

Hospital Wastewater Treatment by Membrane Bioreactor: Performance and Efficiency for Organic Micropollutant Elimination

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ABSTRACT: A pilot-scale membrane bioreactor (MBR) was installed and operated for one year at a Swiss hospital. It was fed an influent directly from the hospital's sanitary collection system. To study the efficiency of micropollutant elimination in raw hospital wastewater that comprises a complex matrix with micropollutant concentrations ranging from low ng/L to low mg/L, an automated on-line SPE-HPLC-MS/MS analytical method was developed. Among the 68 target analytes were: 56 pharmaceuticals (antibiotics, antimycotics, antivirals, iodinated X-ray contrast media, antiinflammatory, cytostatics, diuretics, beta blockers, anesthetics, analgesics, antiepileptics, antidepressants, and others), 10 metabolites, and 2 corrosion inhibitors. The MBR influent contained the majority of those target analytes. The micropollutant elimination efficiency was assessed through continuous flow-proportional sampling of the MBR influent and continuous time-proportional sampling of the MBR effluent. An

1 overall load elimination of all pharmaceuticals and metabolites in the MBR was 22%, as over 80% of
2 the load was due to persistent iodinated contrast media. No inhibition by antibacterial agents or
3 disinfectants from the hospital was observed in the MBR. The hospital wastewater was found to be a
4 dynamic system in which conjugates of pharmaceuticals de-conjugate and biological transformation
5 products are formed, which in some cases are pharmaceuticals themselves.
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13 **KEYWORDS:** 4-acetamidoantipyrine, 4-aminoantipyrine, 4-dimethylaminoantipyrine,
14 4-formylaminoantipyrine, 4-methylaminoantipyrine, acetaminophen, amidotrizoic acid,
15 aminophenazone, aminopyrine, analgesic, anesthetic, antibiotic, antidepressant, antiepileptic,
16 antiinfective, antiinflammatory, antimycotic, antipyretic, antipyrine, antirheumatic, antiviral, anxiolytic,
17 atenolol, atenolol acid, azithromycin, barbiturate, benzalkonium chloride, benzotriazole, beta blocker,
18 bezafibrate, biodegradation, biological degradation, carbamazepine, cilastatin, ciprofloxacin,
19 clarithromycin, clindamycin, clofibrate, clofibric acid, conjugate, corticosteroid, cyclophosphamide,
20 cytostatic, D617, depletion, dexamethasone, diatrizoate, diatrizoic acid, diazepam, diclofenac,
21 dimethyldidecylammonium chloride, diuretic, elimination efficiency, erythromycin, fluconazole,
22 fluoroquinolone, fluoxetine, furosemide, gabapentin, hormonal preparation, hospital wastewater
23 treatment, HPLC-MS/MS, hydrochlorothiazide, ICM, ifosfamide, indometacin, industrial chemical,
24 internal standard, iodinated X-ray contrast media, iohexol, iomeprol, iopamidol, iopromide, ioxitalamic
25 acid, levetiracetam, lidocaine, macrolide, MBR, mefenamic acid, membrane bioreactor, metabolite,
26 metamizole, methylbenzotriazole methylprednisolone, metoprolol, metoprolol acid, metronidazole,
27 micro-pollutant, morphine, N4-acetylsulfamethoxazole, naproxen, non-steroidal antiinflammatory,
28 norfloxacin, NSAID, online SPE, opioid, oseltamivir, oseltamivir carboxylate, oxazepam, paracetamol,
29 pharmaceutical, phenazone, primidone, propranolol, psychostimulant, QAC, quaternary ammonia,
30 ranitidine, removal, ritalin, ritalinic acid, ritonavir, roxithromycin, sampling, sotalol, sulfadiazine,
31 sulfamethoxazole, sulfapyridine, sulfonamide, Switzerland, thiopental, tolyltriazole, tramadol,
32 transformation product, trimethoprim, valsartan, venlafaxine, verapamil.
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INTRODUCTION

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3 While in some countries (e.g. Japan, China, Greece)¹⁻³ wastewater from big hospitals is pre-treated or
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5 biologically treated on-site, in many other countries, including Switzerland, it is connected directly to a
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7 municipal sewer and treated at municipal wastewater treatment plants (WWTP). Treatment of the
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9 wastewater at the source has advantages of avoiding dilution due to mixing with the urban sewage and
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11 avoiding losses into the environment due to sewer leakage and combined sewer overflows.^{4, 5} In case of
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13 hospital wastewater, concerns are to avoid spread of (multi-resistant or pathogenic) bacteria, viruses,
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15 and parasite eggs as well as to avoid input of pharmaceuticals, diagnostic agents and disinfectants.
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19 Membrane bioreactors (MBR) can remove by retention more than 5 and 2 log units of bacteria and
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21 viruses, respectively.³ Hospital wastewater treatment by MBR showed to be feasible in a German full-
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23 scale pilot study and over 95% elimination for four out of nine measured pharmaceuticals was achieved,
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25 although effluent concentrations were often higher than the target value of 100 ng/L.⁶ Apart from the
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27 recent study from Germany, only scarce elimination efficiency data for few micropollutants in hospital
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29 wastewaters are currently available,⁷ whereas considerably more hospital wastewater data is available on
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31 occurrence and contribution of hospitals to pharmaceutical loads.^{1, 8-16} Even in other environmental
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33 studies than those focused on hospital wastewater, only a small subset of all pharmaceuticals that are in
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35 use has thus far been investigated.^{17, 18} An ecotoxicological evaluation of the investigated hospital
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37 wastewater was published earlier.¹⁷
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43 To collect robust data on elimination efficiencies of multiple micropollutants from the complex matrix
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45 of hospital wastewater, proper tools need to be available. First, a suitable sampling strategy needs to be
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47 employed to ensure representative results. Secondly, the analytics should enable high sample throughput
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49 and ideally incorporate numerous micropollutants of interest. Nowadays, with the availability of
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51 sensitive mass spectrometers, pharmaceuticals and other micropollutants can be analyzed in
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53 environmental waters down to low or even sub-nanogram per liter levels. One of the trends in the
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55 environmental analysis of micropollutants is replacement of classical solid phase extraction by
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57 automated alternatives, such as on-line solid-phase extraction high performance liquid chromatography
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1 coupled with tandem mass spectrometry (SPE-HPLC-MS/MS)¹⁹⁻²¹ or large-volume injection HPLC-
2 MS/MS^{22, 23}. The large volume injection and the on-line SPE are conceptually very similar (see SI,
3 Figure S1). The advantages over classical SPE are cost-efficiency due to reusable pre-columns or
4 cartridges, minimal sample handling, time-efficiency, and elimination of analyte losses and human
5 malfunctioning in a multi-step SPE procedure - all resulting in easily achievable high sample
6 throughput. The choice between large-volume injection and on-line SPE-HPLC-MS/MS is thus often
7 only a question of the lab's experience and equipment.
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17 Performance of biological treatment of hospital effluents can be influenced by inhibition of the
18 biomass by disinfectants and antibacterial agents present in much higher concentrations in hospital
19 wastewater than in municipal wastewater. A typical hospital uses tons of disinfectants per year. In Swiss
20 hospitals ethanol, propanol, methyl ethyl ketone, and glutaraldehyde are used in the highest amounts (SI,
21 Table S15). Beside these mostly easily degradable compounds the quaternary ammonia compounds
22 (QACs) are important disinfectants - strongly adsorbing cationic compounds. QACs are eliminated by
23 more than 90% in sewage treatment; however, they have also been shown to have cytotoxic effects.²⁴⁻²⁶
24 QACs commonly used are benzalkonium chloride (BAC-C12-18, technical mixture of isomers C12,
25 C14, C16, and C18) and dimethyldidecylammonium chloride (DDAC-C10) - see SI, Table S15. As
26 reported for those compounds in an Austrian study, QAC-derived risk to sensitive non-target organisms
27 cannot be excluded.²⁶
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42 The objective of this paper was to provide data on the general performance and micropollutant
43 elimination efficiency of an on-site biological wastewater treatment at hospitals. To meet this objective:
44 (i) a pilot-scale MBR was installed to receive and treat wastewater originating in a Swiss hospital and
45 was in continuous operation for one year; (ii) an efficient and representative sampling campaign was
46 designed and employed to representatively collect influent and effluent samples from the MBR; and (iii)
47 an automated and robust on-line SPE-HPLC-MS/MS analytical method was developed and optimized to
48 quantify the concentrations of approximately 68 target analytes including pharmaceuticals, human
49 metabolites, and industrial chemicals. As disinfectants present in hospital wastewater at high levels have
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1 the potential to inhibit biological treatment, maximal tolerable concentration of the abundant QAC
2 benzyldimethyldodecylammonium (BAC-C12) to prevent inhibition of nitrification was determined in a
3 batch study and several QACs measured in the wastewater. The elimination efficiency of the present
4 target micropollutants was determined from the loads of the MBR influent and effluent sampled
5 continuously and flow-proportionally over 3 weeks. Sampling was adjusted to the hydraulic residence
6 time of the MBR. De-conjugation and transformation processes in the sewer and the MBR were
7 specifically addressed, as these abundant mechanisms are sometimes neglected or overlooked when
8 evaluating elimination efficiencies of micropollutants.
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21 MATERIALS AND METHODS

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23 **Pilot-scale MBR.** The pilot plant was installed at a representative Swiss hospital, the Cantonal
24 hospital of Baden (346 beds, water consumption $673 \text{ L}\cdot\text{bed}^{-1}\cdot\text{day}^{-1}$ in 2009) with the help of Holinger
25 AG (Liestal, Switzerland). It was operating continuously for one year from April 2009 to March 2010
26 with an average influent of 1.2 m^3 of wastewater per day (app. 0.5% of the hospital wastewater amount),
27 pumped directly from the hospital sewer collection system. The influent was pumped flow-
28 proportionally based on real-time measurements of hospital drinking water consumption, which are
29 proportional to the wastewater level in the sewer (Figure S3). The pilot plant, as shown in Figure 1,
30 consisted of a primary clarifier and an MBR with an anoxic and an aerobic compartment for
31 denitrification and nitrification, respectively (Picotech Huber AG, Kriens, Switzerland). Submerged
32 ultrafiltration flat sheet membrane plates (Huber MembraneClearBox[®], PP carrier, PES membrane, 7 m^3 ,
33 $15\text{-}30 \text{ L}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$, 38 nm pore size, 150 kDa) were used. Wastewater from the aerobic compartment was
34 recycled to the anoxic compartment ($6\text{-}8 \text{ m}^3/\text{day}$). The excess sludge amount accounted for $20\text{-}50 \text{ L}/\text{day}$.
35 The sludge concentration in the MBR was on average $2 \text{ g}/\text{L}$, the sludge age 30-50 days (organic sludge
36 load $0.06\text{-}0.1 \text{ gCOD}\cdot\text{gTSS}^{-1}\cdot\text{d}^{-1}$ corresponding to $0.03\text{-}0.05 \text{ gBOD5}\cdot\text{gTSS}^{-1}\cdot\text{d}^{-1}$), the average operating
37 temperature was 29°C (temperature of wastewater $27\text{-}28^\circ\text{C}$), pH 7.8, and the conductivity $1100 \mu\text{S}/\text{cm}$.
38 Oxygen concentration in the aerobic compartment was maintained by aeration at $3\pm 2 \text{ mg}/\text{L}$.
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Sampling. A peristaltic pump (average flow rate <2 mL/min) was used for continuous, flow-proportional sampling of the pilot plant influent. Sampling flow was synchronized with the real-time drinking water consumption at the hospital. Fresh MBR-effluent was sampled continuously and time-proportionally by a peristaltic pump before it entered the MBR permeate tank. The flow from the sampling pumps was directed into cooled glass bottles located in a refrigerator at 4°C. Cooling elements were used during 1-hour sample transport from the pilot plant to the lab.

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Several sampling campaigns took place: a preliminary over two days in April 1-2, 2009 (12-h composite samples); one over 2 weeks in June 15–29, 2009; and the main campaign over three weeks in August 10 – September 1, 2009. Daily 24-hours composite samples (9:00-9:00) were mixed to obtain flow-proportional composite samples over 48 h (Mon/Tue), 72 h (Wed/Thu/Fri) and 48 h (Sat/Sun) for the pilot plant influent. For the main sampling campaign, the samples of the MBR permeate were sampled with one day delay relative to the samples in the raw influent of the MBR to account for the hydraulic residence time of waste water in the MBR. The hydraulic behavior of the pilot was determined by a bromide tracer experiment (see SI, Page S42).

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Sample Preparation. Within 3-6 hours after each sampling day, the wastewater samples were filtered through a 0.7- μm GF/F glass-fiber filter (Whatman, Dassel, Germany) and further through a 0.2- μm regenerated cellulose filter (Sartorius AG, Göttingen, Germany). For the analysis of 68 micropollutants (Table S1 and S4), samples were diluted in ratio 1:100 and 1:10 with nanopure water or left undiluted, depending on the matrix (Table S9). Subsequently, 50 isotope labeled internal standards in three mixtures were spiked (Table S2). Prepared samples were stored at 4°C in the dark for 1-20 days before they were analyzed. For analysis, 20 mL of the filtered and internal standard containing sample in an amber glass vial was inserted into a cooled auto-sampler rack, and automatically acidified by formic acid (0.1% formic acid in a sample, v/v) just before injection into the on-line SPE-HPLC-MS/MS system to avoid hydrolysis.

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Analytical method. The analytical hardware and the final SPE-HPLC-MS/MS method are described in SI, Page S18-S19. Shortly, in-house filled on-line SPE cartridge contained Isolute ENV⁺ and Oasis

1 HLB solid phase materials, HPLC column Atlantis T3 (Waters) was used for chromatographic
2 separation, and a triple quadrupole mass spectrometer detector TSQ Quantum Ultra (Thermo Fisher
3 Scientific) for detection. Loading of the sample to a loop, enrichment onto the SPE cartridge, elution and
4 chromatographic run was automated simultaneously in three time steps with an already existing SPE-
5 HPLC-MS/MS set-up used earlier for sulfonamides in surface waters.²⁰ Quality control (QC) was
6 assured by measuring two transitions for each analyte and each internal standard, comparing retention
7 time of an analyte with the retention time of the internal standard in each sample, duplicates, numerous
8 blanks, and QC standards. Details on QC, uncertainty, relative recoveries, and limits of quantification
9 can be found in the SI.

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11 **Elimination Efficiency Calculation.** The term “elimination” refers in this study to the change in the
12 load of a given substance in the effluent (SP-3) compared to the load in the influent (SP-1) usually
13 determined over 3 weeks regardless of whether it is mineralized, transformed, or even formed in the
14 system. Negative elimination efficiencies arise if a substance shows higher loads in the effluent
15 compared to the influent (e.g. in case of de-conjugation of conjugates). Figure S4 contains a detailed
16 description of the elimination efficiency calculation.

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18 **Disinfectants.** Quaternary ammonia disinfectants were analyzed during one week in August 24 –
19 September 1, 2009 at the Austrian Federal Environmental Agency as previously described.²⁷ The set-up
20 of the inhibition batch study is described in the SI. Shortly, seven aerobic batches of activated sludge
21 were spiked with the disinfectant BAC-C12 at increasing concentration in the range of 1-50 mg/L.
22 Oxygen consumption rates were used to assess the effect of a disinfectant on the nitrification process.

23 RESULTS AND DISCUSSION

24 **Hospital wastewater specificity and the general performance of the MBR.**

25 DOC and COD in the wastewater of the Cantonal hospital of Baden (120 and 380 mg/L, respectively)
26 are higher than values of receiving municipal wastewater by factors of 4 – 5. Verlicchi et al. also
27 reported differing values for COD between hospital and municipal wastewaters as well as for BOD,
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1 suspended solids, and chlorides, however other general parameters were similar.⁵ Significantly bigger
2 differences than for general parameters are in the concentrations of certain pharmaceutical classes. For
3 example in hospital wastewater we detected in average 32 $\mu\text{g/L}$ of the antibiotic ciprofloxacin and up to
4 2600 $\mu\text{g/L}$ of iodinated X-ray contrast media (ICM), which is around 70-times more than the
5 concentrations reported in the municipal wastewater for ciprofloxacin, or hundreds of times for ICM.^{28,}
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²⁹ Elevated concentrations of antibiotics but also disinfectants that are used in hospitals in large amounts could cause bacterial inhibition in case of hospital wastewater treatment on-site. Most relevant disinfectant in that respect are QACs (Table S15), as the alcohols, aldehydes and ketones used for disinfection in the highest amounts are well biodegradable.

Our results of the inhibition study with the quaternary ammonia disinfectant BAC-C12 show that its maximal tolerable concentration to prevent inhibition in the given hospital wastewater is 150 $\mu\text{g/L}$ (SI, Page S54). Comparable results were obtained in an Austrian study where the tests were done with activated sludge from a municipal WWTP with no significant contribution of hospitals or industry.³⁰ The measured concentration of the mixture BAC-C12-18 in the wastewater of the Cantonal hospital of Baden was 49 ± 11 $\mu\text{g/L}$, of which 34 ± 9 $\mu\text{g/L}$ was BAC-C12. Those concentrations are more than 3-times below the determined maximal tolerable concentration and thus no inhibition of nitrification by BAC-C12 is expected. DDAC-C10 was found in concentrations 102 ± 9 $\mu\text{g/L}$, also below inhibition concentrations.³⁰ Nevertheless, this might not apply to other hospitals as the disinfectant use routine and water consumption can vary greatly between hospitals and countries and a broad range of yearly loads of disinfectants per bed has been reported for European hospitals (SI, Table S16).³¹

The on-site wastewater treatment MBR was in operation at the Cantonal hospital of Baden for one year and ran under stable conditions during the entire period. The DOC and COD removal was 94% and 92%, respectively (SI, Table S11). Full nitrification (>99% N-elimination) and denitrification (>85%) was achieved. This shows that the hospital wastewater specificity does not significantly disturb biological processes in the MBR and a good performance can be maintained.

Tools for obtaining robust elimination data.

1 1. Analytical on-line SPE-HPLC-MS/MS method. The analytes of our study listed in Tables S1 and S3
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3 contain very polar compounds such as ICM (log K_{ow} around -2) as well as apolar compounds such as
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5 valsartan or ritonavir (log K_{ow} above 4), and anionic, cationic, zwitterionic, and uncharged species are
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7 present at an arbitrary pH (Table S5). The aim of the study was to analyze all of the compounds with one
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9 automated multi-compound method to allow high sample throughput. When designing the method, the
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11 challenge was to find conditions that would provide satisfactory results for the majority of the analytes.
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13 The conditions to be considered were: the SPE material or material combination, pH of the sample at
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15 loading, suitable SPE eluent which serves also as part of the HPLC mobile phase, SPE cartridge
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17 washing solvents, the time of valve switching, the HPLC column material and column dimensions, the
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19 HPLC mobile phase composition and the gradient. Additionally, the parameter that could be varied
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21 depending on the sample matrix was the volume of loaded sample (alternatively the dilution factor).
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26 The major problems were encountered with the acidic ICM, as they were not retained well on most of
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28 the SPE materials, they eluted from the SPE cartridge in broad peaks and due to weak interactions with a
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30 chromatographic material they were not subject to sufficient focusing on the HPLC column. An
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32 improvement in the SPE retention was achieved by using combination of two SPE materials: an Isolute
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34 ENV⁺ material as a main sorbent, on which problematic ICM retained well, but loading the sample first
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36 through the Oasis HLB material, on which most of the other analytes retained.
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40 In total seven different SPE materials in eight different combinations and at three different pH values
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42 (pH 3, 7, 9) were investigated. The optimized method used a layer of ENV⁺ and Oasis HLB materials,
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44 samples were loaded at pH 3, and eluted with methanol. The chromatographic separation run with
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46 methanol and water at pH 3 on an Atlantis T3 column (6 column materials tested). As the method was
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48 based on satisfactory conditions for all analytes, rather than on optimal conditions for each of the
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50 analytes, using available labeled internal standards for the majority of the analytes was crucial to obtain
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52 reliable results.
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56 The relative recoveries of the final analytical method were in the range 80-120% for the majority of
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58 compounds in various wastewater matrices (SI, Table S9). Limits of quantification are listed in Table
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1 S10 - the sensitivity of the method satisfied or exceeded the requirements of the study for all the analytes
2 with exception of three: dexamethasone, methylprednisolone, and fluoxetine (as discussed in SI, Page
3 S41). Over 40 analytes could be quantified with an expanded analytical uncertainty smaller than 30% at
4 a confidence interval of 95% (SI, Table S7 and Page S29). The compounds with high uncertainty were
5 in general compounds without matching internal standards, for which the bias of relative recoveries and
6 intraday precision are higher. Extremely high uncertainties were calculated for metamizole metabolites
7 for which no matching internal standards were available and transformation in stock solutions in minor
8 extend over longer time was observed. Further, iomeprol and iopamidol showed poor intraday precision
9 due to poor interaction with SPE and chromatographic material. The uncertainty range for each
10 compound is stated in the main result table (Table 1).
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23 2. Wastewater sampling. For pharmaceuticals that are used relatively rarely and by a few patients (e.g.
24 cytostatic cyclophosphamide) non-representative sampling can occur when a single toilet flush
25 generating an input pulse is missed or when the sampling event by chance happens only during a single
26 toilet flush input pulse. As discussed in previous studies, even flow-proportional sampling with
27 insufficient sampling frequency intervals can miss a toilet flush pulse and a sampling frequency of less
28 than 1 min for hospital wastewater (less than 5 min for municipal wastewater), or continuous flow-
29 proportional sampling is recommended.^{16, 32}
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40 We opted for long-term continuous flow-proportional sampling in this study. A tracer test showed that
41 the MBR behaved like a fully mixed reactor and the hydraulic residence time in the MBR between the
42 primary clarifier and the permeate tank was up to 98 hours (95 percentile, Figure S2). Due to long
43 hydraulic residence time in the MBR, sampling of at least one week is necessary for calculation of
44 reliable elimination efficiencies for pharmaceuticals that exhibit variable influent concentrations and are
45 not easily biodegradable, e.g. iopromide or tramadol (Figure 2, and Figure S4). As shown in Figure 2 for
46 iopromide, even 2- or 3-days composite flow-proportional samples with a 24-hour shift between influent
47 and effluent are not representative enough for an MBR elimination calculation, and longer sampling
48 time is required. Iopromide elimination efficiencies of nine individual samples (each 48 or 72-hour
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1 composite) vary due to strong variation of concentrations within week days from apparent no
2 elimination to elimination of 60% with very high standard deviation. Such an influence of sampling
3 strategies on the obtained results is crucial but often neglected.³² If the elimination is calculated from
4 weekly composite loads, it varies only between 29% and 33%, with standard deviation of less than 2%.
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6 On the other hand, for easily degradable compounds like paracetamol, the individual composite samples
7 of 48 or 72-hours show the same elimination efficiency as the elimination calculated from weekly
8 influent and effluent loads, which is in both cases more than 99% elimination (see Figure 2).
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19 **Conjugates and Transformation Products**

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21 *1. Conjugates.* Negative elimination efficiencies were observed for some pharmaceuticals that form
22 conjugates and are not easily biodegradable, e.g. sulfadiazine, furosemide or propranolol (see Table S14
23 for the list of pharmaceuticals which form conjugates that are excreted in urine or feces). In general,
24 conjugates of some pharmaceuticals with glucuronic acid, sulfate, glutathione, or acetyl coenzyme A are
25 formed in the human body during phase 2 metabolism to increase solubility and to facilitate excretion.
26 Excreted conjugates then decompose in the wastewater and during the wastewater treatment back into
27 the parent pharmaceuticals and the effluent concentrations of the parent compound can be higher than
28 those in the influent. Such an increase in the loads of the parent pharmaceuticals has been previously
29 observed for sulfamethoxazole and others in municipal wastewater treatment plants^{33, 34} and is expected
30 to be even more common in hospital wastewater, as the travel time of conjugates between the source and
31 the treatment is much shorter than it usually is for municipal WWTPs. Sulfamethoxazole as well as its
32 conjugate N4-acetylsulfametaxazole were measured in this study. The elimination efficiency of
33 sulfamethoxazole without consideration of de-conjugation is 7%, while the real elimination efficiency of
34 sulfamethoxazole calculated from the sum of sulfamethoxazole and N4-acetylsulfamethoxazole in the
35 influent and effluent is 36% (Table 1). Literature studies report -280% to >98% elimination of
36 sulfamethoxazole in municipal wastewater³⁵, which suggests that several previous studies have ignored
37 de-conjugation processes when calculating elimination efficiencies of sulfamethoxazole.
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1 For easily biodegradable compounds that form conjugates (e.g. paracetamol or morphine) significant
2 decreases of load after the MBR treatment is observed, despite de-conjugation. However, we observed
3 an increase of paracetamol loads due to de-conjugation in the primary clarifier, where the microbial
4 activity is lower compared to the MBR (Figure 2). A study of Mohle et al.³⁶ showed that in a batch
5 experiment with 1:10 diluted activated sludge, paracetamol glucuronide was completely de-conjugated
6 to paracetamol within 5 hours. The paracetamol concentration reached a maximum after 8 hours and
7 was further biologically degraded within 18 hours after the beginning of the experiment.
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17 2. Transformation products. Besides the formation of pharmaceuticals by de-conjugation, loads of
18 human drugs or metabolites can increase during biological treatment if biological or abiotic reaction
19 processes result in their formation from other compounds. For example, atenolol acid is formed during
20 wastewater treatment as a microbially-mediated reaction product of atenolol^{37, 38} whereas it is also found
21 in the influent as the human metabolite of metoprolol³⁹. Another example is the human metabolite
22 D617 which is also formed during wastewater treatment from verapamil^{40, 41}. Oseltamivir carboxylate, a
23 human active metabolite of oseltamivir, is also the biodegradation products formed during the
24 wastewater treatment⁴². The same could also apply to oxazepam – a pharmaceutical on its own but also
25 a human metabolite of diazepam. The true elimination of such substances is difficult to assess, as both
26 formation and elimination are occurring. Further, we also observed load increases of clindamycin,
27 oseltamivir, phenazone, and primidone after biological treatment. The compounds are pharmaceuticals
28 for which formation of conjugates is not reported. For oseltamivir, the negative elimination efficiency
29 exhibits a high variation due to the lack of data points and therefore no further conclusion can be drawn.
30 An increase of clindamycin and phenazone loads after municipal wastewater treatment has been
31 previously reported without giving a possible cause.⁴³⁻⁴⁵ We assume that phenazone is formed from
32 metamizole or metamizole metabolites (e.g. 4-methylaminoantipyrine). Such transformation could be
33 just a minor reaction path, although significant when regarding loads of detected metamizole
34 metabolites in the hospital wastewater in hundreds of g/day and phenazone loads in the order of few
35 mg/day. Phenazone is not widely used in Switzerland (only as an eardrop solution in combination with
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1 other pharmaceuticals⁴⁶), and is biologically degradable under aerobic conditions.⁴⁷ The measured loads
2 of phenazone in the hospital wastewater were too high to come from the phenazone consumption, which
3 further supports the hypothesis of phenazone formation from metamizole, or metamizole metabolites.
4 This means that phenazone is either a minor human metabolite of metamizole or it is formed in the
5 sewer before reaching the MBR. As for clindamycin formation, we assume transformation of the main
6 human metabolite clindamycin sulfoxide back to clindamycin in the denitrification process, as an
7 analogous process was previously described for biodegradation of dimethyl to dimethyl sulfide sulfoxide
8 by *Hyphomicrobium denitrificans*.⁴⁸

19 MBR Elimination Efficiencies.

20 It is known that many factors can influence reported elimination efficiencies. Among them are the
21 sludge age and concentration, existence of anoxic and anaerobic compartments, composition of the
22 wastewater, inoculum source and character, technical set-up (conventional activated sludge treatment or
23 MBR), operating temperature, pH, and conductivity - as previously addressed in literature and not
24 discussed further here.³⁵ Additionally, as already discussed above, the sampling strategy, although often
25 neglected, plays a major role. Keeping this in mind, comparison of elimination data has to be handled
26 with care.

27 Literature on elimination efficiencies for the target analytes investigated within this study in hospital
28 wastewater treatment is available for a few compounds in an MBR system with a sludge age of 100
29 days,⁶ and are compared in the following accordingly. Furthermore, results of this study are compared to
30 available literature values for conventional activated sludge and MBR municipal wastewater treatment
31 (SI, Table S12). Additionally to data comparison, it is pointed out for individual compounds whether the
32 elimination is due to biological elimination or sorption (SI, Table S12) according to available K_d and
33 K_{biol} values that were summarized in a literature review (SI, Table S13). In the following text,
34 elimination within pharmaceutical groups is discussed. This could, if desired, lead the medical
35 professionals to more environmentally friendly alternatives for interchangeable active substances, e.g.
36 ICM or anti-inflammatory preparations.

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1. Iodinated X-ray contrast media. This group of pharmaceuticals used in remarkably high quantities consists of compounds with similar structures which are persistent to biological treatment. Out of 6 measured substances all with the exception of iohexol were detected in the majority of influent samples and in $\mu\text{g/L}$ - mg/L levels, with effluent concentrations of the same order of magnitude. The highest elimination efficiency within this pharmaceutical class was detected for iopromide (31%), while the elimination was negligible for the other ICM. Detected elimination is rather due to biodegradation than due to sorption to activated sludge, as the compounds are too polar to adsorb readily and biotransformation products have been previously identified.^{49, 50} Reported data from municipal wastewater treatment are in a broad range³⁵ into which elimination efficiencies from this study fit. While ICM are not toxic themselves, the main environmental concern is their persistency and the formation of toxic disinfection by-products in case of chlorination or chloramination, as ICM are found in ambient waters used as feed water for drinking water production.⁵¹

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2. Antibiotics and other antiinfectives. Eleven out of twelve measured antibiotics (antibacterials) were eliminated with efficiencies below 60%, while 96% elimination was detected for trimethoprim. All available literature data for trimethoprim report much lower elimination than determined in our study, as well as low K_d and K_{biol} values (Tables S12 and S13). Trimethoprim elimination was found to be dependent on the sludge age,⁵² what can explain the high elimination observed in the hospital MBR. . Clindamycin and the sulfonamides are formed, rather than eliminated, as discussed in the section on transformation products and conjugates, respectively. Reported elimination of sulfamethoxazole varies to a high extend from one WWTP to another, even if de-conjugation from N4-acetylsulfamethoxazole is taken into account (Table S12). Norfloxacin, ciprofloxacin, and metronidazole were eliminated by around 50%. Fluoroquinolones are mainly eliminated by sorption to sludge,⁵³ and the lower elimination in the hospital MBR is probably due to the lower sludge production in MBR than in conventional activated sludge. Ciprofloxacin and metronidazole elimination of 60-96% and <30%, respectively was previously reported for municipal wastewaters,^{35, 54} and 80% and 100% for hospital wastewater MBR⁶. The elimination of macrolides azithromycin, clarithromycin, and erythromycin (sum of erythromycin

and erythromycin-H₂O) was ranging from 20% to less than 60%. Complete elimination of clarithromycin in hospital wastewater MBR was reported,⁶ pointing to an influence of sludge age as previously observed for clarithromycin.⁵² Minor or no elimination was observed for the antiviral oseltamivir, its metabolite oseltamivir carboxylate, and the antimycotic fluconazole, while the antiviral ritonavir was eliminated by over 78%. The reported elimination efficiencies are comparable with the available municipal WWTP data (Table S12), although no data were found for ritonavir, distinguished by Escher et al. to have a high risk quotient.¹⁷

3. Non-steroidal antiinflammatory drugs (NSAIDs) and analgesics. This group of pharmaceuticals, some of which are used and excreted in high quantities, consists of numerous chemically diverse drugs and contains persistent as well as easily degradable pharmaceuticals. Diclofenac, tramadol, and indomethacin loads in the influent are almost identical to those in the effluent, while mefenamic acid, paracetamol (acetaminophen), morphine, and metamizole metabolites are easily biodegradable and their loads were decreased by more than 92% after the MBR treatment. Mefenamic acid was found to also partly sorb to sludge.⁵⁵ Our results are well comparable with municipal WWTP literature data for the majority of pharmaceuticals from this class (Table S12). Tramadol elimination up to 35% was previously reported for municipal wastewaters⁵⁶ and 75% for a hospital wastewater MBR.⁶ Elimination of diclofenac varies to a high extent from one WWTP to another (see Table S12), and Beier et al. also found only 20% elimination in their hospital wastewater MBR.⁶ Sludge age itself was found to have no influence on the elimination in some studies,^{6, 57} whereas others found diclofenac elimination to increase with higher sludge age;^{5, 58, 59} the factors that influence elimination are therefore still unclear. Phenazone formation, possibly from metamizole metabolites, is discussed in the section on transformation products. No literature for biological elimination efficiencies of 4-methylaminoantipyrine from wastewater was found, although a study with river water and natural river water biofilms reports its rapid degradation.⁶⁰

4. Beta-blockers and other cardiovascular system preparations. Beta-blockers (with the exception of atenolol and atenolol acid), diuretics, and the verapamil metabolite D617 were not eliminated well in our MBR system, with efficiencies of less than 55%. Other cardiovascular system preparations

1 (bezafibrate, verapamil, and valsartan) were eliminated by over 80%. These data mostly compare well
2 with the municipal WWTP literature data (Table S12). Propranolol is the only beta-blocker forming
3 conjugates⁴⁶ and was rather formed than eliminated in our MBR system. For atenolol a relative high
4 elimination was observed (99%), and elimination in conventional municipal WWTP (0-96%) and in
5 municipal MBR (57-97%) was found to vary a lot from site to site (Table S12). Alder et al. observed
6 higher elimination efficiencies in municipal WWTPs with a sand filter than without.⁶¹ They concluded
7 that a highly effective biofilm might be present on the sand particles of these WWTPs, what could also
8 be the case in the hospital MBR with higher sludge age. No literature data were found on biological
9 elimination efficiencies of D617, although Trautwein et al. pointed out that D617 presumably possesses
10 microbial toxic properties or is persistent and recommended its further research.⁴¹

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24 5. Anesthetics and other nervous system preparations. The anesthetics thiopental and lidocaine are
25 eliminated by 91% and 56%, respectively in the MBR. To our best knowledge, no literature on the
26 biological elimination efficiencies of the two anesthetics is available for comparison. Our results for
27 ritalinic acid, oxazepam and venlafaxine show poor elimination and are in a good agreement with data
28 for municipal WWTPs.⁶²⁻⁶⁴ The measured antiepileptics, with exception of levetiracetam (95%), were
29 very persistent to biological treatment (<23%). Carbamazepine was not eliminated at all, as observed in
30 municipal WWTPs³⁵ and in the hospital wastewater MBR.⁶ Gabapentin elimination (23%) differs from
31 municipal WWTP literature data, where elimination of above 99% is reported.³⁵

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43 6. Other pharmaceutical classes. From the two measured cytostatics only cyclophosphamide was
44 used in the hospital during the main sampling campaign, showing poor elimination. The target
45 pharmaceuticals from the group of hormonal preparations were not detected. The enzyme inhibitor
46 cilastatin and H₂-receptor antagonist ranitidine were eliminated by more than 90% and by 71%,
47 respectively.

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54 7. Industrial chemicals. The target industrial chemicals, the corrosion inhibitors benzotriazole and
55 methylbenzotriazole (tolyltriazole), detected in the MBR influent summed up to 0.82 kg/day.
56 Benzotriazole and methylbenzotriazole were better eliminated in this study (57% and 82%, respectively,
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1 or 80% together) than in municipal WWTP studies where the elimination was less than 40% in a
2 conventional activated sludge treatment, however similar in MBR (61%).^{65, 66} Methylbenzotriazole was
3 measured as the sum of 4-methylbenzotriazole and 5-methylbenzotriazole because the two isomers were
4 chromatographically not fully separated. The peak height ratio between the easily degradable 5-
5 methylbenzotriazole and more persistent 4-methylbenzotriazole was 1.33 ± 0.06 and 1.34 ± 0.07 in SP-1
6 and SP-2, respectively, while after the MBR treatment only the peak of 4-methylbenzotriazole was
7 visible. In the technical mixture of methylbenzotriazole the two isomers occur in the ratio of 1.2 – 1.3.
8 This suggests that if the hospital uses only the technical mixture with the given isomer ratio, the two
9 isomers in the studied hospital wastewater did not degrade during the short traveling time in the hospital
10 sewer or during approximately 1 day in the primary clarifier. These findings for hospital wastewater
11 differ from the findings in municipal wastewater, where the isomer ratio of 0.62 was found in a German
12 study.⁶⁶ Further, while we found 9-times more methylbenzotriazole than benzotriazole, two studies of
13 influents of municipal WWTPs from Germany and Switzerland report 3 – 70 times more benzotriazole
14 than methylbenzotriazole.^{66, 67}

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33 8. Sum of all measured pharmaceuticals and metabolites. All target pharmaceuticals and metabolites
34 detected in the MBR influent summed up to 1.15 kg/day and an overall elimination of 22% was
35 observed. However, 0.94 kg/day of this load (82%) is due to ICM which are generally persistent to
36 biological treatment and only 2% elimination was found for their sum. When only pharmaceuticals and
37 metabolites without ICM are considered, there is an overall mass elimination of 90%. Such a high
38 elimination was achieved despite the fact that only 11 compounds were eliminated by more than 90%,
39 while 20 compounds were eliminated by less than 20%. This was caused by the presence of a few
40 compounds occurring in high quantities which were eliminated well e.g. paracetamol, ciprofloxacin, and
41 the metamizole metabolites which sum up to almost 90% of the load.

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54 The on-site biological wastewater treatment of hospital wastewater by MBR is insufficient to
55 eliminate the majority number of the target pharmaceuticals (especially ICM, antibiotics, and
56 antiepileptics) as well as insufficient to eliminate most of the pharmaceutical load (due to ICM).
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Nevertheless, biological treatment is necessary as a step for DOC elimination, if further on-site treatment of hospital wastewater is considered (e.g. ozonation, advanced oxidation processes, membrane processes, activated carbon, or photo-degradation).

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SUPPORTING INFORMATION: Analyzed micropollutants, analytical method, parameters of the hospital pilot plant, elimination in the MBR and comparison with literature, conjugates in human metabolism, disinfectants (Table S1-S17, Figure S1-S5). This material is available free of charge via the Internet at <http://pubs.acs.org>.

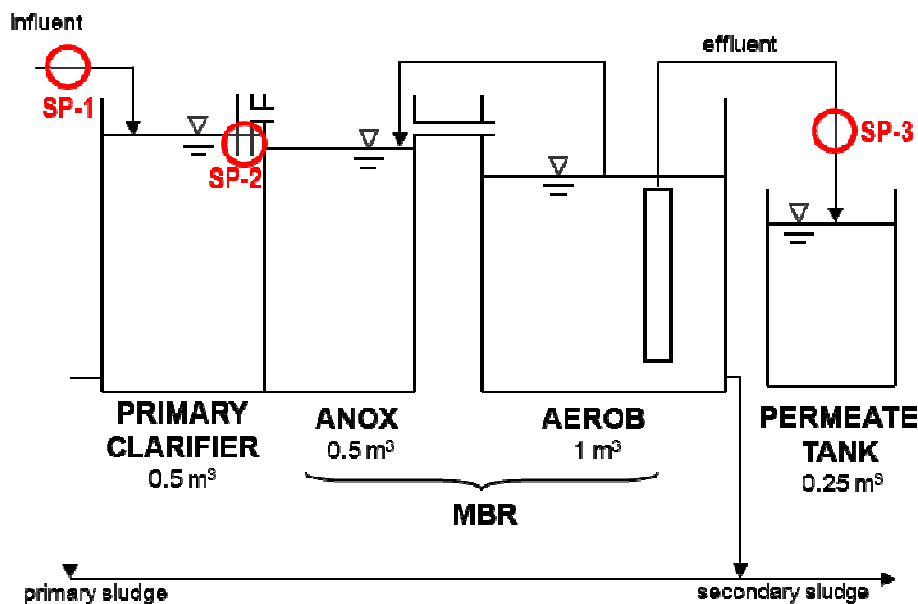


Figure 1. The wastewater treatment pilot plant. Sampling points are represented by circles (SP-1 – SP-3).

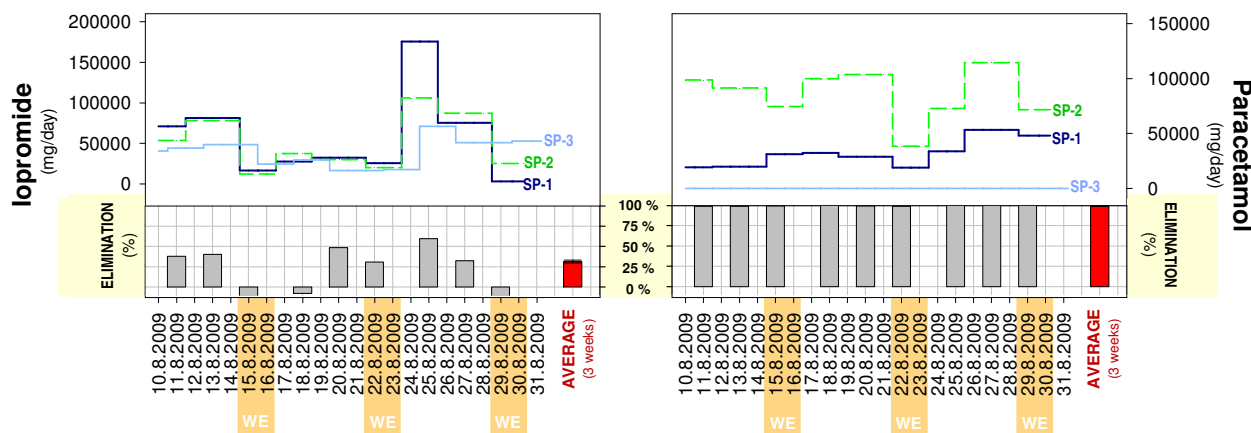


Figure 2. Mass-flow and elimination of iopromide and paracetamol in hospital wastewater during 3 weeks (SP-1 influent, SP-2 after primary clarifier, SP-3 MBR effluent). Elimination was calculated between SP-1 and SP-3.

Table 1. Influent concentrations and eliminations achieved in the hospital MBR.

	Freq ⁽¹⁾ (%)	Influent ⁽²⁾ (ug/L)	Elimination ⁽³⁾ (%)		Freq ⁽¹⁾ (%)	Influent ⁽²⁾ (ug/L)	Elimination ⁽³⁾ (%)
4-Acetamidoantipyrine ^c	100	225 ± 89	95 ± 1	Iopromide ^c	100	170.6 ± 156.3	31 ± 2
4-Aminoantipyrine ^d	100	101 ± 44	83 ± 3	Ioxitalamic acid ^c	88	342.0 ± 197.0	0 ± 15
4-Dimethylaminoantipyrine ^c	0	< 0.14	n.a.	Levetiracetam ^a	100	11.02 ± 6.546	95 ± 1
4-Formylaminoantipyrine ^c	100	47.88 ± 12.39	90 ± 1	Lidocaine ^a	100	9.133 ± 8.071	56 ± 13
4-Methylaminoantipyrine ^d	88	218 ± 208	99 ± 1	Mefenamic acid ^a	100	6.140 ± 1.779	92 ± 0
4/5-Methylbenzotriazole ^d	100	223 ± 132	82 ± 8	Methylprednisolone ^a	12	1.420 ± 0.768 ^{Apr}	n.a.
Atenolol ^a	94	2.315 ± 0.632	99 ± 1	Metoprolol ^a	100	1.325 ± 0.330	55 ± 13
Atenolol acid ^a	100	9.840 ± 1.859	81 ± 3	Metronidazole ^c	100	3.388 ± 1.322	45 ± 56
Azithromycin ^b	59	0.139 ± 0.156	21 ± 95	Morphine ^b	100	3.679 ± 1.834	>96
Benzotriazole ^b	100	23.57 ± 9.09	57 ± 6	N4-Acetylsulfamethoxazole ^a	100	2.394 ± 2.261	81 ± 4
Bezafibrate ^a	29	0.063 ± 0.075	>91	Naproxen ^c	0	< 5.6	n.a.
Carbamazepine ^b	94	0.222 ± 0.118	-6 ± 12	Norfloxacin ^c	88	5.933 ± 3.390	47 ± 5
Cilastatin ^c	76	1.037 ± 1.032	>90	Oseltamivir ^c	35	0.025 ± 0.018	-42 ± 149
Ciprofloxacin ^c	100	31.98 ± 14.06	51 ± 13	Oseltamivir carboxylate ^b	35	0.151 ± 0.081	18 ± 62
Clarithromycin ^a	100	2.555 ± 1.558	50 ± 12	Oxazepam ^a	100	1.123 ± 0.335	6 ± 12
Clindamycin ^c	100	0.983 ± 0.945	-18 ± 40	Paracetamol ^b	88	107.0 ± 85.7	>99
Clofibric acid ^b	0	< 0.07	n.a.	Phenazone ^c	100	0.162 ± 0.079	-158 ± 99
Cyclophosphamide ^a	71	0.161 ± 0.026	<20	Primidone ^b	41	0.383 ± 0.390	-57 ± 87
D617 ^b	100	0.155 ± 0.114	8 ± 31	Propranolol ^b	88	0.116 ± 0.041	-20 ± 39
Dexamethasone ^a	18	0.147 ± 0.013	n.a.	Ranitidine ^b	100	1.565 ± 0.763	71 ± 7
Diatrizoate ^c	100	348.7 ± 241.0	-5 ± 16	Ritalinic acid ^b	76	0.295 ± 0.142	28 ± 4
Diazepam ^a	6	0.069	n.a.	Ritonavir ^b	47	0.108 ± 0.094	78 ± 16
Diclofenac ^b	100	0.833 ± 0.179	-5 ± 3	Roxithromycin ^b	6	0.023	n.a.
Erythromycin + Eryt.-H ₂ O ^c	24	0.188 ± 0.297	<60	Sotalol ^a	82	0.700 ± 0.551	18 ± 17
Fluconazole ^a	100	3.445 ± 1.569	-8 ± 7	Sulfadiazine ^a	41	1.896 ± 4.003	-23 ± 235
Fluoxetine ^b	0	< 0.03	n.a.	Sulfamethoxazole ^a	100	3.476 ± 4.588	7 ± 57
Furosemide ^b	100	2.037 ± 0.595	-21 ± 11	SMX + N4-AcSMX ^{a (4)}	100	5.870 ± 6.849	36 ± 28
Gabapentin ^b	88	19.40 ± 24.15	23 ± 8	Sulfapyridine ^c	6	0.251	n.a.
Hydrochlorothiazide ^a	100	1.995 ± 0.547	8 ± 6	Thiopental ^c	53	0.763 ± 0.860	91 ± 2
Ifosfamide ^b	12	0.895 ± 0.293	n.a.	Tramadol ^a	100	0.958 ± 0.264	7 ± 17
Indomethacin ^a	53	0.069 ± 0.080	7 ± 44	Trimethoprim ^c	94	0.930 ± 0.890	96 ± 1
Iohexol ^c	0	< 12	n.a.	Valsartan ^b	100	3.032 ± 1.282	85 ± 2
Iomeprol ^d	100	439.0 ± 443.9	2 ± 38	Venlafaxine ^a	100	0.811 ± 0.316	16 ± 9
Iopamidol ^d	100	2599 ± 1512	-29 ± 218	Verapamil ^a	94	0.030 ± 0.022	82 ± 3

⁽¹⁾ Frequency of detection, n=17 samples (April n=2, June n=6, August n=9). Morphine, Oxazepam, Thipental: n=15. ⁽²⁾ Average concentration in MBR influent during 2 weeks in June and 3 weeks in August ± variation within this period. Samples in which micropollutant was not detected were not taken into average (max. n=15). ⁽³⁾ Total load elimination of 3 weeks in August ± variation among weeks (n=3). The term “elimination” refers in this study to the change in the load of a given substance in the effluent compared to the load in the influent, regardless of whether it is mineralized, transformed, or even formed in the system.

⁽⁴⁾ Sum of sulfamethoxazole and N4-acetylsulfamethoxazole (^{Apr}) Preliminary sampling campaign April. n.a. – value not available.

Analytical uncertainty: ^a less than 14%; ^b 15 - 29%; ^c 30 - 100%; ^d above 100%.

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