jenkins, D. and Parker, D.S. (1978) A Unified Theory of Filamentous Activated Sludge Bulking. *Journal (Water Pollution Control Federation)* 50(2), 362-381.
- Shade, A., Peter, H., Allison, S.D., Baho, D.L., Berga, M., Bürgmann, H., Huber, D.H., Langenheder, S., Lennon, J.T., Martiny, J.B., Matulich, K.L., Schmidt, T.M. and Handelsman, J. (2012) Fundamentals of microbial community resistance and resilience. *Frontiers in Microbiology* 3, 417.
- Siripong, S. and Rittmann, B.E. (2007) Diversity study of nitrifying bacteria in full-scale municipal wastewater treatment plants. *Water Research* 41(5), 1110-1120.
- Soler-Jofra, A., Perez, J. and van Loosdrecht, M.C.M. (2020) Hydroxylamine and the nitrogen cycle: A review. *Water Research* 190, 116723.
- Song, M.J., Choi, S., Bae, W.B., Lee, J., Han, H., Kim, D.D., Kwon, M., Myung, J., Kim, Y.M. and Yoon, S. (2020) Identification of primary effectors of N<sub>2</sub>O emissions from full-scale biological nitrogen removal systems using random forest approach. *Water Research* 184, 116144.
- Speirs, L.B.M., Rice, D.T.F., Petrovski, S. and Seviour, R.J. (2019) The Phylogeny, Biodiversity, and Ecology of the Chloroflexi in Activated Sludge. *Frontiers in Microbiology* 10, 2015.

- Stenstrom, M.K. and Poduska, R.A. (1980) The effect of dissolved oxygen concentration on nitrification. *Water Research* 14(6), 643-649.
- Su, Q., Domingo-Felez, C., Jensen, M.M. and Smets, B.F. (2019) Abiotic Nitrous Oxide (N<sub>2</sub>O) Production Is Strongly pH Dependent, but Contributes Little to Overall N<sub>2</sub>O Emissions in Biological Nitrogen Removal Systems. *Environmental Science & Technology* 53(7), 3508-3516.
- Van den Abbeele, P., Van de Wiele, T., Verstraete, W. and Possemiers, S. (2011) The host selects mucosal and luminal associations of coevolved gut microorganisms: a novel concept. *FEMS Microbiology Reviews* 35(4), 681-704.
- Vasilaki, V., Massara, T.M., Stanchev, P., Fatone, F. and Katsou, E. (2019) A decade of nitrous oxide (N<sub>2</sub>O) monitoring in full-scale wastewater treatment processes: A critical review. *Water Research* 161, 392-412.
- Vasilaki, V., Volcke, E.I.P., Nandi, A.K., van Loosdrecht, M.C.M. and Katsou, E. (2018) Relating N<sub>2</sub>O emissions during biological nitrogen removal with operating conditions using multivariate statistical techniques. *Water Research* 140, 387-402.
- Von Schulthess, R. and Gujer, W. (1996) Release of nitrous oxide (N<sub>2</sub>O) from denitrifying activated sludge: verification and application of a mathematical model. *Water Research* 30(3), 521-530.
- Vuono, D.C., Benecke, J., Henkel, J., Navidi, W.C., Cath, T.Y., Munakata-Marr, J., Spear, J.R. and Drewes, J.E. (2015) Disturbance and temporal partitioning of the activated sludge metacommunity. *ISME Journal* 9(2), 425-435.
- Wrage-Mönnig, N., Horn, M.A., Well, R., Müller, C., Velthof, G. and Oenema, O. (2018) The role of nitrifier denitrification in the production of nitrous oxide revisited. *Soil Biology and Biochemistry* 123, A3-A16.
- Wu, L., Ning, D., Zhang, B., Li, Y., Zhang, P., Shan, X., Zhang, Q., Brown, M.R., Li, Z., Van Nostrand, J.D., Ling, F., Xiao, N., Zhang, Y., Vierheilig, J., Wells, G.F., Yang, Y., Deng, Y., Tu, Q., Wang, A., Global Water Microbiome, C., Zhang, T., He, Z., Keller, J., Nielsen, P.H., Alvarez, P.J.J., Criddle, C.S., Wagner, M., Tiedje, J.M., He, Q., Curtis, T.P., Stahl, D.A., Alvarez-Cohen, L., Rittmann, B.E., Wen, X. and Zhou, J. (2019) Global diversity and biogeography of bacterial communities in wastewater treatment plants. *Nature Microbiology* 4(7), 1183-1195.
- Wunderlin, P., Lehmann, M.F., Siegrist, H., Tuzson, B., Joss, A., Emmenegger, L. and Mohn, J. (2013) Isotope signatures of N<sub>2</sub>O in a mixed microbial population system: constraints on N<sub>2</sub>O producing pathways in wastewater treatment. *Environmental Science & Technology* 47(3), 1339-1348.

# **Chapter 7**

## **N<sub>2</sub>O formation and reduction are depending on organic substrate variations and process control in wastewater treatment**

Thesis chapter, submitted to Water Research

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## Abstract

Nitrous oxide (N<sub>2</sub>O) dominates greenhouse gas emissions in wastewater treatment plants (WWTPs). Formation of N<sub>2</sub>O occurs during biological nitrogen removal, involves multiple microbial pathways, and is typically very dynamic. Consequently, N<sub>2</sub>O mitigation strategies require an improved understanding of nitrogen transformation pathways and their modulating controls. Analyses of the nitrogen (N) and oxygen (O) isotopic composition of N<sub>2</sub>O and its substrates at natural abundance have been shown to provide valuable information on formation and reduction pathways in laboratory settings but have never been applied to full-scale WWTPs.

Here we show that N-species isotope ratio measurements at natural abundance level, combined with long-term N<sub>2</sub>O monitoring, allow identification of the N<sub>2</sub>O production pathways in a full-scale plug-flow WWTP (Hofen, Switzerland). The invented approach can be applied to other activated sludge systems. Heterotrophic denitrification appears as the main N<sub>2</sub>O production pathway under all tested process conditions, while the importance of nitrifier denitrification appeared to be of lower or variable influence. N<sub>2</sub>O production by hydroxylamine oxidation was not observed. Fractional N<sub>2</sub>O elimination by reduction to dinitrogen (N<sub>2</sub>) during anoxic conditions was clearly indicated by a concomitant increase in SP,  $\delta^{18}\text{O}(\text{N}_2\text{O})$  and  $\delta^{15}\text{N}(\text{N}_2\text{O})$ . The extent of N<sub>2</sub>O reduction correlated with the availability of dissolved inorganic N and organic substrates, which explains the link between diurnal N<sub>2</sub>O emission dynamics and organic substrate fluctuations. Consequently, dosing ammonium-rich reject water under low-organic-substrate conditions is unfavourable, as it is very likely to cause high net N<sub>2</sub>O emissions.

Our results demonstrate that monitoring of the N<sub>2</sub>O isotopic composition holds a high potential to disentangle N<sub>2</sub>O formation mechanisms in engineered systems, such as full-scale WWTP. Our study serves as a starting point for advanced campaigns in the future combining isotopic technologies in WWTP with complementary approaches, such as mathematical modelling of N<sub>2</sub>O formation or microbial assays to develop efficient N<sub>2</sub>O mitigation strategies.



## 7.1 Introduction

Nitrous oxide is the third most important greenhouse gas and the dominant ozone depleting substance in the stratosphere (IPCC 2013, Ravishankara et al. 2009). Wastewater treatment plants are potent point sources and significant contributors to global anthropogenic  $\text{N}_2\text{O}$  emissions (Tian et al. 2018, Vasilaki et al. 2019).  $\text{N}_2\text{O}$  emissions from WWTP exhibit strong temporal dynamics (Gruber et al. 2020). The underlying drivers of these dynamics, however, remain partially unclear, and are likely linked to the complexity of the different nitrogen-cycle reactions involved in  $\text{N}_2\text{O}$  production in wastewater treatment system (Domingo-Félez and Smets 2020, Schreiber et al. 2012). Three main metabolic pathways performed by two different groups of bacteria have been identified in WWTPs: (i) hydroxylamine ( $\text{NH}_2\text{OH}$ ) oxidation (Ni) and (ii) nitrifier denitrification (nD) by ammonia oxidizing bacteria (AOB), as well as (iii) bacterial denitrification (hD) by heterotrophic denitrifying bacteria (HET) (Ren et al. 2019, Wunderlin et al. 2012). However, given a sufficient supply of organic carbon, HET are also able to reduce  $\text{N}_2\text{O}$  to  $\text{N}_2$ , the target product of N elimination in WWTP (Conthe et al. 2018a, Pan et al. 2013a).

The systematic and efficient mitigation of  $\text{N}_2\text{O}$  emissions in WWTP is a challenging task and requires both long-term monitoring of emissions to identify emission peaks, as well as a mechanistic understanding of  $\text{N}_2\text{O}$  formation mechanisms in the wastewater treatment process (Vasilaki et al. 2019). A number of approaches have been successfully applied in full-scale WWTPs to reduce  $\text{N}_2\text{O}$  emissions, such as the control of the dissolved oxygen (DO) through different aeration rates and timing (Rodriguez-Caballero et al. 2015, Sun et al. 2017), or step / intermittent feeding maintaining in situ low ammonium concentrations (Hu et al. 2013). However, given the intricacy of  $\text{N}_2\text{O}$  production and turnover, methods to quantify and to mechanistically understand the pathways involved are essential to explain emission dynamics and develop robust mitigation strategies (Duan et al. 2021).

Differences in stable isotopic substitution of the  $\text{N}_2\text{O}$  molecule and the bulk isotopic composition of reactive nitrogen substrates ammonium ( $\text{NH}_4^+$ ), nitrite ( $\text{NO}_2^-$ ), and nitrate ( $\text{NO}_3^-$ ), provide valuable information on dissolved N species and  $\text{N}_2\text{O}$  transformation processes, since distinct microbial and/or abiotic pathways exhibit

characteristic isotopic signatures (Sutka et al. 2006, Yoshida and Toyoda 2000). The four most abundant N<sub>2</sub>O isotopocules are, <sup>14</sup>N<sup>14</sup>N<sup>16</sup>O, <sup>14</sup>N<sup>15</sup>N<sup>16</sup>O (<sup>15</sup>N at central, α position), <sup>15</sup>N<sup>14</sup>N<sup>16</sup>O (<sup>15</sup>N at terminal, β position), and <sup>14</sup>N<sup>14</sup>N<sup>18</sup>O (Toyoda and Yoshida 1999). The abundances of N and O stable isotopes are reported relative to a standard in the δ-notation in per mil (‰) (Werner and Brand 2001):

$$\delta(\text{‰}) = \frac{(R_{\text{sample}} - R_{\text{standard}})}{R_{\text{standard}}} * 1000 \quad (7.1)$$

where X refers to the rare isotopocule (<sup>14</sup>N<sup>14</sup>N<sup>18</sup>O (abbreviated as <sup>18</sup>O), <sup>14</sup>N<sup>15</sup>N<sup>16</sup>O (<sup>15</sup>N<sup>α</sup>) and <sup>15</sup>N<sup>14</sup>N<sup>16</sup>O (<sup>15</sup>N<sup>β</sup>), and R<sub>sample</sub> and R<sub>standard</sub> are the ratios of the abundance of the least and the most abundant isotopic species in the sample and the standard, respectively. The international scales for nitrogen and oxygen isotope ratios are atmospheric N<sub>2</sub> (AIR-N<sub>2</sub>) and Vienna Standard Mean Ocean Water (VSMOW) (Mohn et al. 2016, Toyoda and Yoshida 1999). The average <sup>15</sup>N composition is referred to as δ<sup>15</sup>N<sup>bulk</sup>(N<sub>2</sub>O) (δ<sup>15</sup>N<sup>bulk</sup>(N<sub>2</sub>O) ≡ (δ<sup>15</sup>N<sup>α</sup> + δ<sup>15</sup>N<sup>β</sup>)/2) and the difference between δ<sup>15</sup>N<sup>α</sup> and δ<sup>15</sup>N<sup>β</sup> is termed as site preference (SP ≡ δ<sup>15</sup>N<sup>α</sup> – δ<sup>15</sup>N<sup>β</sup>).

The N and O isotopic compositions of N<sub>2</sub>O are controlled by (1) the composition of the substrate, (2) kinetic isotope effects that occur during N<sub>2</sub>O formation, and (3) kinetic isotope effects associated with N<sub>2</sub>O reduction to N<sub>2</sub> (Denk et al. 2017, Toyoda et al. 2017, Yu et al. 2020). In addition, the O isotope ratio in the N<sub>2</sub>O pool is influenced by O-atom exchange reactions between water and N intermediate molecules, especially NO<sub>2</sub><sup>-</sup> (Casciotti et al. 2002, Lewicka-Szczebak et al. 2016). SP is independent of substrate isotopic composition and therefore an especially sensitive tool for distinguishing mechanisms of N<sub>2</sub>O production and consumption. N<sub>2</sub>O from HET (hD) and nD typically exhibiting low SP values (hD: -7.5 to +3.7 ‰, nD: -13.6 to +1.9 ‰), and from Ni yielding consistently higher values (+32.0 to +38.7 ‰). Moreover, reduction of N<sub>2</sub>O to N<sub>2</sub> by hD increases SP of the residual N<sub>2</sub>O pool, as the <sup>15</sup>N-O bond is more stable than <sup>14</sup>N-O (summarized in Denk et al. (2017), Ostrom and Ostrom (2017), Yu et al. (2020)). Mathematically, changes in SP by N<sub>2</sub>O reduction (analogous to changes in δ<sup>15</sup>N<sup>bulk</sup> and δ<sup>18</sup>O) can be approximated by ΔSP = εSP x ln f (Jinuntuya-Nortman et al. 2008, Lewicka-Szczebak et al. 2017, Mariotti

et al. 1981), with  $\varepsilon$  being the enrichment factor (-8.2 to -2.9 ‰, (Yu et al. 2020)), and  $f$  the fraction of unreacted  $\text{N}_2\text{O}$ . The isotopic enrichment factor between product  $P$  and substrate  $S$  is defined as  $\varepsilon_{X_{P/S}} = \alpha_{X_{P/S}} - 1 = \delta X_P / \delta X_S - 1$ , where  $\alpha$  is the isotopic fractionation factor. In combination, the processes of hD, nD, and Ni, respectively, leave  $\text{N}_2\text{O}$  with distinct but partly overlapping ranges of values for the isotopic composition.

The most powerful way to use isotopic composition to constrain the formation and processing of  $\text{N}_2\text{O}$  is a *dual isotope mapping approach*, where SP values are plotted against either  $\Delta\delta^{15}\text{N}^{\text{bulk}}(\text{N}_2\text{O}, \text{substrate})$  or  $\Delta\delta^{18}\text{O}(\text{N}_2\text{O}, \text{H}_2\text{O})$  and compared to the isotope signatures known for particular processes (Yu et al. 2020). In this approach the  $\delta^{15}\text{N}^{\text{bulk}}(\text{N}_2\text{O})$  values are compared to  $\delta^{15}\text{N}$  of possible N substrates ( $\text{NH}_4^+$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ ) with the expression  $\Delta\delta^{15}\text{N}^{\text{bulk}}(\text{N}_2\text{O}, \text{substrate}) = \delta^{15}\text{N}^{\text{bulk}}(\text{N}_2\text{O}) - \delta^{15}\text{N}_{\text{substrate}}$ , while  $\delta^{18}\text{O}(\text{N}_2\text{O})$  is compared to  $\delta^{18}\text{O}(\text{H}_2\text{O})$  by  $\Delta\delta^{18}\text{O}(\text{N}_2\text{O}, \text{H}_2\text{O}) = \delta^{18}\text{O}(\text{N}_2\text{O}) - \delta^{18}\text{O}(\text{H}_2\text{O})$ . Wunderlin et al. (2013) followed this approach with SP values and  $\Delta\delta^{15}\text{N}^{\text{bulk}}(\text{N}_2\text{O})$  to verify process conditions that are most conducive to distinct production pathways (e.g., hD, nD, Ni) during batch experiments in a laboratory-scale reactor with activated sludge. Alternatively, Lewicka-Szczebak et al. (2016) showed that a dual isotope mapping approach with SP versus  $\Delta\delta^{18}\text{O}(\text{N}_2\text{O}, \text{H}_2\text{O})$  is especially suitable to elucidate mixing of  $\text{N}_2\text{O}$  produced by hD or Ni and partial  $\text{N}_2\text{O}$  reduction by denitrification.  $\text{N}_2\text{O}$  produced by Ni typically bears oxygen isotope values of  $\delta^{18}\text{O}(\text{N}_2\text{O}) \sim 25$  ‰, inherited from atmospheric  $\text{O}_2$  (Frame and Casciotti 2010). For  $\text{N}_2\text{O}$  produced from hD or nD the parameter  $\Delta\delta^{18}\text{O}(\text{N}_2\text{O}, \text{H}_2\text{O})$  offers additional insights over  $\delta^{18}\text{O}$  alone. In both hD and nD,  $\text{N}_2\text{O}$  is often produced from nitrite that has undergone complete exchange with water, whether during nitrite synthesis or nitrite reduction, and whose  $\delta^{18}\text{O}$  therefore matches the value predicted for thermodynamic equilibrium (Casciotti et al. 2007, Casciotti et al. 2010, Kool et al. 2007). Then, the conversion of  $\text{NO}_2^-$  to  $\text{N}_2\text{O}$  carries a branching kinetic isotope effect generated by the nitrite reductase and nitric oxide reductase enzymes (Casciotti et al. 2007, Casciotti et al. 2002). The identity of the nitrite reductase enzyme (NirK, NirS) controls the size of this branching isotope effect and thus  $\delta^{18}\text{O}(\text{N}_2\text{O}, \text{H}_2\text{O})$ .  $\text{N}_2\text{O}$  produced from nitrite, with an equilibrium value of  $\delta^{18}\text{O}(\text{NO}_2^-$

, H<sub>2</sub>O) by bacteria with NirS is associated with a larger oxygen isotope effect and will have  $\Delta\delta^{18}\text{O}(\text{N}_2\text{O}, \text{H}_2\text{O})$  of  $28 \pm 6$  ‰, compared to bacteria with the copper containing NirK, with  $\Delta\delta^{18}\text{O}(\text{N}_2\text{O}, \text{H}_2\text{O}) = 24 \pm 6$  ‰ (Martin and Casciotti 2016). Various hD species are known to have either NirK or NirS, but only NirK has been found in nD (Kozłowski et al. 2016, Nikaido 2003, Zumft 1997). Therefore, N<sub>2</sub>O associated with nD and hD exhibits overlapping ranges for  $\Delta\delta^{18}\text{O}(\text{N}_2\text{O}, \text{H}_2\text{O})$ , but values greater 30 ‰ are likely to be associated with hD. The only pure culture constraint on  $\Delta\delta^{18}\text{O}(\text{N}_2\text{O}, \text{H}_2\text{O})$  for N<sub>2</sub>O generated by nD, with a value of 22 ‰ (Frame and Casciotti 2010), is in the low end of this range, consistent with this interpretive framework. Finally, subsequent N<sub>2</sub>O reduction by HET causes both  $\Delta\delta^{18}\text{O}(\text{N}_2\text{O}, \text{H}_2\text{O})$  and SP to increase, but along a slope that is distinct from that caused by mixing of Ni and nD/hD (Lewicka-Szczebak et al. 2017). Despite the potential that natural abundance N<sub>2</sub>O isotope measurements offer for pathway characterization, past applications have been limited to laboratory scale reactors ((Tumendelger et al. 2016, Wunderlin et al. 2013a)).

In this study, we tested, for the first time, whether natural abundance stable isotope measurements in a full-scale WWTP can be applied to characterize N<sub>2</sub>O production pathways. In particular, we investigated how variations in relative N<sub>2</sub>O reduction, under changing inflow composition and process operation, are reflected in the observed N<sub>2</sub>O isotope signatures. To further support the estimated contributions of different production pathways and N<sub>2</sub>O reduction, we used measurements of the <sup>15</sup>N/<sup>14</sup>N and <sup>18</sup>O/<sup>16</sup>O isotope ratios of N substrates, NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, and NO<sub>2</sub><sup>-</sup>. Additionally, we performed both spatially and temporally resolved process monitoring of N<sub>2</sub>O emissions and aqueous nitrogen species to interpret the process dynamics during the experiments. Finally, we propose reduction strategies based on the observed emission patterns and attributed pathways.

## 7.2 Materials & Methods

### 7.2.1 Field site

The Hofen WWTP (Switzerland, 47°27'57.3"N 9°23'49.1"E) treats the wastewater of roughly 70,000 population equivalents. After mechanical treatment by screening, grit chambers, and primary clarification, the wastewater enters the biological treatment stage, consisting of six activated-sludge plug-flow reactors, each comprising three cascaded stirred reactors (3 x 530 m<sup>3</sup>, Figure 1). The biological treatment is equipped with multiple liquid-phase sensors for continuous DO (LDO sc, Hach, USA), NH<sub>4</sub><sup>+</sup> (AN\_ISE sc, Hach, USA), NO<sub>2</sub><sup>-</sup> (CA80NO, Endress + Hauser, Germany), and NO<sub>3</sub><sup>-</sup> (AN\_ISE sc, Hach, USA)) monitoring (Figure 7.1).

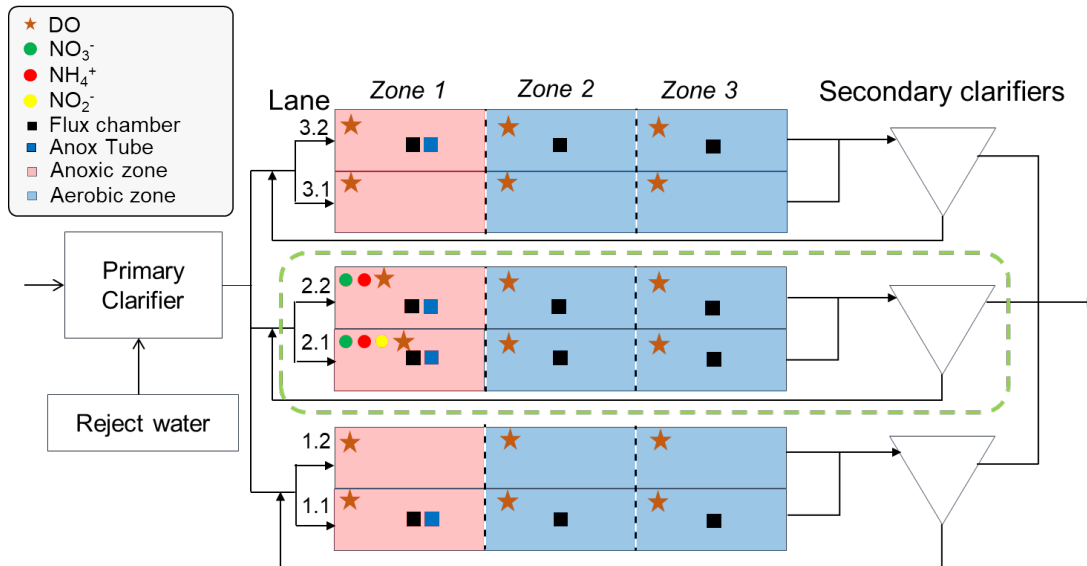


Figure 7.1: Schematic overview of the Hofen WWTP and installed sensors. The green dotted line indicates the lane used for experiments.

The wastewater is evenly distributed over the six treatment lanes. The N removal process is anoxic – oxic, i.e. NH<sub>4</sub><sup>+</sup> oxidation and denitrification to N<sub>2</sub>. The DO concentration is controlled at distinct set-points for each compartment. The first zones are generally operated anoxically and stirred but can be aerated, as soon as the air consumption in zone 3 exceeds a defined threshold. This primarily happens during wet weather conditions and in the winter seasons at low temperatures. The second and the third zone are obligatory oxic, i.e. are continuously aerated. Even under generally non-anoxic conditions, denitrification can proceed within anoxic

microsites/microaggregates. After the biological treatment to eliminate fixed N, the wastewater enters the secondary clarifiers. Two activated sludge lanes share one secondary clarifier, respectively, and therefore receive the same return sludge (Figure 7.1). The biological treatment is operated with a fixed total-solids retention time (SRT) of 13 days. Excess activated sludge is treated in an anaerobic digestion process (not shown in Figure 1), delivering ammonium-rich reject water to the biological treatment. Reject water is dosed into the primary clarifier to make sure that the N load is equally distributed among the lanes. Typically, reject water from sludge treatment is added overnight from 11 pm to 7 am in batches, every 30 minutes.

### **7.2.2 Continuous N<sub>2</sub>O monitoring**

Continuous N<sub>2</sub>O emission monitoring was done using the flux chamber approach, as described in Gruber et al. (2020). Flux chambers were installed in zone 1, 2 and 3 according to Figure 7.1. Additionally, 1.5-meter-long columns, called anox tubes, were installed in zone 1 of selected lanes (1.1, 2.1, 2.2, 3.2) to sample N<sub>2</sub>O from the mixed liquor during non-aerated operation by gas stripping with a blower. This technique provides qualitative information on temporal fluctuations of dissolved N<sub>2</sub>O concentrations for zone 1. N<sub>2</sub>O concentrations from the anox tubes are not quantitative, since the efficiency of the stripping process was not quantified. A sketch of the tube is provided in the SI (Figure D.1). A small share of the off-gas from the chambers and anox tubes was diverted to a central N<sub>2</sub>O measuring unit, consisting of an automated valve system, preceding a dehumidifier and a non-dispersive infra-red sensor (X-stream, Emerson, St. Louis MO, USA). The N<sub>2</sub>O monitoring system was installed in October 2019, and since then is running continuously.

### **7.2.3 Campaigns with isotope measurements**

In 2019, 2020, and 2021 three intensive sampling campaigns supported by N<sub>2</sub>O isotopic measurements were performed on two selected lanes (2.1 and 2.2, Table 7.1). Campaigns were conducted on days with rather dry weather conditions on the day of sampling. Details on the experiments are given in Table 7.1.

Table 7.1: Dates, experimental details, samples taken and hypotheses for the three campaigns conducted at the Hofen WWTP.

Campaign	Weather conditions	Date	Experiment	Sampling of gas and liquid phase for isotope analysis in zones	Research focus (results section)
1	Short and light rain before and after the experiment	28.11.2019 (09:00-12:00)	Lane 2.1, zone 1: aerated Lane 2.2, zone 1: not aerated	Lane 2.1: 1 per zone 1-3 Lane 2.2: 1 per zone 1-3 = 6 samples	Impact of process control (zone 1 aeration) on N <sub>2</sub> O emissions and processes (3.4)
2	Dry weather	08.12.2020 (13:00-15:00)	Lane 2.1, zone 1: not aerated Lane 2.2, zone 1: not aerated	Lane 2.1: 1 per zone 1-3 Lane 2.2: 1 per zone 1-3 = 6 samples	Identify N <sub>2</sub> O production processes under standard operation (3.2)
3	Dry weather	24.02.2021 (6:00-15:30)	Lane 2.1, zone 1: not aerated Lane 2.2, zone 1: aerated	Lane 2.1: Temporal profile, 5 samples in zone 1-2 = 10 samples	Impact of daily COD and N inflow variation on N <sub>2</sub> O production processes (3.3)

#### 7.2.4 Collection of gaseous and aqueous samples and isotopic analyses

Gas samples for N<sub>2</sub>O isotopocule analyses were collected from the sampling lines of the N<sub>2</sub>O monitoring system. For this, the respective line was disconnected from the automated multiport inlet system (Gruber et al. 2020) of the off-gas monitoring device, and the sample gas was extracted with a membrane pump (model PM25032-022, KNF Neuberger AG, Switzerland). Gas samples were dehumidified by permeation drying (model PD-50T-72MSS, Perma Pure LLC, USA) and stored in 40 litre aluminium coated gas bags (model GSB-P/44, Wohlgroth AG, Switzerland) until analysis at the Laboratory for Air Pollution / Environmental Technology, Empa. For the analysis of  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  in the dissolved N species ( $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{NH}_4^+$ ), mixed liquor samples from the wastewater reactors collected in parallel with gas samples, were filtered with 0.45 and 0.2  $\mu\text{m}$  single-use membrane filters, and stored refrigerated until further processing (Magyar et al. 2021). Nitrogen and oxygen isotope analyses of  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ , and  $\text{NH}_4^+$  were conducted at the Department of Environmental Sciences, University of Basel, Switzerland.  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  in

wastewater were measured at the Stable Isotope Laboratory of the Department of Environmental System Sciences, ETH Zurich.

#### *N<sub>2</sub>O isotope measurement (gas phase)*

N<sub>2</sub>O sample gas concentrations were determined with a non-dispersive infrared spectrometer (X-stream, Emerson, St. Louis MO, USA). Thereafter, sample gases were diluted to ambient N<sub>2</sub>O concentrations (approx. 330 ppb) with high-purity synthetic air using mass flow controllers (Vögtlin Instruments GmbH, Switzerland), and the dilution ratio adjusted after CRDS analysis (G5131-i, Picarro Inc., USA). The isotopocule abundances in the samples were measured using quantum cascade laser absorption spectroscopy (QCLAS), preceded by preconcentration (TREX), as described in Ibraim et al. (2018). For calibration a two-point delta calibration approach was implemented (CG1:  $\delta^{15}\text{N}^{\alpha} = 2.06 \pm 0.05 \text{ ‰}$ ,  $\delta^{15}\text{N}^{\beta} = 1.98 \pm 0.20 \text{ ‰}$ ,  $\delta^{18}\text{O} = 36.12 \pm 0.32 \text{ ‰}$ ; CG2:  $\delta^{15}\text{N}^{\alpha} = -82.14 \pm 0.49 \text{ ‰}$ ,  $\delta^{15}\text{N}^{\beta} = -78.02 \pm 0.52 \text{ ‰}$ ,  $\delta^{18}\text{O} = 21.64 \pm 0.12 \text{ ‰}$ ), and instrumental drift, as well as differences in N<sub>2</sub>O concentration corrected (Harris et al. 2020b).

#### *Isotope analysis in dissolved N species*

The N and O isotopic abundances in NO<sub>2</sub><sup>-</sup> were determined using the azide method, where NO<sub>2</sub><sup>-</sup> is chemically converted to gaseous N<sub>2</sub>O at low pH (4 to 4.5) (Magyar et al. 2021, McIlvin and Altabet 2005). For the conversion, a sample volume equivalent to 40 or 10 nmol of NO<sub>2</sub><sup>-</sup> (depending on the concentration in the sample) was added to 3 ml of nitrite-free seawater in a 20 ml headspace vial, and crimp-sealed. The seawater is used to maximize N<sub>2</sub>O yield and minimize oxygen exchange during the reaction (Granger et al. 2020). Then, 300 µl of acetic acid-sodium azide solution (1:1 mixture of 2 M NaN<sub>3</sub> with 20% acetic acid) were injected in the vial, and the mixture was shaken. The reaction was stopped using 200 µl 10 M NaOH after at least 30 minutes. The pre-processing was conducted on the sampling day, and the samples were stored upside-down at room temperature until analysis. The N and O isotopic composition in the concentrated and purified N<sub>2</sub>O samples were measured using a Delta V Plus gas chromatograph isotope ratio mass spectrometer (GC-IRMS, Thermo Scientific, Germany) interfaced with a customized purge-and-trap system



and a GC PAL autosampler (CTC, Switzerland), and standardized using the nitrite reference materials N-7373 and N-10219 (Casciotti et al. 2007) prepared and measured alongside the samples.

The N isotopic composition of  $\text{NH}_4^+$  was determined using the hypobromite method, where  $\text{NH}_4^+$  is chemically converted to  $\text{N}_2\text{O}$  via  $\text{NO}_2^-$  (Zhang et al. 2007). Briefly, a sample volume equivalent to 40 nmol of  $\text{NH}_4^+$  was converted to  $\text{NO}_2^-$  by reaction with 0.5 mL of a 50  $\mu\text{M}$  alkaline hypobromite in a 20 ml headspace vial. Then, this  $\text{NO}_2^-$  sample was converted to  $\text{N}_2\text{O}$  by reaction with sodium azide, and the  $\text{N}_2\text{O}$  was analysed as described in the preceding section. In addition to the nitrite standards N-7373 and N-10219, international ammonium reference materials (IAEA-N1 and USGS26) were prepared, measured alongside the samples and used to standardize the measurements.

The isotopic composition (N, O) of  $\text{NO}_3^-$  was measured by conversion to  $\text{N}_2\text{O}$  with the denitrifier method (Casciotti et al. 2002, Sigman et al. 2001). Prior to the  $\text{NO}_3^-$  isotope analysis, 1 ml of the filtered sample was pre-treated with 40  $\mu\text{l}$  0.6 M sulfamic acid in 2 ml Eppendorf tubes for  $\text{NO}_2^-$  removal. The preparation was neutralized by adding 9  $\mu\text{l}$  2.5 M NaOH after at least 15 minutes and before the end of the day. Until further processing, the samples were stored at  $-20^\circ\text{C}$ . Then,  $\text{NO}_3^-$  sample equivalent to 20 nmole was converted to  $\text{N}_2\text{O}$  by a pure culture of denitrifying bacteria (*Pseudomonas chlororaphis* ATCC 13985) lacking the NosZ enzyme for  $\text{N}_2\text{O}$  reduction. The N and O isotopic composition in the concentrated and purified  $\text{N}_2\text{O}$  samples were measured using a Delta V GC-IRMS (Thermo Scientific, Germany) interfaced with a customized purge-and-trap system and a GC PAL autosampler (CTC, Switzerland), and standardized using international nitrate reference materials (IAEA-N3, USGS32, and USGS34) prepared and measured alongside the samples.

### *H<sub>2</sub>O isotope measurement*

In experiment 3, aqueous samples were analyzed for  $\delta^{18}\text{O}\text{-H}_2\text{O}$  using the high-temperature carbon reduction method. For that purpose, a high-temperature elemental analyzer (TC/EA; Finnigan MAT, Germany) was coupled to a Delta<sup>plus</sup>XP isotope ratio mass spectrometer via a ConFlo III interface (Finnigan MAT, Germany;

(Werner et al. 1999)). The TC/EA was additionally equipped with a custom-made Nafion-trap followed by a 4-port valve (Werner 2003) between the carbon reduction tube and the GC column. The set-up of the carbon reduction tube follows the “MPI-BGC method” described by Gehre et al. (2004). Water was injected automatically with a GC PAL autosampler (CTC, Switzerland) equipped with a 10 µl gas-tight syringe. Preparation for injection of 0.5 µl of water was made with three washing cycles (3 µl) and five pull-ups. All results were normalized to VSMOW and SLAP, assigning consensus values of 0 and 55.5 ‰ for δ<sup>18</sup>O and 0 and 428 ‰ for δ<sup>2</sup>H to VSMOW and SLAP reference waters, respectively (Coplen 1988).

#### **7.2.5 Analyses of reactive N-species**

Concentrations of cations (NH<sub>4</sub><sup>+</sup>-N) and anions (NO<sub>2</sub><sup>-</sup>-N, NO<sub>3</sub><sup>-</sup>-N) were analyzed using flow injection analysis (Foss, FIAstar flow injection 5000 analyzer, Denmark) and anion chromatography (Methrom 881 compact IC, Switzerland), respectively.

## 7.3 Results and discussion

### 7.3.1 N<sub>2</sub>O emissions at the Hofen WWTP

The average N<sub>2</sub>O emissions of lane 2.1 and 2.2 at the Hofen WWTP were 0.8 kg N<sub>2</sub>O-N/d (0.2% of the total nitrogen load) (Table 7.2). Emissions from both lanes displayed similar temporal patterns, with high emissions in winter and lower emissions during the summer season (Figure 7.2). The highest N<sub>2</sub>O emissions were observed over several weeks starting in January 2021. The emission peak occurred in parallel with increased NO<sub>2</sub><sup>-</sup> concentrations in the effluent of the WWTP, which is known to enhance N<sub>2</sub>O emissions via both nD and hD pathways (Ren et al. 2019). In fact, all lanes were fully aerated during the peak emission phase to increase NO<sub>2</sub><sup>-</sup> oxidation capacities of the biological treatment, which in turn favours N<sub>2</sub>O stripping. The detrimental effect of aeration of zone 1 (in terms of N<sub>2</sub>O production) compared to anoxic operation was also shown in campaigns 1 and 3, where the first zone of lane 2.1 or 2.2 were aerated. Similarly, in April 2020 only zone 1 of lane 2.1 was aerated, which led to substantially higher net N<sub>2</sub>O emissions as compared to lane 2.2 (Figure 7.2).

The mean SP value for N<sub>2</sub>O emitted from oxygen-replete zones in all three experiment was  $-1.7 \pm 2.7$  ‰, which is somewhat lower than results (4.5 ‰) from a previous full-scale WWTP study (Toyoda et al. 2011), but fully in the range of isotopic signatures measured for nD and hD at a lab-scale WWTP (Wunderlin et al. 2013), as well as in pure culture studies (Yu et al. 2020). In contrast, N<sub>2</sub>O liberated from zone 1 under anoxic operation, using the anox tube, displayed significantly higher SP values of  $12.3 \pm 2.2$  ‰.

Emissions of both lanes were correlated, if the same operational modes were applied (Figure 7.2, Table 7.2). The highest emissions were observed over several weeks starting in January 2021. The emission peak occurred in parallel with increased NO<sub>2</sub><sup>-</sup> concentrations in the effluent of the WWTP. NO<sub>2</sub><sup>-</sup> accumulation is known to lead to increased N<sub>2</sub>O emissions via both nD and hD pathways. Hence, we expect that these pathways were causing the emission increase at the Hofen WWTP. Additionally, all lanes were fully aerated during the peak phase to increase NO<sub>2</sub><sup>-</sup> oxidation capacities

of the biological treatment, which in turn led to higher emission due to stripping and incomplete hD. The detrimental effect of aeration in zone 1, compared to anoxic operation, was also shown in campaigns 1 and 3 (Table 7.2). In addition, in April 2020 only lane 2.1 was aerated which led to substantially higher emissions as compared to lane 2.2 (Figure 7.2, and Figure 5.5).

Table 2: Daily averaged N<sub>2</sub>O emissions on lanes 2.1 and 2.2 for the complete study period, the high emission peak phase, and the single sampling campaigns. Redox conditions in zone 1, i.e. aeration vs. anoxic, is indicated in brackets.

Phase	Emissions lane 2.1 (kg N <sub>2</sub> O-N/d)	Emissions lane 2.2 (kg N <sub>2</sub> O-N/d)
Average (Nov 2019-Mar 2021)	0.8 (standard operation, variable)	0.8 (standard operation, variable)
Peak phase (Jan 2021)	3.6 (aerated)	4.4 (aerated)
Campaign 1	1.9 (aerated)	0.4 (anoxic)
Campaign 2	0.1 (anoxic)	0.3 (anoxic)
Campaign 3	0.7 (anoxic)	1.7 (aerated)

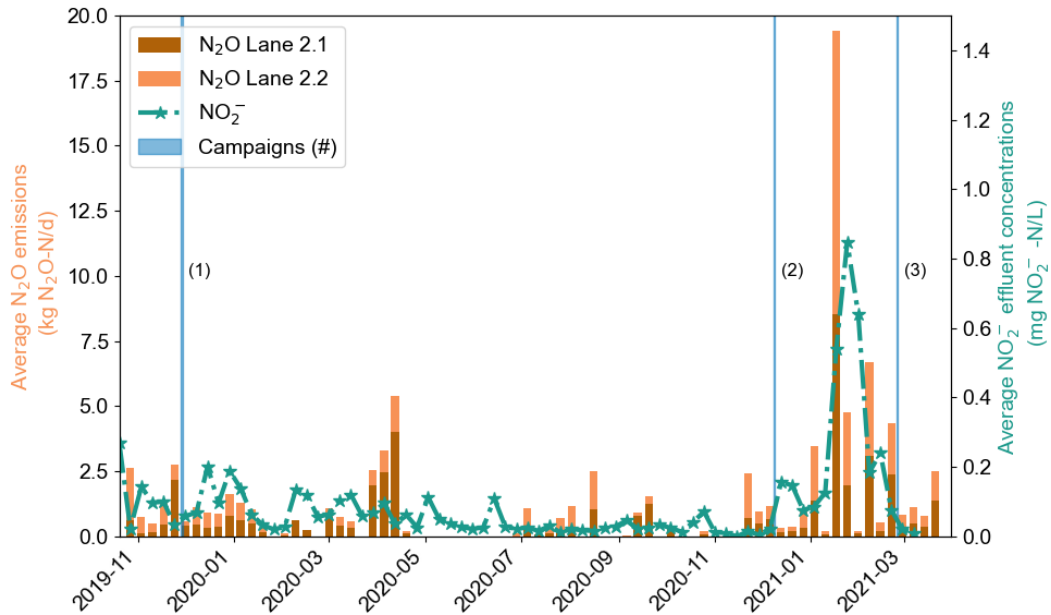


Figure 7.2: Weekly average N<sub>2</sub>O emissions from lanes 2.1 and 2.2 and weekly average NO<sub>2</sub><sup>-</sup> concentrations in the effluent of the WWTP. Blue lines indicate dates of experiments and numbers in brackets refer to the campaign number.

Table 7.2: Average daily N<sub>2</sub>O emissions on lanes 2.1 and 2.2 over different time scales. Redox conditions during the campaigns are indicated in brackets.

Phase	Emissions lane 2.1 (kgN <sub>2</sub> O-N/d)	Emissions lane 2.2 (kgN <sub>2</sub> O-N/d)
Average (Nov 2019-Mar 2021)	0.8	0.8
Peak phases (January 21)	3.6	4.4
Campaign 1	1.9 (aerobic)	0.4 (anoxic)
Campaign 2	0.1 (anoxic)	0.3 (anoxic)
Campaign 3	0.7 (anoxic)	1.7 (aerobic)

### 7.3.2 Identification of N<sub>2</sub>O production pathways using dual isotope mapping approaches

The isotope sampling campaigns at the Hofen WWTP were conducted during different seasons and times of day and under aerobic/anoxic operation of zone 1 (Table 7.1, Table 7.2). The mean SP during aerobic conditions in all three experiment was  $-1.7 \pm 2.7$  ‰, which is lower than the value (4.5 ‰) from a previous full-scale WWTP study (Toyoda et al. 2011) and in the range of isotopic signatures measured for nD and hD at a lab-scale WWTP (Wunderlin et al. 2013a). The low values indicate that Ni was not relevant as a N<sub>2</sub>O production pathway during the experiments conducted (Figure 7.3). The mean SP during anoxic conditions (zone 1 without aeration) was  $12.3 \pm 2.2$  ‰, which is significantly higher than under aerobic conditions.

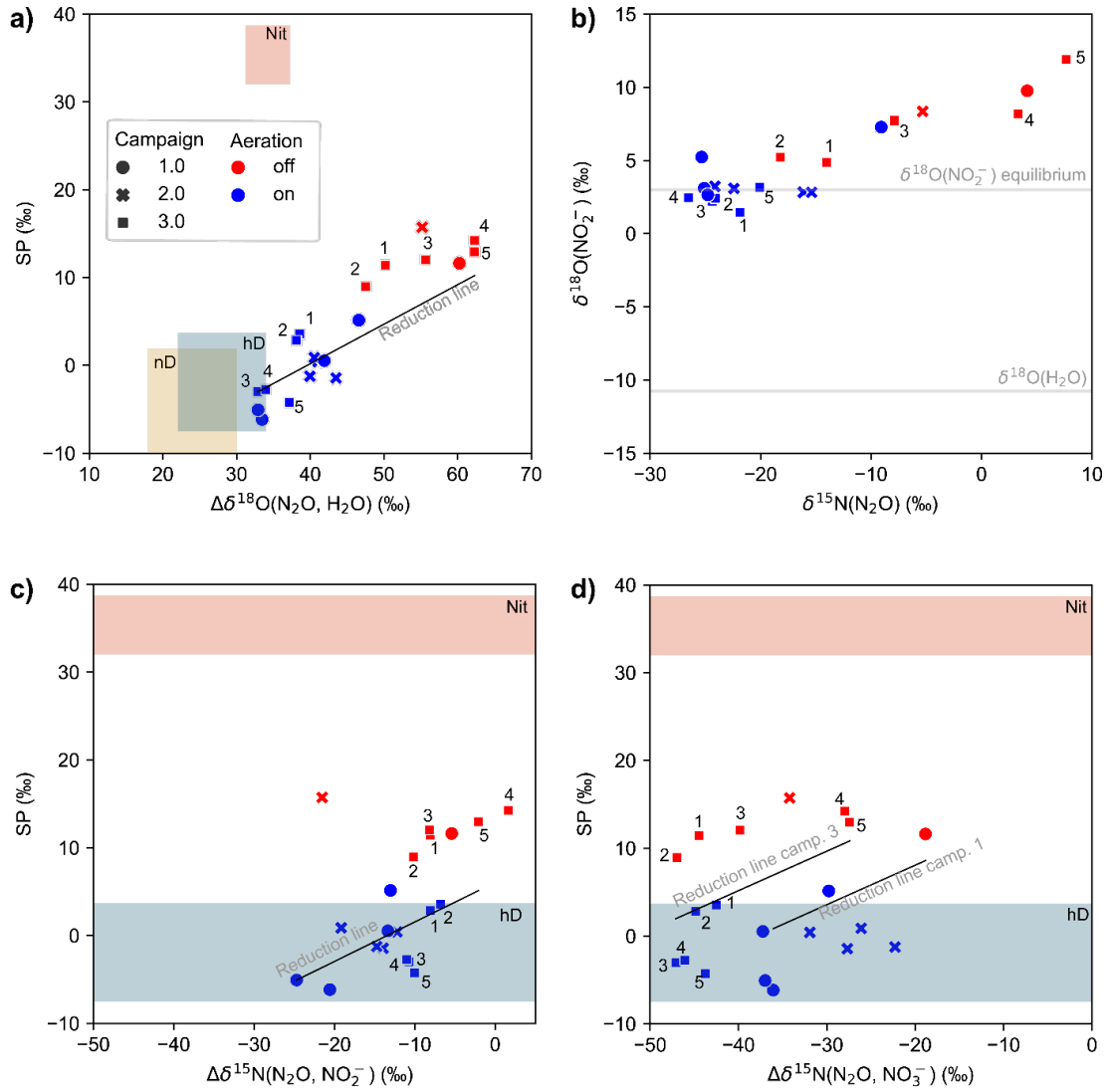


Figure 7.3: Isotopic signatures of N<sub>2</sub>O liberated from aerated (blue symbols) and anoxic (red symbols) zones of the WWTP Hofen, normalized for the substrate isotopic composition (H<sub>2</sub>O, NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>) for the three campaigns that included isotopic measurements. Dual-isotope plots for SP and Δδ<sup>18</sup>O(N<sub>2</sub>O, H<sub>2</sub>O) (panel a), Δδ<sup>18</sup>O(N<sub>2</sub>O, NO<sub>2</sub><sup>-</sup>) (panel b), and Δδ<sup>15</sup>N(N<sub>2</sub>O, NO<sub>2</sub><sup>-</sup>) (panel c) are provided. Colored areas indicate expected isotopic signatures for N<sub>2</sub>O production pathways (Nit = hydroxylamine oxidation, nD = nitrifier denitrification, hD = heterotrophic denitrification) according to Yu et al. (2020). The expected change in isotopic composition during partial reduction of N<sub>2</sub>O to N<sub>2</sub> is indicated by black "reduction lines". For panel (a), all data points fall on one line, while for panel (c) data points of individual days present individual reduction lines for campaigns 1 and 3. Numbers next to data points of campaign 3 (stars) indicate the sampling sequence (t1: 6 – 7 am, t2: 8 – 9 am, t3 = 10 – 11 am, t4 = 1 – 2 pm, t5 = 2:30 – 3:30 pm).

The SP values of  $\text{N}_2\text{O}$  emitted under aerated conditions indicate nD or hD as main  $\text{N}_2\text{O}$  production pathways. The relationship of SP with  $\Delta\delta^{18}\text{O}(\text{N}_2\text{O}, \text{H}_2\text{O})$  (Figure 7.3 a) displays a considerable decrease in both SP and  $\Delta\delta^{18}\text{O}(\text{N}_2\text{O}, \text{H}_2\text{O})$  during the change from anoxic to aerobic conditions. This corresponds to the well-established fractionation in favour of  $\text{N}^{15}\text{NO}$  and  $\text{NN}^{18}\text{O}$  during partial reduction, resulting in the progressive increase of  $\delta^{15}\text{N}^\alpha$  and  $\delta^{18}\text{O}$  for the residual  $\text{N}_2\text{O}$  pool (Lewicka-Szczebak et al. 2014, Ostrom and Ostrom 2017, Yu et al. 2020). The concomitant increase in  $\delta^{18}\text{O}(\text{NO}_2^-)$  and  $\delta^{15}\text{N}(\text{N}_2\text{O})$  also supports the concurrent reduction of nitrite and  $\text{N}_2\text{O}$  through hD (Figure 7.3 b). Interpreting the  $\Delta\delta^{18}\text{O}(\text{N}_2\text{O}, \text{H}_2\text{O})$  signatures of  $\text{N}_2\text{O}$  emitted in the aerobic zone (i.e., concomitant with low SP values) requires a more nuanced interpretation, but yields additional information. The  $\Delta\delta^{18}\text{O}(\text{N}_2\text{O}, \text{H}_2\text{O})$  value is controlled by both equilibrium isotope effects during O-exchange of precursors with water and branching isotope effects during O-abstraction. Both effects depend strongly on the bacterial community that performs denitrification and can differ substantially among systems (Kool et al. 2007, Martin and Casciotti 2016).  $\delta^{18}\text{O}(\text{NO}_2^-)$  is consistent with complete exchange between  $\text{NO}_2^-$  and water for samples in the aerated zone, which yields a composition of  $\sim 3\text{‰}$  at  $15^\circ\text{C}$  to  $20^\circ\text{C}$  (Buchwald and Casciotti 2013) (Figure 7.3 b). Complete exchange can be associated with nitrite produced in nitrification (Buchwald et al. 2012, Casciotti et al. 2010), but also mediated by the iron-containing nitrite reductase NirS, which is present in many heterotrophic denitrifiers (Casciotti et al. 2007, Casciotti et al. 2002, Kool et al. 2007). Then, the final  $\Delta\delta^{18}\text{O}(\text{N}_2\text{O}, \text{H}_2\text{O})$  of  $\text{N}_2\text{O}$  is determined by the branching kinetic isotope effects associated with nitrite reduction to  $\text{NO}$ , followed by  $\text{NO}$  reduction to  $\text{N}_2\text{O}$  (Casciotti et al. 2007, Casciotti et al. 2002, Martin and Casciotti 2016, Rohe et al. 2017).

$\Delta\delta^{18}\text{O}(\text{N}_2\text{O}, \text{H}_2\text{O})$  values for  $\text{N}_2\text{O}$  emitted from the aerated zones of WWTP Hofen fall into the range observed only for bacteria featuring nitrite reduction using the NirS enzyme (30 to 34 ‰, Figure 7.3). This indicates a major contribution of hD, since nD is always associated with NirK (Frame and Casciotti 2010, Kozłowski et al. 2016, Wei et al. 2015), with  $\Delta\delta^{18}\text{O}(\text{N}_2\text{O}, \text{H}_2\text{O})$  values 4 to 16 ‰ lower than observed at the aerated zones of WWTP Hofen (Martin and Casciotti 2016). This result is also concordant with the observation of Orschler et al. (2021), that although

hD can involve either NirK or NirS, in activated sludge systems it is predominantly performed via NirS.  $\Delta\delta^{18}\text{O}(\text{N}_2\text{O}, \text{H}_2\text{O})$  values from the aerated zones are about 10‰ higher than those reported by Lewicka-Szczebak et al. (2016), 16.7 to 23.3 ‰. The observed discrepancy may be explained by the fact that the underlying values reported by Lewicka-Szczebak et al. (2016) were derived from soil systems that likely differ significantly in terms of the active microbial communities and expressed enzymes, as compared to wastewater systems (Wu et al. 2019).

Hence,  $\Delta\delta^{18}\text{O}(\text{N}_2\text{O}, \text{H}_2\text{O})$  values ~35 ‰ point towards a high contribution of hD, rather than nD, to total N<sub>2</sub>O production during oxic phases. The prevalence of anaerobic hD in oxic conditions can readily be rationalized by anoxic microsites in sludge flocs even in aerated zones (Sexstone et al. 1985). Nevertheless, given the variability seen in  $\Delta\delta^{18}\text{O}(\text{N}_2\text{O}, \text{H}_2\text{O})$ , we cannot exclude a variable contribution from nD under certain conditions, which could be what drives difference between aerobic samples in Figure 7.3 a. Slightly lower SP and lower  $\Delta\delta^{18}\text{O}(\text{N}_2\text{O}, \text{H}_2\text{O})$  values may be due to an increased contribution of nD. Alternatively, the higher values may also be caused by a partial reduction of N<sub>2</sub>O also during aerobic phases, assuming that organic substrate is not fully consumed in zone 1 and leaks into zone 2. Furthermore, N<sub>2</sub>O with high SP and  $\Delta\delta^{18}\text{O}(\text{N}_2\text{O}, \text{H}_2\text{O})$  might be transported and mixed in from zone 1 as discussed in section 3.3 in more detail.

Plotting SP values relative to  $\Delta\delta^{15}\text{N}(\text{N}_2\text{O}, \text{NO}_3^-)$  indicates a higher variability among the three intensive sampling campaigns (Figure 7.3 d). Co-variations in SP and  $\Delta\delta^{15}\text{N}(\text{N}_2\text{O}, \text{NO}_3^-)$  values between N<sub>2</sub>O from aerated and anoxic zones during individual campaigns were driven by the anticipated partial N<sub>2</sub>O reduction, displayed by the reduction line. Differences in  $\Delta\delta^{15}\text{N}(\text{N}_2\text{O}, \text{NO}_3^-)$  between experiments, e.g., 31.6 ‰ (campaign 1 and 2) versus 41.1 ‰ (campaign 3), were possibly caused by concentration-dependent variations (affecting cell-specific rates) in the isotope effects associated with denitrification (Kritee et al. 2012). More precisely, the higher NO<sub>3</sub><sup>-</sup> concentrations during experiment 3 (10-18 mg NO<sub>3</sub><sup>-</sup>-N/L) compared to experiment 1 and 2 (0-7 mg NO<sub>3</sub><sup>-</sup>-N/L) may manifest in substantially higher isotope effects. The increased nitrate concentrations were due to the full aeration of all zones over multiple weeks before experiment 3. The operation led to reduced denitrification activity and NO<sub>3</sub><sup>-</sup> accumulation in the biological treatment.



Interestingly,  $\Delta\delta^{15}\text{N}(\text{N}_2\text{O}, \text{NO}_2^-)$  was more consistent than  $\Delta\delta^{15}\text{N}(\text{N}_2\text{O}, \text{NO}_3^-)$  between campaigns, i.e., isotope effects seemed less strongly affected by N substrate concentrations (Figure 7.3 c). Therefore, isotopic signatures for samples from aerated and anoxic compartments cluster significantly closer to the predicted reduction line (Figure 7.3 c). The observed correlation of delta values for individual campaigns supports the notion that the isotopic composition of  $\text{NO}_3^-$ ,  $\text{NO}_2^-$  and  $\text{N}_2\text{O}$  are mostly controlled by the sequential reduction of  $\text{NO}_3^-$  to  $\text{N}_2$  during complete denitrification.

In summary, the isotopic composition of  $\text{N}_2\text{O}$ ,  $\text{NO}_2^-$ , and  $\text{NO}_3^-$  consistently point towards a high contribution of hD to  $\text{N}_2\text{O}$  production during aeration on all days. nD may be of variable relevance, yet Ni can be excluded as a significant contributor. hD was previously shown to govern  $\text{N}_2\text{O}$  production during aeration under low C:N conditions (Domingo-Felez et al. 2016). Our data confirm that obligate anoxic process, such as hD, play an important role even during aerobic conditions, supported by strong oxygen gradients and anoxic microniches in sludge flocs (Daigger et al. 2007). For zones under anoxic process conditions, observed isotope patterns provide clear evidence for substantial  $\text{N}_2\text{O}$  reduction. To diagnose the contribution of different production pathways, the relation of SP and  $\Delta\delta^{18}\text{O}(\text{N}_2\text{O}, \text{H}_2\text{O})$  turned out to be more sensitive than the  $\Delta\delta^{15}\text{N}(\text{N}_2\text{O}, \text{substrate})$  approaches. However, combining both approaches as shown here, has the benefit of being able to additionally validate interpretations, and to provide independent process information to assess the full process complexity of  $\text{N}_2\text{O}$  formation and reduction.

### 7.3.3 Diurnal variation in $\text{N}_2\text{O}$ emissions and production pathways

The main focus of the third campaign was to investigate the effect of the diurnal patterns in N loading (controlled by reject water dosage) and COD substrate inflow on  $\text{N}_2\text{O}$  emissions and variations in  $\text{N}_2\text{O}$  reduction. For this, we analysed the isotopic signatures of  $\text{N}_2\text{O}$  and nitrogenous substrates in zone 1 and 2 for five different time points during one day at lane 2.1 (Figure 7.4).  $\text{N}_2\text{O}$  emissions exhibited a clear diurnal pattern, with a peak at 9 am, right before the reject water dosage was stopped (Figure 7.4 a).  $\text{N}_2\text{O}$  concentration changes in the anoxic zone, measured with the anox tube, were consistent with changes in the  $\text{N}_2\text{O}$  flux from zone 2 and 3. While

NH<sub>4</sub><sup>+</sup> concentrations also exhibited a clear diurnal variation pattern, NO<sub>3</sub><sup>-</sup> concentrations were relatively stable throughout the study period (Figure 7.4 c, Figure D.4, Figure D.6, SI). NO<sub>2</sub><sup>-</sup> was highest in zone 1 and gradually decreased in zone 2 and 3, respectively (Figure D.5 (SI)).

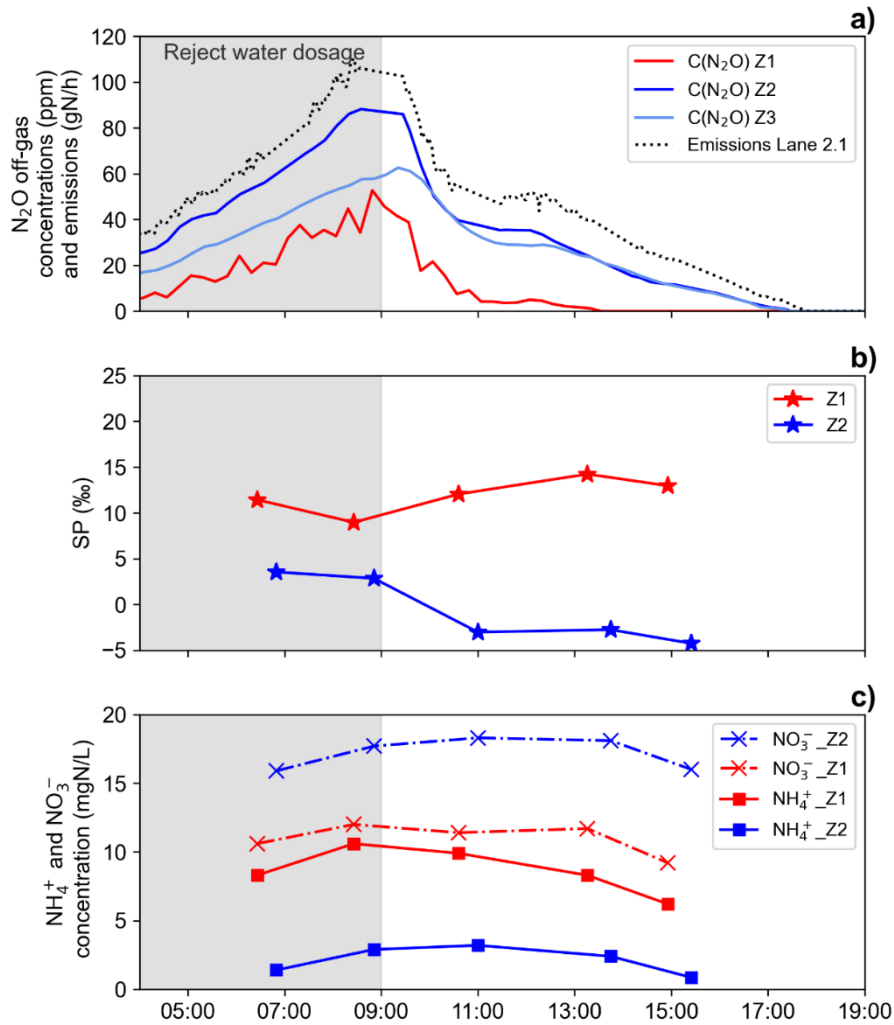


Figure 7.4: (a) N<sub>2</sub>O concentrations and emissions measured at different zones of lane 2.1. Notably, measured gas concentrations in zone 1 are not in equilibrium with the gas phase due to reduced stripping in the anox tube. (b) N<sub>2</sub>O SP in zone 1 and 2, indicating a minimum in N<sub>2</sub>O reduction in zone 1 around 9 am, while N<sub>2</sub>O SP in zone 2 is generally low but increased at high concentrations in zone 1 due to transport. (c) NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> concentrations in zone 1 and 2 of lane 2.1 are stable despite higher NO<sub>3</sub><sup>-</sup> inflow (Figure S.8), pointing towards high denitrifying activity at 11 am. The grey shaded area shows the period of reject water dosage. The timing of gas and liquid sampling is indicated by markers in Fig. 4 b and c: t1: 6 – 7 am, t2: 8 – 9 am, t3 = 10 – 11 am, t4 = 1 – 2 pm, t5 = 2:30 – 3:30 pm.

The diurnal trend of the N<sub>2</sub>O site preference in zone 1 indicates a decreasing importance of N<sub>2</sub>O reduction from 7 am to 9 am (sampling points 1 and 2), also

shown in the *dual isotope mapping approach*, e.g., for SP vs.  $\Delta\delta^{18}\text{O}(\text{N}_2\text{O}, \text{H}_2\text{O})$  (Figure 7.3 a). After 10 am, SP and  $\Delta\delta^{18}\text{O}(\text{N}_2\text{O}, \text{H}_2\text{O})$  values for  $\text{N}_2\text{O}$  from zone 1 increased along the reduction line, which suggests an increasing return to relevance of  $\text{N}_2\text{O}$  reduction for samples 3 to 5.  $\text{NO}_3^-$  concentrations remain stable in zone 1 (Figure 7.4 c) despite an increase of  $\text{NO}_3^-$  inflow from the return sludge (Figure D.4, SI), confirming that heterotrophic nitrate reduction (hD) was very active after 9 am. We suggest two main causes for the strong daily variation in  $\text{N}_2\text{O}$  emissions and N removal.

First, the dosage of reject water and the morning peak in N inflow, typically seen in WWTPs, led to a  $\text{NH}_4^+$  concentration increase (Figure 7.4 c, t1 – t2), while the  $\text{N}_2\text{O}$  reduction capacity of the WWTP was lower due to the increased supply of  $\text{NO}_3^-$ . Second, and more importantly, the availability of organic substrate typically exhibits daily fluctuations. Therefore, despite high  $\text{NH}_4^+$  loads from 10 am to 2 pm (t3 – t4), high availability of organic substrate led to increasing nitrogen removal and in turn increased fractional  $\text{N}_2\text{O}$  reduction rates. Notably, COD concentrations were not measured during the campaign, but are expected to correlate with the inflow rate to the wastewater treatment plant, which exhibits reproducible daily variation (Figure D.3 (SI)).

The  $\text{N}_2\text{O}$  SP in zone 2 is at its maximum between 6 and 9 am, probably due to transport of  $\text{N}_2\text{O}$  produced in zone 1, where both  $\text{N}_2\text{O}$  production and reduction were high in this period as described above (Figure 7.4 b). This would imply that  $\text{N}_2\text{O}$  emissions from zone 2 before and during the peak phase, i.e., the end of the reject water dosage, have a substantial contribution from zone 1. A main contribution of hD is supported by high  $\Delta\delta^{18}\text{O}(\text{N}_2\text{O}, \text{NO}_2^-)$  values ( $-25.7 \pm 2.2\text{‰}$ ). Alternatively, high SP values in zone 2 before 9 am can be explained by partial  $\text{N}_2\text{O}$  reduction, but this is unlikely given the suspected COD limitation during reject water dosage. Moreover, transport of  $\text{N}_2\text{O}$  produced in an anoxic zone to an aerobic zone has been reported earlier for other WWTPs (Mampaey et al. 2016). After 10 am, the difference in SP values between zone 1 and 2 was increasing again, indicating that  $\text{N}_2\text{O}$  transport and mixing was less important.

In addition, the contribution of nD to  $\text{N}_2\text{O}$  formation might have increased after 10 am in zone 2, which could further explain the lower SP and  $\Delta\delta^{18}\text{O}(\text{N}_2\text{O}, \text{H}_2\text{O})$  here.

Nevertheless, we believe that hD also contributed a major part to the emissions in the aerobic zones between 11 am and 4 pm, given the still-high  $\Delta\delta^{18}\text{O}(\text{N}_2\text{O}, \text{H}_2\text{O})$  values.

#### **7.3.4 N<sub>2</sub>O emissions depending on process operation**

The seasonal dynamics in N<sub>2</sub>O emissions indicate that phases where the air consumption in zone 3 exceeds a defined threshold and thus when zone 1 was aerated were generally characterized by high net N<sub>2</sub>O production (Figure 7.2). To better understand the effect of aerobic conditions in the first zone on overall N<sub>2</sub>O formation, we compared the isotopic signatures of N<sub>2</sub>O produced along a fully aerated lane (2.1) and a lane under standard operation, i.e., with anoxic conditions in the zone 1 (2.2) (Table 7.2). The episodes of reject-water dosage in the morning had a high impact on the emissions (i.e., high N<sub>2</sub>O emissions in campaign 3), but N<sub>2</sub>O emissions were even higher from the fully aerated lane (Table 7.2). The difference between lanes was primarily driven by emissions in zone 1 and 2, while emissions in the third zone were comparable (Figure 5 a).

The explanation for increased N<sub>2</sub>O emissions from the fully aerated lane 2.1 can be assessed when comparing isotopic signatures of the N<sub>2</sub>O released from zone 1 of both lanes (Figure 7.5 b, campaign 1). The N<sub>2</sub>O isotopic signature measured in the zone 1 of lane 2.2, with conventional operation, i.e., zone 1 mostly anoxic, indicates a substantial reduction of N<sub>2</sub>O. In contrast, for lane 2.1, with zone 1 aerated, the share of N<sub>2</sub>O reduction was substantially lower. Applying isotopic fractionation factors according to Yu et al. (2020) yields an estimate of 92% of N<sub>2</sub>O (84 to 99% using max and min fractionation factors) reduced for the anoxic zone 1 of lane 2.2, while only 68% (56 to 90% using max and min fractionation factors) is reduced in the aerated zone 1 of lane 2.1 (assuming that the SP values for N<sub>2</sub>O from zone 2 are representative for the N<sub>2</sub>O production process). As during campaign 3, N<sub>2</sub>O production was very likely driven by hD, given the increased  $\Delta\delta^{18}\text{O}(\text{N}_2\text{O}, \text{NO}_2^-)$  values ( $-26.3 \pm 2.8\text{‰}$ ) in the aerobic zones.

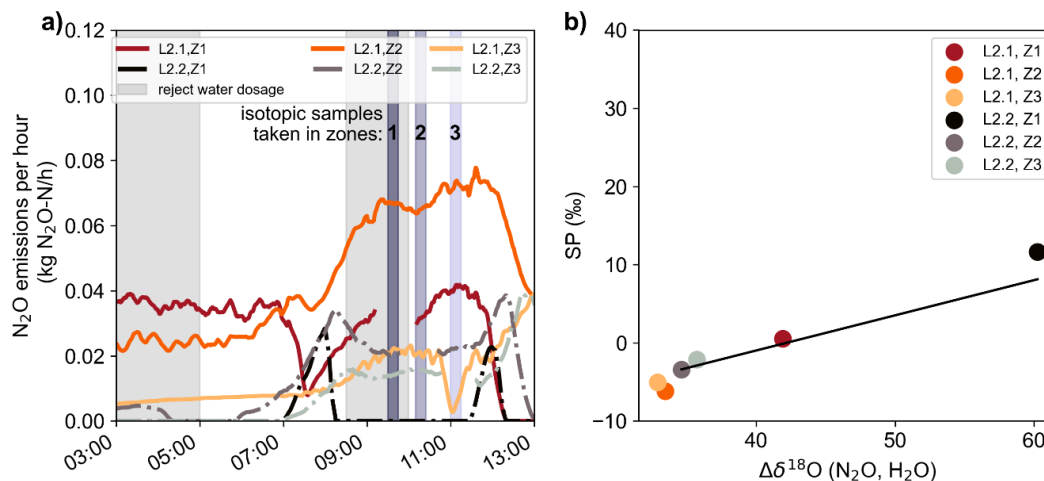


Figure 7.5: N<sub>2</sub>O emissions during campaign 1, indicating higher emissions for lane 2.1, where zone 1 was aerated as compared to conventional operation in lane 2.2 (zone 1 anoxic), the vertical lines indicate timing for isotopic samples. Lane 2.2. zone 1 was aerated for a short period at 7am (panel a). SP and  $\Delta\delta^{18}\text{O}(\text{N}_2\text{O}, \text{H}_2\text{O})$  for N<sub>2</sub>O emitted from lanes 2.1 (zone 1 aerated) and 2.2 (zone 1 anoxic), indicate a higher share of N<sub>2</sub>O reduction for zone 1 of lane 2.2, consistent with lower emissions. The indicated straight line represents the expected change in isotopic signatures with progressive N<sub>2</sub>O reduction, the so-called “reduction line” (panel b).

Campaigns 1 and 3 revealed that organic carbon availability, aeration of zone 1, and reject-water N dosage are the most important modulators of N<sub>2</sub>O emissions during standard operation at the Hofen WWTP, and at a given time of the year. Notably, emissions were lowest in campaign 2 (Table 7.2), with anoxic conditions in zone 1 of both lanes, without reject-water dosage and sampling times in the afternoon, where increased organic substrate concentrations are expected. While it seems relatively clear that aerobic conditions in zone 1 and low organic substrate availability both lead to higher emissions by impairing a more efficient N<sub>2</sub>O reduction, the mechanism behind the increased production of N<sub>2</sub>O caused by elevated reject-water dosage (which leads to an increase in  $\text{NH}_4^+$  concentrations) is not fully understood (Gruber et al. 2020). Most plausibly, elevated N<sub>2</sub>O emissions are directly linked to the high  $\text{NH}_4^+$  concentrations (following substrate- vs- intermediate product systematics). Alternatively, it is possible that the composition of the reject water is somehow unfavourable for HET and AOB. Further research is needed to unravel underlying mechanisms, e.g., by comparing the effects of dosages of reject-water  $\text{NH}_4^+$  versus  $(\text{NH}_4)_2\text{SO}_4$  solution in activated sludge. Nevertheless, our results already yield important information regarding efficient strategies to reduce N<sub>2</sub>O emissions during normal operation at the Hofen WWTP. The guiding principle for

the mitigation of N<sub>2</sub>O emissions is to maximize N<sub>2</sub>O reduction by avoiding aeration of zone 1, and dosing reject-water primarily during periods with high organic carbon load, e.g. in the afternoon. The adaptation of the feeding strategy to optimize organic carbon utilization towards most efficient N<sub>2</sub>O reduction has been successfully applied in side-stream treatment (Peng et al. 2017). However, changing reject-water dosage operation strategies should be critically evaluated as the effects of the NH<sub>4</sub><sup>+</sup> loading are multifaceted. That is, besides potential impacts of the NH<sub>4</sub><sup>+</sup> dosage on net N<sub>2</sub>O emissions, other constraints need to be considered. For example, increased NH<sub>4</sub><sup>+</sup> peak concentrations can lead to ammonia breakthrough, and load equilibration in the diurnal pattern is beneficial for the nitrification performance (Meyer and Wilderer 2004). We propose to apply conventional ASM modelling and full-scale testing and monitoring to optimize reject-water dosage in terms of effluent quality and maximized reduction capacities for N<sub>2</sub>O mitigation (Henze et al. 2000).

Isotopic technologies were successfully applied to analyse the contribution of N<sub>2</sub>O production pathways at the Hofen WWTP, and provided mechanistic understanding to support mitigation strategies. A major advantage to characterize contributions of N<sub>2</sub>O reduction and production pathways at the Hofen WWTP involved the cascaded lanes, with clearly defined redox conditions in each zone. We expect that the application in flow-through, non-compartmented activated sludge systems can be more challenging due to increased mixing over a whole lane, leading to a higher exchange of the nitrogen pools. Furthermore, continuous long-term monitoring is important for the extrapolation and interpretation of the data and the characterization of the seasonal emission peaks. The lion's share of the total annual N<sub>2</sub>O emissions can be attributed to the January peak emission period (Figure 7.2; 50% of the total emissions) in association with elevated NO<sub>2</sub><sup>-</sup> concentration levels. Seasonally impaired NO<sub>2</sub><sup>-</sup> oxidation in WWTPs, leading to NO<sub>2</sub><sup>-</sup> accumulation, has been linked to low abundances of nitrite oxidizing bacteria (NOB) and drastic changes in the whole activated sludge microbial community (Gruber et al. 2021). However, the NOB loss observed by Gruber et al. (2021) at the Uster WWTP led to NO<sub>2</sub><sup>-</sup> accumulation over a periods of 1-2 months and it is unclear whether similar process were also responsible for the accumulation of nitrite over a few weeks at the Hofen WWTP.

## 7.4 Conclusions

- Measurements of relative  $^{15}\text{N}$  and  $^{18}\text{O}$  abundances in nitrogen-bearing molecules were successfully applied to characterize dynamics of  $\text{N}_2\text{O}$  formation pathways under normal operation in a full-scale activated sludge WWTP.  $\text{N}_2\text{O}$  was mainly produced by heterotrophic denitrification, while nitrifier denitrification appeared to be of lower and variable influence, and  $\text{NH}_2\text{OH}$  oxidation was negligible.
- Seasonal emission peaks occurred during winter when  $\text{NO}_2^-$  accumulates, and so the whole biological treatment is operated with full aeration, but NOB activity still appears impaired.
- $\text{N}_2\text{O}$  reduction was identified under anoxic conditions, and to lesser extent also under oxic conditions, when it is restricted to anoxic micro-niches. Fractional  $\text{N}_2\text{O}$  reduction was most pronounced under organic-substrate-replete conditions, while  $\text{N}_2\text{O}$  accumulation in the anoxic zone was primarily observed when organic substrate was limiting. Hence, the daily variation of organic substrate has a strong impact on the reduction of  $\text{N}_2\text{O}$ , and in turn, diurnal  $\text{N}_2\text{O}$  emission fluctuations.
- The dosage of reject-water and full aeration of the biological treatment significantly increased  $\text{N}_2\text{O}$  emissions, since  $\text{N}_2\text{O}$  reduction was strongly impeded. Hence, an efficient mitigation strategy is to optimize  $\text{N}_2\text{O}$  reduction by shifting reject-water dosage to periods with high organic substrate availabilities, as well as by avoiding full aeration of the biological treatment.
- Coupling isotopic technologies with continuous long-term monitoring of  $\text{N}_2\text{O}$  emissions is a powerful tool for qualitative  $\text{N}_2\text{O}$  pathway identification and the development of  $\text{N}_2\text{O}$  mitigation strategies in full-scale WWTPs. However, clearly defined conditions in a reactor system are required to interpret the data.

## 7.5 References

- Buchwald, C. and Casciotti, K.L. (2013) Isotopic ratios of nitrite as tracers of the sources and age of oceanic nitrite. *Nature Geoscience* 6(4), 308-313.
- Buchwald, C., Santoro, A.E., McIlvin, M.R. and Casciotti, K.L. (2012) Oxygen isotopic composition of nitrate and nitrite produced by nitrifying cocultures and natural marine assemblages. *Limnology and Oceanography* 57(5), 1361-1375.
- Casciotti, K.L., Böhlke, J.K., McIlvin, M.R., Mroczkowski, S.J. and Hannon, J.E. (2007) Oxygen Isotopes in Nitrite: Analysis, Calibration, and Equilibration. *Analytical Chemistry* 79(6), 2427-2436.
- Casciotti, K.L., McIlvin, M. and Buchwald, C. (2010) Oxygen isotopic exchange and fractionation during bacterial ammonia oxidation. *Limnology and Oceanography* 55(2), 753-762.
- Casciotti, K.L., Sigman, D.M., Hastings, M.G., Böhlke, J.K. and Hilkert, A. (2002) Measurement of the Oxygen Isotopic Composition of Nitrate in Seawater and Freshwater Using the Denitrifier Method. *Analytical Chemistry* 74(19), 4905-4912.
- Conthe, M., Lycus, P., Arntzen, M.O., Ramos da Silva, A., Frostegard, A., Bakken, L.R., Kleerebezem, R. and van Loosdrecht, M.C.M. (2018) Denitrification as an N<sub>2</sub>O sink. *Water Research* 151, 381-387.
- Coplen, T.B. (1988) Normalization of oxygen and hydrogen isotope data. *Chemical Geology: Isotope Geoscience section* 72(4), 293-297.
- Daigger, G.T., Adams, C.D. and Steller, H.K. (2007) Diffusion of oxygen through activated sludge flocs: experimental measurement, modeling, and implications for simultaneous nitrification and denitrification. *Water Environment Research* 79(4), 375-387.
- Denk, T.R.A., Mohn, J., Decock, C., Lewicka-Szczebak, D., Harris, E., Butterbach-Bahl, K., Kiese, R. and Wolf, B. (2017) The nitrogen cycle: A review of isotope effects and isotope modeling approaches. *Soil Biology and Biochemistry* 105, 121-137.
- Domingo-Felez, C., Pellicer-Nacher, C., Petersen, M.S., Jensen, M.M., Plosz, B.G. and Smets, B.F. (2016) Heterotrophs are key contributors to nitrous oxide production in activated sludge under low C-to-N ratios during nitrification-Batch experiments and modeling. *Biotechnology and Bioengineering* 114(1), 132-140.
- Domingo-Félez, C. and Smets, B.F. (2020) Modelling N<sub>2</sub>O dynamics of activated sludge biomass: Uncertainty analysis and pathway contributions. *Chemical Engineering Journal* 379.
- Duan, H., Zhao, Y., Koch, K., Wells, G.F., Zheng, M., Yuan, Z. and Ye, L. (2021) Insights into Nitrous Oxide Mitigation Strategies in Wastewater Treatment and Challenges for Wider Implementation. *Environmental Science & Technology*.



- Frame, C.H. and Casciotti, K.L. (2010) Biogeochemical controls and isotopic signatures of nitrous oxide production by a marine ammonia-oxidizing bacterium. *Biogeosciences* 7(9), 2695-2709.
- Gehre, M., Geilmann, H., Richter, J., Werner, R.A. and Brand, W.A. (2004) Continuous flow  $2\text{H}/1\text{H}$  and  $18\text{O}/16\text{O}$  analysis of water samples with dual inlet precision. *Rapid Communications in Mass Spectrometry* 18(22), 2650-2660.
- Granger, J., Boshers, D.S., Böhlke, J.K., Yu, D., Chen, N. and Tobias, C.R. (2020) The influence of sample matrix on the accuracy of nitrite N and O isotope ratio analyses with the azide method. *Rapid Communications in Mass Spectrometry* 34(1), e8569.
- Gruber, W., Niederdorfer, R., Ringwald, J., Morgenroth, E., Bürgmann, H. and Joss, A. (2021) Linking seasonal  $\text{N}_2\text{O}$  emissions and nitrification failures to microbial dynamics in a SBR wastewater treatment plant. *Water Research X* 11, 100098.
- Gruber, W., Villez, K., Kipf, M., Wunderlin, P., Siegrist, H., Vogt, L. and Joss, A. (2020)  $\text{N}_2\text{O}$  emission in full-scale wastewater treatment: Proposing a refined monitoring strategy. *Science of The Total Environment* 699, 134157.
- Harris, S.J., Liisberg, J., Xia, L., Wei, J., Zeyer, K., Yu, L., Barthel, M., Wolf, B., Kelly, B.F.J., Cendón, D.I., Blunier, T., Six, J. and Mohn, J. (2020)  $\text{N}_2\text{O}$  isotopocule measurements using laser spectroscopy: analyzer characterization and intercomparison. *Atmospheric Measurement Techniques* 13(5), 2797-2831.
- Henze, M., Gujer, W., Takahashi, M. and Van Loosdrecht, M.C. (2000) Activated sludge models ASM1, ASM2, ASM2d and ASM3, IWA, London.
- Hu, Z., Zhang, J., Xie, H., Liang, S. and Li, S. (2013) Minimization of nitrous oxide emission from anoxic–oxic biological nitrogen removal process: Effect of influent  $\text{COD}/\text{NH}_4^+$  ratio and feeding strategy. *Journal of Bioscience and Bioengineering* 115(3), 272-278.
- Ibraim, E., Harris, E., Eyer, S., Tuzson, B., Emmenegger, L., Six, J. and Mohn, J. (2018) Development of a field-deployable method for simultaneous, real-time measurements of the four most abundant  $\text{N}_2\text{O}$  isotopocules. *Isotopes in Environmental and Health Studies* 54(1), 1-15.
- IPCC (2013) *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*, Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
- Jinuntuya-Nortman, M., Sutka, R.L., Ostrom, P.H., Gandhi, H. and Ostrom, N.E. (2008) Isotopologue fractionation during microbial reduction of  $\text{N}_2\text{O}$  within soil mesocosms as a function of water-filled pore space. *Soil Biology and Biochemistry* 40(9), 2273-2280.
- Kool, D.M., Wrage, N., Oenema, O., Dolfing, J. and Van Groenigen, J.W. (2007) Oxygen exchange between (de)nitrification intermediates and  $\text{H}_2\text{O}$  and its implications for source determination of  $\text{NO}$  and  $\text{N}_2\text{O}$ : a review. *Rapid Communications in Mass Spectrometry* 21(22), 3569-3578.

- Kozłowski, J.A., Kits, K.D. and Stein, L.Y. (2016) Comparison of Nitrogen Oxide Metabolism among Diverse Ammonia-Oxidizing Bacteria. *Frontiers in Microbiology* 7, 1090.
- Kritee, K., Sigman, D.M., Granger, J., Ward, B.B., Jayakumar, A. and Deutsch, C. (2012) Reduced isotope fractionation by denitrification under conditions relevant to the ocean. *Geochimica et Cosmochimica Acta* 92, 243-259.
- Lewicka-Szczebak, D., Augustin, J., Giesemann, A. and Well, R. (2017) Quantifying N<sub>2</sub>O reduction to N<sub>2</sub> based on N<sub>2</sub>O isotopocules – validation with independent methods (helium incubation and <sup>15</sup>N gas flux method). *Biogeosciences* 14(3), 711-732.
- Lewicka-Szczebak, D., Dyckmans, J., Kaiser, J., Marca, A., Augustin, J. and Well, R. (2016) Oxygen isotope fractionation during N<sub>2</sub>O production by soil denitrification. *Biogeosciences* 13(4), 1129-1144.
- Lewicka-Szczebak, D., Well, R., Köster, J.R., Fuß, R., Senbayram, M., Dittert, K. and Flessa, H. (2014) Experimental determinations of isotopic fractionation factors associated with N<sub>2</sub>O production and reduction during denitrification in soils. *Geochimica et Cosmochimica Acta* 134, 55-73.
- Magyar, P.M., Hausherr, D., Niederdorfer, R., Stöcklin, N., Wei, J., Mohn, J., Bürgmann, H., Joss, A. and Lehmann, M.F. (2021) Nitrogen isotope effects can be used to diagnose N transformations in wastewater anammox systems. *Scientific Reports* 11(1).
- Mampaey, K.E., De Kreuk, M.K., van Dongen, U.G., van Loosdrecht, M.C. and Volcke, E.I. (2016) Identifying N<sub>2</sub>O formation and emissions from a full-scale partial nitrification reactor. *Water Research* 88, 575-585.
- Mariotti, A., Germon, J.C., Hubert, P., Kaiser, P., Letolle, R., Tardieux, A. and Tardieux, P. (1981) Experimental determination of nitrogen kinetic isotope fractionation: Some principles; illustration for the denitrification and nitrification processes. *Plant and Soil* 62(3), 413-430.
- Martin, T.S. and Casciotti, K.L. (2016) Nitrogen and oxygen isotopic fractionation during microbial nitrite reduction. *Limnology and Oceanography* 61(3), 1134-1143.
- McIlvin, M.R. and Altabet, M.A. (2005) Chemical Conversion of Nitrate and Nitrite to Nitrous Oxide for Nitrogen and Oxygen Isotopic Analysis in Freshwater and Seawater. *Analytical Chemistry* 77(17), 5589-5595.
- Meyer, S.S. and Wilderer, P.A. (2004) Reject Water: Treating of Process Water in Large Wastewater Treatment Plants in Germany—A Case Study. *Journal of Environmental Science and Health, Part A* 39(7), 1645-1654.
- Mohn, J., Gutjahr, W., Toyoda, S., Harris, E., Ibraim, E., Geilmann, H., Schleppi, P., Kuhn, T., Lehmann, M.F., Decock, C., Werner, R.A., Yoshida, N. and Brand, W.A. (2016) Reassessment of the NH<sub>4</sub>NO<sub>3</sub> thermal decomposition technique for calibration of the N<sub>2</sub>O isotopic composition. *Rapid Communications in Mass Spectrometry* 30(23), 2487-2496.

- Nikaido, H. (2003) Molecular basis of bacterial outer membrane permeability revisited. *Microbiology and Molecular Biology Reviews* 67(4), 593-656.
- Orschler, L., Agrawal, S. and Lackner, S. (2021) Targeted metagenomics reveals extensive diversity of the denitrifying community in partial nitrification anammox and activated sludge systems. *Biotechnol Bioeng* 118(1), 433-441.
- Ostrom, N.E. and Ostrom, P.H. (2017) Mining the isotopic complexity of nitrous oxide: a review of challenges and opportunities. *Biogeochemistry* 132(3), 359-372.
- Pan, Y., Ni, B.J., Bond, P.L., Ye, L. and Yuan, Z. (2013) Electron competition among nitrogen oxides reduction during methanol-utilizing denitrification in wastewater treatment. *Water Research* 47(10), 3273-3281.
- Peng, L., Carvajal-Arroyo, J.M., Seuntjens, D., Prat, D., Colica, G., Pintucci, C. and Vlaeminck, S.E. (2017) Smart operation of nitrification/denitrification virtually abolishes nitrous oxide emission during treatment of co-digested pig slurry centrate. *Water Research* 127, 1-10.
- Ravishankara, A.R., Daniel, J.S. and Portmann, R.W. (2009) Nitrous Oxide (N<sub>2</sub>O): The Dominant Ozone-Depleting Substance Emitted in the 21st Century. *Science* 326(5949), 123-125.
- Ren, Y., Ngo, H.H., Guo, W., Ni, B.-J. and Liu, Y. (2019) Linking the nitrous oxide production and mitigation with the microbial community in wastewater treatment: A review. *Bioresource Technology Reports* 7.
- Rodriguez-Caballero, A., Aymerich, I., Marques, R., Poch, M. and Pijuan, M. (2015) Minimizing N<sub>2</sub>O emissions and carbon footprint on a full-scale activated sludge sequencing batch reactor. *Water Research* 71, 1-10.
- Rohe, L., Well, R. and Lewicka-Szczebak, D. (2017) Use of oxygen isotopes to differentiate between nitrous oxide produced by fungi or bacteria during denitrification. *Rapid Communications in Mass Spectrometry* 31(16), 1297-1312.
- Schreiber, F., Wunderlin, P., Udert, K.M. and Wells, G.F. (2012) Nitric oxide and nitrous oxide turnover in natural and engineered microbial communities: biological pathways, chemical reactions, and novel technologies. *Frontiers in Microbiology* 3, 372.
- Sexstone, A.J., Revsbech, N.P., Parkin, T.B. and Tiedje, J.M. (1985) Direct Measurement of Oxygen Profiles and Denitrification Rates in Soil Aggregates. *Soil Science Society of America Journal* 49(3), 645-651.
- Sigman, D.M., Casciotti, K.L., Andreani, M., Barford, C., Galanter, M. and Böhlke, J.K. (2001) A Bacterial Method for the Nitrogen Isotopic Analysis of Nitrate in Seawater and Freshwater. *Analytical Chemistry* 73(17), 4145-4153.
- Sun, S., Bao, Z., Li, R., Sun, D., Geng, H., Huang, X., Lin, J., Zhang, P., Ma, R., Fang, L., Zhang, X. and Zhao, X. (2017) Reduction and prediction of N<sub>2</sub>O emission from an Anoxic/Oxic wastewater treatment plant upon DO control and model simulation. *Bioresource Technology* 244(Pt 1), 800-809.

- Sutka, R.L., Ostrom, N.E., Ostrom, P.H., Breznak, J.A., Gandhi, H., Pitt, A.J. and Li, F. (2006) Distinguishing nitrous oxide production from nitrification and denitrification on the basis of isotopomer abundances. *Applied Environmental Microbiology* 72(1), 638-644.
- Tian, H., Yang, J., Lu, C., Xu, R., Canadell, J.G., Jackson, R.B., Arneeth, A., Chang, J., Chen, G., Ciais, P., Gerber, S., Ito, A., Huang, Y., Joos, F., Lienert, S., Messina, P., Olin, S., Pan, S., Peng, C., Saikawa, E., Thompson, R.L., Vuichard, N., Winiwarter, W., Zaehle, S., Zhang, B., Zhang, K. and Zhu, Q. (2018) The Global N<sub>2</sub>O Model Intercomparison Project. *Bulletin of the American Meteorological Society* 99(6), 1231-1251.
- Toyoda, S., Suzuki, Y., Hattori, S., Yamada, K., Fujii, A., Yoshida, N., Kouno, R., Murayama, K. and Shiomi, H. (2011) Isotopomer analysis of production and consumption mechanisms of N<sub>2</sub>O and CH<sub>4</sub> in an advanced wastewater treatment system. *Environmental Science & Technology* 45(3), 917-922.
- Toyoda, S. and Yoshida, N. (1999) Determination of Nitrogen Isotopomers of Nitrous Oxide on a Modified Isotope Ratio Mass Spectrometer. *Analytical Chemistry* 71(20), 4711-4718.
- Toyoda, S., Yoshida, N. and Koba, K. (2017) Isotopocule analysis of biologically produced nitrous oxide in various environments. *Mass Spectrometry Reviews* 36(2), 135-160.
- Tumendelger, A., Toyoda, S., Yoshida, N., Shiomi, H. and Kouno, R. (2016) Isotopocule characterization of N<sub>2</sub>O dynamics during simulated wastewater treatment under oxic and anoxic conditions. *Geochemical Journal* 50(2), 105-121.
- Vasilaki, V., Massara, T.M., Stanchev, P., Fatone, F. and Katsou, E. (2019) A decade of nitrous oxide (N<sub>2</sub>O) monitoring in full-scale wastewater treatment processes: A critical review. *Water Research* 161, 392-412.
- Wei, W., Isobe, K., Nishizawa, T., Zhu, L., Shiratori, Y., Ohte, N., Koba, K., Otsuka, S. and Senoo, K. (2015) Higher diversity and abundance of denitrifying microorganisms in environments than considered previously. *ISME Journal* 9(9), 1954-1965.
- Werner, R. (2003) The Online 18O/ 16O Analysis: Development and application. *Isotopes in Environmental and Health Studies* 39(2), 85-104.
- Werner, R.A. and Brand, W.A. (2001) Referencing strategies and techniques in stable isotope ratio analysis. *Rapid Communications in Mass Spectrometry* 15(7), 501-519.
- Wu, L., Ning, D., Zhang, B., Li, Y., Zhang, P., Shan, X., Zhang, Q., Brown, M.R., Li, Z., Van Nostrand, J.D., Ling, F., Xiao, N., Zhang, Y., Vierheilig, J., Wells, G.F., Yang, Y., Deng, Y., Tu, Q., Wang, A., Global Water Microbiome, C., Zhang, T., He, Z., Keller, J., Nielsen, P.H., Alvarez, P.J.J., Criddle, C.S., Wagner, M., Tiedje, J.M., He, Q., Curtis, T.P., Stahl, D.A., Alvarez-Cohen, L., Rittmann, B.E., Wen, X. and Zhou, J. (2019) Global diversity and biogeography of bacterial communities in wastewater treatment plants. *Nature Microbiology* 4(7), 1183-1195.
- Wunderlin, P., Lehmann, M.F., Siegrist, H., Tuzson, B., Joss, A., Emmenegger, L. and Mohn, J. (2013) Isotope signatures of N<sub>2</sub>O in a mixed microbial population system:

- constraints on N<sub>2</sub>O producing pathways in wastewater treatment. *Environmental Science & Technology* 47(3), 1339-1348.
- Wunderlin, P., Mohn, J., Joss, A., Emmenegger, L. and Siegrist, H. (2012) Mechanisms of N<sub>2</sub>O production in biological wastewater treatment under nitrifying and denitrifying conditions. *Water Research* 46(4), 1027-1037.
- Yoshida, N. and Toyoda, S. (2000) Constraining the atmospheric N<sub>2</sub>O budget from intramolecular site preference in N<sub>2</sub>O isotopomers. *Nature* 405(6784), 330-334.
- Yu, L., Harris, E., Lewicka-Szczebak, D., Barthel, M., Blomberg, M.R.A., Harris, S.J., Johnson, M.S., Lehmann, M.F., Liisberg, J., Muller, C., Ostrom, N.E., Six, J., Toyoda, S., Yoshida, N. and Mohn, J. (2020) What can we learn from N<sub>2</sub>O isotope data? - Analytics, processes and modelling. *Rapid Communications in Mass Spectrometry* 34(20), e8858.
- Zhang, L., Altabet, M.A., Wu, T. and Hadas, O. (2007) Sensitive Measurement of NH<sub>4</sub><sup>+</sup> <sup>15</sup>N/<sup>14</sup>N (δ<sup>15</sup>NH<sub>4</sub><sup>+</sup>) at Natural Abundance Levels in Fresh and Saltwaters. *Analytical Chemistry* 79(14), 5297-5303.
- Zumft, W.G. (1997) Cell biology and molecular basis of denitrification. *Microbiology and Molecular Biology Reviews* 61(4), 533-616.

# **Chapter 8**

## **Estimation and mitigation of greenhouse gas emissions from WWTP – a technical perspective**

Thesis chapter

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## Abstract

Wastewater treatment plants (WWTPs) are relevant point sources of greenhouse gas emissions. While  $\text{N}_2\text{O}$  emissions are produced during biological nitrogen removal,  $\text{CH}_4$  emissions originate from anaerobic process in wastewater treatment, such as anaerobic digestion of sludge. Although substantial amounts of  $\text{N}_2\text{O}$  and  $\text{CH}_4$  can be produced and emitted from processes in WWTPs, electricity consumption is still the most often optimized variable to reduce greenhouse gas emission. Here we show that electricity is causes less than 20% of the total emissions and optimization of electricity consumption has a reduction potential of less than 10% for an average WWTP with full nitrogen removal and an average European electricity mix. The most efficient strategy, yielding emission reductions of around 40%, is the treatment of collected off-gas from side-stream processes, such as reject water treatment ( $\text{N}_2\text{O}$ ), sludge treatment ( $\text{CH}_4$ ), and sludge incineration ( $\text{N}_2\text{O}$ ). Reduction of  $\text{N}_2\text{O}$  emissions from biological treatment has the highest impact, but is still difficult given the limited understanding of  $\text{N}_2\text{O}$  production processes. Our results demonstrate that electricity should only be optimized, if other greenhouse gas emissions are not increased. Additionally, monitoring or estimating emissions on a particular WWTP is crucial to define reduction potentials, given the high variabilities for emission factors reported.

## 8.1 Introduction

Atmospheric concentrations of important greenhouse gases (GHG), such as CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O, have increased substantially due to anthropogenic activities since the beginning of the industrial revolution (Petit et al. 1999). A wide range of different sources and activities leading to emissions of greenhouse gases have been identified (Meinshausen et al. 2011). To compare the different gases and the importance of emission sources, greenhouse gas potentials have been calculated based on the residence times in the atmosphere and the radiative forcing caused (Prather et al. 2012). GHG inventories show that CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O are globally the most important GHG in terms of caused radiative forcing with respective contributions of 64%, 17% and 6% (IPCC 2013).

Wastewater treatment plants (WWTP) are important point sources of GHG emissions (Daelman et al. 2013b). Emissions can occur over the whole treatment process and mainly concern the gases CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O (Parravicini et al. 2016). While CO<sub>2</sub> emissions result from energy consumption and are therefore considered as indirect emission, CH<sub>4</sub> and N<sub>2</sub>O are produced during wastewater treatment and are direct emissions from WWTPs (Remy et al. 2013). N<sub>2</sub>O originates from i) biological nitrogen removal (BNR) and ii) thermal oxidation of nitrogen compounds during sludge incineration (Marias et al. 2015, Ren et al. 2019). In the IPCC methodology, production pathway ii) is not attributed to wastewater treatment but to the energy sector, since energy is extracted of the process and biomass burning is listed separately in GHG inventories (IPCC 2006). CH<sub>4</sub> is produced during anaerobic processes in wastewater treatment, as present in the sewer system (unintended by the operators) and during anaerobic digestion (intended by the operators). Emissions are either caused by direct emissions from the sewer, stripping of dissolved methane originating from the sewer system in the water line of a WWTP, or by leakage in the anaerobic digestion of the sewage sludge (Daelman et al. 2012). Direct emissions of CO<sub>2</sub> in the biological treatment by oxidation of organic compound are considered as biogenic and therefore not reported in GHG inventories according to the IPCC guidelines (IPCC 2006).



GHG emissions from WWTP have been monitored in numerous studies and high variability are typically found (Delre et al. 2017, Vasilaki et al. 2019). Monitoring duration and methods should be selected in dependence of the process monitored and the variability expected (Daelman et al. 2013a).  $\text{N}_2\text{O}$  emissions from the biological treatment typically exhibit a high variation in the yearly emissions pattern, requiring at least year-long monitoring studies (Gruber et al. 2020). While  $\text{CH}_4$  emissions are to some extent seasonal, the energy demand is quite stable over a year (Daelman et al. 2013b). Despite the good availability of monitoring studies, a compilation of the different GHG emissions and their sources in WWTP is missing.

Energy consumption is a frequent focus in the optimization of the carbon footprint in WWTPs (Clos et al. 2020). However, an integral approach considering also direct emissions of  $\text{N}_2\text{O}$  and  $\text{CH}_4$  is required to efficiently reduce GHG from WWTP, due to potentially high impact of direct emissions on the carbon footprint of a WWTP (Kosonen et al. 2016). Mitigation strategies can cover an extensive set of solutions, ranging from process optimization to catalytic destruction of produced GHG (Duan et al. 2020, Jabłońska et al. 2019). Hence, a guideline to prioritize mitigation options covering quantitative importance of all relevant emission sources and their technical feasibility would be valuable.

The objectives of this chapter are to i) summarize the processes causing GHG emissions in WWTP, ii) present most recent emission factors (EF) assessed from full-scale monitoring campaigns for different process stages and benchmarking studies, and iii) propose mitigation strategies. For data collection of energy consumption, we rely on energy benchmarking studies (Clos et al. 2020).  $\text{N}_2\text{O}$  emissions factors are assessed according to a literature review (Vasilaki et al. 2019) and results from own extensive long-term monitoring data-set (Chapter 5). For the assessment of  $\text{CH}_4$  emissions, we conducted a short literature review. We focus this chapter on an activated sludge systems with a Modified Ludzak-Ettinger (MLE) process, given the good data availability for this type of WWTP. However, the emission data can be partly transferred to other processes and WWTP. Finally, we propose mitigation strategies.

## 8.2 Materials and Methods

### 8.2.1 Model WWTP system

The model WWTP (Figure 8.1) has a size of 100'000 PE. Physical pre-treatment includes pumping, screening, sand removal, fat removal and primary clarification. The biological treatment consists of a conventional activated sludge process with pre-denitrification followed by secondary clarification. Sludge treatment consists of an anaerobic digester and an open sludge storage tank. Digested sludge is dewatered and incinerated on the WWTP. Reject water is treated in a partial nitrification-anammox system.

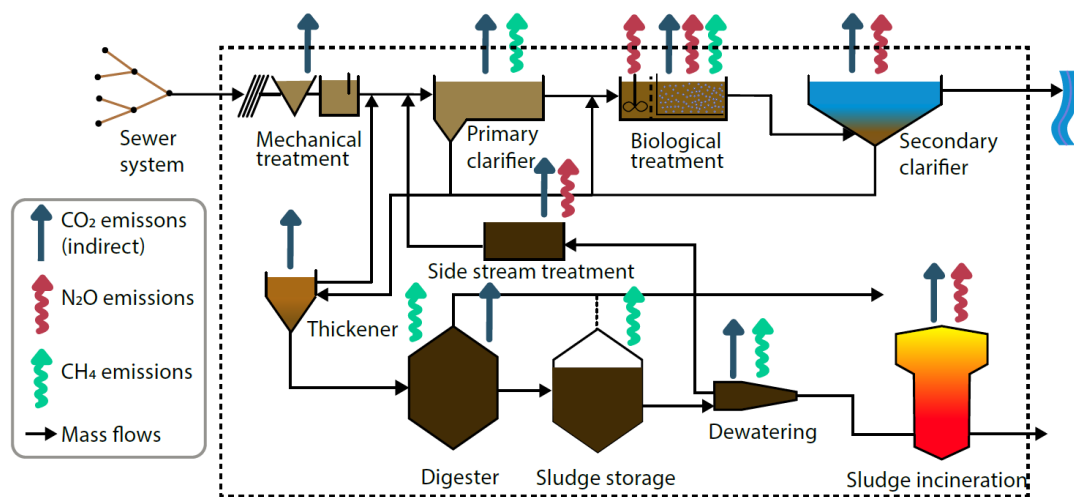


Figure 8.1: Schematic of the elements of the model wastewater treatment plant (WWTP) including sludge incineration and its major greenhouse gas sources. The dotted line represents the boundary of the system.

### 8.2.2 WWTP performance and inflow composition

The influent composition and plant performance were defined according to typical values in (Gujer 2007). Calculated mass flows can be found in the Table E.1 in the SI. Energy demand was assumed according to a Swiss energy benchmark study for WWTP, where a WWTP of the same size (100,000 PE) was chosen as an example (Clos et al. 2020, Müller 2010). Energy consumption of reject water treatment and sludge incineration was estimated according to (Houillon and Jolliet 2005, Lackner et al. 2014). Performance data and GHG emissions are evaluated per population equivalent and per year. Influent indices and performance indicators relevant for the

estimation of the GHG emissions are summarized in Table 8.1. A detailed overview of the assumed mass flows can be found in Table E.2 (SI).

Table 8.1: Key values for inflow composition and WWTP performance assumed for the model plant.

Variable	Numeric value	Unit
COD in inflow	44	kgCOD/PE/a
COD primary sludge	11	kgCOD/PE/a
COD secondary sludge	20	kgCOD/PE/a
COD oxidized in biological treatment	9	kgCOD/PE/a
N in inflow	4.3	kgN/PE/a
N denitrified	1.1	kgN/PE/a
N reject water	0.5	kgN/PE/a
TS in sludge to incineration	35	kgTS/PE/a
Electricity consumption: pre-treatment	2.9	kWh/PE/a
Electricity consumption: biological treatment	17.2	kWh/PE/a
Electricity consumption: sludge treatment	4.2	kWh/PE/a
Electricity consumption: reject water treatment	1	kWh/PE/a
Electricity consumption: sludge incineration	11.7	kWh/PE/a

### 8.2.3 Review of CH<sub>4</sub> emissions in WWTPs

We considered 14 studies with a total of 26 investigated WWTPs to calculate  $EF_{CH_4, \text{sludge\_treat}}$  and  $EF_{CH_4, \text{bio}}$  (Table E.2 (SI)). Different monitoring methods have been applied in the studies analyzed: In two studies, emissions were determined using a tracer gas method (Delre et al. 2017, Samuelsson et al. 2018). Three studies continuously measured emissions in the off-gas of the WWTP with a CH<sub>4</sub> analyzer (Daelman et al. 2012, Ribera-Guardia et al. 2019, Rodriguez-Caballero et al. 2014b) and in eight studies grab-samples were analyzed with gas chromatography, e.g. (Bao et al. 2016, Ren et al. 2013).

While six studies measured CH<sub>4</sub> emissions only during wastewater treatment, eight studies provided values for total emissions of the entire WWTP (including sludge treatment). Two of the eight studies measured the emissions for the total WWTP (Daelman et al. 2013b, Delre et al. 2017). The other six studies provided data on the wastewater treatment, sewage sludge treatment and estimated the total emissions of

the WWTPs. Only two of the studies, providing measurements or estimation for the whole WWTP, were conducted on WWTPs with an anaerobic digester (Daelman et al. 2012, Samuelsson et al. 2018).

#### 8.2.4 GHG emission calculations and emission factors

GHG potentials for N<sub>2</sub>O and CH<sub>4</sub>, 265 gCO<sub>2</sub>-e/gN<sub>2</sub>O and 28 gCO<sub>2</sub>-e/gCH<sub>4</sub>, were defined for a period of 100 years according to IPCC assessment report 5 (IPCC 2014). Carbon footprint of the electricity demand was set to the average value of European Union countries (312 gCO<sub>2</sub>-e/kWh). We considered the following process for greenhouse gas emissions (Figure 8.1) and estimated the emissions (EM) according to the following equations:

*Indirect CO<sub>2</sub> emissions caused by the energy demand in mechanical pretreatment, biological treatment, side-stream treatment (Eq. (8.1))*

$$EM_{CO_2,elec} = EF_{elec} * E_{elec} \quad (8.1)$$

Where EM<sub>CO<sub>2</sub>,elec</sub> are the GHG emissions caused by electricity consumption (gCO<sub>2</sub>-e/PE/y), EF<sub>elec</sub> is the emission factor of the electricity consumed (gCO<sub>2</sub>-e/kWh), and E<sub>elec</sub> is the electricity consumed (kWh/PE/y).

*N<sub>2</sub>O emissions from biological treatment (Eq. (8.2))*

$$EM_{N_2O,bio} = EF_{N_2O,bio} * N_{Load,in} * GHG_{N_2O} * \frac{44}{28} \quad (8.2)$$

Where EM<sub>N<sub>2</sub>O,bio</sub> are the yearly N<sub>2</sub>O emissions from biological treatment (gCO<sub>2</sub>-e/PE/y), EF<sub>N<sub>2</sub>O,bio</sub> is the EF for the biological treatment (kgN<sub>2</sub>O-N/kgN), N<sub>Load,in</sub> is the nitrogen load in the inflow of the WWTP (kgN/PE/y), and GHG<sub>N<sub>2</sub>O</sub> is the GHG potential of N<sub>2</sub>O (gCO<sub>2</sub>-e/gN<sub>2</sub>O).

*N<sub>2</sub>O emissions from secondary clarifier (Eq. (8.3))*

$$EM_{N_2O,secondary} = EF_{N_2O,secondary} * N_{Load,in} * GHG_{N_2O} * \frac{44}{28} \quad (8.3)$$

Where  $EM_{N_2O,secondary}$  are the yearly  $N_2O$  emissions from the secondary clarifier (gCO<sub>2</sub>-e/PE/y),  $EF_{N_2O,secondary}$  is the EF for the secondary clarifier (kgN<sub>2</sub>O-N/kgN),  $N_{Load,in}$  is the nitrogen load in the inflow of the WWTP (kgN/y), and  $GHG_{N_2O}$  is the GHG potential of  $N_2O$ .

*N<sub>2</sub>O emissions from side-stream treatment (Eq. (8.4))*

$$EM_{N_2O,side\_stream} = EF_{N_2O,side\_stream} * N_{Load,reject} * GHG_{N_2O} * \frac{44}{28} \quad (8.4)$$

Where  $EM_{N_2O,side\_stream}$  are the yearly  $N_2O$  emissions from the side-stream treatment (gCO<sub>2</sub>-e/PE/y),  $EF_{N_2O,side\_stream}$  is the EF for the side-stream treatment (kgN<sub>2</sub>O-N/kgN),  $N_{Load,in}$  is the nitrogen load in the inflow of the WWTP (kgN/y), and  $GHG_{N_2O}$  is the GHG potential of  $N_2O$ .

*N<sub>2</sub>O emissions from sludge incineration (Eq. (8.5))*

$$EM_{N_2O,sludge\_inc} = EF_{N_2O,sludge\_inc} * TS_{Dig\_sludge} * GHG_{N_2O} * \frac{44}{28} \quad (8.5)$$

Where  $EM_{N_2O,sludge\_inc}$  are the yearly  $N_2O$  emissions from the sludge incineration (gCO<sub>2</sub>-e/PE/y),  $EF_{N_2O,sludge\_inc}$  is the EF for the sludge incineration (kgN<sub>2</sub>O-N/kgTS),  $TS_{Dig\_sludge}$  is the amount of dry substance in the digested sludge (kgTS/y), and  $GHG_{N_2O}$  is the GHG potential of  $N_2O$ .

*Methane emissions from biological treatment (Eq. (8.6))*

$$EM_{CH_4,bio} = EF_{CH_4,bio} * COD_{Load,in} * GHG_{CH_4} \quad (8.6)$$

Where  $EM_{CH_4,bio}$  are the yearly  $CH_4$  emissions from the biological treatment ( $gCO_2$ -e/PE/y),  $EF_{CH_4,bio}$  is the EF for the biological treatment ( $kgCH_4$ -N/kgCOD),  $COD_{Load,in}$  is the COD in the inflow of the WWTP ( $kgO_2$ /y), and  $GHG_{CH_4}$  is the GHG potential of  $CH_4$

*Methane emissions from sludge treatment and sludge storage (Eq. (8.7))*

$$EM_{CH_4,sludge\_treat} = EF_{CH_4,sludge\_treatment} * COD_{Load,in} * GHG_{CH_4} \quad (8.7)$$

Where  $EM_{CH_4,sludge\_treat}$  are the yearly  $CH_4$  emissions from the sludge treatment ( $gCO_2$ -e/PE/y),  $EF_{CH_4,sludge\_treat}$  is the EF for the sludge treatment ( $kgCH_4$ -N/kgCOD),  $COD_{Load,in}$  is the COD in the inflow of the WWTP ( $kgO_2$ /y), and  $GHG_{CH_4}$  is the GHG potential of  $CH_4$ .

EFs used in this chapter were estimated according to Table 8.2. Other potential emission sources, such as transportation of waste material and chemicals, construction, emissions outside the system boundaries were excluded. Electricity production in anaerobic digestion and carbon credits for energy gain of the WWTP were not considered in this chapter. However, heat autarky was assumed and heat consumption was not included in the GHG balance.

Table 8.2: Emission factors (EF) applied for the GHG balance.

EFs	Value	Unit	Source
EF <sub>elec</sub>	312	gCO <sub>2</sub> -e/kWh	(IEA 2020, IPCC 2014)
EF <sub>N<sub>2</sub>O,bio</sub>	0.009	kgN <sub>2</sub> O-N/kgN	(Chapter 4)
EF <sub>N<sub>2</sub>O,secondary</sub>	0.003	kgN <sub>2</sub> O-N/kgN	(Kosonen et al. 2016)
EF <sub>N<sub>2</sub>O,side_stream</sub>	0.025	kgN <sub>2</sub> O-N/kgN	(Vasilaki et al. 2019)
EF <sub>N<sub>2</sub>O,sludge_inc</sub>	0.038	kgN <sub>2</sub> O-N/kgN	(Soda et al. 2010, Wunderlin 2013)
EF <sub>CH<sub>4</sub>,bio</sub>	0.001	kgCH <sub>4</sub> /kgCOD	This chapter
EF <sub>CH<sub>4</sub>,sludge_treat</sub>	0.009	kgCH <sub>4</sub> /kgCOD	This chapter

### 8.2.5 Mitigation strategies

To show the effects of different carbon footprint optimization options on WWTP, we evaluated five scenarios and compared it to the base case (Table 8.3). We assume that all strategies can be retrofitted on the model WWTP without requiring significant changes in the plant scheme. Hence, we applied the same mass-flow model for all scenarios and changed only the emission factors.

Table 8.3: Assumptions and modified emission factors applied in GHG reduction scenarios.

Scenario	Assumption	Change of EF
Electricity consumption	Substantial Reduction of electricity consumption in all processes	Reduction of consumption by 40%.
Electricity mix	Electricity mix from renewable production (50% wind, 50% solar)	$EF_{elec} = 5 \text{ gCO}_2\text{-e/kWh}$
Off-gas treatment	Implementation of retrofit off-gas treatment on reject-water treatment, sludge treatment and incineration	$EF_{N_2O, side\_stream} = 0.001$ $EF_{N_2O, sludge\_inc} = 0.001$ $EF_{CH_4, sludge\_treat} = 0.001$
N <sub>2</sub> O reduction	Reduction of all N <sub>2</sub> O emissions by optimization (Sludge incineration, reject-water and biological treatment)	$EF_{N_2O, side\_stream} = 0.001$ $EF_{N_2O, sludge\_inc} = 0.001$ $EF_{N_2O, bio} = 0.001$
All measures	Combination of all previous scenarios	As described in the other scenarios



## 8.3 Results and discussion

### 8.3.1 N<sub>2</sub>O emission monitoring and EF

N<sub>2</sub>O emissions from biological treatment have been monitored in numerous studies (Vasilaki et al. 2019). However, continuous monitoring campaigns of at least one year are necessary to assess representative EFs (Daelman et al. 2013a). To date, fourteen continuous long-term monitoring campaigns have been reported, exhibiting a wide range of EFs (0.1-8% of the total nitrogen load; Chapter 5). The EFs have been shown to strongly correlate with the effluent nitrite of the biological treatment. WWTPs with a higher nitrogen removal rate yielded lower N<sub>2</sub>O emission. The average EF assessed in continuous, long-term monitoring campaigns for WWTP with full-nitrogen removal over the whole year is 0.9% (Chapter 4), which we applied in this chapter ( $EF_{N_2O,bio}$ ). N<sub>2</sub>O emissions from secondary clarifiers have been investigated in a number studies (Foley et al. 2010, Mikola et al. 2014). Up to 30% of the total N<sub>2</sub>O emissions could result from stripping in the secondary clarifiers. A strong correlation was found with the emissions from the biological treatment, indicating that the emitted N<sub>2</sub>O is produced in the biological treatment. In this chapter, we applied an  $EF_{N_2O,secondary}$  of 30% relative to the  $EF_{N_2O,bio}$ .

N<sub>2</sub>O EF from biological processes for side-stream treatment, such as partial nitrification-anammox and nitrification-denitrification, are on average higher as the EF in the main-stream biological treatment (Vasilaki et al. 2019). The variability of the emissions over a year is typically not as significant as in main-stream treatment, but exhibit strong variation over a day and between cycles (Vasilaki et al. 2020a). Hence, processes can be monitored over a shorter period of time (days to months) to assess representative EF. An average  $EF_{N_2O,side\_stream}$  of 2.1% was thus applied in this chapter (Vasilaki et al. 2019). Notably, EF reported in side-stream processes vary substantially. Values lower than 1% (Joss et al. 2009) and above 5% have been reported (Stenstrom et al. 2014). A key driver for increased N<sub>2</sub>O EF is nitrite accumulation, which may vary depending on the specific process applied (Schaubroeck et al. 2015).

Incineration of sewage sludge is a source of N<sub>2</sub>O emissions due to the high nitrogen content of the raw material (Svoboda et al. 2006). EF range from 1 to 6.4 gN<sub>2</sub>O/kg

dry sludge for fluidized bed incineration processes (Soda et al. 2010, Suzuki et al. 2003, Wunderlin 2013). Emissions are governed by the process temperature, i.e., higher temperatures generally decrease emissions (Suzuki et al. 2003). In this chapter, we applied 3 gN<sub>2</sub>O/kgTS as an EF<sub>N<sub>2</sub>O,sludge\_inc</sub>.

### **8.3.2 CH<sub>4</sub> emission monitoring and EF**

CH<sub>4</sub> is the product of the anaerobic digestion of organic matter. Emissions at WWTP occur due to unintentional stripping of CH<sub>4</sub> produced in the sewer system (Daelman et al. 2012). Further, emissions can occur during anaerobic sludge treatment, where CH<sub>4</sub> is intentionally produced in large quantities and energetically used for electricity and heat production. A small part of the produced CH<sub>4</sub> is emitted to the atmosphere due to leakages, release of residual dissolved CH<sub>4</sub> from the digestate handling during post treatment processes, and production of CH<sub>4</sub> during storage caused by the residual gas potential in the digested sludge (Tauber et al. 2019).

CH<sub>4</sub> emissions from WWTP have been monitored in multiple studies (). The studies reported a wide range of EF for wastewater treatment, from less than 10<sup>-4</sup> to 10<sup>-2</sup> kgCH<sub>4</sub>/kgCOD (Figure 8.2). In some studies, emissions were estimated for individual plant units within the wastewater treatment line, of which the highest emissions were attributed to the biological treatment in all these studies (Daelman et al. 2012, Liu et al. 2014, Ren et al. 2013, Yan et al. 2014). Therefore, in this chapter we applied an EF<sub>CH<sub>4</sub>,bio</sub> of 0.001 kgCH<sub>4</sub>/kgCOD (median) for the biological treatment. The emissions for sewage sludge treatment are substantially different for WWTPs without and with an anaerobic digestion. The two monitoring campaigns on WWTPs with anaerobic digestion, report higher CH<sub>4</sub> emissions from sludge treatment than from wastewater treatment (Daelman et al. 2012). Similarly, the results for whole-plant show that WWTP with anaerobic digestion have generally higher emissions than WWTP without anaerobic digestion. In summary, there is a general agreement that the sludge treatment was identified as the main CH<sub>4</sub> emissions source in a WWTP (Delre et al. 2017). The median of the EFs for total WWTP, for all plants with anaerobic digestion is approximately 0.01 kgCH<sub>4</sub>/kgCOD. Therefore, we applied an EF<sub>CH<sub>4</sub>,sludge\_treat</sub> of 0.009 kgCH<sub>4</sub>/kgCOD.

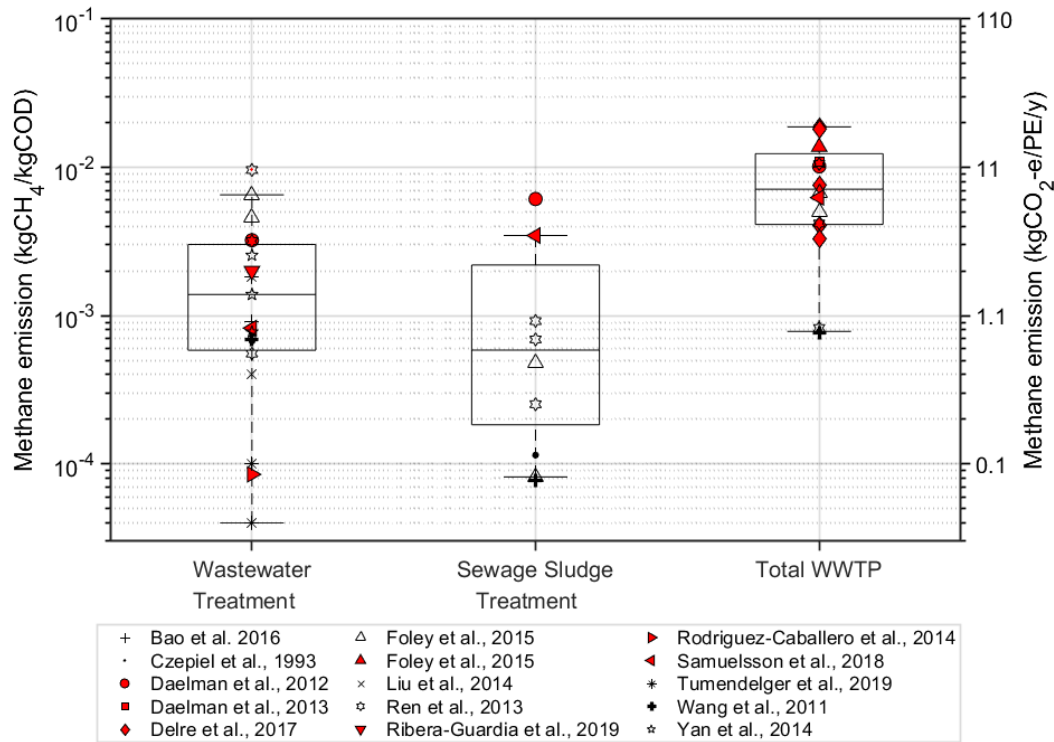


Figure 8.2: Normalized methane emissions for studies found in literature. The EFs on the right axis ( $\text{kg CO}_2\text{-eq/PE/y}$ ) are calculated assuming a COD load of 120 gCOD per inhabitant and day (Gujer 2007). Red data points denote WWTPs with an anaerobic digestion for sludge treatment.

### 8.3.3 Estimation of total GHG Emissions

The total emissions of the model WWTP result in 60  $\text{kg CO}_2\text{-e/PE/y}$ . GHG emissions occur on every stage of the model WWTP (Figure 8.3). While mechanical treatment of the wastewater causes the lowest emissions (1%), the biological treatment contributes the highest share to the total emissions (46%). Each one of the other process stages (reject water treatment, sludge treatment and incineration) emits about 10-25% of the total emission.

$\text{N}_2\text{O}$  is the most important GHG of the model WWTP with important sources in the biological treatment, the reject water treatment and the sludge incineration. Methane and indirect  $\text{CO}_2$  emissions contribute roughly equally to the total emissions. Methane emissions are mainly originating from sludge treatment. Sludge incineration and biological treatment are the main cause for indirect  $\text{CO}_2$  emissions. Direct  $\text{CO}_2$  emissions in the biological treatment and sludge incineration, which are not displayed in Figure 8.3, sum up to 54  $\text{kgCO}_2\text{-e/PE/y}$  or 91% of the total GHG

emissions from all other sources. If included GHG balance, direct CO<sub>2</sub> emissions would be clearly the most important single GHG emissions source.

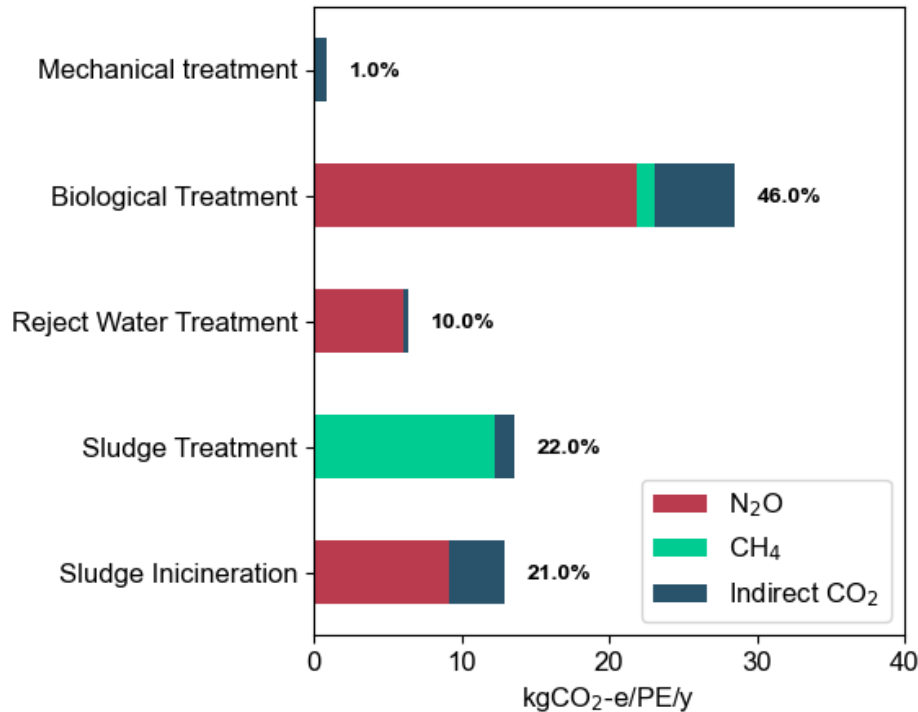


Figure 8.3: Estimated GHG emissions in the model full scale WWTP for different process stages.

The total GHG emissions calculated in this chapter emphasize the relevance of WWTPs for the total emissions of a country. GHG emissions from WWTP (13.9 Mio PE, total Swiss GHG emissions: ~50 Mt) are estimated to contribute 1.7% of the total emissions in Switzerland, assuming that the model WWTP in this chapter is representative for the whole country. Likely, the contribution is even higher, given that the EF of 0.9% for N<sub>2</sub>O emissions from biological treatment applied in this chapter is at the very low end of the countrywide estimation (Chapter 5). The relevance of GHG emissions from WWTPs links to the question of possible mitigation options.

#### 8.3.4 Mitigation strategies

The sources of GHG emissions in WWTP are spread over the whole wastewater treatment process (Figure 8.1). Hence, a versatile set of strategies for GHG mitigation is required depending on the emission target set. Here, we discuss four

different categories of strategies applicable on full-scale WWTP: i) process optimization, ii) off-gas treatment solutions, iii) carbon capture, and iv) economic measures. In the following, we will explain how the categories can be implemented in WWTP and give quantitative estimations of the emission reductions (Figure 8.4). Process optimization (category i) is often applied to reduce electricity consumption. It is feasible yet rarely done so far to optimize the WWTP in terms of  $\text{N}_2\text{O}$  emissions (biological treatment, reject water treatment and sludge incineration). While a temperature increase during sludge incineration is sufficient to reduce  $\text{N}_2\text{O}$  emissions (Sänger et al. 2001), the optimization of biological processes is more challenging due to limited understanding biological  $\text{N}_2\text{O}$  production in WWTP (Song et al. 2020). Off-gas treatment solutions (ii) for GHG reduction on WWTP refer to off-gas treatment from different process steps of WWTP, which allows avoiding direct GHG emissions in WWTPs ( $\text{N}_2\text{O}$ ,  $\text{CH}_4$ ). Technical solutions for GHG destruction are catalytic oxidation (Konsolakis 2015), thermal oxidation (Galle et al. 2001), or combustion of the off-gas in combined heat and power plant (CHP) or sludge incineration. Catalytic oxidation can be only used for  $\text{N}_2\text{O}$  emissions. Off-gas treatment solutions require roofed WWTP facilities with an off-gas collection system. Carbon capture (iii) in WWTP is understood as valorizing organic compounds instead of combustion for heat and power production. Multiple processes have been proposed for carbon capture, such as microbial electrolytic carbon capture, PHA production or biochar production (Lu et al. 2018, Morgan-Sagastume et al. 2013). However, full-scale implementations of carbon capture facilities are rare and technical feasibility needs to be evaluated (Lu et al. 2018). Economic measures (iv) summarize strategies that do not lead to a reduction of GHG emissions on the WWTP directly, such as carbon off-setting or purchase of renewable electricity. While carbon off-setting is reasonable if on-site emission reductions are very costly, purchase of renewable electricity is an operator's decision with a direct effect on the GHG balance of the WWTP.

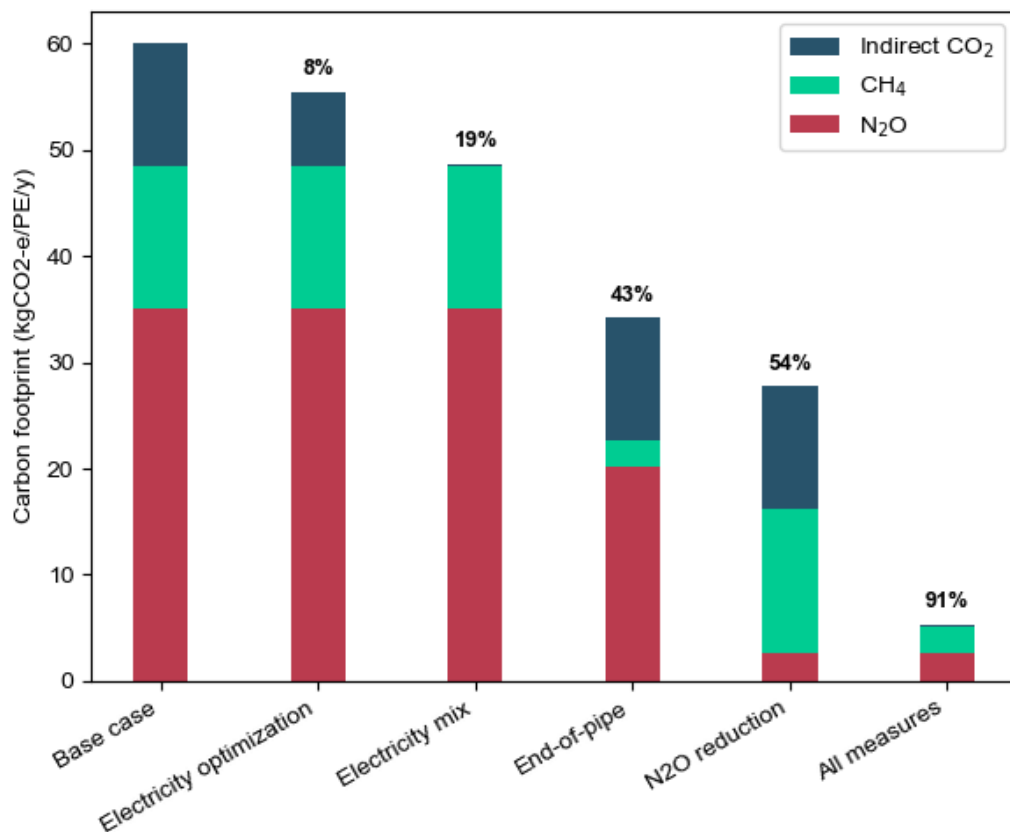


Figure 8.4: Comparison of different scenarios for reducing climate impact of wastewater treatment based on the model WWTP of 100'000 person equivalents (PE). Numbers in plot correspond to the percent reduction of GHG emissions in response to different scenarios from Table 8.3.

The effectiveness of different mitigation measure varies substantially in terms of reducing the carbon footprint of the model WWTP (Figure 8.4). Strategies targeting the electricity consumption have the lowest reduction potential. In contrast to recent studies (Nakkasunchi et al. 2021), optimization of electricity consumption (40% in this chapter) is not effective to reduce the carbon footprint (less than 10% impact reduction). Hence, tools for optimizing electricity consumption should be ranked correspondingly lower compared to other GHG emissions. In terms of carbon footprint, changing to a fully renewable electricity mix should be prioritized, since it leads to a twice as high impact on the carbon footprint (20% reduction). Notably, electricity production is expected to move towards climate neutral technologies (IEA 2020) in the next decades and the importance of electricity consumption for the carbon footprint will further drop. Significantly more effective are off-gas treatment solutions. More than 40% of the emissions can be reduced with technical solutions

that are straight forward to implement on an existing WWTP and do not require a change of operational strategy. Reducing the formation of  $\text{N}_2\text{O}$  in biological treatment, reject-water treatment and sludge incineration by process optimization has a high reduction potential (54% reduction) but is challenging to realize, as discussed above. The implementation of all strategies together would reduce the carbon footprint by 90%. In summary, this analysis shows that off-gas treatment is the most promising strategy to reduce GHG from WWTP. Potentially, even emissions from biological treatment can be collected and reduced in an off-gas treatment system. However, technical feasibility and justifiability of involved costs has to be demonstrated. The same is true for carbon capture systems, which were not included for the optimization analysis, since they require a fundamental redesign of the WWTP. The expected emission reduction are nevertheless substantial and a negative carbon footprint could be reached for WWTP (Lu et al. 2018).

## 8.4 Conclusions

- For an average WWTP, supplied with an average European Union electricity mix and featuring with full-nitrogen removal, N<sub>2</sub>O is the most important GHG, with source in the biological treatment, the reject water treatment and the sludge incineration. CH<sub>4</sub> and indirect CO<sub>2</sub> contribute roughly equal shares to the carbon footprint. Direct CO<sub>2</sub> emissions through the oxidation of organic carbon would be the most important source of GHG, but are often not included in the carbon footprint because the carbon source is primarily renewable.
- More than 40% of the GHG emissions can be reduced by implementing off-gas treatment solutions on the reject water treatment, the anaerobic digestion (sludge storage tanks) and sludge incineration. Such techniques for reduction of GHG are applied in industry and can be retrofitted to existing WWTP facilities.
- Reducing N<sub>2</sub>O formation in biological process has a high potential but is challenging due to limited understanding of the emission variability and entailed difficulties in the development of systematic, reproducible mitigation strategies.
- Optimization of electricity consumption has a very low potential for GHG emission reduction (less than 10%) and replacement of the electricity mix by renewables is more efficient. Carbon capture would be the most impactful strategy to reduce GHG emissions from WWTP. However, applicability in full-scale has to be proven.



## 8.5 References

- Bao, Z., Sun, S. and Sun, D. (2016) Assessment of greenhouse gas emission from A/O and SBR wastewater treatment plants in Beijing, China. *International Biodeterioration & Biodegradation* 108, 108-114.
- Clos, I., Krampe, J., Alvarez-Gaitan, J.P., Saint, C.P. and Short, M.D. (2020) Energy Benchmarking as a Tool for Energy-Efficient Wastewater Treatment: Reviewing International Applications. *Water Conservation Science and Engineering* 5(1-2), 115-136.
- Czepiel, P.M., Crill, P.M. and Harriss, R.C. (1993) Methane emissions from municipal wastewater treatment processes. *Environmental Science & Technology* 27(12), 2472-2477.
- Daelman, M.R., De Baets, B., van Loosdrecht, M.C. and Volcke, E.I. (2013a) Influence of sampling strategies on the estimated nitrous oxide emission from wastewater treatment plants. *Water Research* 47(9), 3120-3130.
- Daelman, M.R., van Voorthuizen, E.M., van Dongen, L.G., Volcke, E.I. and van Loosdrecht, M.C. (2013b) Methane and nitrous oxide emissions from municipal wastewater treatment - results from a long-term study. *Water Science and Technology* 67(10), 2350-2355.
- Daelman, M.R., van Voorthuizen, E.M., van Dongen, U.G., Volcke, E.I. and van Loosdrecht, M.C. (2012) Methane emission during municipal wastewater treatment. *Water Research* 46(11), 3657-3670.
- Delre, A., Monster, J. and Scheutz, C. (2017) Greenhouse gas emission quantification from wastewater treatment plants, using a tracer gas dispersion method. *Science of The Total Environment* 605-606, 258-268.
- Duan, H., van den Akker, B., Thwaites, B.J., Peng, L., Herman, C., Pan, Y., Ni, B.J., Watt, S., Yuan, Z. and Ye, L. (2020) Mitigating nitrous oxide emissions at a full-scale wastewater treatment plant. *Water Research* 185, 116196.
- Foley, J., de Haas, D., Yuan, Z. and Lant, P. (2010) Nitrous oxide generation in full-scale biological nutrient removal wastewater treatment plants. *Water Research* 44(3), 831-844.
- Foley, J., Yuan, Z., Keller, J., Senante, E., Chandran, K., Willis, J., Shah, A., van Loosdrecht, M.C. and van Voorthuizen, E. (2015) N<sub>2</sub>O and CH<sub>4</sub> Emission from Wastewater Collection and Treatment Systems: State of the Science Report and Technical Report.
- Galle, M., Agar, D.W. and Watzenberger, O. (2001) Thermal N<sub>2</sub>O decomposition in regenerative heat exchanger reactors. *Chemical Engineering Science* 56(4), 1587-1595.
- Gruber, W., Villez, K., Kipf, M., Wunderlin, P., Siegrist, H., Vogt, L. and Joss, A. (2020) N<sub>2</sub>O emission in full-scale wastewater treatment: Proposing a refined monitoring strategy. *Science of The Total Environment* 699, 134157.

- Gujer, W. (2007) Siedlungswasserwirtschaft, Springer, Berlin.
- Houillon, G. and Jolliet, O. (2005) Life cycle assessment of processes for the treatment of wastewater urban sludge: energy and global warming analysis. *Journal of Cleaner Production* 13(3), 287-299.
- IEA (2020) Global Energy Review 2020, Paris
- IPCC (2006) Chapter 5.6 Wastewater treatment and discharge. 2006 IPCC Guidelines for National Greenhouse Gas Inventories, <http://www.ipcc-nggip.iges.or.jp/public/2006gl/index.html>.
- IPCC (2013) Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
- IPCC (2014) Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, p. 151, IPCC, Geneva, Switzerland.
- Jabłońska, M., Arán, M.A., Beale, A.M., Góra-Marek, K., Delahay, G., Petitto, C., Pacultová, K. and Palkovits, R. (2019) Catalytic decomposition of N<sub>2</sub>O over Cu–Al–Ox mixed metal oxides. *RSC Advances* 9(7), 3979-3986.
- Joss, A., Salzgeber, D., Eugster, J., König, R., Rottermann, K., Burger, S., Fabijan, P., Leumann, S., Mohn, J. and Siegrist, H. (2009) Full-Scale Nitrogen Removal from Digester Liquid with Partial Nitritation and Anammox in One SBR. *Environmental Science & Technology* 43(14), 5301-5306.
- Konsolakis, M. (2015) Recent Advances on Nitrous Oxide (N<sub>2</sub>O) Decomposition over Non-Noble-Metal Oxide Catalysts: Catalytic Performance, Mechanistic Considerations, and Surface Chemistry Aspects. *ACS Catalysis* 5(11), 6397-6421.
- Kosonen, H., Heinonen, M., Mikola, A., Haimi, H., Mulas, M., Corona, F. and Vahala, R. (2016) Nitrous Oxide Production at a Fully Covered Wastewater Treatment Plant: Results of a Long-Term Online Monitoring Campaign. *Environmental Science & Technology* 50(11), 5547-5554.
- Lackner, S., Gilbert, E.M., Vlaeminck, S.E., Joss, A., Horn, H. and van Loosdrecht, M.C. (2014) Full-scale partial nitritation/anammox experiences--an application survey. *Water Research* 55, 292-303.
- Liu, Y., Cheng, X., Lun, X. and Sun, D. (2014) CH<sub>4</sub> emission and conversion from A2O and SBR processes in full-scale wastewater treatment plants. *Journal of Environmental Sciences* 26(1), 224-230.
- Lu, L., Guest, J.S., Peters, C.A., Zhu, X., Rau, G.H. and Ren, Z.J. (2018) Wastewater treatment for carbon capture and utilization. *Nature Sustainability* 1(12), 750-758.
- Marias, F., Benzaoui, A., Vaxelaire, J., Gelix, F. and Nicol, F. (2015) Fate of Nitrogen during Fluidized Incineration of Sewage Sludge. Estimation of NO and N<sub>2</sub>O Content in the Exhaust Gas. *Energy and Fuels* 29(7), 4534-4548.

- Meinshausen, M., Smith, S.J., Calvin, K., Daniel, J.S., Kainuma, M.L.T., Lamarque, J., Matsumoto, K., Montzka, S.A., Raper, S.C.B., Riahi, K., Thomson, A., Velders, G.J.M. and van Vuuren, D.P.P. (2011) The RCP greenhouse gas concentrations and their extensions from 1765 to 2300. *Climatic Change* 109(1), 213-241.
- Mikola, A., Heinonen, M., Kosonen, H., Leppanen, M., Rantanen, P. and Vahala, R. (2014) N<sub>2</sub>O emissions from secondary clarifiers and their contribution to the total emissions of the WWTP. *Water Science and Technology* 70(4), 720-728.
- Morgan-Sagastume, F., Valentino, F., Hjort, M., Cirne, D., Karabegovic, L., Gerardin, F., Johansson, P., Karlsson, A., Magnusson, P., Alexandersson, T., Bengtsson, S., Majone, M. and Werker, A. (2013) Polyhydroxyalkanoate (PHA) production from sludge and municipal wastewater treatment. *Water Science and Technology* 69(1), 177-184.
- Müller, E.A. (2010) *Energie in ARA*. VSA (ed), Glattbrugg.
- Nakkasunchi, S., Hewitt, N.J., Zoppi, C. and Brandoni, C. (2021) A review of energy optimization modelling tools for the decarbonisation of wastewater treatment plants. *Journal of Cleaner Production* 279.
- Parravicini, V., Svardal, K. and Krampe, J. (2016) Greenhouse Gas Emissions from Wastewater Treatment Plants. *Energy Procedia* 97, 246-253.
- Petit, J.R., Jouzel, J., Raynaud, D., Barkov, N.I., Barnola, J.M., Basile, I., Bender, M., Chappellaz, J., Davis, M., Delaygue, G., Delmotte, M., Kotiyakov, V.M., Legrand, M., Lipenkov, V.Y., Lorius, C., Pépin, L., Ritz, C., Saltzman, E. and Stievenard, M. (1999) Climate and atmospheric history of the past 420,000 years from the Vostok ice core, Antarctica. *Nature* 399(6735), 429-436.
- Prather, M.J., Holmes, C.D. and Hsu, J. (2012) Reactive greenhouse gas scenarios: Systematic exploration of uncertainties and the role of atmospheric chemistry. *Geophysical Research Letters* 39(9).
- Remy, C., Lesjean, B. and Waschnewski, J. (2013) Identifying energy and carbon footprint optimization potentials of a sludge treatment line with Life Cycle Assessment. *Water Science and Technology* 67(1), 63-73.
- Ren, Y., Ngo, H.H., Guo, W., Ni, B.-J. and Liu, Y. (2019) Linking the nitrous oxide production and mitigation with the microbial community in wastewater treatment: A review. *Bioresource Technology Reports* 7.
- Ren, Y.G., Wang, J.H., Li, H.F., Zhang, J., Qi, P.Y. and Hu, Z. (2013) Nitrous oxide and methane emissions from different treatment processes in full-scale municipal wastewater treatment plants. *Environmental Technology* 34(21-24), 2917-2927.
- Ribera-Guardia, A., Bosch, L., Corominas, L. and Pijuan, M. (2019) Nitrous oxide and methane emissions from a plug-flow full-scale bioreactor and assessment of its carbon footprint. *Journal of Cleaner Production* 212, 162-172.
- Rodriguez-Caballero, A., Aymerich, I., Poch, M. and Pijuan, M. (2014) Evaluation of process conditions triggering emissions of green-house gases from a biological wastewater treatment system. *Science of The Total Environment* 493, 384-391.

- Samuelsson, J., Delre, A., Tumlin, S., Hadi, S., Offerle, B. and Scheutz, C. (2018) Optical technologies applied alongside on-site and remote approaches for climate gas emission quantification at a wastewater treatment plant. *Water Research* 131, 299-309.
- Sänger, M., Werther, J. and Ogada, T. (2001) NO<sub>x</sub> and N<sub>2</sub>O emission characteristics from fluidised bed combustion of semi-dried municipal sewage sludge. *Fuel* 80(2), 167-177.
- Schaubroeck, T., De Clippeleir, H., Weissenbacher, N., Dewulf, J., Boeckx, P., Vlaeminck, S.E. and Wett, B. (2015) Environmental sustainability of an energy self-sufficient sewage treatment plant: improvements through DEMON and co-digestion. *Water Research* 74, 166-179.
- Soda, S., Iwai, Y., Sei, K., Shimod, Y. and Ike, M. (2010) Model analysis of energy consumption and greenhouse gas emissions of sewage sludge treatment systems with different processes and scales. *Water Science and Technology* 61(2), 365-373.
- Song, M.J., Choi, S., Bae, W.B., Lee, J., Han, H., Kim, D.D., Kwon, M., Myung, J., Kim, Y.M. and Yoon, S. (2020) Identification of primary effectors of N<sub>2</sub>O emissions from full-scale biological nitrogen removal systems using random forest approach. *Water Research* 184, 116144.
- Stenstrom, F., Tjus, K. and la Cour Jansen, J. (2014) Oxygen-induced dynamics of nitrous oxide in water and off-gas during the treatment of digester supernatant. *Water Science and Technology* 69(1), 84-91.
- Suzuki, Y., Ochi, S.-I., Kawashima, Y. and Hiraide, R. (2003) Determination of Emission Factors of Nitrous Oxide from Fluidized Bed Sewage Sludge Incinerators by Long-term Continuous Monitoring. *JOURNAL OF CHEMICAL ENGINEERING OF JAPAN* 36(4), 458-463.
- Svoboda, K., Baxter, D. and Martinec, J. (2006) Nitrous oxide emissions from waste incineration. *Chemical Papers* 60(1), 78-90.
- Tauber, J., Parravicini, V., Svoldal, K. and Krampe, J. (2019) Quantifying methane emissions from anaerobic digesters. *Water Science and Technology* 80(9), 1654-1661.
- Tumendelger, A., Alshboul, Z. and Lorke, A. (2019) Methane and nitrous oxide emission from different treatment units of municipal wastewater treatment plants in Southwest Germany. *PLoS One* 14(1), e0209763.
- Vasilaki, V., Conca, V., Frison, N., Eusebi, A.L., Fatone, F. and Katsou, E. (2020) A knowledge discovery framework to predict the N<sub>2</sub>O emissions in the wastewater sector. *Water Research* 178, 115799.
- Vasilaki, V., Massara, T.M., Stanchev, P., Fatone, F. and Katsou, E. (2019) A decade of nitrous oxide (N<sub>2</sub>O) monitoring in full-scale wastewater treatment processes: A critical review. *Water Research* 161, 392-412.

- Wang, J., Zhang, J., Xie, H., Qi, P., Ren, Y. and Hu, Z. (2011) Methane emissions from a full-scale A/A/O wastewater treatment plant. *Bioresource Technology* 102(9), 5479-5485.
- Wunderlin, P. (2013) Mechanisms Of N<sub>2</sub>O Production In Biological Wastewater Treatment From Pathway Identification To Process Control. Cumulative thesis, ETH Zurich, Zurich.
- Yan, X., Li, L. and Liu, J. (2014) Characteristics of greenhouse gas emission in three full-scale wastewater treatment processes. *Journal of Environmental Sciences* 26(2), 256-263.

# **Chapter 9**

## **Conclusions & Research Outlook**

N<sub>2</sub>O emissions from the biological treatment stage of WWTP were shown to be the dominant GHG emission from wastewater treatment in a number of case studies (Daelman et al. 2013b, Kosonen et al. 2016). However, a substantial variation of N<sub>2</sub>O EFs for the biological treatment were previously reported leading to high uncertainties in the EF estimation (Cadwallader and Van Briesen 2017, Chen et al. 2019). The duration of monitoring campaigns was often too short to assess representative emissions factors (Chapter 1, 4), since the high seasonal variability requires continuous, year-long campaigns (Daelman et al. 2013a). Prior to this thesis, only three continuous year-long monitoring campaigns have been reported and the underlying causes of the high emission dynamics remained mostly unclear (Daelman et al. 2015, Vasilaki et al. 2019). Therefore, this thesis investigated the quantities, variability and drivers of N<sub>2</sub>O emission in full-scale systems and developed monitoring methods for this purpose. The following chapter is divided in general conclusions, their implications for the operation of WWTP and future research needs.

## 9.1 General conclusions

*What are essential components and important strategies for continuous, long-term off-gas monitoring campaigns on full-scale WWTP based on the flux-chamber approach?*

The first aim of this thesis was to provide strategies and methods for representative N<sub>2</sub>O emission monitoring campaigns on full-scale WWTP based on the floating flux chamber approach. The assessment of representative EFs requires a spatially and temporally highly resolved monitoring approach, due to the substantial emission variation in space and time reported in this thesis (Chapter 4). Lanes and reactors with comparable inflow characteristics and operational strategies exhibited similar N<sub>2</sub>O emission patterns. Thus monitoring one lane is sufficient in such case. Monitoring of all lanes is required, if different operational strategies or differences in inflow characteristics are to be expected. Hence, a monitoring system is required that has multiple monitoring channels, a quick response time, and is reliable for achieving year-long campaigns. Key components of such a system are a pre-suction

pump to allow constant flushing of the sample tubes and representative measurements within one minute, multiple barriers for liquids to avoid measurement failure, and full-automation to reduce maintenance and operational costs (Chapter 3).

*What is the contribution of N<sub>2</sub>O emissions from WWTP to the total GHG emissions in a country and how are these emissions optimally estimated?*

The second aim of the thesis was to review the current method for the estimation of N<sub>2</sub>O emission from wastewater treatment for Switzerland and propose a refined methodology. The review revealed that the current emission factor strongly underestimates the N<sub>2</sub>O emissions from WWTP and that a broader data basis is required to reduce uncertainties of emission estimates (Chapter 2). Hence, we conducted at least year-long monitoring campaigns on ten full-scale WWTPs (Chapter 3, Chapter 5), which resulted in a wide range of yearly emission factors (0.1-8% of the total nitrogen load). The emission factors exhibited a very high and statistically significant correlation with the effluent nitrite compared to influent nitrogen and a good correlation with the nitrogen removal efficiency (Chapter 5). Since nitrite effluent concentrations were not available on a countrywide level, we suggest calculating a countrywide EF based on the weighted, average emission factors of three nutrient removal categories (carbon removal, nitrification only, and nitrogen removal). The new methodology leads to a more realistic representation of emission processes on WWTP. The estimated EF (0.9-3.6%) for Switzerland was strongly depending on the EF estimated for the carbon removal WWTPs, which is highly uncertain due to the insufficient data basis (one continuous, long-term monitoring campaign). Despite the high uncertainties, the estimates confirm that N<sub>2</sub>O is the dominant GHG emission from WWTPs (Chapter 7) and has a relevance for the total countrywide emissions in Switzerland (0.3-1.4%). The refined methodology is applicable to other countries in the temperate climate zone and allows a country specific representation of wastewater treatment compared to the IPCC methodology. For other climate zones, long-term monitoring campaigns are necessary to confirm the emission variability reported in this thesis.



*What are causes for the pronounced N<sub>2</sub>O emissions dynamics over days and over seasons and how do they link to N<sub>2</sub>O mitigation strategies?*

The third aim of the thesis was to evaluate potential drivers for the seasonal emission pattern and discuss mitigation strategies. The seasonal emission pattern exhibited a strong correlation with microbial community dynamics in six full-scale SBR reactors, which could not be explained by standard engineering approaches, focusing on growth conditions of nitrifiers (Chapter 6). Stability and diversity of the microbial communities in the reactors were good predictors for the seasonal N<sub>2</sub>O emission peaks: a higher stability and diversity of the activated sludge microbiome correlated with lower emissions. Abundances of NOB and filamentous bacteria responsible for floc stability (*Chloroflexi*) were significantly reduced during high emission phases. Despite the strong correlations, causal relationships could not be elucidated and strategies to stabilize microbial communities remain unclear. Nevertheless, mitigation strategies should primarily focus on the seasonal emission peak phase, typically occurring in spring (Chapter 5), and possibly include microbial community dynamics. A key focus in N<sub>2</sub>O emission mitigation should be laid on year-round denitrification (Chapter 5) and availability of organic substrate during denitrification (Chapter 7). Implementing reactor conditions for pre-denitrification always lead to an immediate reduction of N<sub>2</sub>O emissions. However, reduction of N<sub>2</sub>O by denitrification was strongly depending on organic carbon availability, causing higher emission if reject water was dosed during nights when organic carbon supply was lower. Measurement of isotopocules confirmed the varying efficiency of N<sub>2</sub>O reduction and showed that heterotrophic denitrification was the main pathway for N<sub>2</sub>O production on the WWTP studied (Chapter 7).

## 9.2 Implications for the operation of WWTPs

N<sub>2</sub>O from biological treatment can be the most important GHG source of WWTP and must be considered when optimizing the carbon footprint of a WWTP (Chapter 8). In case of a low N<sub>2</sub>O EF (<0.5%), other emission sources become dominant, such as the N<sub>2</sub>O emissions from reject water treatment, CH<sub>4</sub> emission from sludge storage,

and indirect GHG emissions from energy consumption. The indirect GHG emissions caused by the electricity consumption strongly depend on the energy consumption of the aeration and on the carbon footprint of the purchased electricity mix. On the other hand,  $\text{N}_2\text{O}$  and  $\text{CH}_4$  from side-stream and sludge treatment can be efficiently reduced using end of pipe solutions, such as catalytic oxidation or destruction in the combined heat and power plant of the WWTP (Chapter 8).

$\text{N}_2\text{O}$  emissions from biological treatment can be reduced by i) roofing WWTPs and treating the off-gas or ii) process optimization. To date, off-gases from biological treatment are often emitted directly to the atmosphere and cannot be treated. In terms of process optimization year-round denitrification is important to achieve low  $\text{N}_2\text{O}$  emissions (Chapter 5), since it is the only biological process to reduce  $\text{N}_2\text{O}$  (Conthe et al. 2018a). Additionally, denitrification can support the reduction of nitrite and avoids nitrite accumulation, a main driver for increased  $\text{N}_2\text{O}$  emissions (Chapter 5, 6).

Reject water addition to the biological treatment in the mainstream can cause high  $\text{N}_2\text{O}$  emissions, if organic carbon is limited, i.e. during nights (Chapter 7), or if the biological treatment is overloaded and exhibits  $\text{NO}_2^-$  accumulation as well as increased  $\text{N}_2\text{O}$  emissions (Chapter 2). To reduce  $\text{N}_2\text{O}$  emissions, reject water dosage should be optimized by taking into account the organic carbon availability in the daily pattern. Alternatively, multiple side-stream processes have been proposed for reject water treatment. However, processes commonly applied, such as partial-nitrification anammox or nitrification-denitrification, in turn can cause very high  $\text{N}_2\text{O}$  emissions (Joss et al. 2009, Kampschreur et al. 2009a, Vasilaki et al. 2020a). Hence, such processes are ideally combined with an off-gas treatment for  $\text{N}_2\text{O}$  decomposition (Chapter 8).

### 9.3 Research outlook

*How can the estimations of countrywide N<sub>2</sub>O emissions from wastewater treatment be refined further?*

A key challenges towards more accurate countrywide emission estimations from WWTP is the limited availability of representative monitoring campaigns for certain processes as well as in most climate zones (Cadwallader and Van Briesen 2017) (Chapter 5). A strong link between NO<sub>2</sub><sup>-</sup> concentrations and N<sub>2</sub>O emissions has been reported by us and other research groups (Kampschreur et al. 2009b, Massara et al. 2017, Ren et al. 2019). My thesis helped to identify that NO<sub>2</sub><sup>-</sup> effluent loads are a good predictor for N<sub>2</sub>O EFs in WWTP (Chapter 6). However, NO<sub>2</sub><sup>-</sup> effluent loads are usually not available on countrywide levels. In summary, (i) an improved data availability of NO<sub>2</sub><sup>-</sup> effluent loads from WWTPs and (ii) further N<sub>2</sub>O monitoring campaigns on specific WWTPs are needed to achieve better N<sub>2</sub>O emission estimates. NO<sub>2</sub><sup>-</sup> effluent concentrations are typically monitored on Swiss WWTP and measurement should be a general requirement due to the major impacts linked to NO<sub>2</sub><sup>-</sup> accumulation, such as N<sub>2</sub>O emissions and ecotoxicological issues in the receiving waters. A countrywide collection of measured NO<sub>2</sub><sup>-</sup> effluent concentration data is currently lacking. Further monitoring campaigns would be particularly important for carbon removal WWTP and biofilm systems (e.g. fixed bed, IFAS, MBBR, aerobic granular sludge), since for such reactor types the current data availability is insufficient. Additionally, continuous long-term monitoring campaigns have been uniquely conducted in European countries (Vasilaki et al. 2019). Monitoring N<sub>2</sub>O emissions from non-BNR systems in other climate zones is of high importance, since the share of carbon removing plants can be quite high in many countries and the N<sub>2</sub>O emissions are potentially quite significant.

To further improve N<sub>2</sub>O emission estimates from WWTP, two minor points should be considered:

- i. Side-stream treatment processes of ammonium-rich reject water, such as partial-nitrification anammox or nitrification-denitrification, can cause very high N<sub>2</sub>O emissions (Vasilaki et al. 2019). National inventories of WWTP with side-stream treatment steps would be helpful.

- ii. The EF for the effluent nitrogen transformed to  $N_2O$  in the environment used by the IPCC (0.5%) is a very rough estimate and scientific literature is quite sparse. More case studies would be helpful to quantify the uncertainties of the EF currently applied.

*Why and how should  $N_2O$  monitoring be implemented in wastewater treatment?*

$N_2O$  emission monitoring by the operators of full-scale WWTPs will be increasingly important to i) get accurate emission estimates for specific WWTP and ii) as an early warning system for nitrite accumulation. More importantly,  $N_2O$  mitigation in biological treatment requires long-term emission monitoring (Duan et al. 2020, Gruber et al. 2020). Reporting and reducing  $N_2O$  emissions in WWTP is expected to become even legally binding, as e.g. current environmental policies in Denmark suggest (Chen and Sin 2020). Emission monitoring will remain the main tool for the assessment of emissions, as long as mathematical models cannot be applied for accurate estimations. Black-box models based on data segregation have been proposed to reduce monitoring efforts to a few days every month in a case study (Vasilaki et al. 2020b). However, more such case studies are required to confirm the boundary conditions and the prediction accuracy of this approach. The increasing interest for  $N_2O$  emissions monitoring by operators will further drive the development of appropriate monitoring technologies.

Several approaches exist for long-term monitoring of  $N_2O$  emissions, i.e. i) Clark-type sensors for dissolved  $N_2O$  monitoring (Chen et al. 2019, Marques et al. 2014), ii) flux-chamber based off-gas monitoring (Chandran et al. 2016, Gruber et al. 2020), and iii) whole plant off-gas measurement (only applicable in covered WWTPs) (Daelman et al. 2015, Kosonen et al. 2016). A comparison of monitoring approaches i)-iii) is provided in Table 9.1.

Table 9.1: Comparison of continuous long-term monitoring approaches. Each criterion is ranked with an attribute (Bad, Medium, Good).

<b>Monitoring approach</b>	<b>i) Clark-type electrode</b>	<b>ii) Flux-chamber</b>	<b>iii) Whole plant</b>
Appropriateness for EF estimation	Medium Emissions have to be estimated using a stripping model	Medium-good Emissions are directly measured but only at a few points	Good Emissions are directly measured
Appropriateness for pathway identification and emission mitigation	Good Formation hotspots can be characterized	Good Emissions hotspots can be characterized	Medium Emissions hotspots cannot be spatially resolved
Usage for other parameters relevant in WWTP	Bad Measurement limited to N <sub>2</sub> O	Good Other GHGs or off-gas components can be detected	Good Other GHGs or off-gas components can be detected
Flexibility (reactor systems applicable)	Good No limitations	Medium-good Large distances between sampling point and monitoring station as well as systems with fluctuating water levels (SBR) are challenging	Bad Only covered WWTP
Investment costs/Scalability	Bad-medium Medium initial costs but expensive to scale-up (linear increase of costs with sampling points)	Bad-medium High initial costs but very cheap to scale-up (only marginal cost increase per sampling point)	Medium-good Moderate initial costs but very cheap to scale-up (only marginal cost increase per sampling point)
Operation costs	Bad Sensors have to be replaced regularly. Regular sensor cleaning and calibration.	Good Calibration can be automated	Good Calibration can be automated
Readiness of technology	Good Commercially available technology	Bad Prototype level	Good Commercially available technology

The comparison in Table 9.1 suggests that Clark type electrode (Approach i) is suitable for monitoring campaigns with a few sampling points, optimal to develop mitigation measures but very expensive in maintenance and for scale-up. Approach iii) is optimal for whole plant measurement and likely offers the cheapest option to monitor emission but is only possible in covered WWTP. The flux-chamber

approach is a good trade-off between approach i) and iii). It offers high scalability, low maintenance cost, a good setup for mitigation measures, and a high level of flexibility, as shown in my thesis. However, the readiness level of the technology is a major disadvantage of the system and should be improved. Moreover, flux measurements for each hood need to be implemented and a validation of the flux estimation over the blower speed, as applied in this study, would be needed. A comparative study providing quantitative data would be a significant advantage for future technology decisions.

The off-gas monitoring approaches additionally offer the possibility to measure other compounds in the off-gas, such as oxygen or methane. I expect a high potential for such additional measures, to quantify aeration performance, nitrification rates, or GHG emissions in real-time with a low-maintenance system (Baeten et al. 2020, Daelman et al. 2012, Groves et al. 1992, Leu et al. 2010).

#### *How is seasonal nitrite accumulation linked to microbial community dynamics?*

$\text{NO}_2^-$  accumulation is a major issue in wastewater treatment, since it is toxic if discharged to water bodies, leads to increased  $\text{N}_2\text{O}$  emissions in the biological treatment and may enhance the growth of filamentous bacteria causing bulking of sludge (Philips et al. 2002, Wunderlin et al. 2012, Yildiz and Benli 2004). Seasonally increased  $\text{NO}_2^-$  effluent concentration have been reported not only in WWTP with low nitrogen removal efficiencies, such as the Uster WWTP (Chapter 6), but, to a lower extent, also on year-round denitrification plants, such as the Lucerne WWTP and the Kralingseveer WWTP (Chapter 5).  $\text{NO}_2^-$  accumulation appears to occur in a yearly repeating pattern, as  $\text{NO}_2^-$  effluent concentrations in two Swiss WWTP suggest (Figure 9.1), similar to our results at the Uster WWTP (Figure C.4 (SI)).

It is assumed that effluent concentration of  $\text{NO}_2^-$  is only an indirect predictor for increased  $\text{N}_2\text{O}$  emissions from a mechanistic point of view, since  $\text{NO}_2^-$  can accumulate at different spots during nitrification-denitrification and get reduced at others. Concentrations can even differ substantially between the bulk phase of a reactor and inside a floc due to diffusion limitation (Chen et al. 2018). Studying the yearly dynamics of  $\text{NO}_2^-$  over a WWTP could help to establish even better correlations with  $\text{N}_2\text{O}$  emissions.

The underlying causes for the yearly recurring  $\text{NO}_2^-$  and  $\text{N}_2\text{O}$  pattern are unknown, but an important link to microbial community dynamics has been discovered and discussed in this thesis and in other researches (Chapter 6) (Gruber et al. 2021, Vieira et al. 2018). The loss of NOB is a common pattern in both studies and obviously leads to  $\text{NO}_2^-$  accumulation and entailed  $\text{N}_2\text{O}$  emissions. Impaired NOB performance may be linked to the absence of other organisms, such as bacteria crucial for floc stability (Chapter 6). Characterizing the causes for the seasonal loss of key functional groups of a WWTP could potentially lead to a mechanistic understanding of the seasonal  $\text{N}_2\text{O}$  pattern, and ultimately lead to the development of novel strategies for  $\text{N}_2\text{O}$  mitigation.

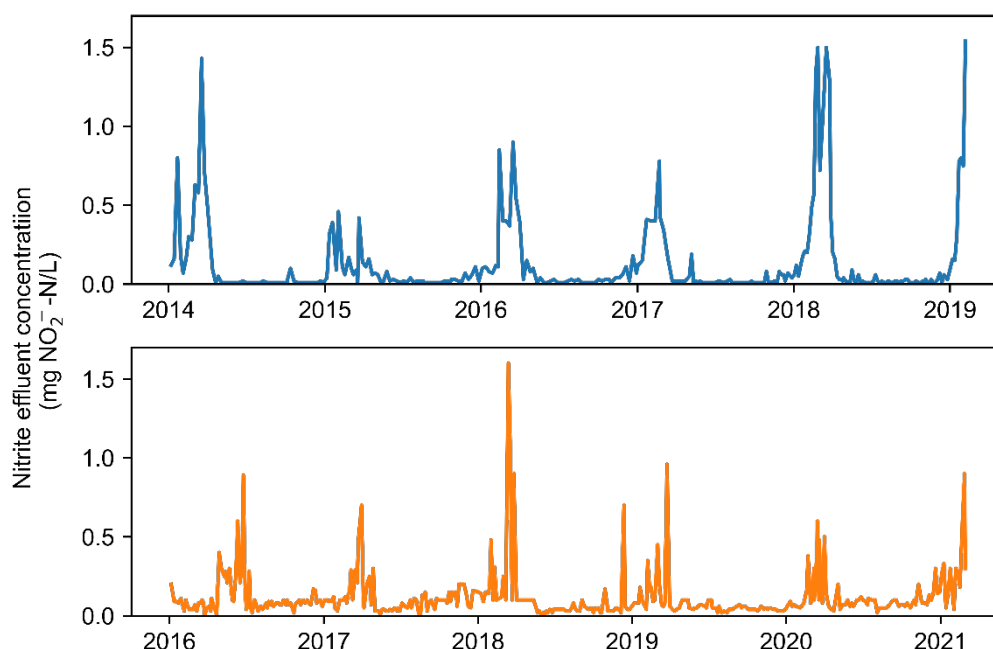


Figure 9.1:  $\text{NO}_2^-$  effluent concentration of the biological treatment at the Bülach WWTP and the Obermarch WWTP.

*How could the measurement of isotopic signatures be applied in future wastewater treatment studies?*

Isotopic techniques are still rarely applied in WWTP studies, despite the great potential for the identification of  $\text{N}_2\text{O}$  formation pathways (Duan et al. 2017, Harris et al. 2015, Wunderlin et al. 2013a). A major obstacle towards a more regular use certainly is the availability and the complexity of the  $\text{N}_2\text{O}$  isotopocules

measurements (Mohn et al. 2014). Additionally, isotopic analysis of dissolved species ( $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{NH}_4^+$ ) are strictly required for every gas sample measured in order to correctly interpret the  $\text{N}_2\text{O}$  isotopic signatures, given the substantial variations of the  $^{15}\text{N}$  and  $^{18}\text{O}$  values in wastewater samples (Archana et al. 2016) (Chapter 7). The additional samples further increase the complexity of the measurement. However, the technologies required are commercially available and collecting grab-samples as well as pre-processing is not a huge additional effort.

Measurement of  $\text{N}_2\text{O}$  isotopocules could be applied in three types of studies, such as i) in combination with long-term  $\text{N}_2\text{O}$  monitoring (Chapter 7), ii) in combination with ASM modeling with  $\text{N}_2\text{O}$  formation extensions (Domingo-Félez and Smets 2016) to cross-validate both approaches with each other, iii) in combination with microbial analysis tools (qPCR, metatranscriptomics, metaproteomics) to cross-validate both approaches with each other, and iv) isotopic tracer studies to quantitatively track microbial  $\text{N}_2\text{O}$  formation pathways (Ma et al. 2017). Options ii)-iv) have been previously proposed (Duan et al. 2017), but two further components are to be added for future study design, i.e., the sampling strategy and the system studied. Newly developed field deployable laser spectrometers can be used for real-time analysis of  $\text{N}_2\text{O}$  isotopocules (Ibraim et al. 2018), allowing different sampling strategies, i.e., continuous long-term (a), continuous short-term (b), and grab-sampling (c). The techniques can be applied to a whole range of reactor systems in full-scale and lab-scale applications. Table 9.2 provides a proposal for future study design.



Table 9.2: Potential for future studies applying isotopic technologies in wastewater treatment systems. Letters for sampling denote the following strategies: a: continuous, long-term; b: continuous, short-term; c: grab-sampling.

<b>Monitoring approach</b>	<b>i) Long-term monitoring and nitrogen mass-balancing</b>	<b>ii) ASM modeling with N<sub>2</sub>O formation extensions</b>	<b>iii) microbial tools</b>	<b>iv) isotopic tracer</b>
Lab-scale	Possible but not of particular interest	Useful for model calibration	Very useful for signature validation in pure cultures as well as mixed cultures.	Very useful for quantitative signature validation
	Sampling: a,b,c	Sampling: a,b,c	Sampling: c) since microbial tools are only grab samples	Sampling: a,b,c
	Complexity: low	Complexity: moderate	Complexity: high	Complexity: high
Full-scale	Very useful to provide mechanistic understanding for N <sub>2</sub> O mitigation strategies	Very useful for N <sub>2</sub> O model identification and calibration Very interesting with continuous long-term sampling (a)	Very useful likely very complex due to substantial temporal variation of both variables	Useful but very likely impossible, due to the amounts of tracer needed.
	Sampling: a,b	Sampling: a,b,c	Sampling: c) since microbial tools are only grab samples	Sampling: b,c
	Complexity: moderate	Complexity: high	Complexity: very high	Complexity: very high

*What are successful strategies to reduce the carbon footprint of WWTP?*

GHG emissions from WWTP, including CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O, can be caused by various processes within a WWTP (Chapter 9). Consequently, reducing the carbon footprint of WWTPs requires different measures. My thesis shows that the highest emission is to be expected from the seasonal N<sub>2</sub>O emissions (Chapter 8). For future studies, the key focus is to be laid on i) off-gas treatment ii) strategies avoiding seasonal N<sub>2</sub>O emissions patterns in biological treatment.

Focus i): catalytic destruction or combustion, are expected to play an important role for GHG mitigation in covered processes with off-gas collection. Such approaches require covering open storage tanks for digested sludge or the off-gas of the biological reject water treatment since both processes can cause high GHG emissions (Chapter 8). Practical research projects on such processes would be very valuable for mitigating emissions.

Focus ii): A practical guideline for N<sub>2</sub>O mitigation from full-scale WWTP has been proposed in Duan et al. 2020. However, advancing the understanding of the seasonal N<sub>2</sub>O emission dynamics is crucial. For this purpose, the application of multiple technologies, such as extensive process monitoring, molecular tools from microbiology, isotopic measurements and modeling is necessary (Chapter 6, 7). I doubt that practical research and case studies will foster reproducible mitigation strategies unless they provide a mechanistic understanding.

## 9.4 References

- Archana, A., Li, L., Shuh-Ji, K., Thibodeau, B. and Baker, D.M. (2016) Variations in nitrate isotope composition of wastewater effluents by treatment type in Hong Kong. *Marine Pollution Bulletin* 111(1-2), 143-152.
- Baeten, J.E., van Loosdrecht, M.C.M. and Volcke, E.I.P. (2020) When and why do gradients of the gas phase composition and pressure affect liquid-gas transfer? *Water Research* 178.
- Cadwallader, A. and Van Briesen, J.M. (2017) Incorporating Uncertainty into Future Estimates of Nitrous Oxide Emissions from Wastewater Treatment. *Journal of Environmental Engineering* 143(8).
- Chandran, K., Volcke, E.I. and Van Loosdrecht, M.C.M. (2016) Experimental Methods in Wastewater Treatment. Van Loosdrecht, M.C.M., Nielen, P.H., López Vázquez, C.M. and Brdjanovic, D. (eds), IWA, London.
- Chen, X., Mielczarek, A.T., Habicht, K., Andersen, M.H., Thornberg, D. and Sin, G. (2019) Assessment of Full-Scale N<sub>2</sub>O Emission Characteristics and Testing of Control Concepts in an Activated Sludge Wastewater Treatment Plant with Alternating Aerobic and Anoxic Phases. *Environmental Science & Technology* 53(21), 12485-12494.
- Chen, X. and Sin, G. (2020) Quantification and Analyses of N<sub>2</sub>O Emission and Testing of Relevant N<sub>2</sub>O Control Technology at Avedøre Wastewater Treatment Plant, <https://www.danva.dk/media/6422/technical-report-final-version-10-lattergasstyring.pdf>.
- Chen, X., Yuan, Z. and Ni, B.J. (2018) Nitrite accumulation inside sludge flocs significantly influencing nitrous oxide production by ammonium-oxidizing bacteria. *Water Research* 143, 99-108.
- Conthe, M., Lycus, P., Arntzen, M.O., Ramos da Silva, A., Frostegard, A., Bakken, L.R., Kleerebezem, R. and van Loosdrecht, M.C.M. (2018) Denitrification as an N<sub>2</sub>O sink. *Water Research* 151, 381-387.
- Daelman, M.R., De Baets, B., van Loosdrecht, M.C. and Volcke, E.I. (2013a) Influence of sampling strategies on the estimated nitrous oxide emission from wastewater treatment plants. *Water Research* 47(9), 3120-3130.
- Daelman, M.R., van Voorthuizen, E.M., van Dongen, L.G., Volcke, E.I. and van Loosdrecht, M.C. (2013b) Methane and nitrous oxide emissions from municipal wastewater treatment - results from a long-term study. *Water Science and Technology* 67(10), 2350-2355.
- Daelman, M.R., van Voorthuizen, E.M., van Dongen, U.G., Volcke, E.I. and van Loosdrecht, M.C. (2012) Methane emission during municipal wastewater treatment. *Water Research* 46(11), 3657-3670.
- Daelman, M.R., van Voorthuizen, E.M., van Dongen, U.G., Volcke, E.I. and van Loosdrecht, M.C. (2015) Seasonal and diurnal variability of N<sub>2</sub>O emissions from a

- full-scale municipal wastewater treatment plant. *Science of The Total Environment* 536, 1-11.
- Domingo-Félez, C. and Smets, B.F. (2016) A consilience model to describe N<sub>2</sub>O production during biological N removal. *Environmental Science: Water Research & Technology* 2(6), 923-930.
- Duan, H., van den Akker, B., Thwaites, B.J., Peng, L., Herman, C., Pan, Y., Ni, B.J., Watt, S., Yuan, Z. and Ye, L. (2020) Mitigating nitrous oxide emissions at a full-scale wastewater treatment plant. *Water Research* 185, 116196.
- Duan, H., Ye, L., Erler, D., Ni, B.J. and Yuan, Z. (2017) Quantifying nitrous oxide production pathways in wastewater treatment systems using isotope technology - A critical review. *Water Research* 122, 96-113.
- Groves, K.P., Daigger, G.T., Simpkin, T.J., Redmon, D.T. and Ewing, L. (1992) Evaluation of oxygen transfer efficiency and alpha-factor on a variety of diffused aeration systems. *Water Environment Research* 64(5), 691-698.
- Gruber, W., Niederdorfer, R., Ringwald, J., Morgenroth, E., Bürgmann, H. and Joss, A. (2021) Linking seasonal N<sub>2</sub>O emissions and nitrification failures to microbial dynamics in a SBR wastewater treatment plant. *Water Research X* 11, 100098.
- Gruber, W., Villez, K., Kipf, M., Wunderlin, P., Siegrist, H., Vogt, L. and Joss, A. (2020) N<sub>2</sub>O emission in full-scale wastewater treatment: Proposing a refined monitoring strategy. *Science of The Total Environment* 699, 134157.
- Harris, E., Joss, A., Emmenegger, L., Kipf, M., Wolf, B., Mohn, J. and Wunderlin, P. (2015) Isotopic evidence for nitrous oxide production pathways in a partial nitrification-anammox reactor. *Water Research* 83, 258-270.
- Ibraim, E., Harris, E., Eyer, S., Tuzson, B., Emmenegger, L., Six, J. and Mohn, J. (2018) Development of a field-deployable method for simultaneous, real-time measurements of the four most abundant N<sub>2</sub>O isotopocules. *Isotopes in Environmental and Health Studies* 54(1), 1-15.
- Joss, A., Salzgeber, D., Eugster, J., König, R., Rottermann, K., Burger, S., Fabijan, P., Leumann, S., Mohn, J. and Siegrist, H. (2009) Full-Scale Nitrogen Removal from Digester Liquid with Partial Nitrification and Anammox in One SBR. *Environmental Science & Technology* 43(14), 5301-5306.
- Kampschreur, M.J., Poldermans, R., Kleerebezem, R., van der Star, W.R., Haarhuis, R., Abma, W.R., Jetten, M.S., Jetten, M.S. and van Loosdrecht, M.C. (2009a) Emission of nitrous oxide and nitric oxide from a full-scale single-stage nitrification-anammox reactor. *Water Science and Technology* 60(12), 3211-3217.
- Kampschreur, M.J., Temmink, H., Kleerebezem, R., Jetten, M.S. and van Loosdrecht, M.C. (2009b) Nitrous oxide emission during wastewater treatment. *Water Research* 43(17), 4093-4103.
- Kosonen, H., Heinonen, M., Mikola, A., Haimi, H., Mulas, M., Corona, F. and Vahala, R. (2016) Nitrous Oxide Production at a Fully Covered Wastewater Treatment Plant:

- Results of a Long-Term Online Monitoring Campaign. *Environmental Science & Technology* 50(11), 5547-5554.
- Leu, S.Y., Libra, J.A. and Stenstrom, M.K. (2010) Monitoring off-gas O<sub>2</sub>/CO<sub>2</sub> to predict nitrification performance in activated sludge processes. *Water Research* 44(11), 3434-3444.
- Ma, C., Jensen, M.M., Smets, B.F. and Thamdrup, B. (2017) Pathways and Controls of N<sub>2</sub>O Production in Nitritation-Anammox Biomass. *Environmental Science & Technology*.
- Marques, R., Oehmen, A. and Pijuan, M. (2014) Novel microelectrode-based online system for monitoring N<sub>2</sub>O gas emissions during wastewater treatment. *Environmental Science & Technology* 48(21), 12816-12823.
- Massara, T.M., Malamis, S., Guisasola, A., Baeza, J.A., Noutsopoulos, C. and Katsou, E. (2017) A review on nitrous oxide (N<sub>2</sub>O) emissions during biological nutrient removal from municipal wastewater and sludge reject water. *Science of The Total Environment* 596-597, 106-123.
- Mohn, J., Wolf, B., Toyoda, S., Lin, C.T., Liang, M.C., Bruggemann, N., Wissel, H., Steiker, A.E., Dyckmans, J., Szvec, L., Ostrom, N.E., Casciotti, K.L., Forbes, M., Giesemann, A., Well, R., Doucett, R.R., Yarnes, C.T., Ridley, A.R., Kaiser, J. and Yoshida, N. (2014) Interlaboratory assessment of nitrous oxide isotopomer analysis by isotope ratio mass spectrometry and laser spectroscopy: current status and perspectives. *Rapid Communications in Mass Spectrometry* 28(18), 1995-2007.
- Philips, S., Laanbroek, H.J. and Verstraete, W. (2002) Origin, causes and effects of increased nitrite concentrations in aquatic environments. *Reviews in Environmental Science & Bio/Technology* 1(2), 115-141.
- Ren, Y., Ngo, H.H., Guo, W., Ni, B.-J. and Liu, Y. (2019) Linking the nitrous oxide production and mitigation with the microbial community in wastewater treatment: A review. *Bioresource Technology Reports* 7.
- Vasilaki, V., Conca, V., Frison, N., Eusebi, A.L., Fatone, F. and Katsou, E. (2020a) A knowledge discovery framework to predict the N<sub>2</sub>O emissions in the wastewater sector. *Water Research* 178, 115799.
- Vasilaki, V., Danishvar, S., Mousavi, A. and Katsou, E. (2020b) Data-driven versus conventional N<sub>2</sub>O EF quantification methods in wastewater; how can we quantify reliable annual EFs? *Computers & Chemical Engineering*.
- Vasilaki, V., Massara, T.M., Stanchev, P., Fatone, F. and Katsou, E. (2019) A decade of nitrous oxide (N<sub>2</sub>O) monitoring in full-scale wastewater treatment processes: A critical review. *Water Research* 161, 392-412.
- Vieira, A., Galinha, C.F., Oehmen, A. and Carvalho, G. (2018) The link between nitrous oxide emissions, microbial community profile and function from three full-scale WWTPs. *Science of The Total Environment* 651(Pt 2), 2460-2472.
- Wunderlin, P., Lehmann, M.F., Siegrist, H., Tuzson, B., Joss, A., Emmenegger, L. and Mohn, J. (2013) Isotope signatures of N<sub>2</sub>O in a mixed microbial population system:

constraints on N<sub>2</sub>O producing pathways in wastewater treatment. *Environmental Science & Technology* 47(3), 1339-1348.

Wunderlin, P., Mohn, J., Joss, A., Emmenegger, L. and Siegrist, H. (2012) Mechanisms of N<sub>2</sub>O production in biological wastewater treatment under nitrifying and denitrifying conditions. *Water Research* 46(4), 1027-1037.

Yildiz, H.Y. and Benli, A.C.K. (2004) Nitrite toxicity to crayfish, *Astacus leptodactylus*, the effects of sublethal nitrite exposure on hemolymph nitrite, total hemocyte counts, and hemolymph glucose. *Ecotoxicology and Environmental Safety* 59(3), 370-375.

# Appendix

## A Supplementary Information: Chapter 4

### A.1 Measurement setup and plant layout

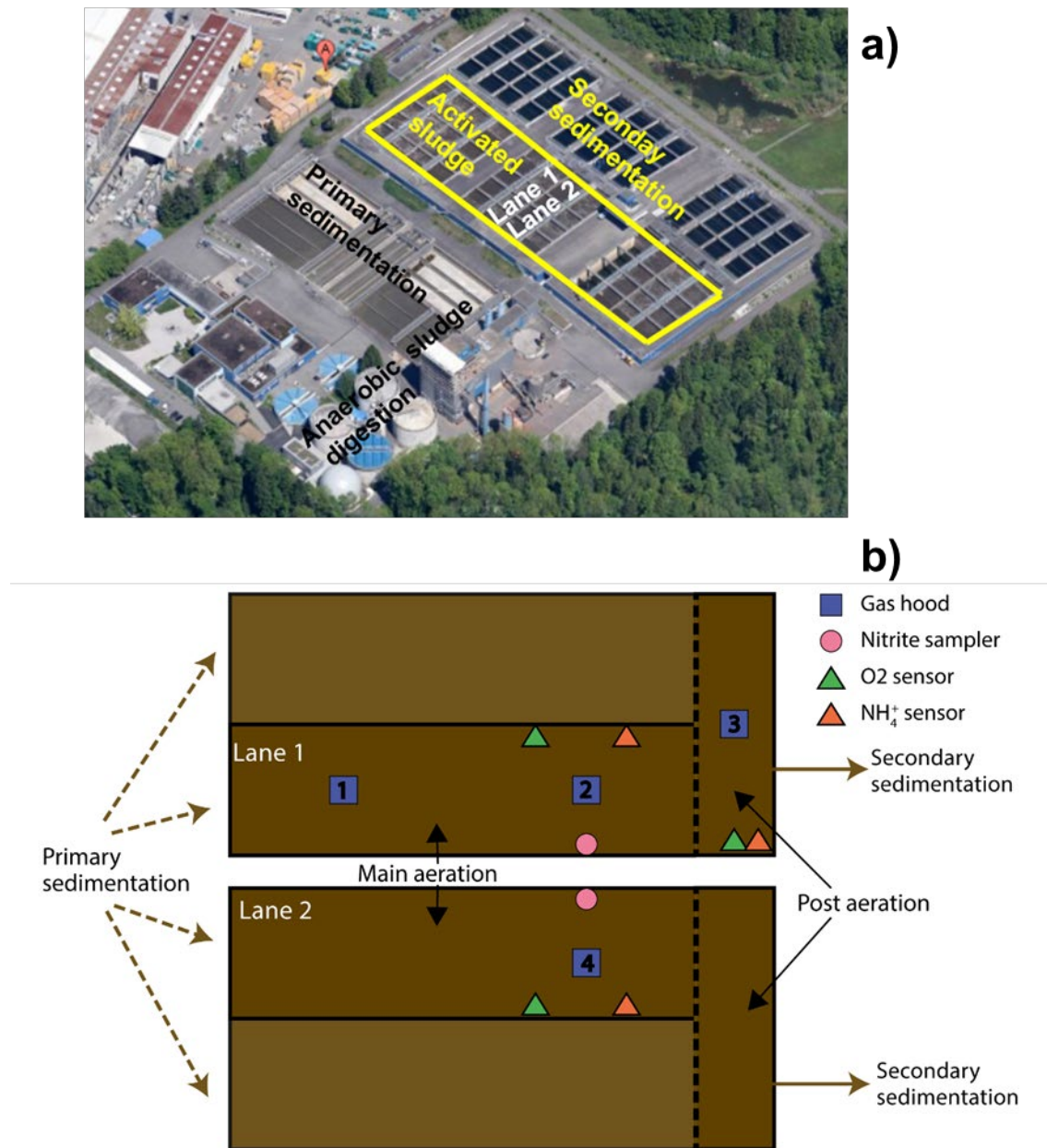


Figure A.1: Plant layout (a) and measurement setup (b) at Lucerne WWTP.



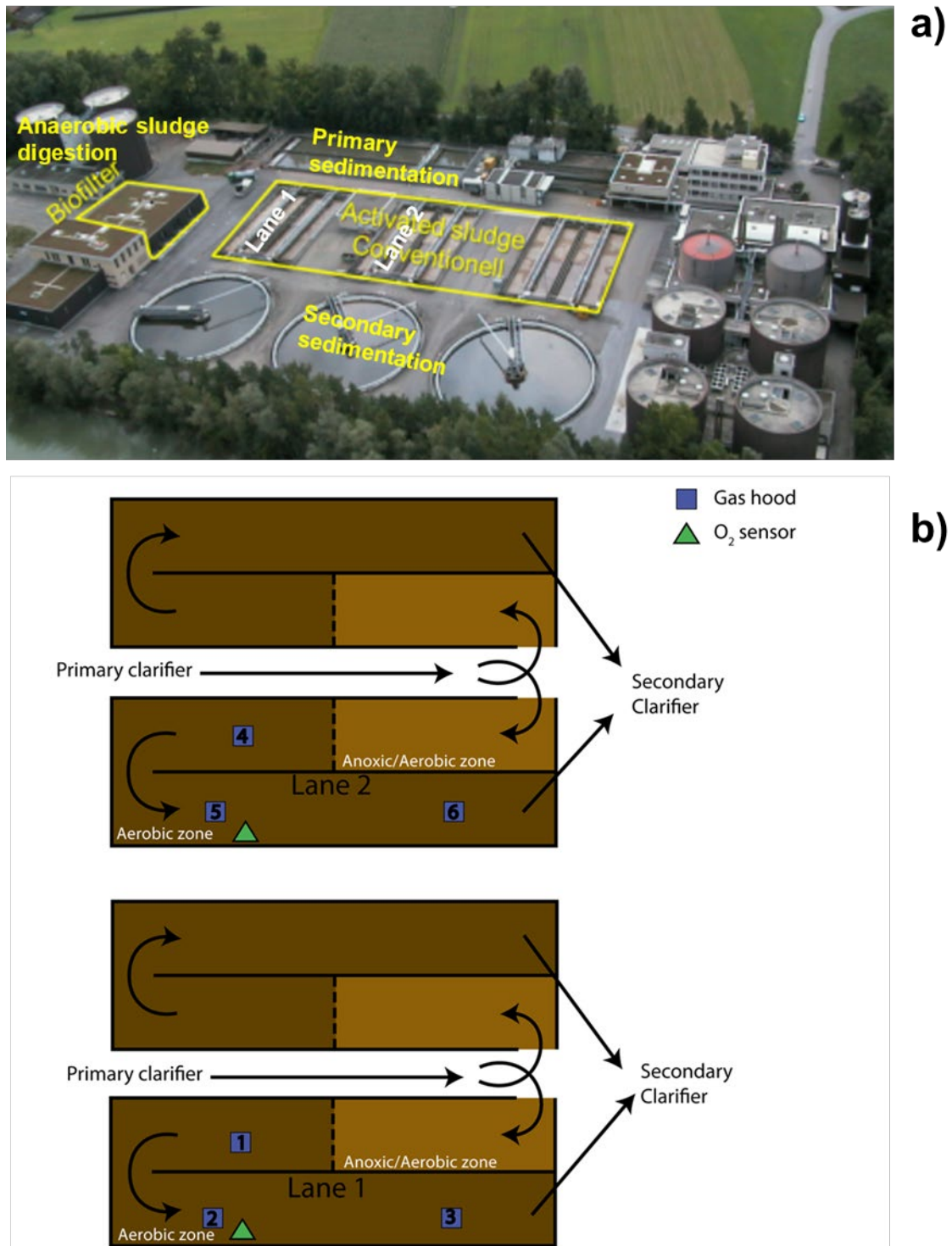


Figure A.2: Plant layout (a) and measurement setup (b) at Altenrhein WWTP.

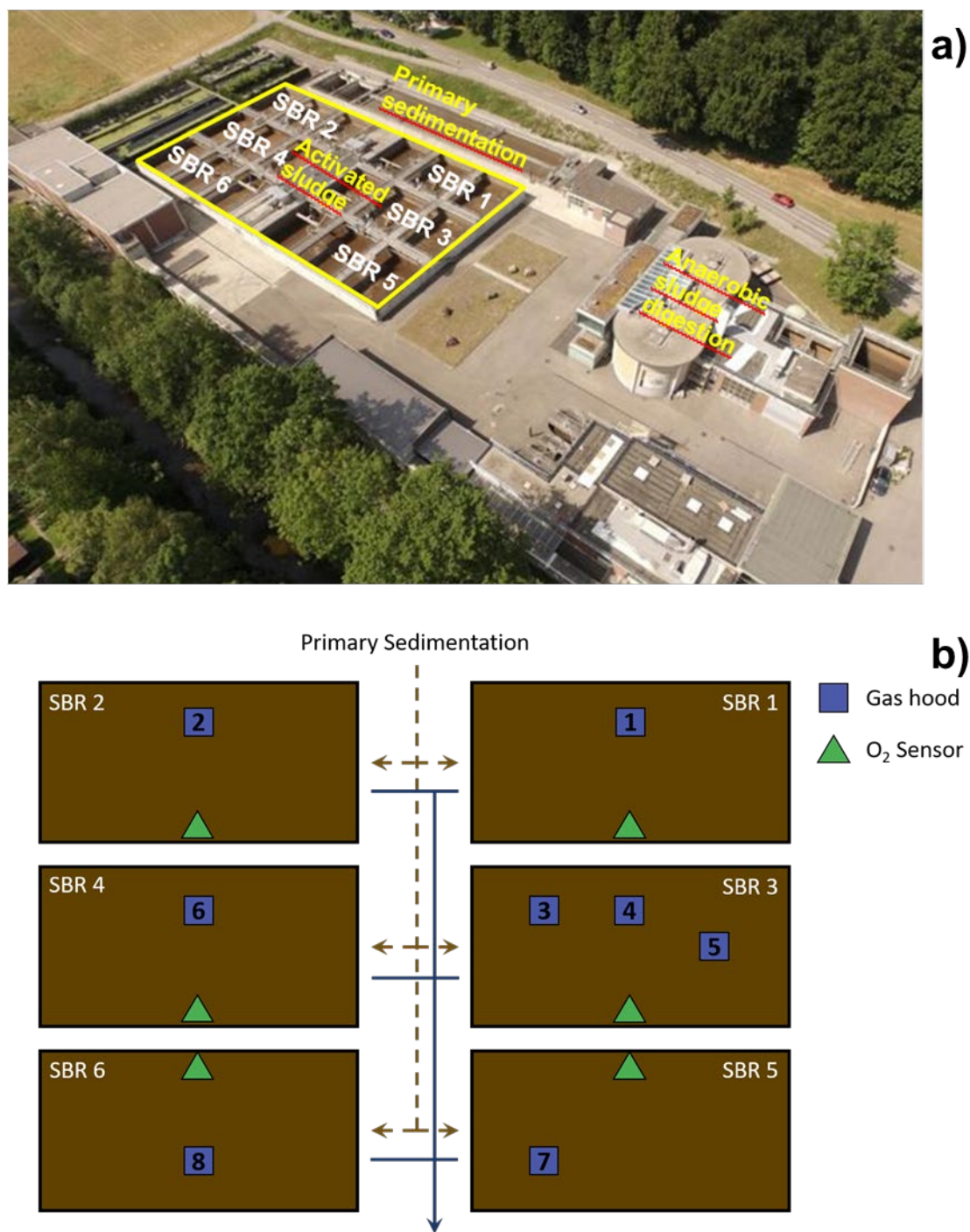


Figure A.3: Plant layout (a) and measurement setup (b) at Uster WWTP.

## A.2 Correlating N<sub>2</sub>O emissions with regression analysis

### A.2.1 Method

Regression was used to analyze the degree to which selected explanatory variables, subsequently called *features*, can explain the N<sub>2</sub>O emissions for three data sets: (1) the Lucerne monitoring campaign, (2) the Altenrhein monitoring campaign, (3) the Kralingseveer monitoring campaign. (Daelman et al. 2015) For each data set, two subsets were analyzed: (1) one data set containing all of the selected features, and (2) one data set containing only the features common to all monitoring campaigns. Finally, a combination normalized by nitrogen load with the common features of all the three monitoring campaigns (Table 1) was analyzed as a joint data set. Two modeling approaches were compared: An ordinary least square linear regression model (Hastie et al. 2009) and the random forest regression model. (Breiman 2001) Overall, this results in a total of four modeling scenarios (linear – all, linear – common, random forest – all, random forest – common) for each monitoring campaign and two modeling scenarios for the joint data set (linear – common, random Forest – common).

Matlab statistical software (Mathworks Inc., Natick, USA) was used to compute two algorithms: the fitlm algorithm for the linear, least-square, model (Mathworks Inc., 2011-2014), and the TreeBagger algorithm for the random forest model (Mathworks Inc., 2011-2014). Both are implemented in the, Statistics and Machine Learning, Toolbox (Mathworks Inc., 2011-2014).

#### *Feature selection and data pre-processing*

The features selected executed according to the current understanding of N<sub>2</sub>O formation processes (Kampschreur et al. 2009b). The selected features for each data set are indicated in Table A.1. For each monitoring campaign two datasets were tested: (1) all features and (2) only common features. For further analyses, the temporal resolution of all input parameters was reduced to daily values. Incorporating daily average measurements in the analysis removed the strong day/night emission variation in order to focus on seasonal emission patterns. Days with missing values were ignored. In total, datasets were prepared with 87 days for the Lucerne campaign, 71 days for Altenrhein, and 61 days for Kralingseveer

campaign. The nitrite measurements at the Kralingseveer campaign were ignored, due to the limited duration of the measurement and the low resulting number of events (10 days).

Table A.1: Features selected for regression modeling in each monitoring campaign (Lu=Lucerne, Alt=Altenrhein, Kra=Kralingseveer): data derived from 24h composite samples taken every 4 to 5 days).

Feature	Abbreviation	Lu	Alt	Kra
Influent	INF	X	X	X
Dissolved oxygen	DO	X	X	X
Total suspended solids	TSS	X	X	X
Temperature	TEMP	X	X	X
COD load in the influent *	COD	X	X	X
C-to-N ration in the influent *	C2N	X	X	X
Nitrogen load in the influent *	Nload	X	X	X
Nitrogen load in effluent *	Neff	X	X	X
NO <sub>3</sub> <sup>-</sup> -N concentration in the reactor	NO3	X		X
NH <sub>4</sub> <sup>+</sup> -N concentration in the reactor	NH4	X		X
Max. NH <sub>4</sub> <sup>+</sup> -N concentration in the reactor	NH4max	X		X
NO <sub>2</sub> <sup>-</sup> -N concentration in the reactor	NO2	X		
Max. NO <sub>2</sub> <sup>-</sup> -N concentration in the reactor	NO2max	X		X
Nitrite load in the effluent *	NO2eff			X
Airflow	AIR	X	X	
Time of aeration	tAIR	X		

#### *Random forest (nonlinear regression)*

Random forest regression was applied according to (Breiman 2001). Random forests are a combination of multiple classification or regression trees. Each decision/regression tree consists of multiple decision nodes at which the value of a given feature is tested and a decision or a value is assigned. In the random forest algorithm, each tree is calibrated for a randomly selected subsample of data, independent of the subsamples of the other trees but with the same distribution for the sample selection. The random selection of predictors ensures less correlation among the trees in the forest, leading to higher accuracy of the predictions (Suchetana et al. 2017). Finally, the ensemble of the all trees votes for a certain decision or output value. The generalization error for random forests converges to a

limit as the number of trees increases (Breiman 2001). The optimal number of trees is lowest number of trees that can achieve the converged generalization error. When the random forest algorithm is used for regression, the response of the random forest is the average response of all trees. Additionally, relative feature importance and goodness of the fit can be estimated in random forest, with the remaining samples not selected in the calibration of a specific tree (out-of-bag samples) (Dürrenmatt and Gujer 2011). Random forest has been applied for feature selection (Suchetana et al. 2017).

### *Bootstrapping*

For both the linear and the random forest modeling approaches the models were calibrated with all the data to characterize the importance of each feature. Bootstrapping was applied for every model scenario in order to assess the model performance and to avoid overfitting the data. Data points were randomly selected in shares of 90% for calibration and 10% for validation of each model. Bootstrapping was performed over 3,000 runs for each model approach to obtain a large number of models. The validation data samples were evaluated with the resulting mean  $R^2$  of all the 3000 runs.

### **A.2.2 Results & discussion**

The random forest algorithm was more accurate than to the linear regression model. The respective averaged coefficient of determination ( $R^2$ ) values from the bootstrapping for each combination of data set (common or all variables) and model (linear or random forest) are shown in Figure A.4. For the Lucerne and Altenrhein datasets, and the dataset combining the variables common to all three datasets (All), the random forest was clearly better, showing that data variations are driven by non-linear processes. Conversely, for the Kralingseveer dataset, none of models performed significantly better, indicating either that non-linear processes play a minor role or that the non-linear effects are not captured in the data available.

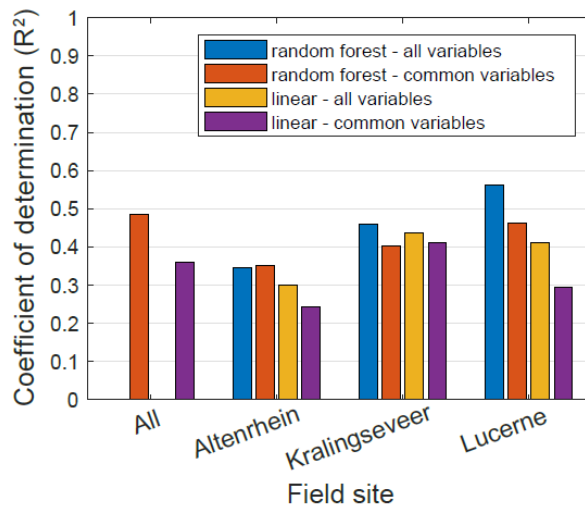


Figure A.4: Mean coefficients of determination ( $R^2$ ) for bootstrapping each modeling scenario with 3000 runs. Samples were randomly selected for calibration (90% of the data) and validation (10% of the data) in each run.

The general predictive power of the modeling approach was relatively low. In the best case (see ,Lucerne,, random forest, all variables), 55% of the variation in the  $N_2O$  emission data could be explained, but only 25% of the variation in the worst case (see Altenrhein, linear, common variables). In another study, standard operational data has been successfully used to detect patterns in the Kralingseveer data set at shorter time-scales of hours and days and for partitioned subsets of data.(Vasilaki et al. 2018). However, we conclude that key drivers governing the high emission variation in the yearly course have not yet been identified. Other features are required to overcome this gap, such as advanced tools from microbiology (Stein 2018), measurement of inorganic carbon (Peng et al. 2016), and monitoring of isotopomers (Ostrom and Ostrom 2017). For example, the measurement of microbial diversity and gene, protein, and metabolites expression has been applied successfully to explain variation of  $N_2O$  emissions at lab-scale (Ge et al. 2018, Perez-Garcia et al. 2014).

The regression analysis highlights the lack of current understanding of the processes that cause the high variation. Similarly, it is questionable whether the seasonal variability shown can be modeled with the current ASM modeling approaches (Ni and Yuan 2015). Such modeling studies have not been conducted, despite the clear results from the Kralingseveer study in 2015, and the Viikinmäki study in 2016. Given the high variability observed and the limited predictive power of the variables

assessed, the identification of new key variables for N<sub>2</sub>O emissions should be based on long-term monitoring campaigns conducted as described above (Ni and Yuan 2015). Short-term experiments should be used to better explain the pathway dynamics and support the long-term campaigns. As long as significant advances in long-term emissions modeling are lacking, monitoring campaigns are necessary to determine emission factors.

### **A.3 Monitoring data**

The monitoring data and the metadata of the monitoring campaigns can be found under the following link: <https://doi.org/10.25678/0003H2>

#### A.4 Daily emission variation

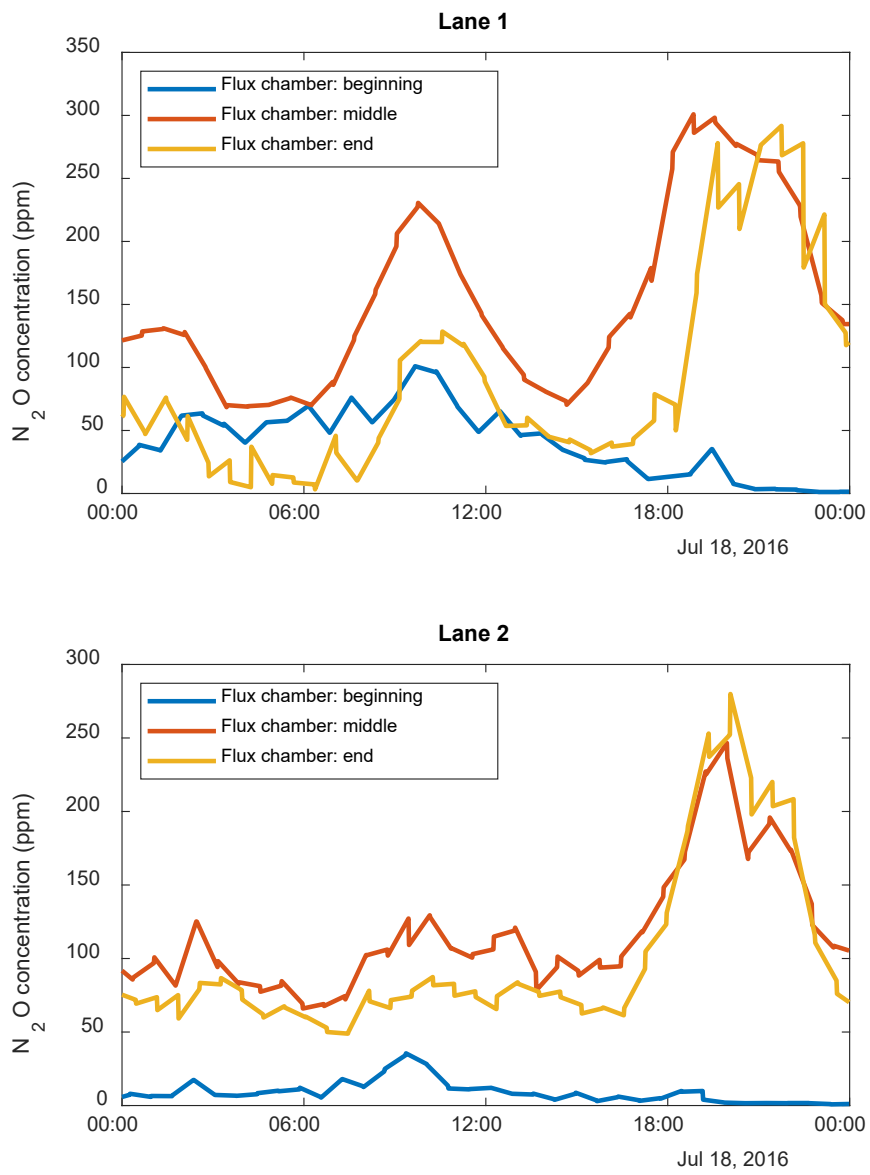


Figure A.5: Typical daily variation profile of  $N_2O$  for one lane at Altenrhein WWTP. The first chamber (beginning) was not aerated.



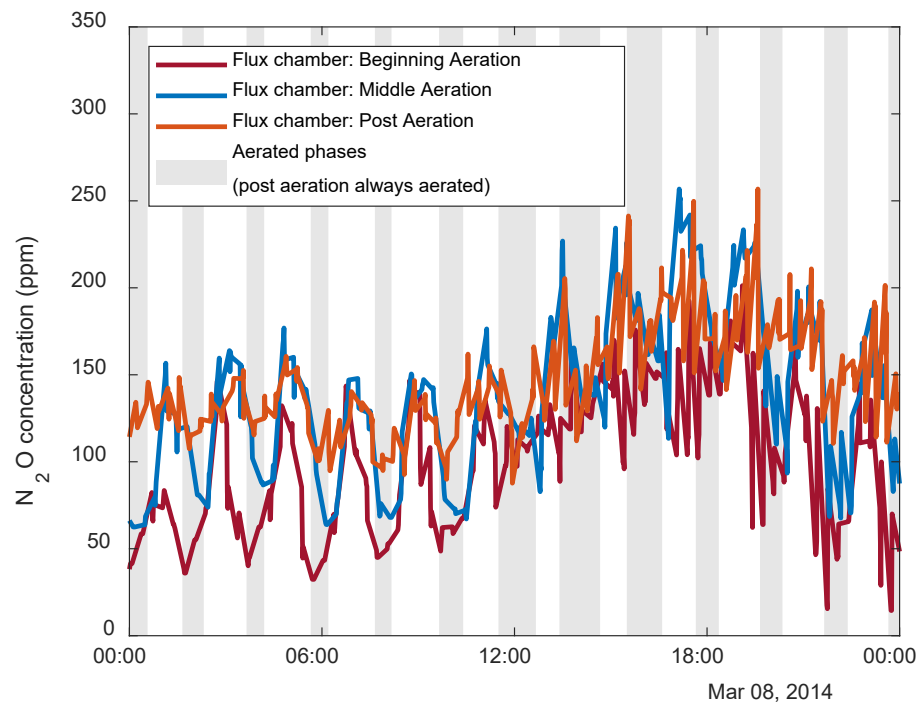


Figure A.6: Typical daily variation profile of N<sub>2</sub>O for different zones on one lane at Lucerne WWTP. Grey areas indicate aerated phases.

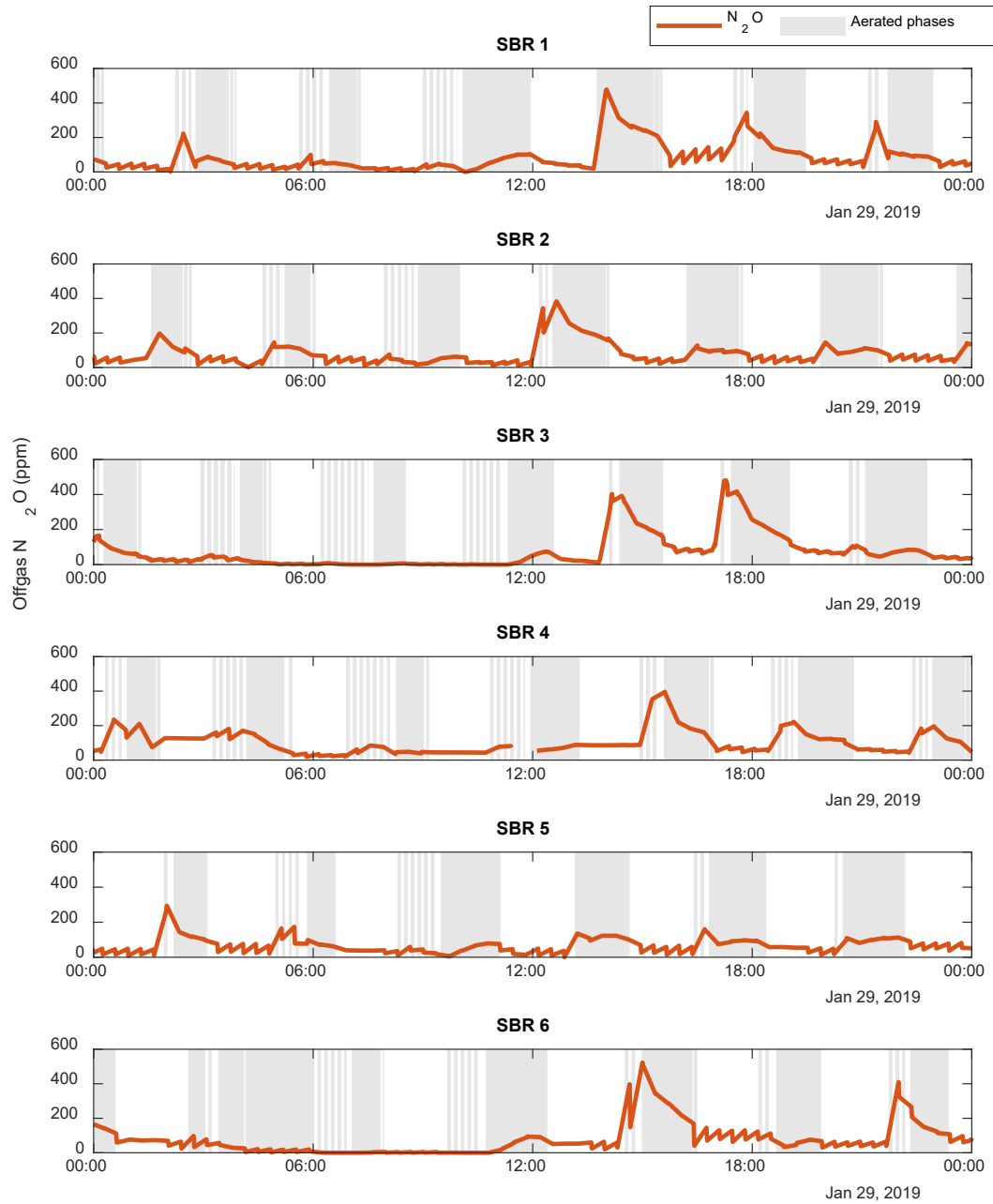


Figure A.7: Typical daily N<sub>2</sub>O variation profile for all SBRs at Uster WWTP. Grey areas indicate aerated phases.

## A.5 Emission factors Uster WWTP

Table A.2: Average over the measurement period of the Emission factor (EF) for each SBR (SBR 1- SBR 6) and the entire WWTP (Uster WWTP).

Treatment Unit	EF [%]
SBR 1	2.3
SBR 2	5.1
SBR 3	1.6
SBR 4	3.3
SBR 5	2.8
SBR 6	3.6
Uster WWTP	2.4

## **B Supplementary Information: Chapter 5**

### **B.1 Monitoring setup**

The description is further described in Chapter 3 and is available from the Eawag Research Data Institutional Collection (Eric) at <https://doi.org/10.25678/0003WD>

The description contains the following elements:

- Description of the system (A)
- P&ID of the system (B)
- Wiring diagram (C)
- Components list (D)
- Software for control and operation (E)
- Technical drawings (F)

### **B.2 Monitoring campaigns**

For each of the newly conducted monitoring campaigns, three files are attached as Supporting information: 1) a data sheet (*Data\_sheet\_WWTP.pdf*) with information on the biological treatment, on the origin of the lab data, and on the monitoring campaign, 2) a lab data sheet (*WWTP\_DATA\_Lab.csv*) with measured or estimated influent and effluent loads for total nitrogen and COD, and 3) a data sheet (*WWTP\_DATA\_Emissions.csv*) with highly resolved N<sub>2</sub>O emissions.

The three types of data files described exist for the monitoring campaigns on the following WWTP:

- Bazenheid
- Birs
- Giubiasco
- Hofen
- Moossee

- Schönauf
- Werdhölzli

The monitoring data is available from the Eawag Research Data Institutional Collection (Eric) at <https://doi.org/10.25678/0003XE>.

### B.3 Data availability

Table B.1: Duration of monitoring campaigns and number of days evaluated (i.e. availability of the monitoring device).

WWTP (location name)	Duration of the campaign (days)	Days evaluated (share of total)
Bazenheid	492	389 (80%)
Birs	369	236 (64%)
Giubiasco	382	325 (85%)
Hofen	419	375 (90%)
Moossee	248	345 (72%)
Schönauf	312	348 (90%)
Werdhölzli	632	728 (87%)

### B.4 Nitrogen mass balance of a primary clarifier

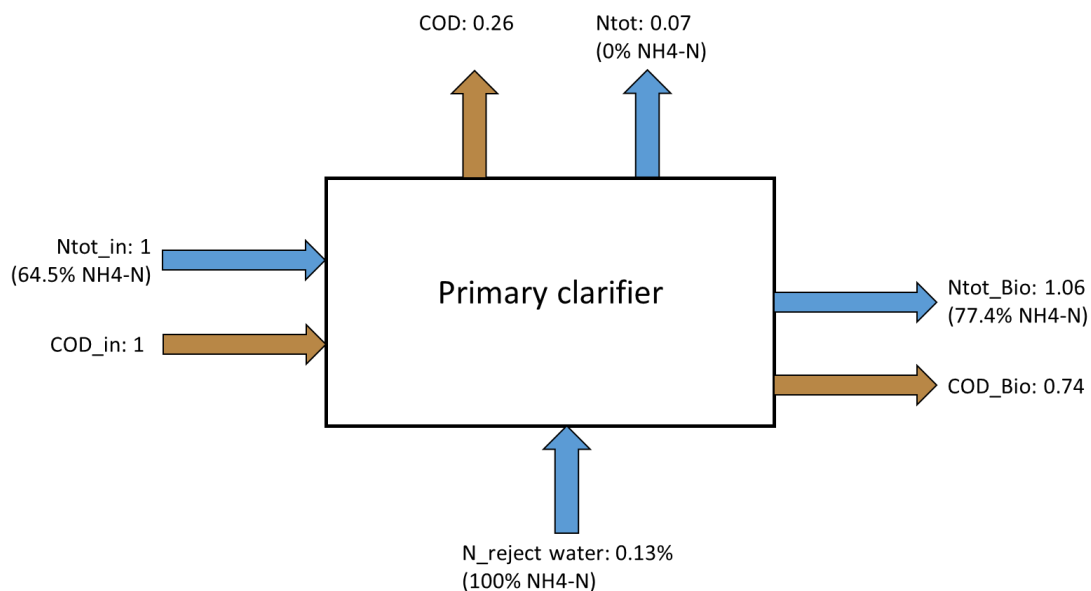


Figure B.1: Nitrogen and COD mass balance of model primary clarifier based on (Gujer 2007) to estimate missing values for monitoring campaigns. Numeric values denote fractions of  $N_{tot\_in}$  and  $COD\_in$ . Percentage values below nitrogen values denote ammonium share of total nitrogen.

## **B.5 Meta Data for correlation analysis**

Data on the monitoring campaigns on all monitoring campaigns analyzed and corresponding data for correlation analysis is shown in Table B.2, Table B.3, and Table B.4.

Table B.2: Design characteristics of the WWTP

Name	Vikinn-	Avedøre	Kralings.	Hofen	Birs	Schoenau	Moossee	Giubia.	Bazen.	Uster	Altenr. FB	Altenr. AS	Zurich	Luzern
Design load (PE)	840000	350000	360000	50000	150000	180000	50000	100000	50000	48000	120000	120000	670000	280000
Nutrient removal goal (-)	N	N	N	N	N	N	N	C	N	NH4	N	NH4	N	N
Process biological treatment (-)	A2O	CARR	CARR	AO	SBR	A2O	AO	CAS	IFAS	SBR	FB	CAS	A/I	A/I
Biomass type (-)	AS	AS	AS	AS	AS	AS	AS	AS	BF	AS	BF	AS	AS	AS
Volume biological treatment (m3)	92000	-	39100	9000	40500	27900	8940	6200	4112	17472	1512	8220	60000	36000
Volume secondary clarification (m3)	120600	-	-	9900	0	17000	4480	1904	0	0	0	7500	72000	38400
Total volume biological treatment (m3)	212600	-	-	18900	40500	44900	13420	8104	4112	17472	1512	15720	132000	74400
Average TS biological treatment (gTS/l)	3	2.3	4.1	4.42	3.04	3.19	3.02	2.4	-	2.41	-	2.45	3.2	2.65
Aerobic SRT (d)	7	-	-	12.78	15.4	13.9	14.3	6	5.3	12.3	-	10	5.2	5.2
SRT (d)	-	-	-	19	25	19.5	21.7	6	5.3	23	-	10	10	12
Supernatant treatment	No	-	No	No	No	Yes	No	No	Yes	No	No	No	Yes	No
Co-digestion (-)	-	-	-	No	No	No	No	No	Yes	No	Yes	Yes	Yes	No
Sandfilter (-)	-	-	No	No	No	Yes	No	No	No	Yes	Yes	Yes	No	No

Table B.3: Inflow and effluent characteristics (Q = water flow, COD = chemical oxygen demand, TN = total nitrogen, NO<sub>2</sub>-N = nitrite load; in = influent, out = effluent, in\_bio = after primary clarifier, out\_bio = effluent biological treatment) WWTP

Name	Vilkinmäki	Avedøre	Kralings.	Hofen	Birs	Schoenau	Moossee	Bellinzona	Bazenheid	Uster	Altenrhein FB	Altenrhein AS	Zürich	Luzern
Q_in (m <sup>3</sup> /d)	289900	70000	88000	25387	24,029	56,099	13,177	17,559	4,897	15,973	8,816	18,436	172,699	93,942
COD_in (kg/d)	176,839	-	25327	8,788	12,090	20,622	4,705	8,996	2,200	4,519	3,804	8,816	81,078	16,783
COD_out (kg/d)	12400	-	3380	493	165	731	251	478	-	249	-	-	3181	1576
TN_in WW (kg/d)	12877	-	3216	825	812	1997	453	714	216	527	231	462	6869	2554
TN_in_DigSup (kg/d)	-	-	-	187	250	0	75	83.1	224	170	85	170	0	349
TN_tot (kg/d)	-	-	-	1012	1062	1997	528	797	440	697	316	632	6869	2903
TN_in_bio (kg/d)	14495	-	3375	959	994	1866	499	692	440	580	336	673	6311	2747
TN_out_bio (kg/d)	-	-	963	279	156	691	167	446	169	229	151	398	1456	620
TN_out_WWT P	1160	308	963	297	156	691	167	446	169	205	151	398	1456	620
NO <sub>2</sub> -N out_bio (kg/d)	-	-	24	2	2	-	1	21	1	11	-	-	37	9



Table B.4: Correlation Data for Pearson's correlation analysis

Name	Viikinkaiki	Avedøre	Kralings.	Hofen	Birs	Schoenau	Moossee	Bellinzona	Bazenheid	Uster	Altenr.F B	Altenr. AS	Zurich	Luzern
N2O EF (%)	1.9	1.1	2.9	0.1	0.2	0.3	0.1	8.0	1.4	2.4	1.4	1.7	0.3	1
Average load	1207917	350000	281250	79917	82833	155500	41583	57667	36667	48333	28000	56083	525917	228917
Specific reactor volume	14.7	-	-	19.7	40.7	24.1	26.9	11.7	9.3	30.1	4.5	23.4	20.9	27.1
Nitrogen removal efficiency	60.0	85.0	81.0	72.4	85.3	65.4	66.5	35.5	61.6	60.5	52.2	52.4	78.8	78.6
C to N ratio (C2N)	12.2	-	7.5	9.2	12.2	11.1	9.4	13.0	5.0	7.8	11.3	13.1	12.8	6.1
(kgO2/kgN)	-	-	0.7	0.2	0.2	-	0.2	3.0	0.2	1.9	-	-	0.6	0.3
Nitrite in effluent as share of	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Specific nitrogen loading of	0.053	-	0.021	0.024	0.008	0.021	0.018	0.047	-	0.014	-	0.033	0.033	0.029
Aerobic SRT biological	7.0	-	-	12.8	15.4	13.9	14.3	6.0	5.3	12.3	-	10	5.2	-
WWTP utilization (Average)	175.4	-	58.6	146.5	67.2	95.5	78.4	75.0	36.7	78.5	63.4	105.0	100.8	49.9

## B.6 Countrywide extrapolation of N<sub>2</sub>O emissions and uncertainties

Table B.5: Monitoring campaigns and emission factors applied in the 2019 IPCC guidelines (<sup>1</sup> AO; Anaerobic-oxic activated sludge process, A2O; Anaerobic-anoxic-oxic activated sludge process, SBR; Sequencing batch reactor, OD; Oxidation ditch, IA; Intermittent aeration process, EA; Extended aeration process, CAS; Conventional activated sludge process, MLE; Modified Ludzack-Ettinger. BNR: Biological nutrient removal)

Type of treatment process <sup>1</sup>	Categories	References	N <sub>2</sub> O emission factor (kg N <sub>2</sub> O-N/kg N)	Monitoring strategy (sampling, duration)
AO	BNR	(Daelman et al. 2015)	0.028	Continuous, long-term
AO	BNR	(Foley et al. 2010)	0.021	Grab, short-term
AO	BNR	(Foley et al. 2010)	0.045	Grab, short-term
A2O	BNR	(Foley et al. 2010)	0.013	Grab, short-term
SBR	BNR	(Foley et al. 2010)	0.023	Grab, short-term
OD	BNR	(Foley et al. 2010)	0.0080	Grab, short-term
IA	BNR	(Kimochi et al. 1998)	0.0005	Grab, short-term
EA	BNR	(Foley et al. 2010)	0.015	Grab, short-term
A2O	BNR	(Wang et al. 2016)	0.013	Grab, short-term
CAS	BNR	(Aboobakar et al. 2013)	0.00036	Continuous, short-term
AO	BNR	(Rodriguez-Caballero et al. 2014a)	0.12	Continuous, short-term
OD	BNR	(Masuda et al. 2018)	0.00016	Grab, short-term
AO	BNR	(Masuda et al. 2018)	0.0013	Grab, short-term
AO	BNR	(Masuda et al. 2018)	0.0049	Grab, short-term
Separate-stage BNR	BNR	(Ahn et al. 2010)	0.00019	Grab, short-term
Bardenpho	BNR	(Ahn et al. 2010)	0.0036	Grab, short-term
Step-feed BNR	BNR	(Ahn et al. 2010)	0.011	Grab, short-term
MLE	BNR	(Ahn et al. 2010)	0.0007	Grab, short-term
MLE	BNR	(Ahn et al. 2010)	0.0006	Grab, short-term
OD	BNR	(Ahn et al. 2010)	0.0003	Grab, short-term
Step-feed BNR	BNR	(Ahn et al. 2010)	0.015	Grab, short-term
Step feed, plug flow	BNR	(Ni et al. 2015, Pan et al. 2016)	0.019	Continuous, short-term
SBR	BNR	(Bao et al. 2016)	0.029	Grab, short-term
SBR	BNR	(Rodriguez-Caballero et al. 2015)	0.038	Continuous, short-term
Plug flow	Non-BNR	(Ahn et al. 2010)	0.004	Grab, short-term
Plug flow	Non-BNR	(Ahn et al. 2010)	0.0062	Grab, short-term
Step-feed non-BNR	Non-BNR	(Ahn et al. 2010)	0.0018	Grab, short-term
Plug flow	Non-BNR	(Masuda et al. 2015)	0.023	Grab, short-term
AO	Non-BNR	(Bao et al. 2016)	0.013	Grab, short-term
IA	Non-BNR	(Mello et al. 2013)	0.0016	Grab, short-term

## B.7 Nitrogen loads to Swiss WWTPs

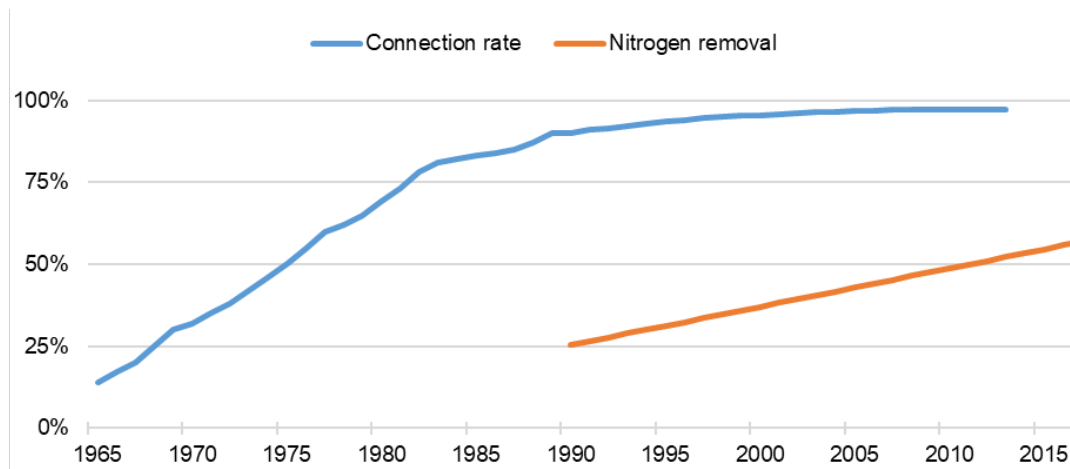


Figure B.2: Connection rate of the Swiss population to WWTP over time and interpolated nitrogen removal.

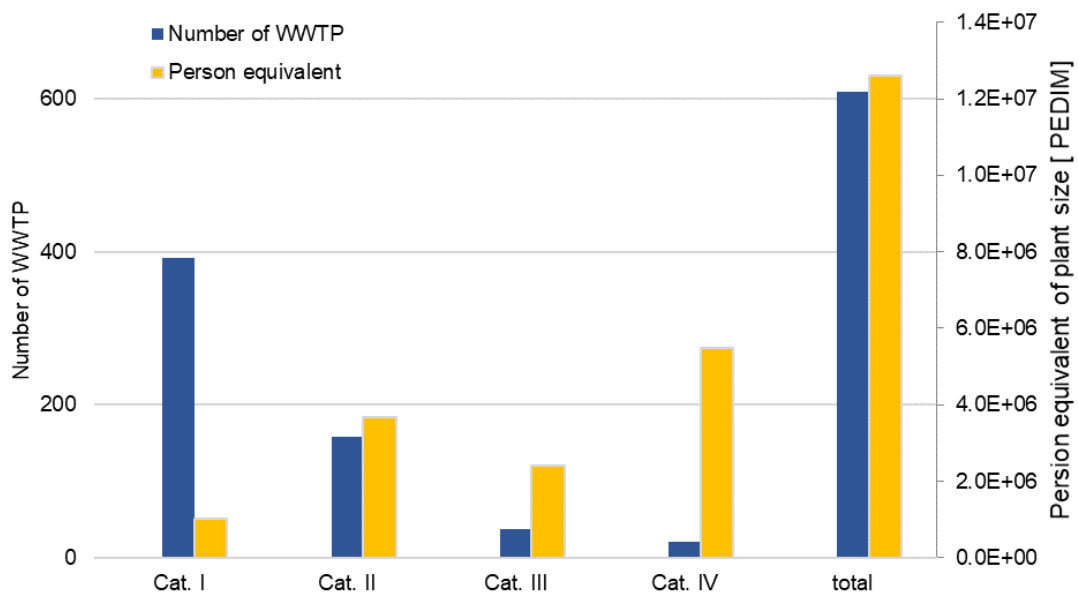


Figure B.3: Size distribution of Swiss WWTPs in the year 2011 with respect to number of plants and persons treated. Cat. I: <50'000 PE; Cat. II: 50'000 - 200'000 PE; Cat. III: 200'000 to 500'000 PE; Cat. IV: >500'000 PE.

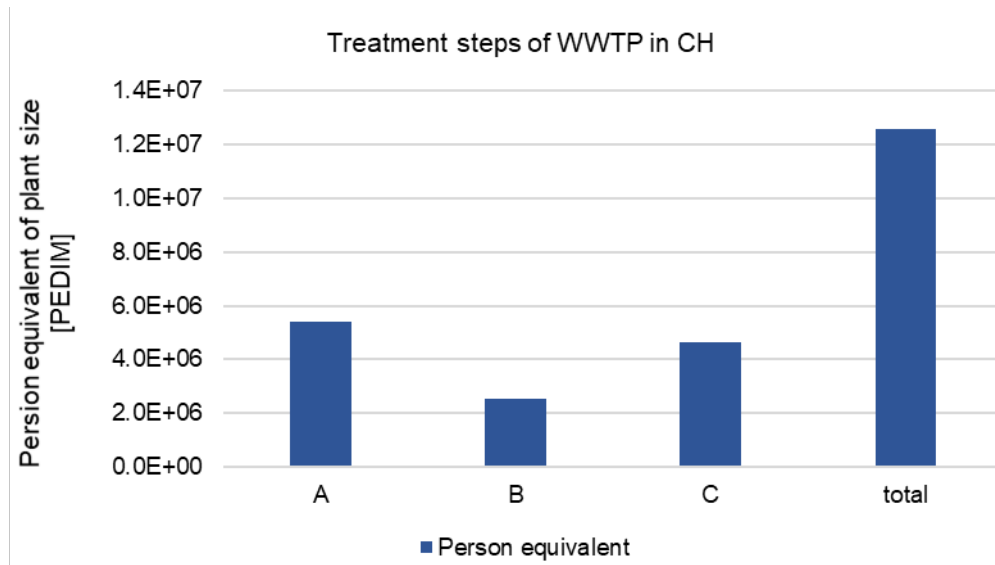


Figure B.4: Amount of person equivalents treated in Swiss WWTPs in 2011 depending on the treatment goal: A = carbon removal; B=nitrification without requirement to denitrify all year; C=nitrify and denitrify all year.

## C Supplementary Information: Chapter 6

### C.1 Influent characteristics, SBR design, and operation

Table C.1: Influent characteristics after primary clarifier

Parameter	Yearly average
Wastewater load	16,000 m <sup>3</sup> /d $\pm$ 7,700
COD	3,360 kgCOD/d $\pm$ 1,200
N <sub>tot</sub>	580 kgN/d $\pm$ 226
NH <sub>4</sub> <sup>+</sup>	391 kgNH <sub>4</sub> <sup>+</sup> -N/d $\pm$ 111
P	56 kgP/d $\pm$ 19

With a total volume of  $6 \times 3,000 \text{ m}^3 = 18,000 \text{ m}^3$ , the WWTP has a design volumetric loading of  $0.2 \text{ kgCOD/m}^3/\text{d}$ , that is in the lower range of typical SBR design (Table 8-19, in (Tchobanoglous et al. 2014)).

Table C.2: Reactor operation conditions

Parameter	Yearly average	Control
pH	$7 \pm 0.14$	Not controlled
O <sub>2</sub>	$2.2 \pm 0.4 \text{ mgO}_2/\text{l}$	Set-point during aeration
SRT	10 d	Set via excess sludge
Temperature	$15.8 \pm 3.5^\circ\text{C}$	Not controlled

Table C.3: Rules for dynamic cycle operation

Phase	Rules	Average time
Filling	Time set by the operators. Calculated based on current settling velocity and nitrification performance.	$45 \pm 20$ min
Pre-denitrification	Fixed time before aeration (0 – 60 min)	$30 \pm 25$ min
Nitrification	If <ol style="list-style-type: none"> <li>1. predefined minimal nitrification time is reached</li> <li>2. <math>\text{NH}_4^+</math> concentration in the reactor &lt; set value (&lt; 0.3 – 0.5 mg/l)</li> <li>3. blowers on lowest power consumption levels</li> <li>4. predefined post-aeration passed (20 – 0 min)</li> </ol> aeration is stopped.	$60 \pm 40$ min
Sedimentation	Depending on the fill level and sedimentation velocity, calculated dynamically. Sedimentation velocity is calculated after each cycle by measuring the time between the start of the sedimentation and when the decanting unit equipped with a TS sensor reaches the sludge bed for the first time. The height between the fill level and the sludge bed divided by the duration result in the settling velocity.	$45 \pm 20$ min
Decanting	Stopped when a defined fill-level is reached. Interrupted when sludge bed is reached and continued after a short interval.	$45 \pm 20$ min

Table C.4: Sensor types &amp; test kits used for variable measurement

Variable	Sensor	Control measurement
ISE Ammonium & Nitrate concentration (R2,4,6)	ISE CAS 0D \$ (Endress & Hauser)	Weekly to bi-weekly Average deviation compared to lab value: +0.15 mg NH <sub>4</sub> <sup>+</sup> -N/L & -0.43 NO <sub>3</sub> <sup>-</sup> -N/L
ISE Ammonium & Nitrate concentration (R1,3,5)	AN-ISE SC (HACH LANGE)	Weekly to bi-weekly Average deviation compared to lab value: +0.27 mg NH <sub>4</sub> <sup>+</sup> -N/L & -0.13 NO <sub>3</sub> <sup>-</sup> -N/L
O <sub>2</sub> concentration (R1-6)	Oxysafe (SWAN)	Weekly to bi-weekly
pH value	Smartpath PH 8320 (KROHNE)	Weekly to bi-weekly
TS concentration (R1,3,5)	CUS 51D (Endress & Hauser)	Weekly to bi-weekly
TS concentration (R2,4,6)	Turbimax CUS 65 (Endress und Hauser)	Weekly to bi-weekly
UV/Vis Nitrate & Nitrate concentration (R1-6)	OPUS -UV-01-VA— D-CTrios (Ensola)	Weekly to bi-weekly
Variable	Test	
N <sub>tot</sub> concentration (effluent)	LCK 238 (HACH LANGE)	
Ammonium concentration (effluent)	LCK 304 (HACH LANGE)	
Nitrite concentration (effluent)	LCK 341 (HACH LANGE)	
Snellen transparency	Sample water is stepwise filled in a water column with a high contrast image below. Evaluate until which column depth the image at the bottom still is visible. The maximum value (= transparent sample water) of the method is 60 cm.	

## C.2 Sequence analysis

Raw sequences from both sequence runs were analyzed within the QIIME2 framework (Caporaso et al. 2010). When necessary (only 1st campaign sequences), primer sequences were removed with the cutadapt QIIME2 plugin. Subsequently, low-quality reads were filtered out and all high-quality reads were analyzed individually with the DADA2 software (Callahan et al. 2016) to produce amplicon sequencing variants (ASV) based on Illumina Miseq/Hiseq error profiles. Taxonomic assignment of the ASVs was performed within QIIME2 environment with a trained naive Bayesian classifier based on the Microbial Database for Activated Sludge (MiDAS3, (Nierychlo et al. 2020)). After filtering of unclassified and contaminated ASVs, the resulting sequence table consisted of 4,145 ASVs from the 1st campaign and 2,264 ASVs from the 2nd campaign, which can be explained by the large differences in number of raw sequences. All subsequent biostatistics analysis were performed individually on the sequence tables (reference provided at the end of the manuscript), derived from the analysis pipeline.

After normalization based on the variance stabilization algorithm within DESEQ2 (Love et al. 2014), we performed a non-metric multidimensional scaling (nMDS) analysis on Bray–Curtis dissimilarity matrices served to visualize patterns of community composition, and PERMANOVA tested for differences among communities over time for each data set using vegan and R software (Oksanen et al. 2007, R-Core-Team 2020). Based on a hierarchic clustering approach (vegdist function, vegan, R) applied on the dissimilarities in community composition, we were able to statistically divide the samples from all reactors into different clusters within each campaign. While community dissimilarities in campaign 1 were statistically most robust when explained by 5 clusters (A, B, C, D, E), campaign 2 could be divided into 4 Clusters (X, Y $\alpha$ , Y $\beta$ , Z). We calculated species richness and evenness parameters for these clusters and between the investigated reactors. Diversity indices for all samples and clusters were calculated within the R environment using the vegan package.

We used Deseq2 (Love et al. 2014) to assess the most significant changes in abundance of ASVs, contributing to the observed community dissimilarities between



the clusters. Furthermore, we assigned, if possible, ASVs to their putative functional role in the wastewater treatment plant based on the Global Database of Microbes in Wastewater Treatment Systems and Anaerobic Digesters (MIDAS) (Nierychlo et al. 2020). Changes in abundance are expressed in log2foldchange between the clusters (Figure S13). The relationship between bacterial functional groups and numerical environmental variables was assessed with a Pearson correlation and plotted into a heatmap (Figure S14).

### C.3 Process data

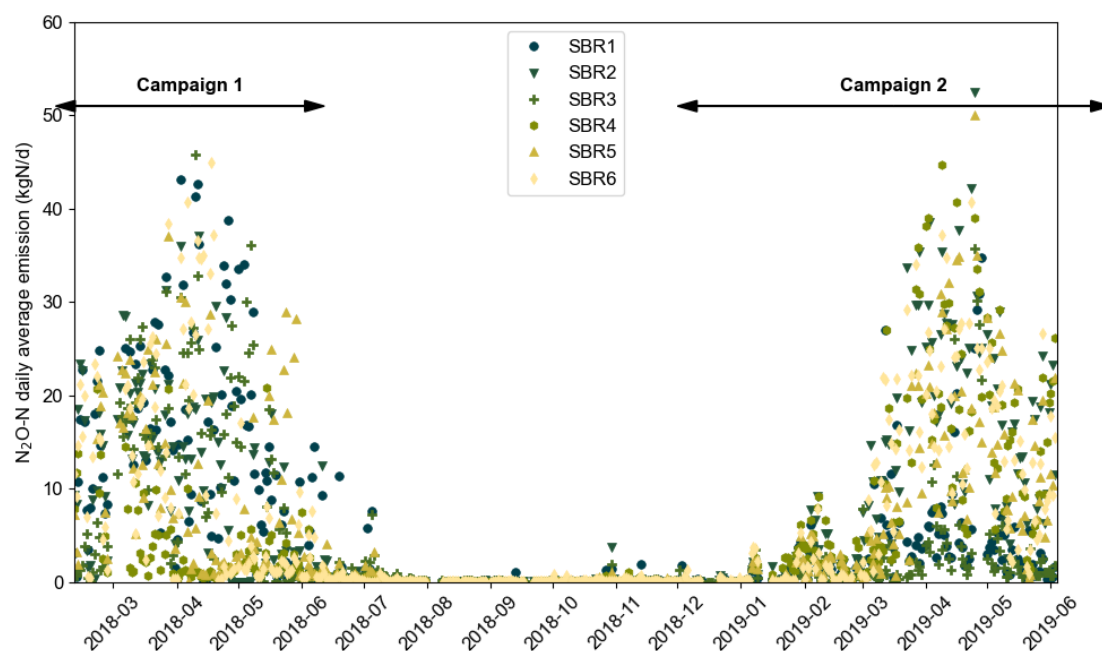


Figure C.1: N<sub>2</sub>O emission of each reactor.

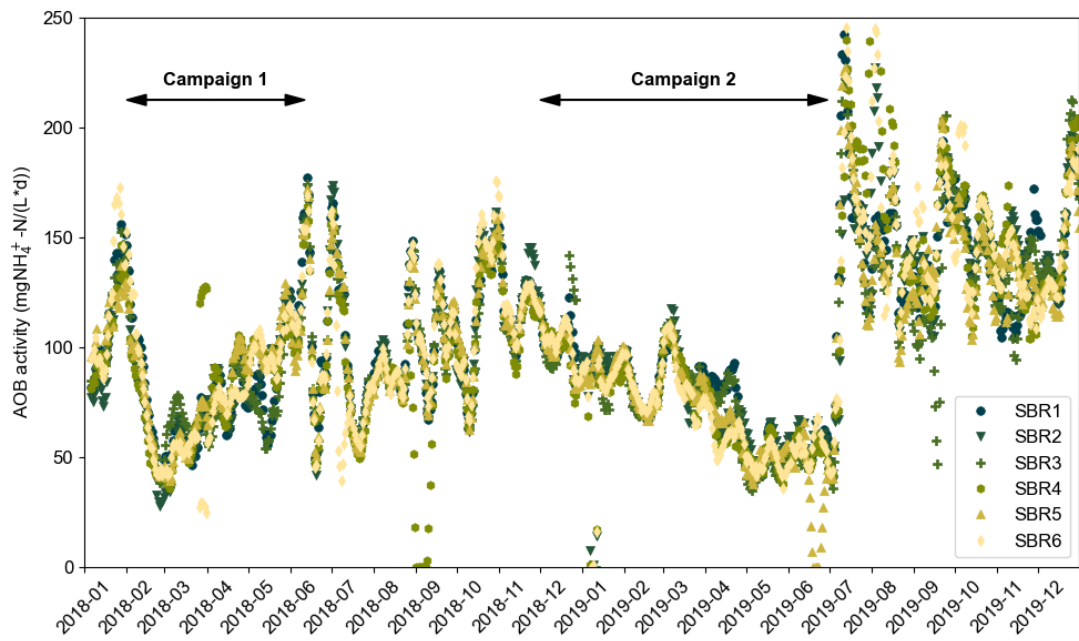


Figure C.2: Daily average AOB activity of each reactor during aeration, estimated based on online data: Concentration at the beginning of the cycle minus minimum concentration divided by aeration time.

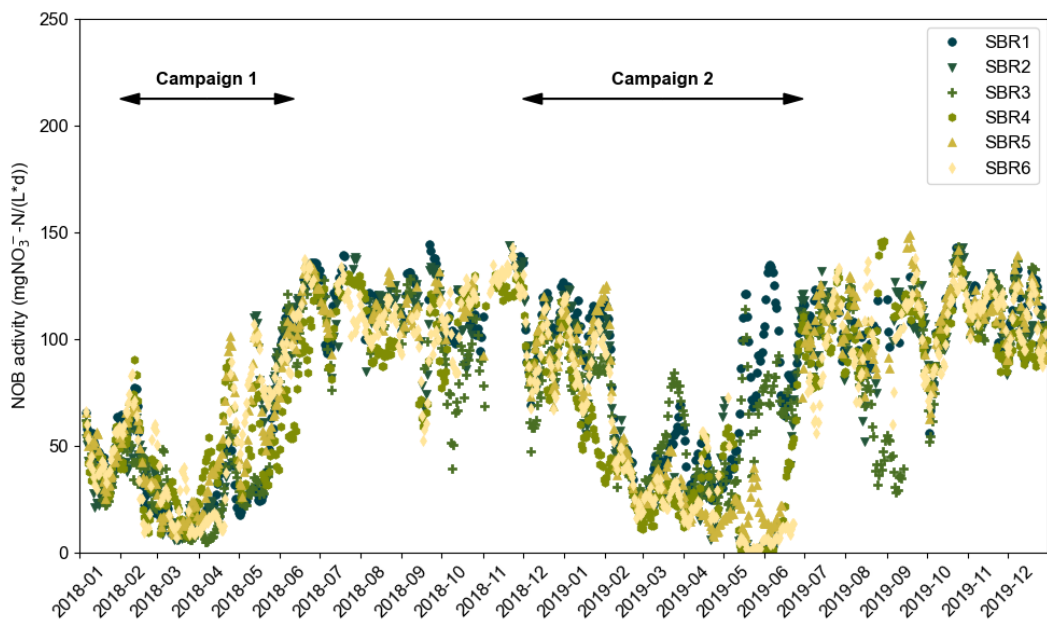


Figure C.3: Daily average NOB activity of each reactor during aeration, estimated based on online data: Concentration at the beginning of the cycle minus minimum concentration divided by aeration time.

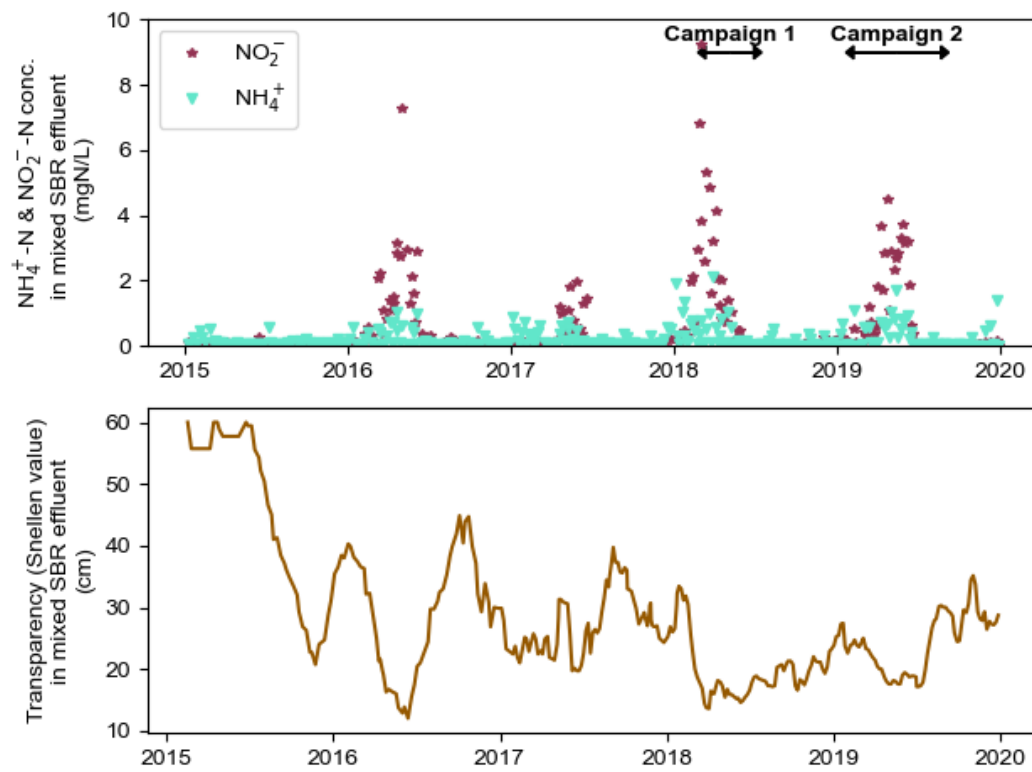


Figure C.4: Effluent concentration of  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_2^-\text{-N}$  and transparency value (Snellen) in 2018 and 2019, measured in 24-h composite samples.

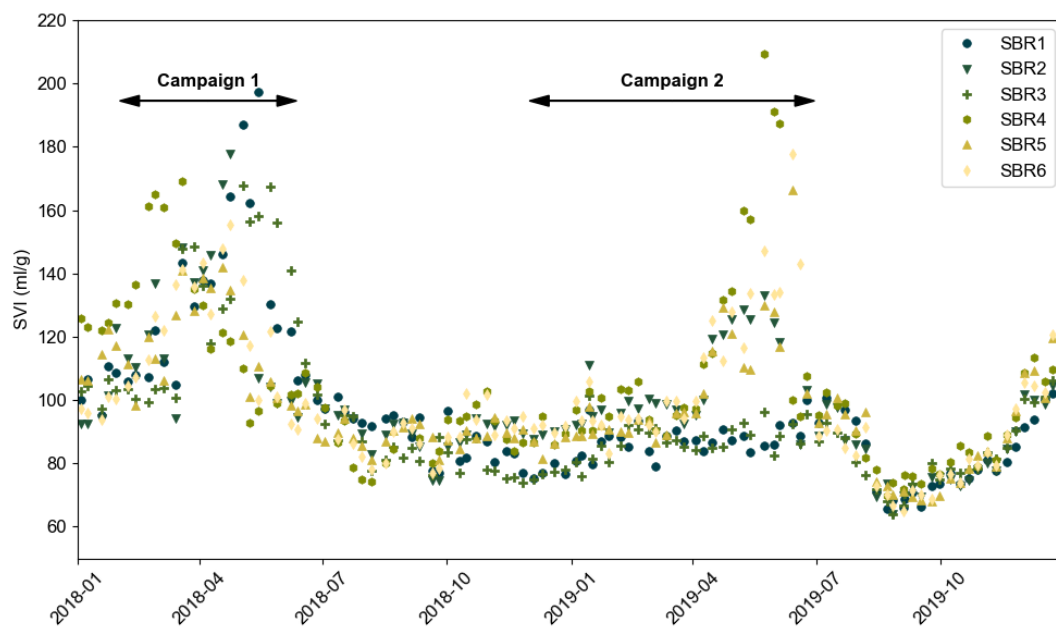


Figure C.5: Sludge volume index (SVI) of each reactor.

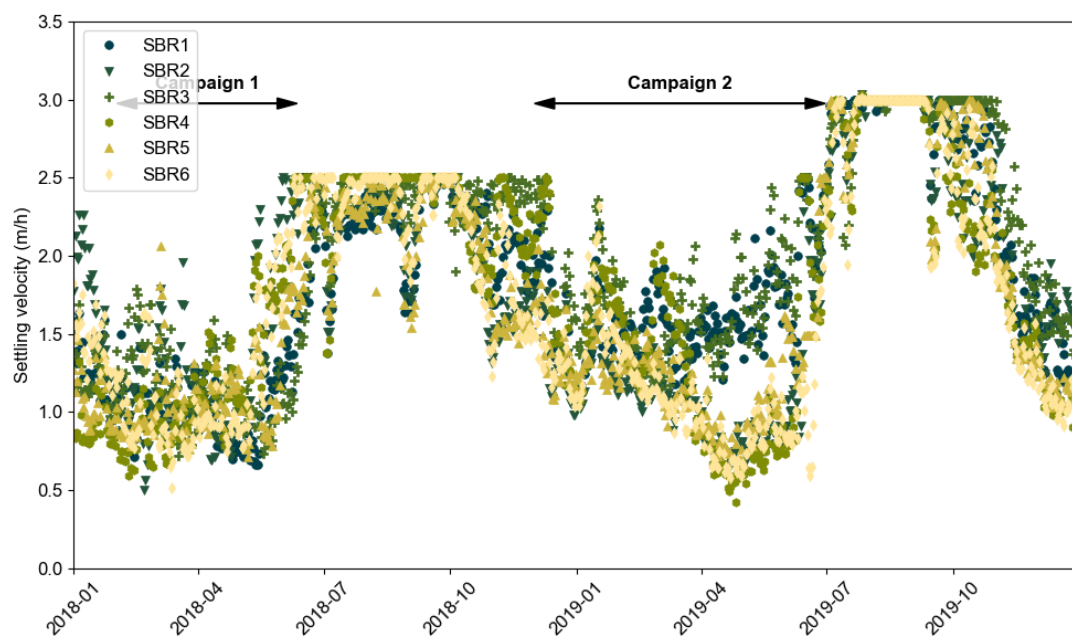


Figure C.6: Daily average settling velocity of each reactor. The method allowed to measure maximally 2.5 and 3.0 m/h in 2018 respectively 2019.

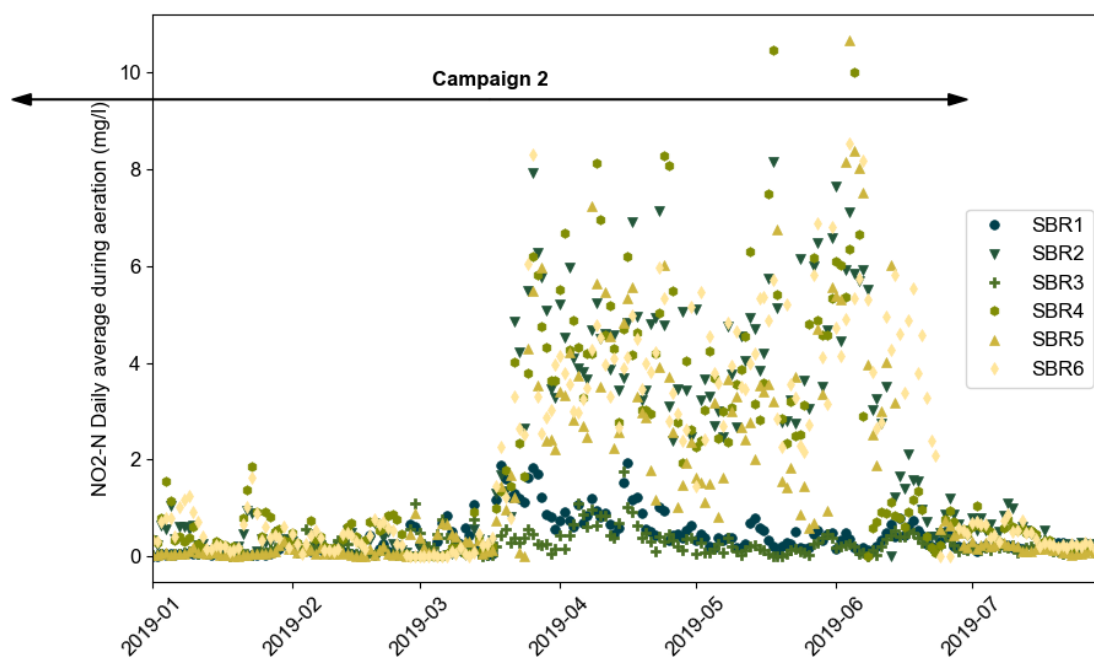


Figure C.7: Daily average nitrite concentration during aeration of each reactor based on online measurement.

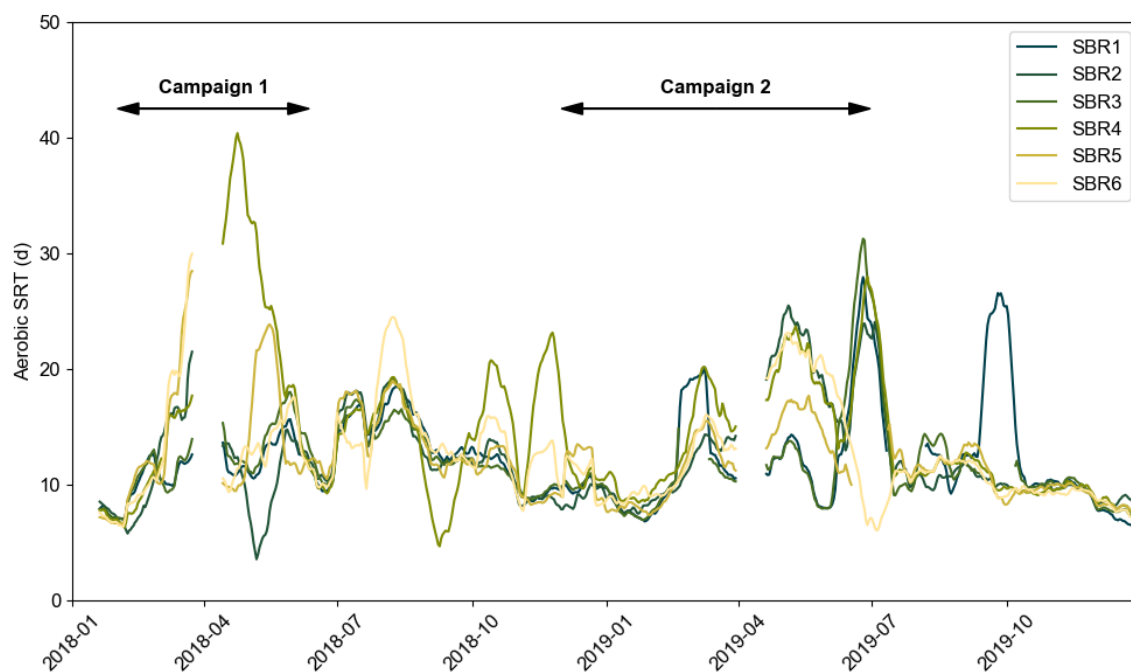


Figure C.8: Average aerobic sludge age (SRT) of each reactor.

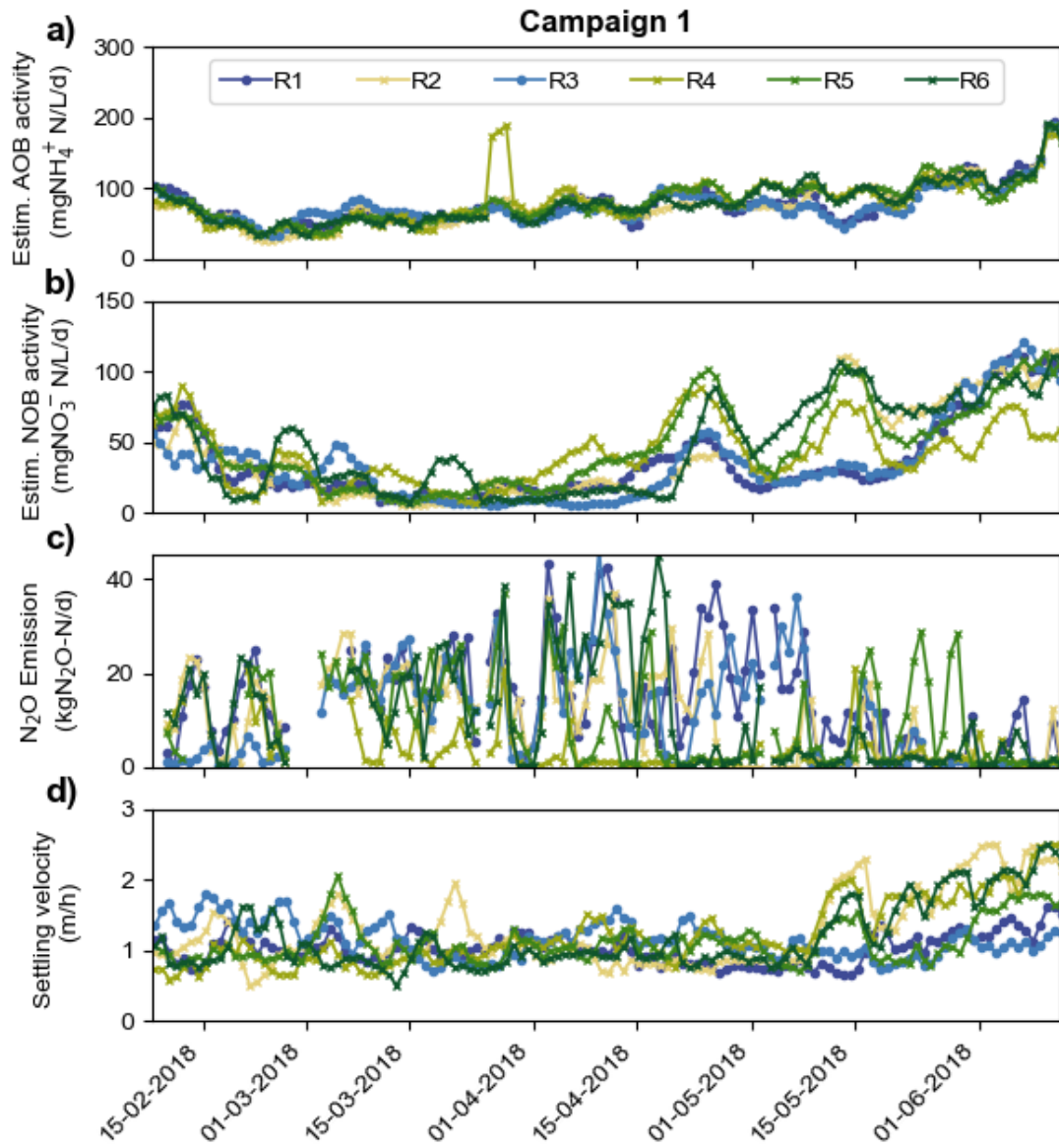


Figure C.9: Comparison of reactor performance and  $\text{N}_2\text{O}$  emissions during campaign 1. Data was smoothed with a moving average of 6 days in panels a), b), and c).

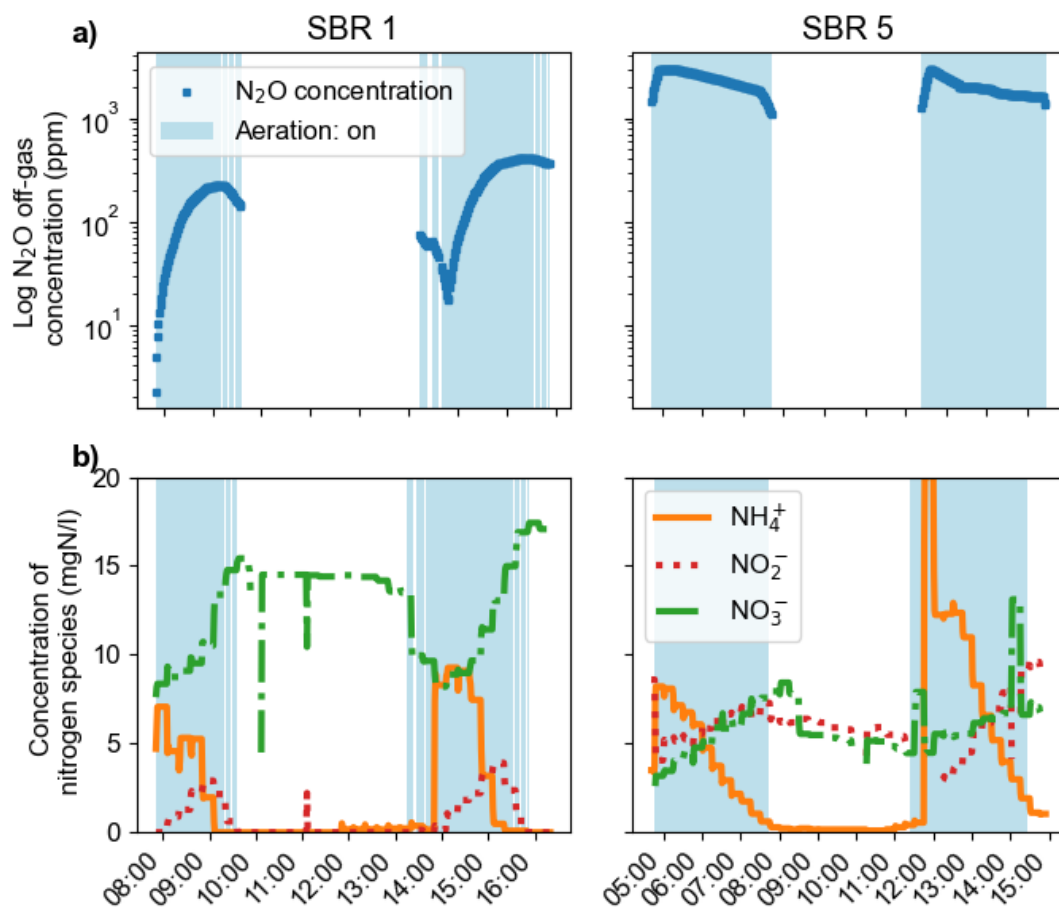


Figure C.10: N<sub>2</sub>O off-gas concentrations (a) and nitrogen species (NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>) concentrations (b) during two batch cycles in R 1 and R 5.

## C.4 Correlation analysis

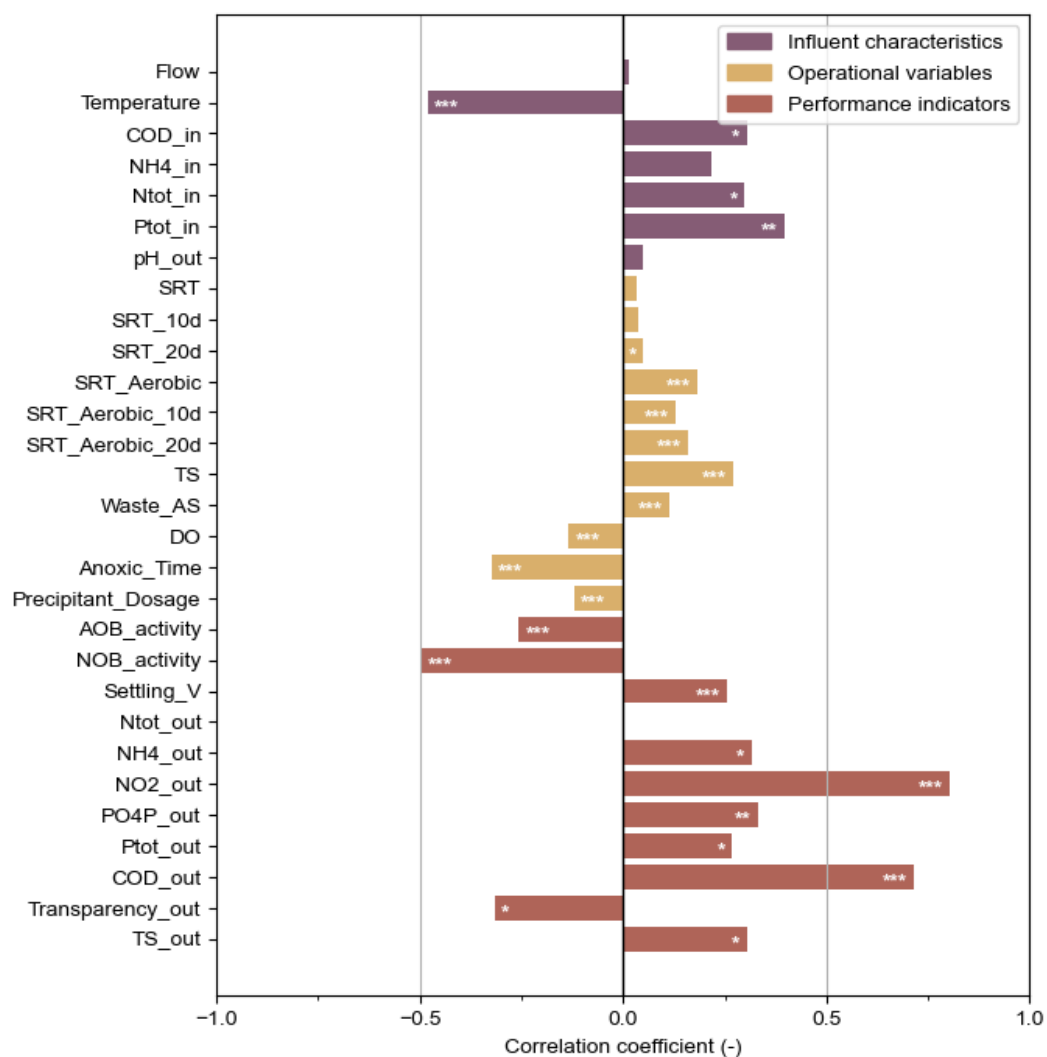


Figure C.11: Correlation between N<sub>2</sub>O emissions, operational parameters, and wastewater indices including all available data of both campaigns. Asterisks highlight significant p-values (\* < 0.05, \*\* < 0.01, \*\*\* < 0.001).



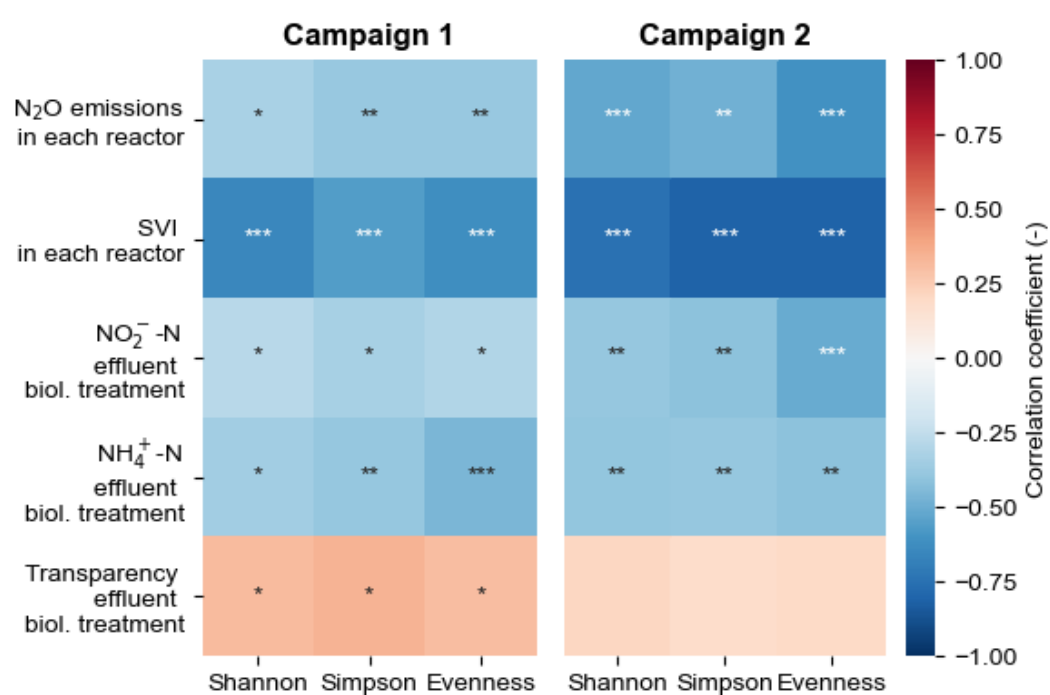


Figure C.12: Correlation between N<sub>2</sub>O emissions and diversity indices for campaign 1 (left) and campaign 2 (right). Color of the heatmap denotes the level of correlation (blue: negative, red: positive). Asterisks highlight significant p-values (\* < 0.05, \*\* < 0.01, \*\*\* < 0.005).

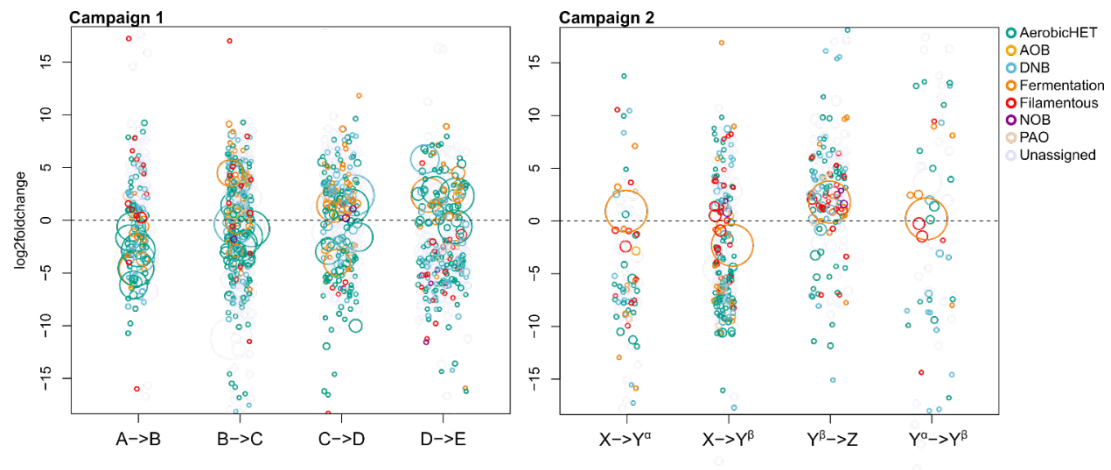


Figure C.13: Amplicon sequencing variants (ASVs) assigned to their putative functional role that displayed the most significant changes in abundances (expressed as log<sub>2</sub>foldchange) between the clusters. The size of bubbles corresponds to the relative abundance.

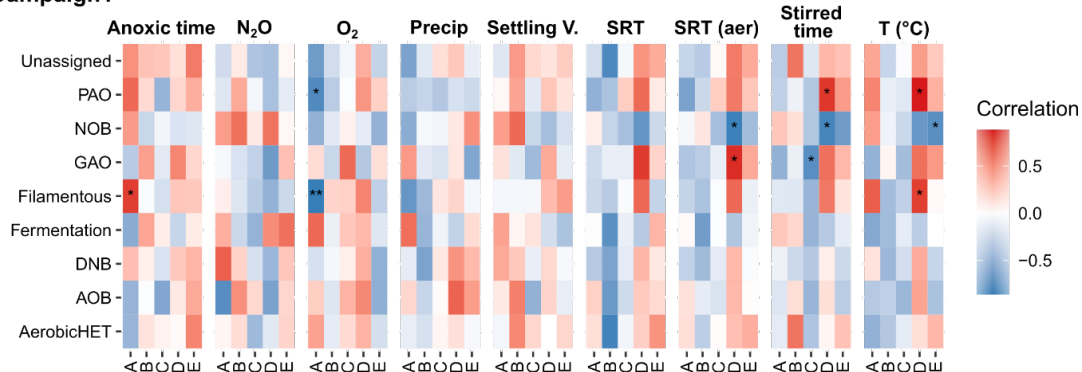
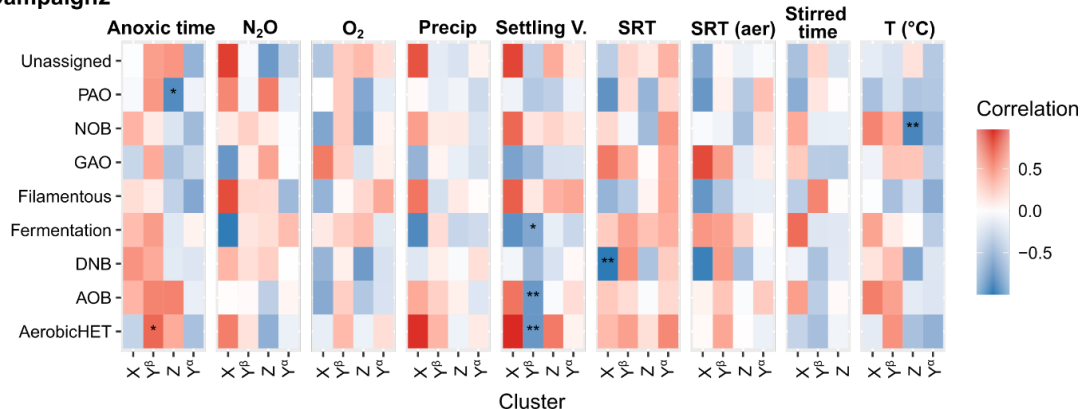
**Campaign1****Campaign2**

Figure C.14: Correlation between prominent operation parameter values, chemical conditions, and relative abundance of functionally categorized ASVs within the different clusters. Color of the heatmap denotes the level of correlation (blue: negative, red: positive). Asterisks highlight significant correlations (p-value adjusted to Benjamini Hochberg). Upper panel campaign 1, lower panel campaign 2.

## D Supplementary Information: Chapter 7

### D.1 Monitoring setup

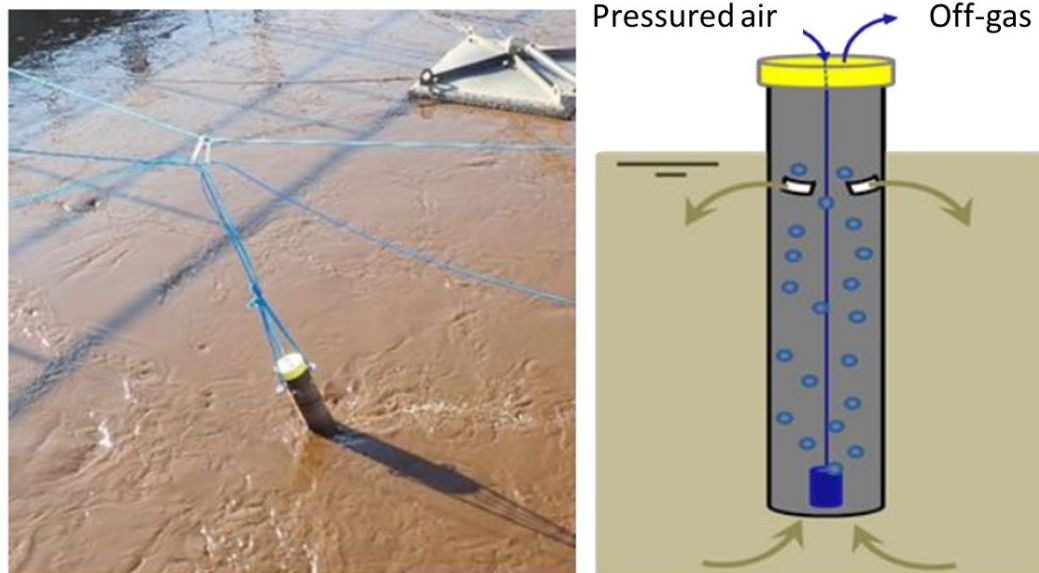


Figure D.1: 'Anox Tube' for off-gas stripping from unaerated zones

## D.2 Isotopic measurements

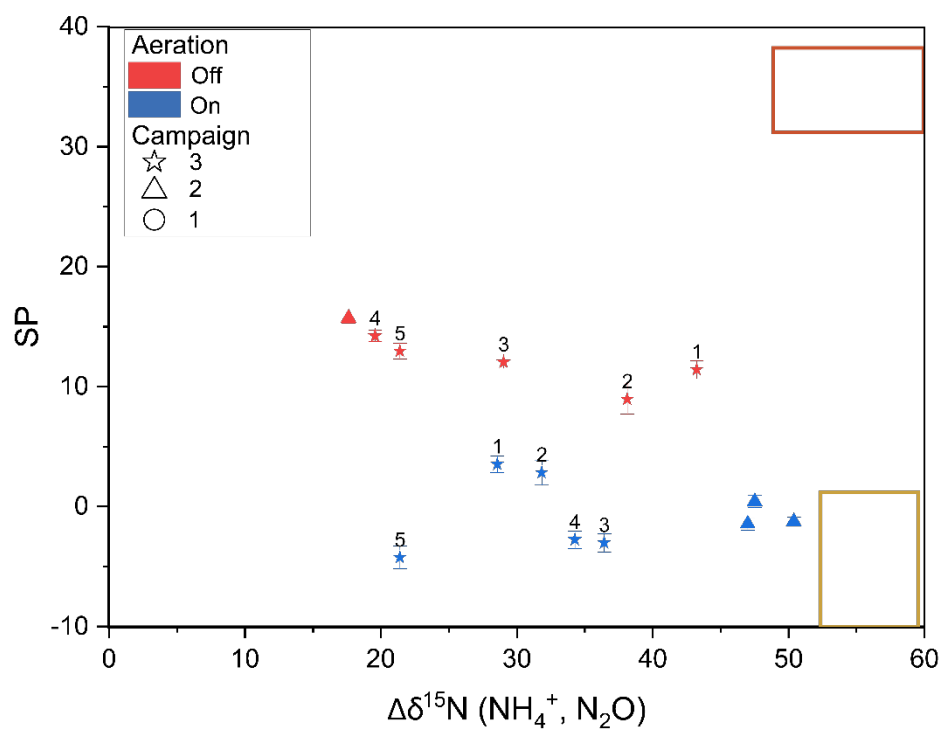


Figure D.2: Relation between  $\Delta\delta^{15}\text{N}(\text{N}_2\text{O}, \text{NH}_4^+)$  and SP in all campaigns conducted. Numbers next to campaign 3 data points (stars) indicate sampling sequence.

### D.3 Monitoring data

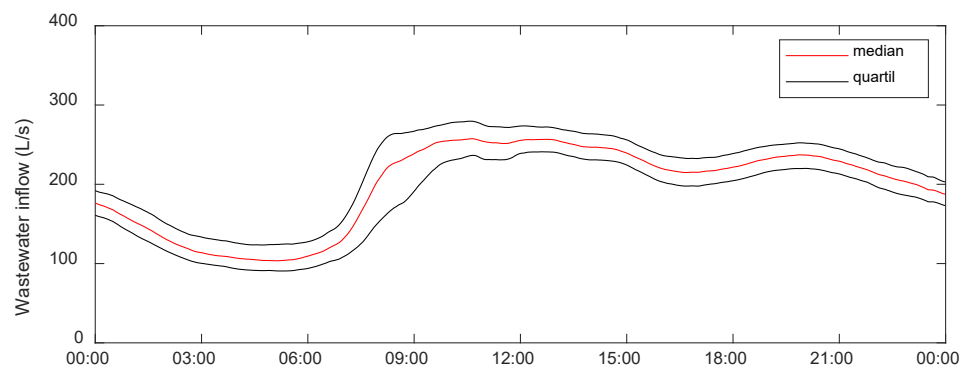


Figure D.3: Wastewater inflow on dry weather days (178 days between 01.11.19 and 27.02.21)

D.4 IC measurement during experiment 3

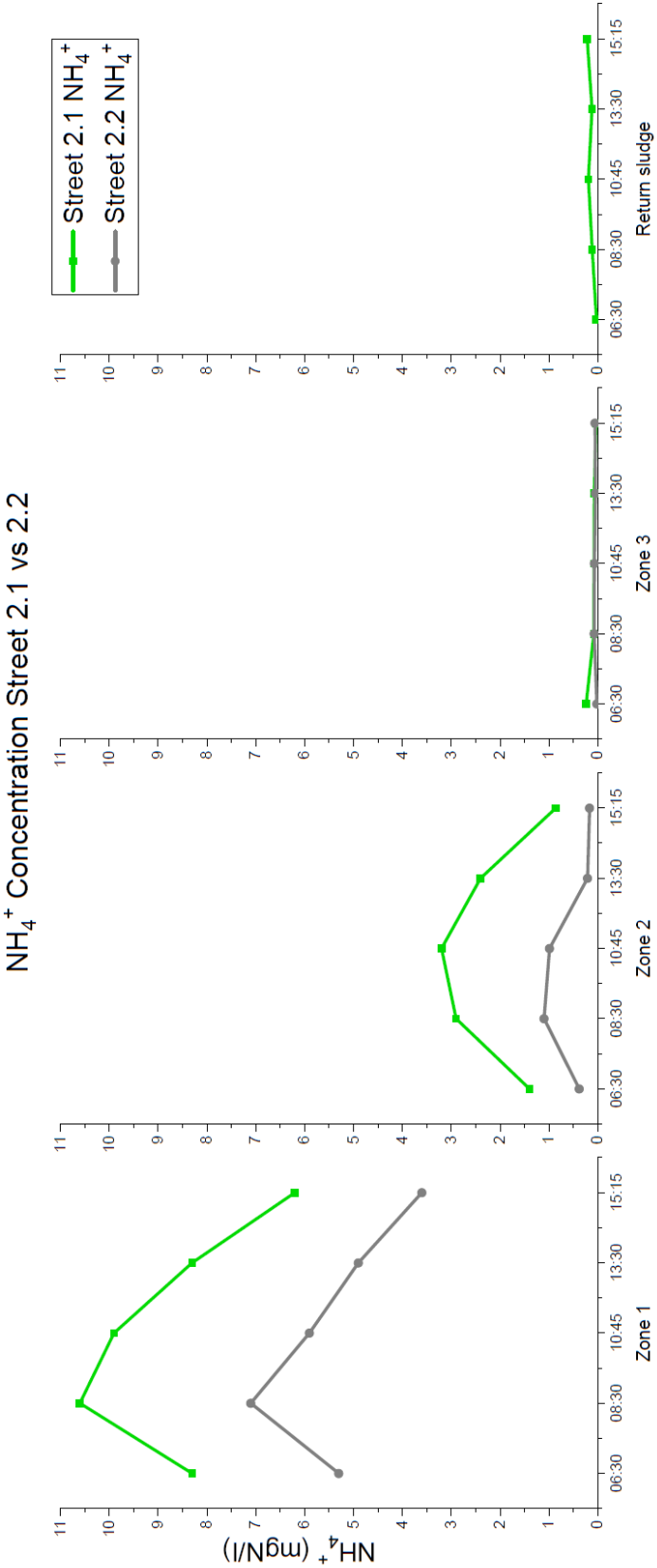


Figure D.4: Measured  $\text{NH}_4^+$  concentrations during experiment 3 in zones 1, 2, 3 and in the return sludge.

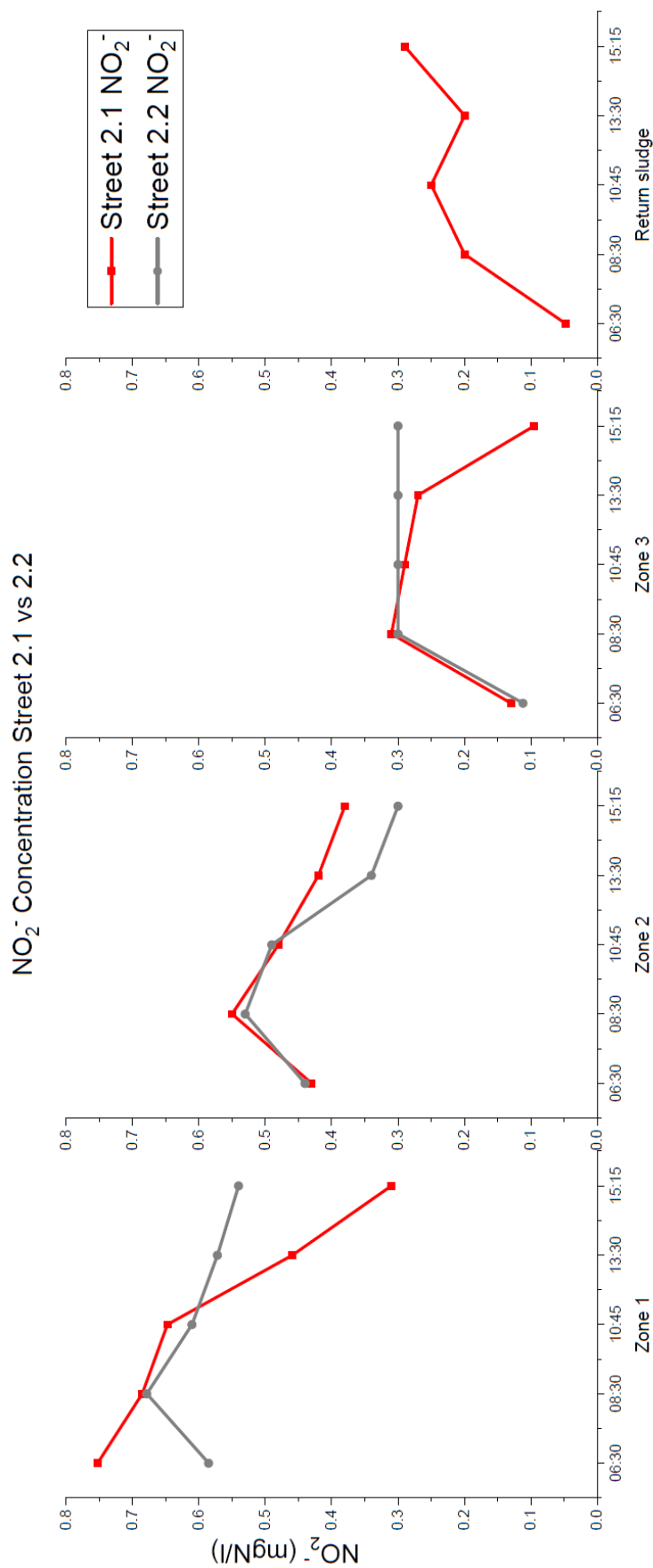


Figure D.5: Measured NO<sub>2</sub><sup>-</sup> concentrations during experiment 3 in zones 1, 2, 3 and in the return sludge.



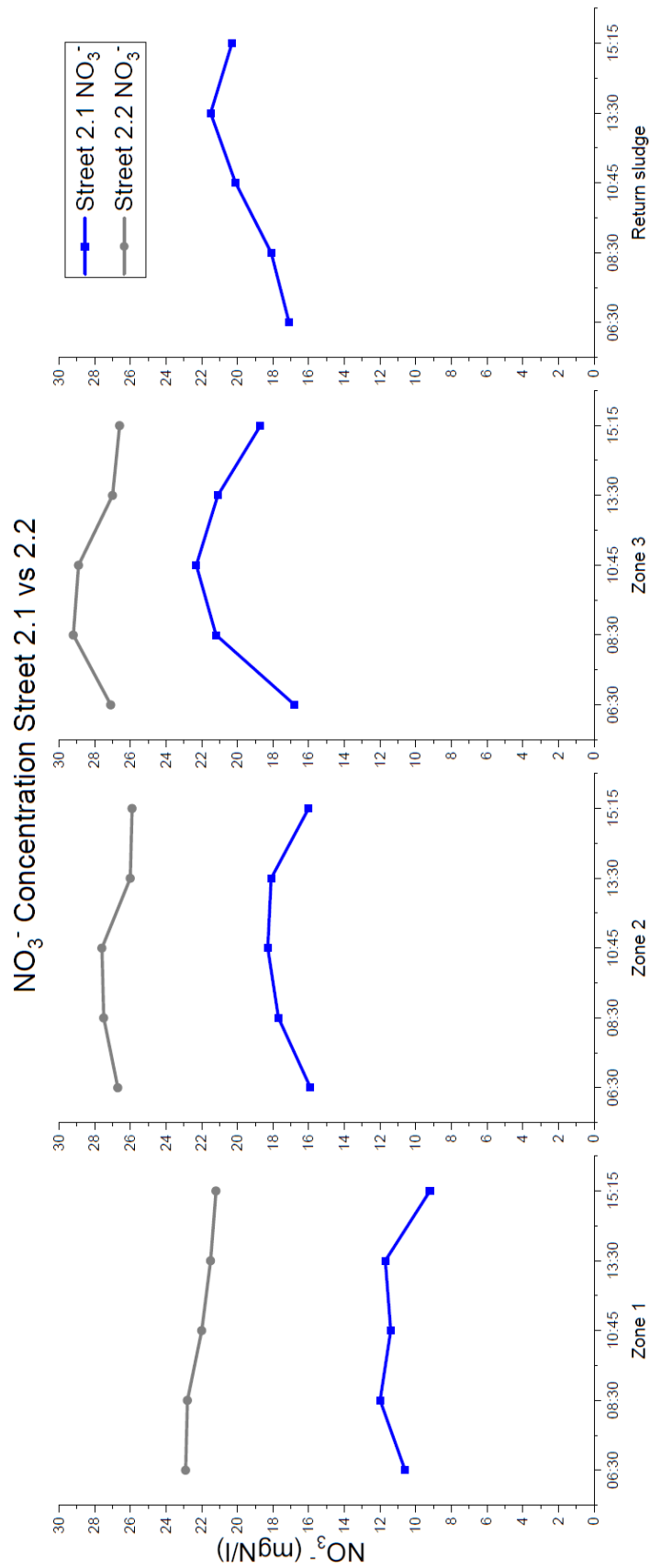


Figure D.6: Measured NO<sub>3</sub><sup>-</sup> concentrations during experiment 3 in zones 1, 2, 3 and in the return sludge.

## E Supplementary Information: Chapter 8

Table E.1: Calculated COD and nitrogen mass flows (Flow) for the model WWTP per person equivalent (PE) based on (Gujer 2007).

Mass flows	COD		N <sub>tot</sub>	
	g/d/PE	kg/y/PE	g/d/PE	kg/y/PE
Inflow	120	44	12	4.4
Primary sludge	30	11	1.2	0.4
Secondary sludge	53	20	2	0.7
Effluent	12	4	1.8	1
Off-gas bio. treatment	25	9	7	2.6
Reject water	0	0	1.6	0.6
Dewatered sludge	83	30	1.6	0.6
Off-gas from incineration	83	30	0.6	0.2
Incinerated sludge	0	0	1	0.4

Table E.2: Emission factors from literature in kgCH<sub>4</sub>/kgCOD with details whether an anaerobic digestion is available and with details about the measurement method applied by the authors. Monitoring methods are indicated by small letters: a) Grab samples analyzed with GC, b) Continuous measurement with infrared gas analyzer, c) Day wise measurement with Tracer gas dispersion method, d) Continuous measurement with a gas analyzer, n/a: not available.

Study	Emissions (kgCH <sub>4</sub> /kgCOD)			Anaerobic digestion	Monitoring method
	Waste-water treatment	Sewage sludge	Total WWTP		
Bao et al. (2016)	0.00091			no	a)
Bao et al. (2016)	0.00182			no	a)
Czepiel et al. (1995)	0.00067	0.00011	0.00078	no	a)
Daelman et al. (2012)	0.00323	0.00612	0.01015	yes	b)
Daelman et al. (2013b)			0.01100	yes	b)
Delre et al. (2017)			0.00756	yes	c)
Delre et al. (2017)			0.01876	yes	c)
Delre et al. (2017)			0.01799	yes	c)
Delre et al. (2017)			0.00405	yes	c)
Delre et al. (2017)			0.00329	yes	c)
Foley et al. (2015)	0.00653	0.00008	0.00669	no	a)
Foley et al. (2015)	0.00457	0.00048	0.00502	no	a)
Foley et al. (2015)			0.01374	yes	a)
Foley et al. (2015)			0.01019	yes	a)
Liu et al. (2015b)	0.00040			no	a)
Liu et al. (2015b)	0.00183			no	a)
Ren et al. (2013)	0.00318	0.00069	0.00418	no	a)
Ren et al. (2013)	0.00960	0.00092	0.01052	no	a)
Ren et al. (2013)	0.00055	0.00025	0.00083	no	a)
Ribera-Guardia et al. (2019)	0.00200			yes	d)
Rodriguez-Caballero et al. (2014a)	0.00008			yes	d)
Samuelsson et al. (2018)	0.00082	0.00347	0.00625	yes	c)
Tumendelger et al. (2019)	0.00004			no	n/a
Tumendelger et al. (2019)	0.00010			yes	n/a
Wang et al. (2011)	0.00069	0.00008	0.00077	no	a)
Yan et al. (2014)	0.00255			no	a)
Yan et al. (2014)	0.00139			no	a)
Yan et al. (2014)	0.00078			no	a)

## F References

- Aboobakar, A., Cartmell, E., Stephenson, T., Jones, M., Vale, P. and Dotro, G. (2013) Nitrous oxide emissions and dissolved oxygen profiling in a full-scale nitrifying activated sludge treatment plant. *Water Research* 47(2), 524-534.
- Ahn, J.H., Kim, S.W., Park, H., Rahm, B., Pagilla, K. and Chandran, K. (2010) N<sub>2</sub>O Emissions from activated sludge Processes, 2008-2009: Results of a national monitoring survey in the United States. *Environmental Science & Technology* 44(12), 4505-4511.
- Archana, A., Li, L., Shuh-Ji, K., Thibodeau, B. and Baker, D.M. (2016) Variations in nitrate isotope composition of wastewater effluents by treatment type in Hong Kong. *Marine Pollution Bulletin* 111(1-2), 143-152.
- Arrhenius, S. (1896) On the Influence of Carbonic Acid in the Air upon the Temperature of the Ground. *Philosophical Magazine and Journal of Science* 41, 237-276.
- Baeten, J.E., van Loosdrecht, M.C.M. and Volcke, E.I.P. (2020) When and why do gradients of the gas phase composition and pressure affect liquid-gas transfer? *Water Research* 178.
- Bao, Z., Sun, S. and Sun, D. (2016) Assessment of greenhouse gas emission from A/O and SBR wastewater treatment plants in Beijing, China. *International Biodeterioration & Biodegradation* 108, 108-114.
- Belda, Belda, M., Holtanová, E., Halenka, T. and Kalvová, J.M. (2014) Climate classification revisited: from Köppen to Trewartha. *Climate Research* 59(1), 1-13.
- Bock, M. (2018) Nationale Expertenreview - Anforderungen und Anregungen, Federal Office for the Environment, Bern.
- Bollon, J., Filali, A., Fayolle, Y., Guerin, S., Rocher, V. and Gillot, S. (2016) N<sub>2</sub>O emissions from full-scale nitrifying biofilters. *Water Research*.
- Breiman, L. (2001) Random Forests. *Machine Learning* 45(1), 5-32.
- Brotto, A.C., Kligerman, D.C., Andrade, S.A., Ribeiro, R.P., Oliveira, J.L., Chandran, K. and de Mello, W.Z. (2015) Factors controlling nitrous oxide emissions from a full-scale activated sludge system in the tropics. *Environmental Science and Pollution Research* 22(15), 11840-11849.
- Buchwald, C. and Casciotti, K.L. (2013) Isotopic ratios of nitrite as tracers of the sources and age of oceanic nitrite. *Nature Geoscience* 6(4), 308-313.
- Buchwald, C., Santoro, A.E., McIlvin, M.R. and Casciotti, K.L. (2012) Oxygen isotopic composition of nitrate and nitrite produced by nitrifying cocultures and natural marine assemblages. *Limnology and Oceanography* 57(5), 1361-1375.
- Burger, W., Krysiak-Baltyn, K., Scales, P.J., Martin, G.J.O., Stickland, A.D. and Gras, S.L. (2017) The influence of protruding filamentous bacteria on floc stability and solid-liquid separation in the activated sludge process. *Water Research* 123, 578-585.

- Bürgmann, H., Jenni, S., Vazquez, F. and Udert, K.M. (2011) Regime shift and microbial dynamics in a sequencing batch reactor for nitrification and anammox treatment of urine. *Applied and Environmental Microbiology* 77(17), 5897-5907.
- Cadwallader, A. and Van Briesen, J.M. (2017) Incorporating Uncertainty into Future Estimates of Nitrous Oxide Emissions from Wastewater Treatment. *Journal of Environmental Engineering* 143(8).
- Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A. and Holmes, S.P. (2016) DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods* 13(7), 581-583.
- Canfield, D.E., Glazer, A.N. and Falkowski, P.G. (2010) The evolution and future of Earth's nitrogen cycle. *Science* 330(6001), 192-196.
- Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J. and Knight, R. (2010) QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* 7(5), 335-336.
- Caranto, J.D. and Lancaster, K.M. (2017) Nitric oxide is an obligate bacterial nitrification intermediate produced by hydroxylamine oxidoreductase. *PNAS* 114(31), 8217-8222.
- Casciotti, K.L., Böhlke, J.K., McIlvin, M.R., Mroczkowski, S.J. and Hannon, J.E. (2007) Oxygen Isotopes in Nitrite: Analysis, Calibration, and Equilibration. *Analytical Chemistry* 79(6), 2427-2436.
- Casciotti, K.L., McIlvin, M. and Buchwald, C. (2010) Oxygen isotopic exchange and fractionation during bacterial ammonia oxidation. *Limnology and Oceanography* 55(2), 753-762.
- Casciotti, K.L., Sigman, D.M., Hastings, M.G., Böhlke, J.K. and Hilkert, A. (2002) Measurement of the Oxygen Isotopic Composition of Nitrate in Seawater and Freshwater Using the Denitrifier Method. *Analytical Chemistry* 74(19), 4905-4912.
- Castro-Barros, C.M., Rodríguez-Caballero, A., Volcke, E.I.P. and Pijuan, M. (2016) Effect of nitrite on the  $N_2O$  and NO production on the nitrification of low-strength ammonium wastewater. *Chemical Engineering Journal* 287, 269-276.
- Chandran, K., Volcke, E.I. and Van Loosdrecht, M.C.M. (2016) *Experimental Methods in Wastewater Treatment*. Van Loosdrecht, M.C.M., Nielen, P.H., López Vázquez, C.M. and Brdjanovic, D. (eds), IWA, London.
- Chen, X., Mielczarek, A.T., Habicht, K., Andersen, M.H., Thornberg, D. and Sin, G. (2019) Assessment of Full-Scale  $N_2O$  Emission Characteristics and Testing of Control Concepts in an Activated Sludge Wastewater Treatment Plant with Alternating Aerobic and Anoxic Phases. *Environmental Science & Technology* 53(21), 12485-12494.
- Chen, X. and Sin, G. (2020) Quantification and Analyses of  $N_2O$  Emission and Testing of Relevant  $N_2O$  Control Technology at Avedøre Wastewater Treatment Plant,

<https://www.danva.dk/media/6422/technical-report-final-version-10-lattergasstyring.pdf>.

- Chen, X., Yuan, Z. and Ni, B.J. (2018) Nitrite accumulation inside sludge flocs significantly influencing nitrous oxide production by ammonium-oxidizing bacteria. *Water Research* 143, 99-108.
- Clos, I., Krampe, J., Alvarez-Gaitan, J.P., Saint, C.P. and Short, M.D. (2020) Energy Benchmarking as a Tool for Energy-Efficient Wastewater Treatment: Reviewing International Applications. *Water Conservation Science and Engineering* 5(1-2), 115-136.
- Colliver, B.B. and Stephenson, T. (2000) Production of nitrogen oxide and dinitrogen oxide by autotrophic nitrifiers. *Biotechnology Advances* 18, 219-232.
- Conthe, M., Lycus, P., Arntzen, M.O., Ramos da Silva, A., Frostegard, A., Bakken, L.R., Kleerebezem, R. and van Loosdrecht, M.C.M. (2018a) Denitrification as an N<sub>2</sub>O sink. *Water Research* 151, 381-387.
- Conthe, M., Wittorf, L., Kuenen, J.G., Kleerebezem, R., van Loosdrecht, M.C.M. and Hallin, S. (2018b) Life on N<sub>2</sub>O: deciphering the ecophysiology of N<sub>2</sub>O respiring bacterial communities in a continuous culture. *ISME Journal* 12(4), 1142-1153.
- Coplen, T.B. (1988) Normalization of oxygen and hydrogen isotope data. *Chemical Geology: Isotope Geoscience section* 72(4), 293-297.
- Cotto, I., Dai, Z., Huo, L., Anderson, C.L., Vilardi, K.J., Ijaz, U., Khunjar, W., Wilson, C., De Clippeleir, H., Gilmore, K., Bailey, E. and Pinto, A.J. (2020) Long solids retention times and attached growth phase favor prevalence of comammox bacteria in nitrogen removal systems. *Water Research* 169, 115268.
- Cunningham, M., Fink, A. and Baier, U. (2015) Methanemissionen Auf Kläranlagen. *Aqua & Gas* 3/2015.
- Cydzik-Kwiatkowska, A. and Zielinska, M. (2016) Bacterial communities in full-scale wastewater treatment systems. *World Journal of Microbiology and Biotechnology* 32(4), 66.
- Czepiel, P., Crill, P. and Harriss, R. (1995) Nitrous oxide emissions from municipal wastewater treatment. *Environmental Science & Technology*, 2352-2356.
- Czepiel, P.M., Crill, P.M. and Harriss, R.C. (1993) Methane emissions from municipal wastewater treatment processes. *Environmental Science & Technology* 27(12), 2472-2477.
- Daelman, M.R., De Baets, B., van Loosdrecht, M.C. and Volcke, E.I. (2013a) Influence of sampling strategies on the estimated nitrous oxide emission from wastewater treatment plants. *Water Research* 47(9), 3120-3130.
- Daelman, M.R., van Voorthuizen, E.M., van Dongen, L.G., Volcke, E.I. and van Loosdrecht, M.C. (2013b) Methane and nitrous oxide emissions from municipal wastewater treatment - results from a long-term study. *Water Science and Technology* 67(10), 2350-2355.

- Daelman, M.R., van Voorthuizen, E.M., van Dongen, U.G., Volcke, E.I. and van Loosdrecht, M.C. (2012) Methane emission during municipal wastewater treatment. *Water Research* 46(11), 3657-3670.
- Daelman, M.R., van Voorthuizen, E.M., van Dongen, U.G., Volcke, E.I. and van Loosdrecht, M.C. (2015) Seasonal and diurnal variability of N<sub>2</sub>O emissions from a full-scale municipal wastewater treatment plant. *Science of The Total Environment* 536, 1-11.
- Daigger, G.T., Adams, C.D. and Steller, H.K. (2007) Diffusion of oxygen through activated sludge flocs: experimental measurement, modeling, and implications for simultaneous nitrification and denitrification. *Water Environment Research* 79(4), 375-387.
- Daims, H., Lebedeva, E.V., Pjevac, P., Han, P., Herbold, C., Albertsen, M., Jehmlich, N., Palatinszky, M., Vierheilig, J., Bulaev, A., Kirkegaard, R.H., von Bergen, M., Rattei, T., Bendinger, B., Nielsen, P.H. and Wagner, M. (2015) Complete nitrification by *Nitrospira* bacteria. *Nature* 528(7583), 504-509.
- Daims, H., Nielsen, J.L., Nielsen, P.H., Schleifer, K.H. and Wagner, M. (2001) In situ characterization of *Nitrospira*-like nitrite-oxidizing bacteria active in wastewater treatment plants. *Applied Environmental Microbiology* 67(11), 5273-5284.
- de Celis, M., Belda, I., Ortiz-Alvarez, R., Arregui, L., Marquina, D., Serrano, S. and Santos, A. (2020) Tuning up microbiome analysis to monitor WWTPs' biological reactors functioning. *Scientific Reports* 10(1), 4079.
- Delre, A., Monster, J. and Scheutz, C. (2017) Greenhouse gas emission quantification from wastewater treatment plants, using a tracer gas dispersion method. *Science of The Total Environment* 605-606, 258-268.
- Denk, T.R.A., Mohn, J., Decock, C., Lewicka-Szczebak, D., Harris, E., Butterbach-Bahl, K., Kiese, R. and Wolf, B. (2017) The nitrogen cycle: A review of isotope effects and isotope modeling approaches. *Soil Biology and Biochemistry* 105, 121-137.
- Domingo-Felez, C., Pellicer-Nacher, C., Petersen, M.S., Jensen, M.M., Plosz, B.G. and Smets, B.F. (2016) Heterotrophs are key contributors to nitrous oxide production in activated sludge under low C-to-N ratios during nitrification-Batch experiments and modeling. *Biotechnology and Bioengineering* 114(1), 132-140.
- Domingo-Félez, C. and Smets, B.F. (2016) A consilience model to describe N<sub>2</sub>O production during biological N removal. *Environmental Science: Water Research & Technology* 2(6), 923-930.
- Domingo-Félez, C. and Smets, B.F. (2020) Modelling N<sub>2</sub>O dynamics of activated sludge biomass: Uncertainty analysis and pathway contributions. *Chemical Engineering Journal* 379.
- Duan, H., van den Akker, B., Thwaites, B.J., Peng, L., Herman, C., Pan, Y., Ni, B.J., Watt, S., Yuan, Z. and Ye, L. (2020) Mitigating nitrous oxide emissions at a full-scale wastewater treatment plant. *Water Research* 185, 116196.

- Duan, H., Ye, L., Erler, D., Ni, B.J. and Yuan, Z. (2017) Quantifying nitrous oxide production pathways in wastewater treatment systems using isotope technology - A critical review. *Water Research* 122, 96-113.
- Duan, H., Ye, L., Lu, X. and Yuan, Z. (2019) Overcoming Nitrite Oxidizing Bacteria Adaptation through Alternating Sludge Treatment with Free Nitrous Acid and Free Ammonia. *Environmental Science & Technology* 53(4), 1937-1946.
- Duan, H., Zhao, Y., Koch, K., Wells, G.F., Zheng, M., Yuan, Z. and Ye, L. (2021) Insights into Nitrous Oxide Mitigation Strategies in Wastewater Treatment and Challenges for Wider Implementation. *Environmental Science & Technology*.
- Dürrenmatt, D.J. and Gujer, W. (2011) Data-driven modeling approaches to support wastewater treatment plant operation. *Environmental Modelling & Software*.
- EEA (2017) Annual European Union greenhouse gas inventory 1990–2015 and inventory report 2017., European Environment Agency, Copenhagen, Denmark.
- Falas, P., Wick, A., Castronovo, S., Habermacher, J., Ternes, T.A. and Joss, A. (2016) Tracing the limits of organic micropollutant removal in biological wastewater treatment. *Water Research* 95, 240-249.
- FEA Germany, F.E.A. (2017) National Inventory Report for the German Greenhouse Gas Inventory 1990 – 2015.
- FOE (2017) Schweizerische Statistik der erneuerbaren Energien. Federal Office for Energy FOE (ed), Bern.
- FOEN (2010) Stickstoffflüsse in der Schweiz 2005. Federal Office for the Environment FOEN (ed), Bern.
- FOEN (2013) Stickstoffflüsse in der Schweiz 2020. Federal Office for the Environment FOEN (ed), Bern.
- FOEN (2016) Annual statistics on waste generation in Switzerland. Federal Office for the Environment FOEN (ed), Bern.
- FOEN (2017) Switzerland's National Inventory Report 2017. Federal Office for the Environment FOEN (ed), Bern.
- FOEN (2018) EMIS-Kommentar zu Sektor 5D. Federal Office for the Environment FOEN (ed), Bern.
- FOEN (2020) Kenngrößen zur Entwicklung der Treibhausgasemissionen in der Schweiz 1990–2018. Federal Office for the Environment, F., Abteilung Klima (ed), Bern.
- FOEN Indicator WS076 (2018) WS076 - Anschlussgrad an Abwasserreinigungsanlage. Federal Office for the Environment, F. (ed), <https://www.bafu.admin.ch/bafu/de/home/themen/thema-wasser/wasser--daten--indikatoren-und-karten/wasser--indikatoren/indikator-wasser.pt.html>.
- Foley, J., de Haas, D., Yuan, Z. and Lant, P. (2010) Nitrous oxide generation in full-scale biological nutrient removal wastewater treatment plants. *Water Research* 44(3), 831-844.



- Foley, J., Yuan, Z., Keller, J., Senante, E., Chandran, K., Willis, J., Shah, A., van Loosdrecht, M.C. and van Voorthuizen, E. (2015) N<sub>2</sub>O and CH<sub>4</sub> Emission from Wastewater Collection and Treatment Systems: State of the Science Report and Technical Report.
- Fourier, J.J. (1827) On the Temperatures of the Terrestrial Sphere and Interplanetary Space. *Mémoires de l'Académie Royale des Sciences* 7, 569-604.
- Fowler, D., Coyle, M., Skiba, U., Sutton, M.A., Cape, J.N., Reis, S., Sheppard, L.J., Jenkins, A., Grizzetti, B., Galloway, J.N., Vitousek, P., Leach, A., Bouwman, A.F., Butterbach-Bahl, K., Dentener, F., Stevenson, D., Amann, M. and Voss, M. (2013) The global nitrogen cycle in the twenty-first century. *Philos Trans R Soc Lond B Biol Sci* 368(1621), 20130164.
- Frame, C.H. and Casciotti, K.L. (2010) Biogeochemical controls and isotopic signatures of nitrous oxide production by a marine ammonia-oxidizing bacterium. *Biogeosciences* 7(9), 2695-2709.
- Galle, M., Agar, D.W. and Watzenberger, O. (2001) Thermal N<sub>2</sub>O decomposition in regenerative heat exchanger reactors. *Chemical Engineering Science* 56(4), 1587-1595.
- Ge, J., Huang, G., Li, J., Sun, X. and Han, L. (2018) Multivariate and Multiscale Approaches for Interpreting the Mechanisms of Nitrous Oxide Emission during Pig Manure-Wheat Straw Aerobic Composting. *Environmental Science & Technology* 52(15), 8408-8418.
- Gehre, M., Geilmann, H., Richter, J., Werner, R.A. and Brand, W.A. (2004) Continuous flow 2H/1H and 18O/16O analysis of water samples with dual inlet precision. *Rapid Communications in Mass Spectrometry* 18(22), 2650-2660.
- Granger, J., Boshers, D.S., Böhlke, J.K., Yu, D., Chen, N. and Tobias, C.R. (2020) The influence of sample matrix on the accuracy of nitrite N and O isotope ratio analyses with the azide method. *Rapid Communications in Mass Spectrometry* 34(1), e8569.
- Griffin, J.S. and Wells, G.F. (2017) Regional synchrony in full-scale activated sludge bioreactors due to deterministic microbial community assembly. *ISME Journal* 11(2), 500-511.
- Groves, K.P., Daigger, G.T., Simpkin, T.J., Redmon, D.T. and Ewing, L. (1992) Evaluation of oxygen transfer efficiency and alpha-factor on a variety of diffused aeration systems. *Water Environment Research* 64(5), 691-698.
- Gruber, W., Niederdorfer, R., Ringwald, J., Morgenroth, E., Bürgmann, H. and Joss, A. (2021) Linking seasonal N<sub>2</sub>O emissions and nitrification failures to microbial dynamics in a SBR wastewater treatment plant. *Water Research X* 11, 100098.
- Gruber, W., Villez, K., Kipf, M., Wunderlin, P., Siegrist, H., Vogt, L. and Joss, A. (2020) N<sub>2</sub>O emission in full-scale wastewater treatment: Proposing a refined monitoring strategy. *Science of The Total Environment* 699, 134157.
- Gujer, W. (2007) *Siedlungswasserwirtschaft*, Springer, Berlin.

- Han, P., Wu, D., Sun, D., Zhao, M., Wang, M., Wen, T., Zhang, J., Hou, L., Liu, M., Klumper, U., Zheng, Y., Dong, H.P., Liang, X. and Yin, G. (2021) N<sub>2</sub>O and NO<sub>y</sub> production by the comammox bacterium *Nitrospira inopinata* in comparison with canonical ammonia oxidizers. *Water Research* 190, 116728.
- Hanaki, K., Hong, Z. and Matsuo, T. (1992) Production of nitrous oxide gas during denitrification of wastewater. *Water Science and Technology* 26(5-6), 1027-1036.
- Hansen, J., Johnson, D., Lacis, A., Lebedeff, S., Lee, P., Rind, D. and Russell, G. (1981) Climate impact of increasing atmospheric carbon dioxide. *Science* 213(4511), 957-966.
- Hare, W., Stockwell, C., Flachslund, C. and Oberthür, S. (2010) The architecture of the global climate regime: a top-down perspective. *Climate Policy* 10(6), 600-614.
- Harris, C.R., Millman, K.J., van der Walt, S.J., Gommers, R., Virtanen, P., Cournapeau, D., Wieser, E., Taylor, J., Berg, S., Smith, N.J., Kern, R., Picus, M., Hoyer, S., van Kerkwijk, M.H., Brett, M., Haldane, A., del Río, J.F., Wiebe, M., Peterson, P., Gérard-Marchant, P., Sheppard, K., Reddy, T., Weckesser, W., Abbasi, H., Gohlke, C. and Oliphant, T.E. (2020a) Array programming with NumPy. *Nature* 585(7825), 357-362.
- Harris, E., Joss, A., Emmenegger, L., Kipf, M., Wolf, B., Mohn, J. and Wunderlin, P. (2015) Isotopic evidence for nitrous oxide production pathways in a partial nitrification-anammox reactor. *Water Research* 83, 258-270.
- Harris, S.J., Liisberg, J., Xia, L., Wei, J., Zeyer, K., Yu, L., Barthel, M., Wolf, B., Kelly, B.F.J., Cendón, D.I., Blunier, T., Six, J. and Mohn, J. (2020b) N<sub>2</sub>O isotopocule measurements using laser spectroscopy: analyzer characterization and intercomparison. *Atmospheric Measurement Techniques* 13(5), 2797-2831.
- Hastie, T., Tibshirani, R. and Friedman, J. (2009) *The elements of statistical learning data mining, inference, and prediction*, Springer, New York, N.Y.
- Hayhurst, A.N. and Lawrence, A.D. (1992) Emissions of nitrous oxide from combustion sources. *Progress in Energy and Combustion Science* 18(6), 529-552.
- Held, D. and Rogger, C. (2018) Three Models of Global Climate Governance: From Kyoto to Paris and Beyond. *Global Policy* 9(4), 527-537.
- Hellinga, C., Schellen, A.A.J.C., Mulder, J.W., van Loosdrecht, M.C.M. and Heijnen, J.J. (1998) The SHARON process: An innovative method for nitrogen removal from ammonium-rich waste water. *Water Science and Technology* 37(9), 135-142.
- Henze, M., Gujer, W., Takahashi, M. and Van Loosdrecht, M.C. (2000) *Activated sludge models ASM1, ASM2, ASM2d and ASM3*, IWA, London.
- Houillon, G. and Joliet, O. (2005) Life cycle assessment of processes for the treatment of wastewater urban sludge: energy and global warming analysis. *Journal of Cleaner Production* 13(3), 287-299.
- Hu, Z., Zhang, J., Xie, H., Liang, S. and Li, S. (2013) Minimization of nitrous oxide emission from anoxic-oxic biological nitrogen removal process: Effect of influent COD/NH<sub>4</sub><sup>+</sup>

- ratio and feeding strategy. *Journal of Bioscience and Bioengineering* 115(3), 272-278.
- Huang, Z., Gedalanga, P.B., Asvapathanagul, P. and Olson, B.H. (2010) Influence of physicochemical and operational parameters on *Nitrobacter* and *Nitrospira* communities in an aerobic activated sludge bioreactor. *Water Research* 44(15), 4351-4358.
- Ibraim, E., Harris, E., Eyer, S., Tuzson, B., Emmenegger, L., Six, J. and Mohn, J. (2018) Development of a field-deployable method for simultaneous, real-time measurements of the four most abundant N<sub>2</sub>O isotopocules. *Isotopes in Environmental and Health Studies* 54(1), 1-15.
- IEA (2020) Global Energy Review 2020, Paris
- IPCC (2006) Chapter 5.6 Wastewater treatment and discharge. 2006 IPCC Guidelines for National Greenhouse Gas Inventories, <http://www.ipcc-nggip.iges.or.jp/public/2006gl/index.html>.
- IPCC (2013) Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
- IPCC (2014) Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, p. 151, IPCC, Geneva, Switzerland.
- IPCC (2019a) 2019 Refinement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories. Buendia, E.C., Guendehou, S., Limmeechokchai, B., Pipatti, R., Rojas, Y., Sturgiss, R., Tanabe, K., Wirth, T., Romano, D., Witi, J., Garg, A., Weitz, M.M., Cai, B., Ottinger, D.A., Dong, H., MacDonald, J.D., Ogle, S.M., Rocha, M.T., Sanchez, M.J.S., Bartram, D. and Towprayoon, S. (eds).
- IPCC (2019b) Chapter 5.6 Wastewater treatment and discharge. 2019 Refinement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories, <https://www.ipcc-nggip.iges.or.jp/public/2019rf/index.html>.
- IPCC Guidelines (2006) Chapter 6 - Wastewater treatment and discharge.
- Jabłońska, M., Arán, M.A., Beale, A.M., Góra-Marek, K., Delahay, G., Petitto, C., Pacultová, K. and Palkovits, R. (2019) Catalytic decomposition of N<sub>2</sub>O over Cu–Al–Ox mixed metal oxides. *RSC Advances* 9(7), 3979-3986.
- Jinuntuya-Nortman, M., Sutka, R.L., Ostrom, P.H., Gandhi, H. and Ostrom, N.E. (2008) Isotopologue fractionation during microbial reduction of N<sub>2</sub>O within soil mesocosms as a function of water-filled pore space. *Soil Biology and Biochemistry* 40(9), 2273-2280.
- Johnston, J. and Behrens, S. (2020) Seasonal Dynamics of the Activated Sludge Microbiome in Sequencing Batch Reactors, Assessed Using 16S rRNA Transcript Amplicon Sequencing. *Applied and Environmental Microbiology* 86(19), e00597-00520.

- Johnston, J., LaPara, T. and Behrens, S. (2019) Composition and Dynamics of the Activated Sludge Microbiome during Seasonal Nitrification Failure. *Scientific Reports* 9(1), 4565.
- Joss, A., Salzgeber, D., Eugster, J., König, R., Rottermann, K., Burger, S., Fabijan, P., Leumann, S., Mohn, J. and Siegrist, H. (2009) Full-Scale Nitrogen Removal from Digester Liquid with Partial Nitrification and Anammox in One SBR. *Environmental Science & Technology* 43(14), 5301-5306.
- Ju, F., Guo, F., Ye, L., Xia, Y. and Zhang, T. (2014) Metagenomic analysis on seasonal microbial variations of activated sludge from a full-scale wastewater treatment plant over 4 years. *Environmental Microbiology Reports* 6(1), 80-89.
- Kampschreur, M.J., Poldermans, R., Kleerebezem, R., van der Star, W.R., Haarhuis, R., Abma, W.R., Jetten, M.S., Jetten, M.S. and van Loosdrecht, M.C. (2009a) Emission of nitrous oxide and nitric oxide from a full-scale single-stage nitrification-anammox reactor. *Water Science and Technology* 60(12), 3211-3217.
- Kampschreur, M.J., Tan, N.C.G., Kleerebezem, R., Picioroneau, C., Jetten, M.S. and Van Loosdrecht, M.C. (2008) Effect of dynamic process conditions on nitrogen oxides emission from a nitrifying culture. *Environmental Science & Technology* 42(2), 429-435.
- Kampschreur, M.J., Temmink, H., Kleerebezem, R., Jetten, M.S. and van Loosdrecht, M.C. (2009b) Nitrous oxide emission during wastewater treatment. *Water Research* 43(17), 4093-4103.
- Keeling, C.D., Bacastow, R.B., Bainbridge, A.E., Ekdahl, C.A., Guenther, P.R. and Waterman, L.S. (1976) Atmospheric carbon dioxide variations at Mauna Loa Observatory, Hawaii. *Tellus* 28(6), 538-551.
- Kimochi, Y., Inamori, Y., Mizuochi, M., Xu, K.-Q. and Matsumura, M. (1998) Nitrogen removal and N<sub>2</sub>O emission in a full-scale domestic wastewater treatment plant with intermittent aeration. *Journal of Fermentation and Bioengineering* 86(2), 202-206.
- Konsolakis, M. (2015) Recent Advances on Nitrous Oxide (N<sub>2</sub>O) Decomposition over Non-Noble-Metal Oxide Catalysts: Catalytic Performance, Mechanistic Considerations, and Surface Chemistry Aspects. *ACS Catalysis* 5(11), 6397-6421.
- Kool, D.M., Wrage, N., Oenema, O., Dolfing, J. and Van Groenigen, J.W. (2007) Oxygen exchange between (de)nitrification intermediates and H<sub>2</sub>O and its implications for source determination of NO and N<sub>2</sub>O: a review. *Rapid Communications in Mass Spectrometry* 21(22), 3569-3578.
- Kosonen, H., Heinonen, M., Mikola, A., Haimi, H., Mulas, M., Corona, F. and Vahala, R. (2016) Nitrous Oxide Production at a Fully Covered Wastewater Treatment Plant: Results of a Long-Term Online Monitoring Campaign. *Environmental Science & Technology* 50(11), 5547-5554.
- Kozłowski, J.A., Kits, K.D. and Stein, L.Y. (2016) Comparison of Nitrogen Oxide Metabolism among Diverse Ammonia-Oxidizing Bacteria. *Frontiers in Microbiology* 7, 1090.

- Kritee, K., Sigman, D.M., Granger, J., Ward, B.B., Jayakumar, A. and Deutsch, C. (2012) Reduced isotope fractionation by denitrification under conditions relevant to the ocean. *Geochimica et Cosmochimica Acta* 92, 243-259.
- Külling, D. (2002) Nährstoffe und Schwermetalle im Klärschlamm 1975 - 1999. *AGRARForschung* 9.
- Kuokkanen, A., Blomberg, K., Heinonen, M. and Mikola, A. (2020) Mainstream Shortcut Nitrification-denitrification Causing Massive N<sub>2</sub>O Emissions In A Continuous Activated Sludge Process, IWA, Helsinki.
- Kuyper, J., Schroeder, H. and Linnér, B.-O. (2018) The Evolution of the UNFCCC. *Annual Review of Environment and Resources* 43(1), 343-368.
- Kuypers, M.M.M., Marchant, H.K. and Kartal, B. (2018) The microbial nitrogen-cycling network. *Nature Reviews Microbiology* 16(5), 263-276.
- Lackner, S., Gilbert, E.M., Vlaeminck, S.E., Joss, A., Horn, H. and van Loosdrecht, M.C. (2014) Full-scale partial nitrification/anammox experiences--an application survey. *Water Research* 55, 292-303.
- Larsen, T.A. (2015) CO<sub>2</sub>-neutral wastewater treatment plants or robust, climate-friendly wastewater management? A systems perspective. *Water Research* 87, 513-521.
- Lau, A.O., Strom, P.F. and Jenkins, D. (1984) Growth Kinetics of *Sphaerotilus natans* and a Floc Former in Pure and Dual Continuous Culture. *Journal (Water Pollution Control Federation)* 56(1), 41-51.
- Law, Y., Ye, L., Pan, Y. and Yuan, Z. (2012) Nitrous oxide emissions from wastewater treatment processes. *Philos Trans R Soc Lond B Biol Sci* 367(1593), 1265-1277.
- Lehmann, M. and Gruber, W. (2018) Personal communication with Moritz Lehmann. Gruber, W. (ed), eawag Dübendorf.
- Leu, S.Y., Libra, J.A. and Stenstrom, M.K. (2010) Monitoring off-gas O<sub>2</sub>/CO<sub>2</sub> to predict nitrification performance in activated sludge processes. *Water Research* 44(11), 3434-3444.
- Lewicka-Szczebak, D., Augustin, J., Giesemann, A. and Well, R. (2017) Quantifying N<sub>2</sub>O reduction to N<sub>2</sub> based on N<sub>2</sub>O isotopocules – validation with independent methods (helium incubation and <sup>15</sup>N gas flux method). *Biogeosciences* 14(3), 711-732.
- Lewicka-Szczebak, D., Dyckmans, J., Kaiser, J., Marca, A., Augustin, J. and Well, R. (2016) Oxygen isotope fractionation during N<sub>2</sub>O production by soil denitrification. *Biogeosciences* 13(4), 1129-1144.
- Lewicka-Szczebak, D., Well, R., Köster, J.R., Fuß, R., Senbayram, M., Dittert, K. and Flessa, H. (2014) Experimental determinations of isotopic fractionation factors associated with N<sub>2</sub>O production and reduction during denitrification in soils. *Geochimica et Cosmochimica Acta* 134, 55-73.
- Liu, Y., Cheng, X., Lun, X. and Sun, D. (2014) CH<sub>4</sub> emission and conversion from A<sub>2</sub>O and SBR processes in full-scale wastewater treatment plants. *Journal of Environmental Sciences* 26(1), 224-230.

- Liu, Y., Ni, B.-J., Ganigué, R., Werner, U., Sharma, K.R. and Yuan, Z. (2015a) Sulfide and methane production in sewer sediments. *Water Research* 70, 350-359.
- Liu, Y., Ni, B.J., Sharma, K.R. and Yuan, Z. (2015b) Methane emission from sewers. *Sci Total Environ* 524-525, 40-51.
- Lu, H., Chandran, K. and Stensel, D. (2014) Microbial ecology of denitrification in biological wastewater treatment. *Water Research* 64, 237-254.
- Lu, L., Guest, J.S., Peters, C.A., Zhu, X., Rau, G.H. and Ren, Z.J. (2018) Wastewater treatment for carbon capture and utilization. *Nature Sustainability* 1(12), 750-758.
- Luck, M. (2018) Expert Review - Additional data. eawag (ed).
- Lucker, S., Schwarz, J., Gruber-Dorninger, C., Spieck, E., Wagner, M. and Daims, H. (2015) Nitrotoxa-like bacteria are previously unrecognized key nitrite oxidizers in full-scale wastewater treatment plants. *ISME Journal* 9(3), 708-720.
- Ma, C., Jensen, M.M., Smets, B.F. and Thamdrup, B. (2017) Pathways and Controls of N<sub>2</sub>O Production in Nitritation-Anammox Biomass. *Environmental Science & Technology*.
- Magyar, P.M., Hausherr, D., Niederdorfer, R., Stöcklin, N., Wei, J., Mohn, J., Bürgmann, H., Joss, A. and Lehmann, M.F. (2021) Nitrogen isotope effects can be used to diagnose N transformations in wastewater anammox systems. *Scientific Reports* 11(1).
- Mampaey, K.E., De Kreuk, M.K., van Dongen, U.G., van Loosdrecht, M.C. and Volcke, E.I. (2016) Identifying N<sub>2</sub>O formation and emissions from a full-scale partial nitritation reactor. *Water Research* 88, 575-585.
- Mampaey, K.E., van Dongen, U.G., van Loosdrecht, M.C. and Volcke, E.I. (2015) Novel method for online monitoring of dissolved N<sub>2</sub>O concentrations through a gas stripping device. *Environmental Science & Technology* 36(13-16), 1680-1690.
- Manabe, S. and Wetherald, R.T. (1967) Thermal Equilibrium of the Atmosphere with a Given Distribution of Relative Humidity. *Journal of Atmospheric Science* 24, 241-258.
- Marescaux, A., Thieu, V. and Garnier, J. (2018) Carbon dioxide, methane and nitrous oxide emissions from the human-impacted Seine watershed in France. *Science of The Total Environment* 643, 247-259.
- Marias, F., Benzaoui, A., Vaxelaire, J., Gelix, F. and Nicol, F. (2015) Fate of Nitrogen during Fluidized Incineration of Sewage Sludge. Estimation of NO and N<sub>2</sub>O Content in the Exhaust Gas. *Energy and Fuels* 29(7), 4534-4548.
- Mariotti, A., Germon, J.C., Hubert, P., Kaiser, P., Letolle, R., Tardieux, A. and Tardieux, P. (1981) Experimental determination of nitrogen kinetic isotope fractionation: Some principles; illustration for the denitrification and nitrification processes. *Plant and Soil* 62(3), 413-430.

- Marques, R., Oehmen, A. and Pijuan, M. (2014) Novel microelectrode-based online system for monitoring N<sub>2</sub>O gas emissions during wastewater treatment. *Environmental Science & Technology* 48(21), 12816-12823.
- Marques, R., Rodriguez-Caballero, A., Oehmen, A. and Pijuan, M. (2016) Assessment of online monitoring strategies for measuring N<sub>2</sub>O emissions from full-scale wastewater treatment systems. *Water Research*.
- Martin, T.S. and Casciotti, K.L. (2016) Nitrogen and oxygen isotopic fractionation during microbial nitrite reduction. *Limnology and Oceanography* 61(3), 1134-1143.
- Massara, T.M., Malamis, S., Guisasola, A., Baeza, J.A., Noutsopoulos, C. and Katsou, E. (2017) A review on nitrous oxide (N<sub>2</sub>O) emissions during biological nutrient removal from municipal wastewater and sludge reject water. *Science of The Total Environment* 596-597, 106-123.
- Masuda, S., Sano, I., Hojo, T., Li, Y.Y. and Nishimura, O. (2018) The comparison of greenhouse gas emissions in sewage treatment plants with different treatment processes. *Chemosphere* 193, 581-590.
- Masuda, S., Suzuki, S., Sano, I., Li, Y.Y. and Nishimura, O. (2015) The seasonal variation of emission of greenhouse gases from a full-scale sewage treatment plant. *Chemosphere* 140, 167-173.
- McIlvin, M.R. and Altabet, M.A. (2005) Chemical Conversion of Nitrate and Nitrite to Nitrous Oxide for Nitrogen and Oxygen Isotopic Analysis in Freshwater and Seawater. *Analytical Chemistry* 77(17), 5589-5595.
- McKinney, W. (2010) *Data Structures for Statistical Computing in Python*, pp. 51-56.
- Meinshausen, M., Smith, S.J., Calvin, K., Daniel, J.S., Kainuma, M.L.T., Lamarque, J., Matsumoto, K., Montzka, S.A., Raper, S.C.B., Riahi, K., Thomson, A., Velders, G.J.M. and van Vuuren, D.P.P. (2011) The RCP greenhouse gas concentrations and their extensions from 1765 to 2300. *Climatic Change* 109(1), 213-241.
- Mello, W.Z.d., Ribeiro, R.P., Brotto, A.C., Kligerman, D.C., Piccoli, A.d.S. and Oliveira, J.L.M. (2013) Nitrous oxide emissions from an intermittent aeration activated sludge system of an urban wastewater treatment plant. *Química Nova* 36, 16-20.
- Meyer, J., Sommer, M. and Von Schulthess, R. (1996) *Stickstoff-Frachten aus Abwasserreinigungsanlagen*. Federal Office for the Environment, F. (ed), Bern.
- Meyer, S.S. and Wilderer, P.A. (2004) Reject Water: Treating of Process Water in Large Wastewater Treatment Plants in Germany—A Case Study. *Journal of Environmental Science and Health, Part A* 39(7), 1645-1654.
- Mikola, A., Heinonen, M., Kosonen, H., Leppanen, M., Rantanen, P. and Vahala, R. (2014) N<sub>2</sub>O emissions from secondary clarifiers and their contribution to the total emissions of the WWTP. *Water Science and Technology* 70(4), 720-728.
- Mohn, J., Gutjahr, W., Toyoda, S., Harris, E., Ibraim, E., Geilmann, H., Schleppi, P., Kuhn, T., Lehmann, M.F., Decock, C., Werner, R.A., Yoshida, N. and Brand, W.A. (2016) Reassessment of the NH<sub>4</sub>NO<sub>3</sub> thermal decomposition technique for calibration of

- the N<sub>2</sub>O isotopic composition. *Rapid Communications in Mass Spectrometry* 30(23), 2487-2496.
- Mohn, J., Wolf, B., Toyoda, S., Lin, C.T., Liang, M.C., Bruggemann, N., Wissel, H., Steiker, A.E., Dyckmans, J., Szvec, L., Ostrom, N.E., Casciotti, K.L., Forbes, M., Giesemann, A., Well, R., Doucett, R.R., Yarnes, C.T., Ridley, A.R., Kaiser, J. and Yoshida, N. (2014) Interlaboratory assessment of nitrous oxide isotopomer analysis by isotope ratio mass spectrometry and laser spectroscopy: current status and perspectives. *Rapid Communications in Mass Spectrometry* 28(18), 1995-2007.
- Montzka, S.A., Dlugokencky, E.J. and Butler, J.H. (2011) Non-CO<sub>2</sub> greenhouse gases and climate change. *Nature* 476(7358), 43-50.
- Morgan-Sagastume, F., Valentino, F., Hjort, M., Cirne, D., Karabegovic, L., Gerardin, F., Johansson, P., Karlsson, A., Magnusson, P., Alexandersson, T., Bengtsson, S., Majone, M. and Werker, A. (2013) Polyhydroxyalkanoate (PHA) production from sludge and municipal wastewater treatment. *Water Science and Technology* 69(1), 177-184.
- Müller, E.A. (2010) *Energie in ARA. VSA* (ed), Glatbrugg.
- Nakkasunchi, S., Hewitt, N.J., Zoppi, C. and Brandoni, C. (2021) A review of energy optimization modelling tools for the decarbonisation of wastewater treatment plants. *Journal of Cleaner Production* 279.
- Ni, B.J., Pan, Y., van den Akker, B., Ye, L. and Yuan, Z. (2015) Full-Scale Modeling Explaining Large Spatial Variations of Nitrous Oxide Fluxes in a Step-Feed Plug-Flow Wastewater Treatment Reactor. *Environ Sci Technol* 49(15), 9176-9184.
- Ni, B.J. and Yuan, Z. (2015) Recent advances in mathematical modeling of nitrous oxides emissions from wastewater treatment processes. *Water Research* 87, 336-346.
- Nierychlo, M., Milobedzka, A., Petriglieri, F., McIlroy, B., Nielsen, P.H. and McIlroy, S.J. (2019) The morphology and metabolic potential of the Chloroflexi in full-scale activated sludge wastewater treatment plants. *FEMS Microbiology Ecology* 95(2), fty228.
- Nikaido, H. (2003) Molecular basis of bacterial outer membrane permeability revisited. *Microbiology and Molecular Biology Reviews* 67(4), 593-656.
- Oppenheimer, M. and Petsonk, A. (2005) Article 2 of the UNFCCC: Historical Origins, Recent Interpretations. *Climatic Change* 73(3), 195-226.
- Örmeci, B. and Vesilind, P.A. (2000) Development of an improved synthetic sludge: a possible surrogate for studying activated sludge dewatering characteristics. *Water Research* 34(4), 1069-1078.
- Orschler, L., Agrawal, S. and Lackner, S. (2021) Targeted metagenomics reveals extensive diversity of the denitrifying community in partial nitrification anammox and activated sludge systems. *Biotechnol Bioeng* 118(1), 433-441.
- Ostrom, N.E. and Ostrom, P.H. (2017) Mining the isotopic complexity of nitrous oxide: a review of challenges and opportunities. *Biogeochemistry* 132(3), 359-372.



- Pan, K.L., Gao, J.F., Fan, X.Y., Li, D.C. and Dai, H.H. (2018) The more important role of archaea than bacteria in nitrification of wastewater treatment plants in cold season despite their numerical relationships. *Water Research* 145, 552-561.
- Pan, Y., Ni, B.J., Bond, P.L., Ye, L. and Yuan, Z. (2013a) Electron competition among nitrogen oxides reduction during methanol-utilizing denitrification in wastewater treatment. *Water Research* 47(10), 3273-3281.
- Pan, Y., Ni, B.J. and Yuan, Z. (2013b) Modeling electron competition among nitrogen oxides reduction and  $\text{N}_2\text{O}$  accumulation in denitrification. *Environmental Science & Technology* 47(19), 11083-11091.
- Pan, Y., van den Akker, B., Ye, L., Ni, B.J., Watts, S., Reid, K. and Yuan, Z. (2016) Unravelling the spatial variation of nitrous oxide emissions from a step-feed plug-flow full scale wastewater treatment plant. *Scientific Reports* 6, 20792.
- Parravicini, V., Svoldal, K. and Krampe, J. (2016) Greenhouse Gas Emissions from Wastewater Treatment Plants. *Energy Procedia* 97, 246-253.
- Parravicini, V., Valkova, T., Haslinger, J., Saracevic, E., Winkelbauer, A., Tauber, J., Svoldal, K., Hohenblum, P., Clara, M., Windhofer, G., Pazdernik, K. and Lampert, C. (2015) Reduktionspotential bei den Lachgasemissionen aus Kläranlagen durch Optimierung des Betriebes Bundesministerium Landwirtschaft, Regionen und Tourismus, Wien.
- Peng, L., Carvajal-Arroyo, J.M., Seuntjens, D., Prat, D., Colica, G., Pintucci, C. and Vlaeminck, S.E. (2017) Smart operation of nitrification/denitrification virtually abolishes nitrous oxide emission during treatment of co-digested pig slurry centrate. *Water Research* 127, 1-10.
- Peng, L., Ni, B.-J., Law, Y. and Yuan, Z. (2016) Modeling  $\text{N}_2\text{O}$  production by ammonia oxidizing bacteria at varying inorganic carbon concentrations by coupling the catabolic and anabolic processes. *Chemical Engineering Science* 144, 386-394.
- Peng, L., Ni, B.J., Ye, L. and Yuan, Z. (2015) The combined effect of dissolved oxygen and nitrite on  $\text{N}_2\text{O}$  production by ammonia oxidizing bacteria in an enriched nitrifying sludge. *Water Research* 73, 29-36.
- Perez-Garcia, O., Villas-Boas, S.G., Swift, S., Chandran, K. and Singhal, N. (2014) Clarifying the regulation of  $\text{NO}/\text{N}_2\text{O}$  production in *Nitrosomonas europaea* during anoxic-oxic transition via flux balance analysis of a metabolic network model. *Water Research* 60, 267-277.
- Petit, J.R., Jouzel, J., Raynaud, D., Barkov, N.I., Barnola, J.M., Basile, I., Bender, M., Chappellaz, J., Davis, M., Delaygue, G., Delmotte, M., Kotiyakov, V.M., Legrand, M., Lipenkov, V.Y., Lorius, C., Pépin, L., Ritz, C., Saltzman, E. and Stievenard, M. (1999) Climate and atmospheric history of the past 420,000 years from the Vostok ice core, Antarctica. *Nature* 399(6735), 429-436.
- Philips, S., Laanbroek, H.J. and Verstraete, W. (2002) Origin, causes and effects of increased nitrite concentrations in aquatic environments. *Reviews in Environmental Science & Bio/Technology* 1(2), 115-141.

- Prather, M.J., Holmes, C.D. and Hsu, J. (2012) Reactive greenhouse gas scenarios: Systematic exploration of uncertainties and the role of atmospheric chemistry. *Geophysical Research Letters* 39(9).
- Prather, M.J., Hsu, J., DeLuca, N.M., Jackman, C.H., Oman, L.D., Douglass, A.R., Fleming, E.L., Strahan, S.E., Steenrod, S.D., Sovde, O.A., Isaksen, I.S., Froidevaux, L. and Funke, B. (2015) Measuring and modeling the lifetime of nitrous oxide including its variability. *Journal of Geophysical Research-Atmospheres* 120(11), 5693-5705.
- Prinn, R., Weiss, R., Arduini, J., Arnold, T., DeWitt, H., Fraser, P., Ganesan, A., Gasore, J., Harth, C., Hermansen, O., Kim, J., Krummel, P., Li, S., Loh, Z., Lunder, C., Maione, M., Manning, A., Miller, B., Mitrevski, B., Muhle, J., O'Doherty, S., Park, S., Reimann, S., Rigby, M., Saito, T., Salameh, P., Schmidt, R., Simmonds, P., Steele, L., Vollmer, M., Wang, H.J., Yao, B., Yokouchi, Y., Young, D. and Zhou, L. (2018) History of chemically and radiatively important atmospheric gases from the Advanced Global Atmospheric Gases Experiment (AGAGE). Carbon Dioxide Information Analysis Center (CDIAC), Oak Ridge National Laboratory (ORNL), Oak Ridge, TN (United States). AGAGE (ed).
- Ramirez-Melgarejo, M., Reyes-Figueroa, A.D., Gasso-Domingo, S. and Guereca, L.P. (2020) Analysis of empirical methods for the quantification of N<sub>2</sub>O emissions in wastewater treatment plants: Comparison of emission results obtained from the IPCC Tier 1 methodology and the methodologies that integrate operational data. *Science of The Total Environment* 747, 141288.
- Randall, C.W. and Buth, D. (1984) Nitrite Build-Up in Activated Sludge Resulting from Temperature Effects. *Journal (Water Pollution Control Federation)* 56(9), 1039-1044.
- Ravishankara, A.R., Daniel, J.S. and Portmann, R.W. (2009) Nitrous Oxide (N<sub>2</sub>O): The Dominant Ozone-Depleting Substance Emitted in the 21st Century. *Science* 326(5949), 123-125.
- Remy, C., Lesjean, B. and Waschnewski, J. (2013) Identifying energy and carbon footprint optimization potentials of a sludge treatment line with Life Cycle Assessment. *Water Science and Technology* 67(1), 63-73.
- Ren, Y., Ngo, H.H., Guo, W., Ni, B.-J. and Liu, Y. (2019) Linking the nitrous oxide production and mitigation with the microbial community in wastewater treatment: A review. *Bioresource Technology Reports* 7.
- Ren, Y.G., Wang, J.H., Li, H.F., Zhang, J., Qi, P.Y. and Hu, Z. (2013) Nitrous oxide and methane emissions from different treatment processes in full-scale municipal wastewater treatment plants. *Environmental Technology* 34(21-24), 2917-2927.
- Revelle, R. and Suess, H.E. (1957) Carbon Dioxide Exchange Between Atmosphere and Ocean and the Question of an Increase of Atmospheric CO<sub>2</sub> during the Past Decades *Tellus* 9(1), 18-27.
- Ribera-Guardia, A., Bosch, L., Corominas, L. and Pijuan, M. (2019) Nitrous oxide and methane emissions from a plug-flow full-scale bioreactor and assessment of its carbon footprint. *Journal of Cleaner Production* 212, 162-172.

- Rodriguez-Caballero, A., Aymerich, I., Marques, R., Poch, M. and Pijuan, M. (2015) Minimizing N<sub>2</sub>O emissions and carbon footprint on a full-scale activated sludge sequencing batch reactor. *Water Research* 71, 1-10.
- Rodriguez-Caballero, A., Aymerich, I., Poch, M. and Pijuan, M. (2014a) Evaluation of process conditions triggering emissions of green-house gases from a biological wastewater treatment system. *Sci Total Environ* 493, 384-391.
- Rodriguez-Caballero, A., Aymerich, I., Poch, M. and Pijuan, M. (2014b) Evaluation of process conditions triggering emissions of green-house gases from a biological wastewater treatment system. *Science of The Total Environment* 493, 384-391.
- Rohe, L., Well, R. and Lewicka-Szczebak, D. (2017) Use of oxygen isotopes to differentiate between nitrous oxide produced by fungi or bacteria during denitrification. *Rapid Communications in Mass Spectrometry* 31(16), 1297-1312.
- Roots, P., Wang, Y., Rosenthal, A.F., Griffin, J.S., Sabba, F., Petrovich, M., Yang, F., Kozak, J.A., Zhang, H. and Wells, G.F. (2019) Comammox Nitrospira are the dominant ammonia oxidizers in a mainstream low dissolved oxygen nitrification reactor. *Water Research* 157, 396-405.
- Samuelsson, J., Delre, A., Tumlin, S., Hadi, S., Offerle, B. and Scheutz, C. (2018) Optical technologies applied alongside on-site and remote approaches for climate gas emission quantification at a wastewater treatment plant. *Water Research* 131, 299-309.
- Sänger, M., Werther, J. and Ogada, T. (2001) NO<sub>x</sub> and N<sub>2</sub>O emission characteristics from fluidised bed combustion of semi-dried municipal sewage sludge. *Fuel* 80(2), 167-177.
- Schaubroeck, T., De Clippeleir, H., Weissenbacher, N., Dewulf, J., Boeckx, P., Vlaeminck, S.E. and Wett, B. (2015) Environmental sustainability of an energy self-sufficient sewage treatment plant: improvements through DEMON and co-digestion. *Water Research* 74, 166-179.
- Schreiber, F., Wunderlin, P., Udert, K.M. and Wells, G.F. (2012) Nitric oxide and nitrous oxide turnover in natural and engineered microbial communities: biological pathways, chemical reactions, and novel technologies. *Frontiers in Microbiology* 3, 372.
- Sexstone, A.J., Revsbech, N.P., Parkin, T.B. and Tiedje, J.M. (1985) Direct Measurement of Oxygen Profiles and Denitrification Rates in Soil Aggregates. *Soil Science Society of America Journal* 49(3), 645-651.
- Sezgin, M., Jenkins, D. and Parker, D.S. (1978) A Unified Theory of Filamentous Activated Sludge Bulking. *Journal (Water Pollution Control Federation)* 50(2), 362-381.
- Shade, A., Peter, H., Allison, S.D., Baho, D.L., Berga, M., Bürgmann, H., Huber, D.H., Langenheder, S., Lennon, J.T., Martiny, J.B., Matulich, K.L., Schmidt, T.M. and Handelsman, J. (2012) Fundamentals of microbial community resistance and resilience. *Frontiers in Microbiology* 3, 417.
- Siegrist, H. and Luck, M. (2018) Personal communication with Hansruedi Siegrist. Luck, M. (ed), eawag Dübendorf.

- Sigman, D.M., Casciotti, K.L., Andreani, M., Barford, C., Galanter, M. and Böhlke, J.K. (2001) A Bacterial Method for the Nitrogen Isotopic Analysis of Nitrate in Seawater and Freshwater. *Analytical Chemistry* 73(17), 4145-4153.
- Siripong, S. and Rittmann, B.E. (2007) Diversity study of nitrifying bacteria in full-scale municipal wastewater treatment plants. *Water Research* 41(5), 1110-1120.
- Soda, S., Iwai, Y., Sei, K., Shimod, Y. and Ike, M. (2010) Model analysis of energy consumption and greenhouse gas emissions of sewage sludge treatment systems with different processes and scales. *Water Science and Technology* 61(2), 365-373.
- Soler-Jofra, A., Perez, J. and van Loosdrecht, M.C.M. (2020) Hydroxylamine and the nitrogen cycle: A review. *Water Research* 190, 116723.
- Song, M.J., Choi, S., Bae, W.B., Lee, J., Han, H., Kim, D.D., Kwon, M., Myung, J., Kim, Y.M. and Yoon, S. (2020) Identification of primary effectors of N<sub>2</sub>O emissions from full-scale biological nitrogen removal systems using random forest approach. *Water Research* 184, 116144.
- Speirs, L.B.M., Rice, D.T.F., Petrovski, S. and Seviour, R.J. (2019) The Phylogeny, Biodiversity, and Ecology of the Chloroflexi in Activated Sludge. *Frontiers in Microbiology* 10, 2015.
- Stein, L.Y. (2018) Insights into the physiology of ammonia-oxidizing microorganisms. *Current Opinion in Chemical Biology* 49, 9-15.
- Stein, L.Y. and Klotz, M.G. (2016) The nitrogen cycle. *Current Biology* 26(3), R94-98.
- Stenstrom, F., Tjus, K. and la Cour Jansen, J. (2014) Oxygen-induced dynamics of nitrous oxide in water and off-gas during the treatment of digester supernatant. *Water Science and Technology* 69(1), 84-91.
- Stenstrom, M.K. and Poduska, R.A. (1980) The effect of dissolved oxygen concentration on nitrification. *Water Research* 14(6), 643-649.
- Stocker, T.F., Qin, D., Plattner, G.K., Tignor, M.M.B., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V. and Midgley, P.M. (2013) Climate change 2013 the physical science basis: Working Group I contribution to the fifth assessment report of the intergovernmental panel on climate change.
- Strähl, S., Ort, C., Siegrist, H., Thomann, M., Obrecht, J. and Kurz, E. (2013) Stickstoffelimination in Schweizer ARA. *Aqua & Gas* 5/2013, 74-84.
- Su, Q., Domingo-Felez, C., Jensen, M.M. and Smets, B.F. (2019) Abiotic Nitrous Oxide (N<sub>2</sub>O) Production Is Strongly pH Dependent, but Contributes Little to Overall N<sub>2</sub>O Emissions in Biological Nitrogen Removal Systems. *Environmental Science & Technology* 53(7), 3508-3516.
- Suchetana, B., Rajagopalan, B. and Silverstein, J. (2017) Assessment of wastewater treatment facility compliance with decreasing ammonia discharge limits using a regression tree model. *Sci Total Environ* 598, 249-257.
- Suess, H.E. (1955) Radiocarbon Concentration in Modern Wood. *Science* 122, 415-417.

- Sun, S., Bao, Z., Li, R., Sun, D., Geng, H., Huang, X., Lin, J., Zhang, P., Ma, R., Fang, L., Zhang, X. and Zhao, X. (2017) Reduction and prediction of N<sub>2</sub>O emission from an Anoxic/Oxic wastewater treatment plant upon DO control and model simulation. *Bioresource Technology* 244(Pt 1), 800-809.
- Sutka, R.L., Ostrom, N.E., Ostrom, P.H., Breznak, J.A., Gandhi, H., Pitt, A.J. and Li, F. (2006) Distinguishing nitrous oxide production from nitrification and denitrification on the basis of isotopomer abundances. *Applied Environmental Microbiology* 72(1), 638-644.
- Suzuki, Y., Ochi, S.-I., Kawashima, Y. and Hiraide, R. (2003) Determination of Emission Factors of Nitrous Oxide from Fluidized Bed Sewage Sludge Incinerators by Long-term Continuous Monitoring. *JOURNAL OF CHEMICAL ENGINEERING OF JAPAN* 36(4), 458-463.
- Svoboda, K., Baxter, D. and Martinec, J. (2006) Nitrous oxide emissions from waste incineration. *Chemical Papers* 60(1), 78-90.
- Tallec, G., Garnier, J., Billen, G. and Gousailles, M. (2006) Nitrous oxide emissions from secondary activated sludge in nitrifying conditions of urban wastewater treatment plants: effect of oxygenation level. *Water Research* 40(15), 2972-2980.
- Tans, P. and Keeling, R. (2021) Trends in Atmospheric Carbon Dioxide. NOAA/GML (ed).
- Tauber, J., Parravicini, V., Svoldal, K. and Krampe, J. (2019) Quantifying methane emissions from anaerobic digesters. *Water Science and Technology* 80(9), 1654-1661.
- Tchobanoglous, G., Stensel, D.H., Tsuchihashi, R. and Burton, F. (2014) *Wastewater Engineering: Treatment and Resource Recovery* McGrawHill, New York.
- Thompson, R.L., Lassaletta, L., Patra, P.K., Wilson, C., Wells, K.C., Gressent, A., Koffi, E.N., Chipperfield, M.P., Winiwarter, W., Davidson, E.A., Tian, H. and Canadell, J.G. (2019) Acceleration of global N<sub>2</sub>O emissions seen from two decades of atmospheric inversion. *Nature Climate Change* 9(12), 993-998.
- Tian, H., Xu, R., Canadell, J.G., Thompson, R.L., Winiwarter, W., Suntharalingam, P., Davidson, E.A., Ciais, P., Jackson, R.B., Janssens-Maenhout, G., Prather, M.J., Regnier, P., Pan, N., Pan, S., Peters, G.P., Shi, H., Tubiello, F.N., Zaehle, S., Zhou, F., Arneth, A., Battaglia, G., Berthet, S., Bopp, L., Bouwman, A.F., Buitenhuis, E.T., Chang, J., Chipperfield, M.P., Dangal, S.R.S., Dlugokencky, E., Elkins, J.W., Eyre, B.D., Fu, B., Hall, B., Ito, A., Joos, F., Krummel, P.B., Landolfi, A., Laruelle, G.G., Lauerwald, R., Li, W., Lienert, S., Maavara, T., MacLeod, M., Millet, D.B., Olin, S., Patra, P.K., Prinn, R.G., Raymond, P.A., Ruiz, D.J., van der Werf, G.R., Vuichard, N., Wang, J., Weiss, R.F., Wells, K.C., Wilson, C., Yang, J. and Yao, Y. (2020) A comprehensive quantification of global nitrous oxide sources and sinks. *Nature* 586(7828), 248-256.
- Tian, H., Yang, J., Lu, C., Xu, R., Canadell, J.G., Jackson, R.B., Arneth, A., Chang, J., Chen, G., Ciais, P., Gerber, S., Ito, A., Huang, Y., Joos, F., Lienert, S., Messina, P., Olin, S., Pan, S., Peng, C., Saikawa, E., Thompson, R.L., Vuichard, N., Winiwarter, W., Zaehle, S., Zhang, B., Zhang, K. and Zhu, Q. (2018) The Global N<sub>2</sub>O Model Intercomparison Project. *Bulletin of the American Meteorological Society* 99(6), 1231-1251.

- Toyoda, S., Suzuki, Y., Hattori, S., Yamada, K., Fujii, A., Yoshida, N., Kouno, R., Murayama, K. and Shiomi, H. (2011) Isotopomer analysis of production and consumption mechanisms of N<sub>2</sub>O and CH<sub>4</sub> in an advanced wastewater treatment system. *Environmental Science & Technology* 45(3), 917-922.
- Toyoda, S. and Yoshida, N. (1999) Determination of nitrogen isotopomers of nitrous oxide on a modified isotope ratio mass spectrometer. *Analytical Chemistry* 71(20), 4711-4718.
- Toyoda, S., Yoshida, N. and Koba, K. (2017) Isotopocule analysis of biologically produced nitrous oxide in various environments. *Mass Spectrometry Reviews* 36(2), 135-160.
- Tumendelger, A., Alshboul, Z. and Lorke, A. (2019) Methane and nitrous oxide emission from different treatment units of municipal wastewater treatment plants in Southwest Germany. *PLoS One* 14(1), e0209763.
- Tumendelger, A., Toyoda, S., Yoshida, N., Shiomi, H. and Kouno, R. (2016) Isotopocule characterization of N<sub>2</sub>O dynamics during simulated wastewater treatment under oxic and anoxic conditions. *Geochemical Journal* 50(2), 105-121.
- Tyndall, J. (1861) XXIII. On the absorption and radiation of heat by gases and vapours, and on the physical connexion of radiation, absorption, and conduction.—The bakerian lecture. *The London, Edinburgh, and Dublin Philosophical Magazine and Journal of Science* 22(146), 169-194.
- Umweltbundesamt Austria (2017) Austria's National Inventory Report 2017, Wien.
- UNEP (1987) Montreal Protocol on Substances that Deplete the Ozone Layer.
- United Nations Climate Change (1992) United Nations Framework Convention on Climate Change. Change, U.N.C. (ed).
- United Nations Climate Change (1997) Kyoto Protocol to the United Nations Framework Convention on Climate Change. Change, U.N.C. (ed).
- United Nations Climate Change (2015) Paris Protocol to the United Nations Framework Convention on Climate Change. Change, U.N.C. (ed).
- Valkova, T., Parravicini, V., Saracevic, E., Tauber, J., Svardal, K. and Krampe, J. (2020) A method to estimate the direct nitrous oxide emissions of municipal wastewater treatment plants based on the degree of nitrogen removal. *Journal of Environmental Management*, 111563.
- Van den Abbeele, P., Van de Wiele, T., Verstraete, W. and Possemiers, S. (2011) The host selects mucosal and luminal associations of coevolved gut microorganisms: a novel concept. *FEMS Microbiology Reviews* 35(4), 681-704.
- Van Drecht, G., Bouwman, A.F., Harrison, J. and Knoop, J.M. (2009) Global nitrogen and phosphate in urban wastewater for the period 1970 to 2050. *Global Biogeochemical Cycles* 23(4), n/a-n/a.
- Van Rossum, G. and Drake, F.L. (2009) Python 3 Reference Manual, CreateSpace, Scotts Valley, CA.

- Vasilaki, V., Conca, V., Frison, N., Eusebi, A.L., Fatone, F. and Katsou, E. (2020a) A knowledge discovery framework to predict the N<sub>2</sub>O emissions in the wastewater sector. *Water Research* 178, 115799.
- Vasilaki, V., Danishvar, S., Mousavi, A. and Katsou, E. (2020b) Data-driven versus conventional N<sub>2</sub>O EF quantification methods in wastewater; how can we quantify reliable annual EFs? *Computers & Chemical Engineering*.
- Vasilaki, V., Massara, T.M., Stanchev, P., Fatone, F. and Katsou, E. (2019) A decade of nitrous oxide (N<sub>2</sub>O) monitoring in full-scale wastewater treatment processes: A critical review. *Water Research* 161, 392-412.
- Vasilaki, V., Volcke, E.I.P., Nandi, A.K., van Loosdrecht, M.C.M. and Katsou, E. (2018) Relating N<sub>2</sub>O emissions during biological nitrogen removal with operating conditions using multivariate statistical techniques. *Water Research* 140, 387-402.
- Vieira, A., Galinha, C.F., Oehmen, A. and Carvalho, G. (2018) The link between nitrous oxide emissions, microbial community profile and function from three full-scale WWTPs. *Science of The Total Environment* 651(Pt 2), 2460-2472.
- Virtanen, P., Gommers, R., Oliphant, T.E., Haberland, M., Reddy, T., Cournapeau, D., Burovski, E., Peterson, P., Weckesser, W., Bright, J., van der Walt, S.J., Brett, M., Wilson, J., Millman, K.J., Mayorov, N., Nelson, A.R.J., Jones, E., Kern, R., Larson, E., Carey, C.J., Polat, İ., Feng, Y., Moore, E.W., VanderPlas, J., Laxalde, D., Perktold, J., Cimrman, R., Henriksen, I., Quintero, E.A., Harris, C.R., Archibald, A.M., Ribeiro, A.H., Pedregosa, F., van Mulbregt, P., Vijaykumar, A., Bardelli, A.P., Rothberg, A., Hilboll, A., Kloeckner, A., Scopatz, A., Lee, A., Rokem, A., Woods, C.N., Fulton, C., Masson, C., Häggström, C., Fitzgerald, C., Nicholson, D.A., Hagen, D.R., Pasechnik, D.V., Olivetti, E., Martin, E., Wieser, E., Silva, F., Lenders, F., Wilhelm, F., Young, G., Price, G.A., Ingold, G.-L., Allen, G.E., Lee, G.R., Audren, H., Probst, I., Dietrich, J.P., Silterra, J., Webber, J.T., Slavič, J., Nothman, J., Buchner, J., Kulick, J., Schönberger, J.L., de Miranda Cardoso, J.V., Reimer, J., Harrington, J., Rodríguez, J.L.C., Nunez-Iglesias, J., Kuczynski, J., Tritz, K., Thoma, M., Newville, M., Kümmerer, M., Bolingbroke, M., Tartre, M., Pak, M., Smith, N.J., Nowaczyk, N., Shebanov, N., Pavlyk, O., Brodtkorb, P.A., Lee, P., McGibbon, R.T., Feldbauer, R., Lewis, S., Tygier, S., Sievert, S., Vigna, S., Peterson, S., More, S., Pudlik, T., Oshima, T., Pingel, T.J., Robitaille, T.P., Spura, T., Jones, T.R., Cera, T., Leslie, T., Zito, T., Krauss, T., Upadhyay, U., Halchenko, Y.O., Vázquez-Baeza, Y. and SciPy, C. (2020) SciPy 1.0: fundamental algorithms for scientific computing in Python. *Nature Methods* 17(3), 261-272.
- Von Schulthess, R. and Gujer, W. (1996) Release of nitrous oxide (N<sub>2</sub>O) from denitrifying activated sludge: verification and application of a mathematical model. *Water Research* 30(3), 521-530.
- Vuono, D.C., Benecke, J., Henkel, J., Navidi, W.C., Cath, T.Y., Munakata-Marr, J., Spear, J.R. and Drewes, J.E. (2015) Disturbance and temporal partitioning of the activated sludge metacommunity. *ISME Journal* 9(2), 425-435.
- Wang, J., Zhang, J., Xie, H., Qi, P., Ren, Y. and Hu, Z. (2011) Methane emissions from a full-scale A/A/O wastewater treatment plant. *Bioresource Technology* 102(9), 5479-5485.

- Wang, W.C., Yung, Y.L., Lacis, A.A., Mo, T. and Hansen, J.E. (1976) Greenhouse Effects Due to Man-Made Perturbations of Trace Gases. *Science* 194(4266), 685-690.
- Wang, Y., Fang, H., Zhou, D., Han, H. and Chen, J. (2016) Characterization of nitrous oxide and nitric oxide emissions from a full-scale biological aerated filter for secondary nitrification. *Chemical Engineering Journal*.
- Ward, B.B., Arp, D.J. and Klotz, M.G. (2011) *Nitrification*, American Society of Microbiology.
- Weart, S.R. (2008) *The Discovery of Global Warming* Revised and Expanded Edition, Harvard University Press.
- Wegen, S., Nowka, B. and Spieck, E. (2019) Low Temperature and Neutral pH Define "Candidatus Nitrotoga sp." as a Competitive Nitrite Oxidizer in Coculture with *Nitrospira defluvii*. *Applied Environmental Microbiology* 85(9).
- Wei, W., Isobe, K., Nishizawa, T., Zhu, L., Shiratori, Y., Ohte, N., Koba, K., Otsuka, S. and Senoo, K. (2015) Higher diversity and abundance of denitrifying microorganisms in environments than considered previously. *ISME Journal* 9(9), 1954-1965.
- Werner, R. (2003) The Online 18O/ 16O Analysis: Development and application. *Isotopes in Environmental and Health Studies* 39(2), 85-104.
- Werner, R.A. and Brand, W.A. (2001) Referencing strategies and techniques in stable isotope ratio analysis. *Rapid Communications in Mass Spectrometry* 15(7), 501-519.
- Wrage-Mönnig, N., Horn, M.A., Well, R., Müller, C., Velthof, G. and Oenema, O. (2018) The role of nitrifier denitrification in the production of nitrous oxide revisited. *Soil Biology and Biochemistry* 123, A3-A16.
- Wu, L., Ning, D., Zhang, B., Li, Y., Zhang, P., Shan, X., Zhang, Q., Brown, M.R., Li, Z., Van Nostrand, J.D., Ling, F., Xiao, N., Zhang, Y., Vierheilig, J., Wells, G.F., Yang, Y., Deng, Y., Tu, Q., Wang, A., Global Water Microbiome, C., Zhang, T., He, Z., Keller, J., Nielsen, P.H., Alvarez, P.J.J., Criddle, C.S., Wagner, M., Tiedje, J.M., He, Q., Curtis, T.P., Stahl, D.A., Alvarez-Cohen, L., Rittmann, B.E., Wen, X. and Zhou, J. (2019) Global diversity and biogeography of bacterial communities in wastewater treatment plants. *Nature Microbiology* 4(7), 1183-1195.
- Wunderlin, P. (2013) *Mechanisms Of N<sub>2</sub>O Production In Biological Wastewater Treatment From Pathway Identification To Process Control*. Cumulative thesis, ETH Zurich, Zurich.
- Wunderlin, P., Lehmann, M.F., Siegrist, H., Tuzson, B., Joss, A., Emmenegger, L. and Mohn, J. (2013a) Isotope signatures of N<sub>2</sub>O in a mixed microbial population system: constraints on N<sub>2</sub>O producing pathways in wastewater treatment. *Environmental Science & Technology* 47(3), 1339-1348.
- Wunderlin, P., Mohn, J., Joss, A., Emmenegger, L. and Siegrist, H. (2012) Mechanisms of N<sub>2</sub>O production in biological wastewater treatment under nitrifying and denitrifying conditions. *Water Research* 46(4), 1027-1037.



- Wunderlin, P., Siegrist, H. and Joss, A. (2013b) Online N<sub>2</sub>O measurement: the next standard for controlling biological ammonia oxidation? *Environmental Science & Technology* 47(17), 9567-9568.
- Yan, X., Li, L. and Liu, J. (2014) Characteristics of greenhouse gas emission in three full-scale wastewater treatment processes. *Journal of Environmental Sciences* 26(2), 256-263.
- Yildiz, H.Y. and Benli, A.C.K. (2004) Nitrite toxicity to crayfish, *Astacus leptodactylus*, the effects of sublethal nitrite exposure on hemolymph nitrite, total hemocyte counts, and hemolymph glucose. *Ecotoxicology and Environmental Safety* 59(3), 370-375.
- Yoshida, H., Monster, J. and Scheutz, C. (2014) Plant-integrated measurement of greenhouse gas emissions from a municipal wastewater treatment plant. *Water Research* 61, 108-118.
- Yoshida, N. and Toyoda, S. (2000) Constraining the atmospheric N<sub>2</sub>O budget from intramolecular site preference in N<sub>2</sub>O isotopomers. *Nature* 405(6784), 330-334.
- Yu, L., Harris, E., Lewicka-Szczebak, D., Barthel, M., Blomberg, M.R.A., Harris, S.J., Johnson, M.S., Lehmann, M.F., Liisberg, J., Muller, C., Ostrom, N.E., Six, J., Toyoda, S., Yoshida, N. and Mohn, J. (2020) What can we learn from N<sub>2</sub>O isotope data? - Analytics, processes and modelling. *Rapid Communications in Mass Spectrometry* 34(20), e8858.
- Zhang, L., Altabet, M.A., Wu, T. and Hadas, O. (2007) Sensitive Measurement of NH<sub>4</sub><sup>+</sup> <sup>15</sup>N/<sup>14</sup>N (δ<sup>15</sup>NH<sub>4</sub><sup>+</sup>) at Natural Abundance Levels in Fresh and Saltwaters. *Analytical Chemistry* 79(14), 5297-5303.
- Zhou, Y., Oehmen, A., Lim, M., Vadivelu, V. and Ng, W.J. (2011) The role of nitrite and free nitrous acid (FNA) in wastewater treatment plants. *Water Research* 45(15), 4672-4682.
- Zumft, W.G. (1997) Cell biology and molecular basis of denitrification. *Microbiology and Molecular Biology Reviews* 61(4), 533-616.

# Curriculum Vitae

## Personal Data

Name	Wenzel Johann Gruber
Date of birth	April 27, 1988
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## Education

2012 – 2014	Master of Science ETH in environmental engineering Major in urban water management Swiss Federal Institute of Technology (ETH), Zürich, Switzerland
2008 – 2011	Bachelor of Science ETH in environmental engineering Swiss Federal Institute of Technology (ETH) Zürich, Switzerland

## Employment

2017 – 2021	PhD student at the Swiss Federal Institute of Aquatic Science and Technology (Eawag), Department of Process Engineering, Dübendorf, Switzerland
2015 – 2017	Scientific assistant at the Swiss Federal Institute of Aquatic Science and Technology (Eawag), Department of Process Engineering, Dübendorf, Switzerland
2014 – 2015	Civilian servant Sérvice social international (SSI), Lomé, Togo
2014	Internship and Junior Project Manager South Pole Carbon, Zurich, Switzerland
2011 – 2012	Internship TBF+Partner AG Zurich, Switzerland
2008	Civilian servant Altersheim Wollishofen Zurich, Switzerland

## Publications

### Articles in peer reviewed journals

Gruber, W., Niederdorfer, R., Ringwald, J., Morgenroth, E., Bürgmann, H. and Joss, A. (2021) Linking seasonal N<sub>2</sub>O emissions and nitrification failures to microbial dynamics in a SBR wastewater treatment plant. *Water Research X* 11, 100098.

Gruber, W., Villez, K., Kipf, M., Wunderlin, P., Siegrist, H., Vogt, L. and Joss, A. (2020) N<sub>2</sub>O emission in full-scale wastewater treatment: Proposing a refined monitoring strategy. *Science of The Total Environment* 699, 134157.

Gruber, W., von Känel, L., Vogt, L., Luck, M., Biolley, L., Feller, K., Moosmann, A., Krähenbühl, N., Kipf, M., Loosli, R., Vogel, M., Morgenroth, E., Braun, D., Joss, A. (2021) Estimation of countrywide N<sub>2</sub>O emissions from wastewater treatment in Switzerland using long-term monitoring data. *Water Research X* 13 (2021) 100122

### Technical reports and other contributions:

Luck, M., Gruber, W., Joss, A. (2018) Review of “Source category 5D – Wastewater treatment and discharge” in Switzerland. Expert review of Switzerland’s ‘National GHG Inventory Report, Sector 5: Waste’ and ‘Chapter 6: Wastewater treatment and discharge - of the 2006 IPCC Guidelines’

Böhler, M., Hernandez, A., Fleiner, J., Gruber, W., Siegris, H., Seyfried, A., Luning, L. (2018) Design and operation of a full-scale plant for membrane ammonia stripping. Powerstep, Project report WP6.

### Conference contributions:

Gruber, W., Von Kaenel, L., Biolley, L., Moosmann, A., Vogt, L., Luck, M., Morgenroth, E., Braun, D., and Joss, A. (2020) Nutrient removal efficiencies govern yearly N<sub>2</sub>O production rates on wastewater treatment plants. IWA Nutrient Removal and Recovery Conference 2020, Helsinki, Finland.