Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/watres

Development of biomass in a drinking water granular active carbon (GAC) filter

Silvana Velten [a], Markus Boller [a], Oliver Köster [b], Jakob Helbing [b], Hans-Ulrich Weilenmann [a], Frederik Hammes [a,*]

[a] Eawag, Swiss Federal Institute of Aquatic Science and Technology, 8600 Duebendorf, Switzerland
[b] Stadt Zurich, Wasserversorgung, Hardhof 9, Postfach 1179, 8021 Zurich, Switzerland

ARTICLE INFO

Article history:
Received 25 January 2011
Received in revised form
4 September 2011
Accepted 6 September 2011
Available online 16 September 2011

Keywords:
Granular activated carbon (GAC)
Biologically activated carbon (BAC)
Drinking water
Biofiltration
Biodegradation

ABSTRACT

Indigenous bacteria are essential for the performance of drinking water biofilters, yet this biological component remains poorly characterized. In the present study we followed biofilm formation and development in a granular activated carbon (GAC) filter on pilot-scale during the first six months of operation. GAC particles were sampled from four different depths (10, 45, 80 and 115 cm) and attached biomass was measured with adenosine tri-phosphate (ATP) analysis. The attached biomass accumulated rapidly on the GAC particles throughout all levels in the filter during the first 90 days of operation and maintained a steady state afterward. Vertical gradients of biomass density and growth rates were observed during start-up and also in steady state. During steady state, biomass concentrations ranged between 0.8–1.83 x 10^{-6} g ATP/g GAC in the filter, and 22% of the influent dissolved organic carbon (DOC) was removed. Concomitant biomass production was about 1.8 × 10^{12} cells/m^2h, which represents a yield of 1.26 × 10^6 cells/μg. The bacteria assimilated only about 3% of the removed carbon as biomass. At one point during the operational period, a natural 5-fold increase in the influent phytoplankton concentration occurred. As a result, influent assimilable organic carbon concentrations increased and suspended bacteria in the filter effluent increased 3-fold as the direct consequence of increased growth in the biofilter. This study shows that the combination of different analytical methods allows detailed quantification of the microbiological activity in drinking water biofilters.

1. Introduction

Granular activated carbon (GAC) filters are commonly used during drinking water treatment for the removal of undesirable dissolved organic carbon (DOC) fractions including biodegradable organic matter, micropollutants, halogenated hydrocarbons and taste and odor compounds by adsorption (Servais et al., 1994; Urfer et al., 1997; Fonseca et al., 2001; Velten et al., 2007). Filters in which the GAC is not regularly replaced or regenerated evolve naturally into biofilters, where most of the DOC removal is a result of biodegradation instead of adsorption (Lee et al., 1981; Servais et al., 1991; Moll et al., 1999; Velten et al., 2007; Hammes et al., 2008). In this case, indigenous microbial communities colonize the surfaces of the GAC particles, and such filters are also referred to in literature as biologically activated carbon (BAC) filters. This transition from a GAC to a BAC filter is a time-dependent process that alters the performance of the system considerably. BAC filters typically

* *Corresponding author*. Tel.: +41 44 823 5350; fax: +41 44 823 5028.
E-mail address: frederik.hammes@eawag.ch (F. Hammes).
0043-1354/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.watres.2011.09.017

remove less total DOC than GAC filters, but targets specifically the biodegradable organic carbon fraction.

GAC/BAC filters are usually placed in a treatment train after an oxidation step, such as ozonation. During ozonation DOC is oxidized to low molecular weight oxygen-containing organic carbon molecules, resulting in an increase of the biodegradable fraction of DOC (Volk and LeChevallier, 2002; von Gunten, 2003; Hammes et al., 2006). Subsequent removal of this biodegradable fraction is essential and contributes to the biological stability of the water, thereby reducing the likelihood of undesirable bacterial re-growth in the water distribution system (van der Kooij et al., 1989). Since the major part of DOC removal during biofiltration can be attributed to biological processes, it is important to be able to quantify and characterize the microbial biomass responsible for this process. Quantitative knowledge of these microbiological processes increases the understanding of the system and the ability to operate and maximize the potential of the biofilter. For example the time required for indigenous bacteria to completely colonize the GAC filter, thus the transition from GAC to BAC filters, as well as the differentiation between adsorbed and biodegraded organic carbon are important parameters for water utilities. Data can also be used as for modeling the performance of biological filters and allow optimization of design and operation (Rittmann and Stilwell, 2002; van der Aa et al., 2006). However, despite several decades of biofiltration research and extensive full-scale application of this technology, relatively little is known about the indigenous microbial communities that colonize biofilter opportunistically and thus contribute to the treatment of drinking water (Simpson, 2008). Some practical limitations in previous studies were the absence of data over extended time periods (Servais et al., 1994; Carlson and Amy, 1998; Urfer and Huck, 2001) or a lack of representative samples from the entire filter bed, with sampling often only feasible from the top (Wang et al., 1995; Magic-Knezev and van der Kooij, 2004; van der Aa et al., 2006; Velten et al., 2007).

The goal of this study was to follow the initial colonization and development of biomass in a pilot-scale GAC drinking water filter over time and vertical filter depth in a long-term experiment subject to uncontrolled changes in raw water parameters. We used a direct method based on ATP-measurements to quantify the biofilm development on the GAC particles and flow cytometry for analyzing suspended bacteria. These microbial data were compared to the organic carbon removal in order to evaluate the filter performance.

2. Materials and methods

2.1. Pilot plant lay-out and operation

The experiments were conducted at a pilot plant that was set up at the Zurich Waterworks (WVZ Lengg, Switzerland) and operated at a capacity of 5.6 m^3/h. The pilot plant consisted of pre-filtration (20 μm), ozonation, GAC filtration and ultrafiltration, treating water from lake Zurich, and has been described previously (Hammes et al., 2008). The GAC filter was operated in down-flow mode with ozonated surface water (Table 1). The design of the GAC filter allowed sampling of both water and GAC particles over the filter bed height, as well as influent and effluent water samples (Fig. 1). GAC was sampled at four sampling points that were distributed over the filter with interspaces of 35 cm. Fig. 1 shows that sample points are labeled from the top of the GAC filter downwards; thus the GAC 1 sample was taken 10 cm below the top of the filter. Similarly, water sample 1 (WS 1) was taken at the same height. The data reported herein cover the first 6 months of operation. For the explicit purpose of studying distribution of biomass, no backwashing was applied to the filter during this period.

Table 1 – GAC filter and water quality parameters.

Carbon type	(–)	Chemviron SGL 8 × 18
Packed bed density	(kg/m^3)	460
Reactor volume	(m^3)	1.47
GAC depth	(m)	1.55
Column diameter	(m)	1.1
Filtration velocity	(m/h)	5.9
Empty bed contact time	(min)	15.76
Influent DOC	(mg/L)	1.1 (±0.04)
Influent pH	(–)	7.79 (±0.14)
Temperature	(°C)	7.05 (±0.7)

2.2. Sampling

For GAC sampling, a metal tube (inner and outer diameter 0.9 and 1.1 cm, respectively) was inserted 0.8 m into the GAC filter from the side through purpose-build sampling ports and about

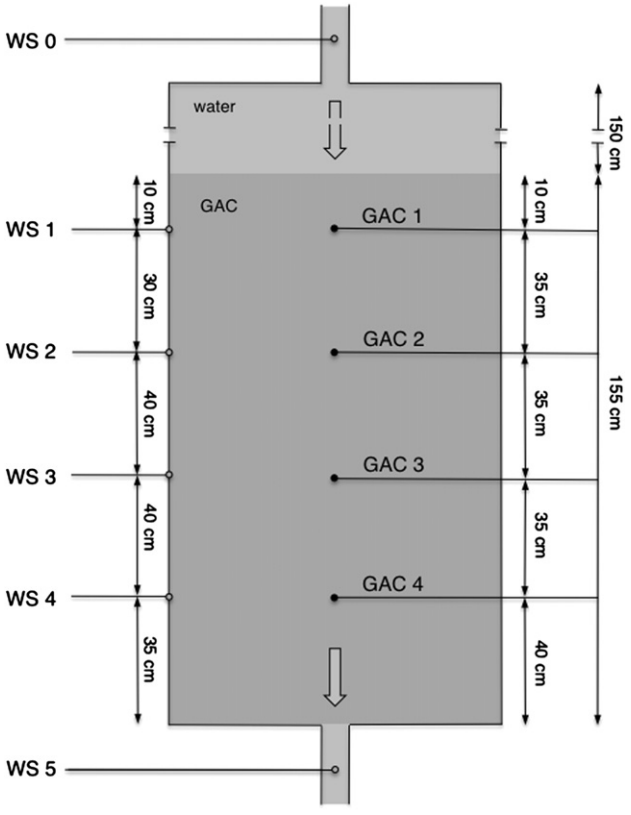

Fig. 1 – Schematic presentation of the investigated pilot-scale granular activated carbon (GAC) filter. Ozonated lake water (Lake Zurich) was used as influent water. Water samples (WS 0–5) and GAC samples (GAC 1–4) were sampled from different depths of the filter.

20 g GAC particles were collected from each sampling point. Water samples (250 mL) were taken from taps located at the side of the GAC filter, as well as before and after the filter. These samples were collected in sterile, carbon-free glassware prepared as described previously (Hammes and Egli, 2005). During the first 70 days, samples were taken twice a week and afterward sampling was reduced stepwise to once every two weeks. A higher sampling intensity was employed during the biofilm development phase to track the dynamic changes expected in this period. The sampling intensity was decreased with decreasing biofilm growth rates. GAC samples were analyzed for biomass concentrations (see below for details) while the water samples were divided and analyzed for microbial abundance with flow cytometry, assimilable organic carbon (AOC) and DOC analysis (see below for details). All samples were transported to the laboratory in cold storage, and analyzed within 3 h of sampling.

2.3. Quantification of biomass on GAC particles

GAC samples were treated as described previously (Velten et al., 2007). In short, the GAC particles were rinsed thrice in phosphate buffer. Thereafter, 200 mg (wet weight) was transferred to an Eppendorf tube together with 100 μL sterile phosphate buffer and 300 μL BacTiterGlo™ (Promega Corporation, Madison, WI, USA), and the resulting luminescence was measured as relative light units (RLU). Results were converted to ATP concentrations using a calibration curve and, where applicable, converted to a corresponding number of bacteria by using a case-specific bacterial ATP concentration (see below). All GAC samples were analyzed in triplicate. For the calculation of total filter biomass, the filter was partitioned into four segments, with the intersection half of the distance between adjacent sampling points. Each sampling point was assumed to give the average ATP concentration of the segment that it represents and was multiplied with the mass of GAC in that particular segment. The sum of ATP in all four segments gives the total ATP content for the entire GAC filter, which was calculated for every time point (Supplementary Information Fig. S1).

2.4. Determining a case-specific bacterial ATP concentration

To obtain the case-specific ATP/cell value, a fraction of the bacteria was removed from the GAC surface by gentle manual shaking for 1 min (from the rinsed GAC sample, see above). The ATP concentration of these suspended bacteria was determined as described previously (Velten et al., 2007). The number of bacteria in the sample was measured with flow cytometry (see below). From the combination of the data, a case-specific ATP/cell value was derived.

2.5. Calculation of growth rate and yield in GAC filter

The biofilm specific growth rate (μ) was calculated from the ATP-measurements as follows (Eq. (1)):

$$\mu = (\ln\ (N_{t2}) - \ln\ (N_{t1}))/\Delta t \quad (1)$$

where N_{t1} and N_{t2} are the biofilm concentrations (cells/g GAC) measured at subsequent time points and Δt is the expired time interval between these points. The yield at steady state was calculated as follows (Eq. (2)):

$$\text{yield} = (\text{biomass production})/(\text{DOC removal}) \quad (2)$$

2.6. Total suspended bacterial concentrations measurements with flow cytometry

The total cell concentration (TCC) in the water samples was measured with SYBR® Green I staining and flow cytometry (FCM) as described previously in detail (Hammes et al., 2008). FCM was performed using a Partec CyFlow Space instrument (Partec GmbH, Münster, Germany). The CyFlow Space is equipped with volumetric counting hardware and has an experimentally determined quantification limit of 1000 cells/mL (Hammes et al., 2008). Every one out of ten water samples was measured in triplicate, to control the standard instrumental error, which never exceeded 5%.

2.7. AOC analysis

AOC was measured with a method comprising the use of a site-specific natural microbial community, fluorescent staining and flow cytometry for growth quantification (Hammes and Egli, 2005; Hammes et al., 2006). In short, a natural microbial community is inoculated into a bacteria-free water sample (0.22 μm filtered) and incubated at 30 °C until stationary phase is reached. The bacteria concentration at stationary phase is converted to a concentration of AOC with a conversion factor of 1 μg AOC giving 1×10^7 cells (Hammes et al., 2006). All samples were measured in triplicate.

2.8. DOC analysis

DOC was detected by an infrared (IR) detector after complete oxidation of DOC to CO_2 in a Graentzel Thin-Film Reactor (DOC-Labor Dr. Huber, Germany). The detection limit was 10 μg/L (Huber and Frimmel, 1996).

2.9. Analysis of phytoplankton

Phytoplankton in the lake water was measured as described in a previous study (Müller et al., 2003). For the analysis, the samples were immediately fixed with Lugol's solution. Phytoplankton genera species were differentiated and counted by means of an inverted microscope (Zeiss AXIOVERT 10, Germany). The biovolume was calculated by multiplying the counts of the different phytoplankton species by their respective biovolume. The biomass was following calculated from the biovolume of the phytoplankton (1 mg/L = 10^6 μm^3/mL).

3. Results and discussion

3.1. Start-up phase: biofilm development and filter transition

The first 11 days prior to the actual filter initialization (day 0) were used for testing the hydraulics of the filter using non-chlorinated drinking water. During that time the virgin GAC

was exposed to the microbial community present in the water, which contributed to a rapid initial colonization. Once the filter was initialized and the ozonation reactor was activated (t = 0 days), suspended bacteria in the influent were completely damaged and inactivated by ozone (Hammes et al., 2008) and all subsequent biomass increase on the GAC is therefore regarded as growth and not as attachment. The biofilm biomass accumulated rapidly on the new GAC particles throughout all levels in the filter during the first three months of operation (t_1: −11–91 days) (Fig. 2). During the initial development period, different biomass concentrations and growth rates were clearly observed in the different levels of the filter (Fig. 2A). The biomass accumulation ceased after about 90 operational days (=8300 empty bed volumes (EBV)) in all sampling points, indicating the establishment of a steady state (t_2: 91–198 days). The steady state was defined as the period when the average biomass growth rate was zero throughout the filter. Importantly, this transition from a GAC to BAC filter requires a paradigm shift in the perception of filter performance: simultaneous to the development of biomass in the filter, organic carbon removal decreases as a result of the saturation of adsorption capacity on the GAC (Fig. 2B). Interestingly, the biological steady state was established during the same period as when the DOC effluent concentration stabilized (Fig. 2B). This was also observed in a previous study and suggests that straightforward DOC data could potentially be used as an easy indicator for the biological steady state in the filter (Velten et al., 2007).

In period (t_1), initial biofilm development (expressed as the growth rate) proceeded at the highest rate in the upper layer of the filter (GAC 1 = 0.0041 h^{-1}) and 54% slower in the bottom layer (GAC 4 = 0.0019 h^{-1}). The higher growth rate in the upper layer resulted in a rapid establishment of attached biomass, whereas the lower growth rates at the bottom of the filter are ascribed to a decreasing availability of organic nutrients downward through the filter. Very little DOC is present in the effluent of the filter in the initial stage of operation (Fig. 2B). Fig. 3 shows an example of the dynamic changes in the DOC profiles through the filter on two days for the first period (t_1) and two days for the second period (t_2), respectively. In the first period (t_1) evidently, less than 20% of the initial DOC concentration reaches the bottom level of the filter, which is directly

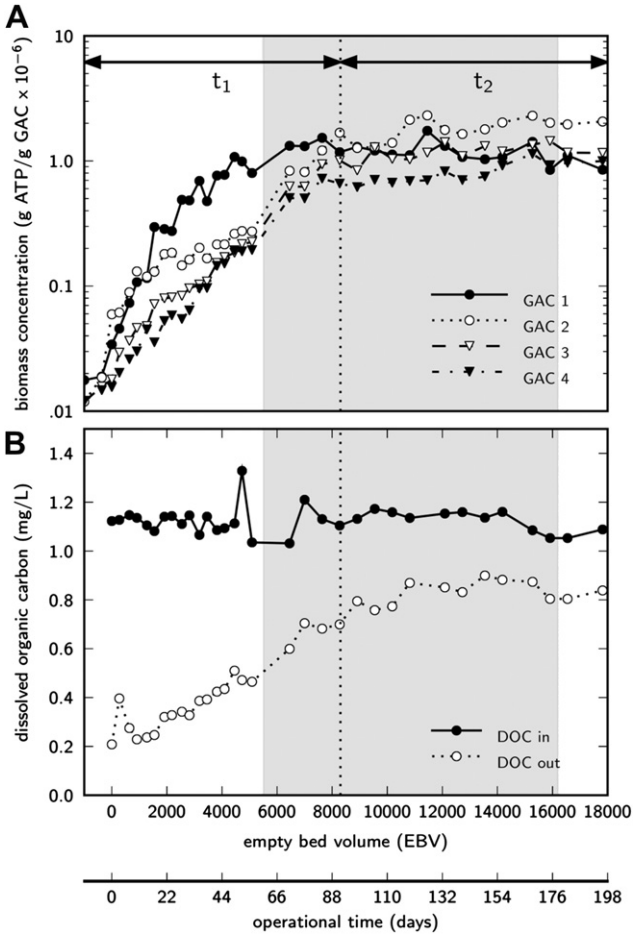

Fig. 2 – (A) Development of attached biofilm biomass (g ATP/g GAC) at different depths of the GAC filter (GAC 1 = 10 cm; GAC 2 = 45 cm; GAC 3 = 80 cm; GAC 4 = 115 cm) as a function of empty bed volume (EBV) and operational time. All data points are average values of triplicate measurements, with an average standard deviation always below 15% (see Fig. 4). (B) Evolution of influent and effluent DOC concentrations of the GAC filter as a function of EBV and operational time. The dotted line indicates the beginning of the steady state whereas the gray zone shows the period of increased phytoplankton concentrations in the raw water.

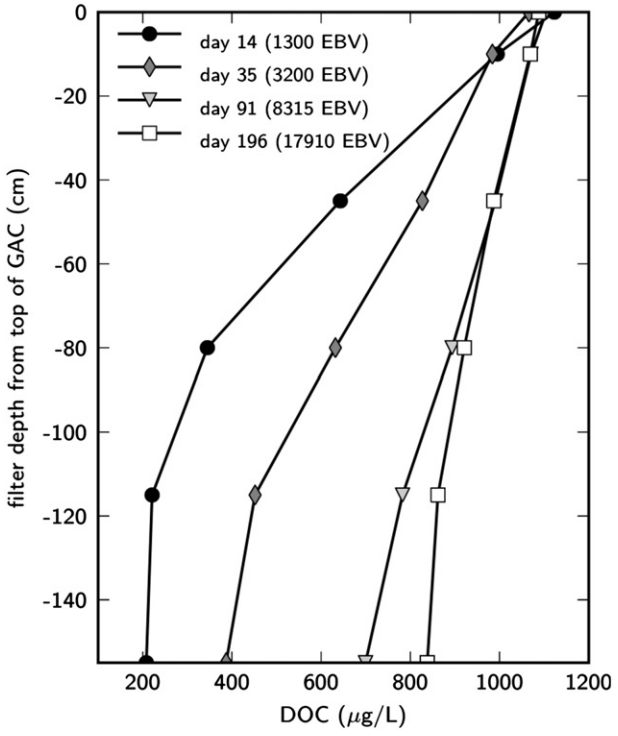

Fig. 3 – Typical DOC filter profiles for four sampling days (day 14, 35, 91 and 196). At day 14 (1300 EBV) a high adsorption capacity for DOC existed still, whereas at day 196 (17906 EBV) the adsorption capacity was decreased and resulted in an increased DOC effluent concentration. The standard error is < 10%.

ascribed to adsorption. At this time point the adsorption capacity of the fresh GAC for DOC is high and one can assume that the GAC filter functions primarily as adsorber. This adsorption process has a double advantage for a faster biofilm development in the upper layers of the filter: while bacteria in the bottom layers are deprived of easily available DOC, adsorption in the upper layers would create a nutrient-rich micro-environment on the surface of the GAC granules that is favorable to biological growth (Li and DiGiano, 1983; Urfer et al., 1997; Herzberg et al., 2003). In the second period the adsorption capacity of the carbon clearly became saturated (Fig. 2B). It is assumed that during this period the GAC filter functions primarily as a BAC filter, where the DOC was removed biologically. However, an abiotic control for this hypothesis was not feasible on such a large scale (5.6 m^3/h) during such a long experimental period (200 days).

The benefit of measuring and calculating biofilter growth rates is that it provides insight into biological development in the biofilter, and allows the modeling and estimation of reactor start-up times, which is essential for water utilities. However, the rate of biofilm development is likely to differ considerably in different situations, and can be influenced by amongst other things water temperature, organic carbon quality and quantity and microbial community composition. Throughout the initial biofilm development period, the growth rates obtained in this study (0.0001–0.0043 h^{-1}) were considerably lower than those reported by Servais et al. (1991) for a full-scale GAC filter. These authors calculated growth rates of 0.038–0.16 h^{-1} for similar empty bed contact times (EBCT). The difference in growth rates can partly be ascribed to the water temperature difference, which was up to 15 °C higher (9–22 °C) than in the present study (7.05 (±0.7) °C). Moll et al. (1999) similarly noted lower biomass concentration and lower growth rates in GAC filters operated at cold (5 °C) temperatures opposed to ambient temperatures. Additionally, the DOC concentrations of 1.7–2.95 mg/L reported by Servais et al. (1991) were much higher than in the investigated system (1.13 (±0.05) mg/L), which would all be supportive of higher growth rates. Further, bacterial biomass was determined by a different method based on $^{14}CO_2$ respiration of added ^{14}C-Glucose that may also have contributed to different interpretation of growth rates.

3.2. Steady state period: biomass concentration and distribution in the GAC filter

In the 2nd period (t_2), differences from 1.5 up to 2.3 times in biomass concentrations occur in the separate levels. The average concentration ($n = 14$) for each level during the steady state period was:

GAC 1 (10 cm) = 1.17 (±0.2) × 10^{-6} g ATP/g GAC;
GAC 2 (45 cm) = 1.83 (±0.4) × 10^{-6} g ATP/g GAC;
GAC 3 (80 cm) = 1.18 (±0.2) × 10^{-6} g ATP/g GAC;
GAC 4 (115 cm) = 0.8 (±0.2) × 10^{-6} g ATP/g GAC.

These values are comparable with previous reported concentrations for GAC filters of 0.3–1.8 × 10^{-6} g ATP/g GAC (van der Aa et al., 2006; Velten et al., 2007).

The highest biomass concentration (1.83 × 10^{-6} g ATP/g GAC) was established at the second sampling point (GAC 2; 45 cm from the top) and decreased thereafter by a factor of 2.3 to the bottom of the filter (Fig. 2A). Evidently a considerable amount of biomass has established over the vertical profile of the filter. This suggests that biological activity occurs throughout a biofilter and is not only limited to the top few centimeters as suggested previously (Urfer and Huck, 2001; van der Aa et al., 2006). Interestingly, Servais et al. (1994) showed a decreasing biomass concentrations over the filter bed only during operational temperatures of 20 °C and not for low temperatures (e.g. 9 °C), as in this study. Regarding the lower concentration at the first sampling point, a previous study explained similar observations by the presence of residual ozone in the influent of the filter (Urfer and Huck, 2001). However, this explanation would contradict the high growth rates observed at the top of the filter in the start-up phase (Fig. 2A). Rather, inhibitive effects as a result of accumulated phytoplankton biomass (discussed below), or the development of a unique microbial community (Boon et al., 2011), can explain the lower biomass concentration at the top of the filter. In both the present study and that previously reported (Urfer et al., 1997), the biomass concentration decreased likewise with the GAC filter depth. The additional benefit of measuring the filter at different levels/depths is that the total amount of filter biomass can be calculated more accurately. Based on the average data (above), the entire GAC filter contained in total approximately 0.8 g ATP, which equates to about 1.8 × 10^{15} cells/m^3, when considering the specific ATP-per-cell values measured for this particular system (on average 3 ± 1.5 × 10^{-16} g ATP/cell; $n = 105$) (for additional information, see Fig. S1, Supplementary Information).

3.3. Filter performance - DOC removal and related biomass production

While the influent DOC concentration remained constant over the investigation period, the DOC concentration in the effluent increased continuously, which is the direct result of a decreasing adsorption capacity of the GAC (Figs. 2B and 3). At the same time the primary function of the GAC filter – adsorption of DOC – is taken over by biological processes. For the purpose of this paper we considered the adsorption after 90 days to be negligible and the combination of bacterial respiration and biomass assimilation predominantly accountable for the DOC removal.

Biomass production was measured as the concentration of detached bacteria suspended in the effluent of the reactor (measured with flow cytometry) as well as the increase of attached biofilm biomass on the GAC particles in the filter (Fig. 4A and Supplementary Information Fig. S1). The average concentration of suspended bacteria in the filter effluent was 2.53 ± 0.6 × 10^5 cells/mL, corresponding to a production of 1.49 × 10^{12} cells/m^2h (Fig. 4B). The attached biomass displayed only a slight increase at a rate of about 0.0001 h^{-1} (=2.74 × 10^{11} cells/m^2h) (Fig. 4A). This implies that detached bacteria represent the majority (84%) of the total bacteria production (1.78 × 10^{12} cells/m^2h) in the GAC filter during steady state. The average DOC removal at steady state was 240 (±24) μg/L ($n = 14$), which equals 22% of the influent DOC concentration and which implies a removal per filter surface

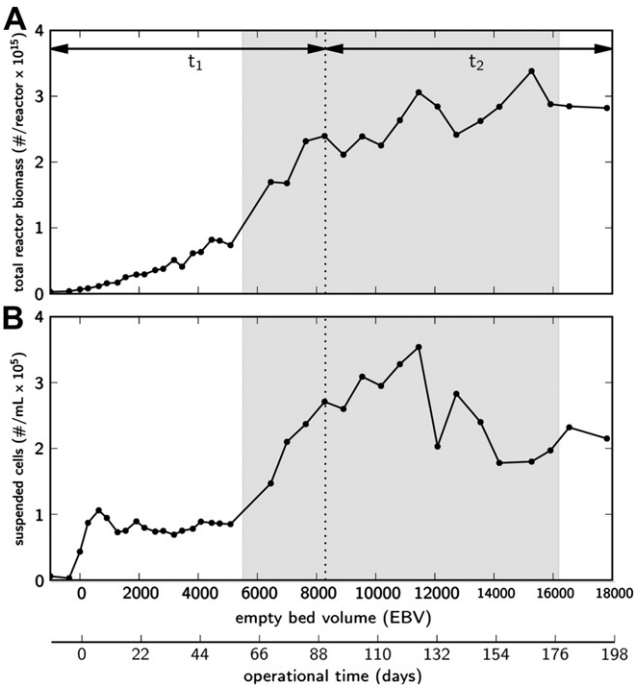

Fig. 4 – Evolution of total filter biomass attached to GAC particles (A) and detached bacteria suspended in the effluent of the filter (B) as a function of EBV and operational time. Attached biomass was measured with ATP analysis while suspended bacteria in the effluent were determined with FCM. The gray zone indicates the period of the higher phytoplankton concentrations in the raw water.

area of 1.41 g/m^{2}h (flow rate of 5.6 m^3/h). This removal is comparable to previous studies, which suggest that in surface waters anything between 5 and 49% of the total DOC can be present as BDOC and be removed biologically (Servais et al., 1991; Volk and LeChevallier, 2000). In this study, the fraction of biologically removed DOC is lower compared to the amount of DOC that was removed by adsorption on fresh GAC (Fig. 2B). However, it is important to realize that BAC filters remove the crucial DOC fraction that is relevant for the biological stability of the water. This also means that GAC/BAC filters should be considered and designed from a biological perspective.

The total biomass production of 1.78×10^{12} cells/m^{2}h, combined with the total DOC removal (1.41 g/m^{2}h) translates to a yield of 1.26×10^6 cells/μg. This value is about 10-times lower than yield values for suspended bacteria of natural microbial communities in drinking water growing under optimal conditions (van der Kooij, 2002; Vital et al., 2008). However, it should be considered that these bacteria grew in biofilms where severe nutrient limitation prevailed. Coupled with the low temperatures and a high dilution rate (Table 1) a lower yield can be expected (Vital et al., 2008). By using an average bacterial carbon content of 2×10^{-14} g/cell (Servais et al., 1991; Batté et al., 2003), it was calculated that 0.035 g/m^{2}h carbon was assimilated as biomass. This fraction is only 3% of the total DOC amount removed in the filter (1.41 g/m^{2}h), which is lower than the findings of Servais et al. (1991) of 8%. The low yield in biofilters is beneficial for operation, since by-product formation (in this case bacterial cells) is low in comparison with target-compound removal (in this case DOC).

3.4. *Changes in influent quality impact the GAC filter performance*

The pilot plant used in the present study was fed with actual lake water (Lake Zürich) and therefore subject to natural changes in the influent. Algae in the raw water increased from an average concentration of 300 μg/L up to 1530 μg/L between day 50–70 (December–January) and the algal concentration remained relatively high until day 198 (Fig. 5A). At the same time the turbidity increased from about 0.2 to 0.5 NTU (Fig. 5B) and the total phosphate concentration from 7 to 15 μg/L (Fig. 5C). Both of the latter parameters were related to the increase in phytoplankton ($R^2 = 0.77$ and $R^2 = 0.54$). Such a dramatic algal increase has potential implications for GAC filtration. In the present system, an ozonation step preceded the GAC filtration. Ozonation severely damages algal cells, causing the release/formation of AOC (Müller et al., 2003; Hammes et al., 2007). Fig. 6 shows average AOC profiles in the filter for the periods before

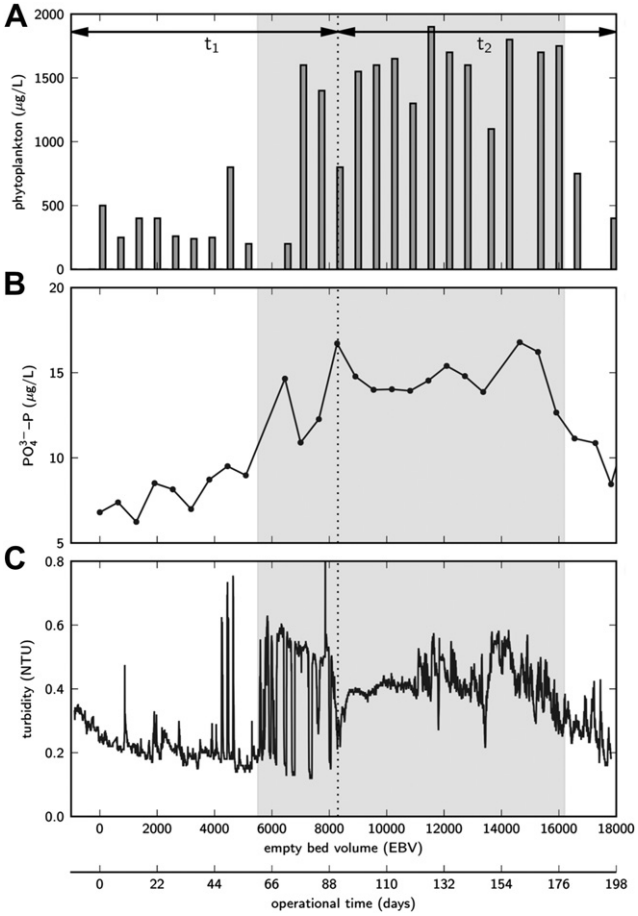

Fig. 5 – The profiles of phytoplankton (A), total phosphate (B) and turbidity (C) show changes in the influent water quality over the investigation period. The gray zone indicates the period of the higher phytoplankton concentration in the raw water.

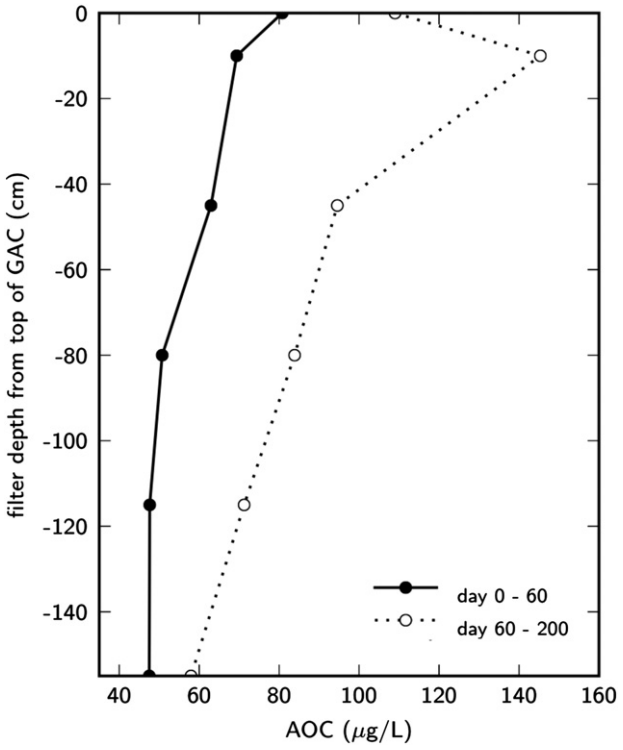

Fig. 6 – AOC filter profiles for two periods, the first average data from 0 to 60 days and the second average data from 60 to 200 days showing the impact of high algae influx. Data points are mean values of 14 data points in steady state.

and during the increased algal influx. During the period of increased algal influx, the AOC concentration in the filter influent was higher (from 80 μg/L to 110 μg/L). Moreover, instead of decreasing, the AOC concentrations increased at level WS 1/GAC 1 (average = 145 μg/L). We propose that this can be ascribed to the absence of backwashing of the filter, resulting in the accumulation of dead algal cells on top of the filter bed, where ongoing chemical degradation (residual ozone is on average 0.22 mg/L) and enzymatic degradation contributed to increased AOC concentrations in the water. A direct correlation between AOC concentrations and biomass in the filter were not observed ($R^2 = 0.03$). However, as shown in Figs. 4 and 5, the increased algae concentration coincided with in an increase of suspended bacteria in the effluent of the filter, as well as an increase in the total biofilm biomass. This indicates that changes in the influent affected the GAC filtration process and these changes could be observed by monitoring the effluent of a biological filter.

3.5. *Practical value of applied methods*

In this study various analytical tools have been applied to increase the understanding of biological filters. One shortcoming was the estimation of the biodegradable carbon fraction, for which we used the AOC assay. AOC analysis detected only about 35% of the DOC that was actually removed during steady state. Moreover, the data in Fig. 6 suggest that organic carbon turnover in a GAC filter can potentially lead to localized variations in AOC concentrations. Also, no correlation between AOC concentrations and biomass growth was obvious. From these observations the conclusion can be drawn that AOC analysis has limited value to study biodegradability and performance in GAC filters and comparable systems. Consequently, consideration should be given to BDOC analysis as an alternative or at least complimentary tool for this purpose (Volk and LeChevallier, 2000; Escobar and Randall, 2001; Prevost et al., 2005). Apart from AOC, the combination of the different analytical tools can be a valuable approach for other researchers who aim to characterize biological filters. Specifically, filter biomass analysis with the ATP method provided meaningful scientific information on biofilm development and distribution in the filters, which in turn can be combined with additional data on the filter performance. However, regular sampling of biomass from different levels of full-scale filters is often not feasible. In such a case, the data have shown that straightforward DOC analysis and FCM total bacterial counts of suspended cells provided meaningful data, which described both the general filter performance as well as specific changes/events in the water quality (e.g. FCM increase during algal intake). These methods are fast and easy to use, and therefore have considerable practical value for both researchers and end-users.

4. Conclusions

- The combination of ATP and FCM total cell counts has been successfully applied to describe the system and can be a valuable tool for the characterization of biological filters.
- A steady state in biofilm concentration was reached after 90 days of operation; in the same period the DOC effluent concentration stabilized.
- The highest biomass concentration was established 45 cm from the filter top (1.83×10^{-6} g ATP/g GAC) and this decreased to the bottom of the filter (0.8×10^{-6} g ATP/g GAC) by a factor of 2.3.
- During steady state, 22% of the total DOC was removed but only 3% of the consumed DOC was assimilated as biomass; 84% of this biomass was measured as suspended cells in the filter effluent.

Acknowledgment

The authors kindly acknowledge financial assistance through the Eawag Wave21 project, BAFU, the European Framework Project Techneau (018320), the Zurich Water Works (WVZ) and Wabag, as well as scientific assistance form Sébastien Meylan, Jacqueline Traber, Iris Hülshoff and Michael Berney.

Appendix. Supplementary material

Supplementary data related to this article can be found online at doi:10.1016/j.watres.2011.09.017.

REFERENCES

Batté, M., Koudjonou, B., Laurent, P., Mathieu, L., Coallier, J., Prévost, M., 2003. Biofilm responses to ageing and to a high phosphate load in a bench-scale drinking water system. Water Res. 37 (6), 1351–1361.

Boon, N., Pycke, B., Marzorati, M., Hammes, F., 2011. Nutrient gradients in a granulated activated carbon biofilter drives bacterial community organization and dynamics. Water Res 45 (19), 6355–6361.

Carlson, K.H., Amy, G.L., 1998. BOM removal during biofiltration. J. AWWA 90 (12), 42–52.

Escobar, I.C., Randall, A.A., 2001. Assimilable organic carbon (AOC) and biodegradable dissolved organic carbon (BDOC): complementary measurements. Water Res. 35 (18), 4444–4454.

Fonseca, A.C., Summers, R.S., Hernandez, M.T., 2001. Comparative measurements of microbial activity in drinking water biofilters. Water Res. 35 (16), 3817–3824.

Hammes, F.A., Egli, T., 2005. New method for assimilable organic carbon determination using flow-cytometric enumeration and a natural microbial consortium as inoculum. Environ. Sci. Technol. 39 (9), 3289–3294.

Hammes, F., Salhi, E., Koster, O., Kaiser, H.-P., Egli, T., von Gunten, U., 2006. Mechanistic and kinetic evaluation of organic disinfection by-product and assimilable organic carbon (AOC) formation during the ozonation of drinking water. Water Res. 40 (12), 2275–2286.

Hammes, F., Meylan, S., Salhi, E., Koester, O., Egli, T., von Gunten, U., 2007. Formation of assimilable organic carbon (AOC) and specific natural organic matter (NOM) fractions during ozonation of phytoplankton. Water Res. 41 (7), 1447–1454.

Hammes, F., Berney, M., Wang, Y., Vital, M., Koster, O., Egli, T., 2008. Flow-cytometric total bacterial cell counts as a descriptive microbiological parameter for drinking water treatment processes. Water Res. 42 (1–2), 269–277.

Herzberg, M., Dosoretz, C.G., Tarre, S., Green, M., 2003. Patchy biofilm coverage can explain the potential advantage of BGAC reactors. Environ. Sci. Technol. 37 (18), 4274–4280.

Huber, S.A., Frimmel, F.H., 1996. Size-Exclusion chromatography with organic carbon detection (LC-OCD): a fast and reliable method for the characterization of hydrophilic organic matter in natural waters. Vom Wasser 86, 277–290.

Li, A.Y.L., DiGiano, F.A., 1983. Availability of sorbed substrate for microbial-degradation on granular activated carbon. J. Water Pollut. Control. Fed. 55 (4), 392–399.

Lee, M.C., Snoeyink, V.L., Crittenden, J.C., 1981. Activated carbon adsorption of humic substances. J. AWWA 73 (8), 440–446.

Magic-Knezev, A., van der Kooij, D., 2004. Optimisation and significance of ATP analysis for measuring active biomass in granular activated carbon filters used in water treatment. Water Res. 38 (18), 3971–3979.

Moll, D.M., Summers, R.S., Fonseca, A.C., Matheis, W., 1999. Impact of temperature on drinking water biofilter performance and microbial community structure. Environ. Sci. Technol. 33 (14), 2377–2382.

Müller, K.C., Forster, R., Gammeter, S., Hambsch, B., 2003. Influence of ozonated cyanobacteria on bacterial growth in rapid sand filters. J. Water SRT-Aqua 52 (5), 333–340.

Prevost, M., Laurent, P., Servais, P., Joret, J.-C., 2005. Biodegradable Organic Matter in Drinking Water Treatment and Distribution. AWWA, Denver, CO.

Rittmann, B.E., Stilwell, D., 2002. Modelling biological processes in water treatment: the integrated biofiltration model. J. Water SRT-Aqua 51 (1), 1–14.

Servais, P., Billen, G., Ventresque, C., Bablon, G.P., 1991. Microbial activity in GAC filters at the Choisy-Le-Roi treatment-plant. J. AWWA 83 (2), 62–68.

Servais, P., Billen, G., Bouillot, P., 1994. Biological colonization of granular activated carbon filters in drinking-water treatment. J. Environ. Eng. 120 (4), 888–899.

Simpson, D.R., 2008. Biofilm processes in biological active carbon water purification. Water. Res. 42 (12), 2839–2848.

Urfer, D., Huck, P.M., Booth, S.D.J., Coffey, B.M., 1997. Biological filtration for BOM and particle removal: a critical review. J. AWWA 89 (12), 83–98.

Urfer, D., Huck, P.M., 2001. Measurement of biomass activity in drinking water biofilters using a respirometric method. Water Res. 35 (6), 1469–1477.

van der Aa, L.T.J., Kolpa, R.J., Magic-Knezev, A., Rietveld, L.C., van Dijk, J.C., 2006. Biomass Development in Biological Activated Carbon Filters. Recent Progress in Slow Sand and Alternative Biofiltration Processes. In: Gimbel, R., Graham, N., Collins, R. (Eds.). IWA Publishing, Mulheim an der Ruhr, Germany, pp. 293–302.

van der Kooij, D., Hijnen, W.A.M., Kruithof, J.C., 1989. The effects of ozonation, biological filtration and distribution on the concentration of easily assimilable organic-carbon (AOC) in drinking-water. Ozone-Sci. Eng. 11 (3), 297–311.

van der Kooij, D., 2002. Assimilable organic carbon (AOC) in treated water: determination and significance. In: Bitton, G. (Ed.), Encyclopedia of Environmental Microbiology. John Wiley & Sons., pp. 312–327.

Velten, S., Hammes, F., Boller, M., Egli, T., 2007. Rapid and direct estimation of active biomass on granular activated carbon through adenosine tri-phosphate (ATP) determination. Water Res. 41 (9), 1973–1983.

Vital, M., Hammes, F., Egli, T., 2008. *Escherichia coli* O157 can grow in natural freshwater at low carbon concentrations. Environ. Microbiol. 10 (9), 2387–2396.

Volk, C.J., LeChevallier, M.W., 2000. Assessing biodegradable organic matter. J. AWWA 92 (5), 64–76.

Volk, C.J., LeChevallier, M.W., 2002. Effects of conventional treatment on AOC and BDOC levels. J. AWWA 94 (6), 112–123.

von Gunten, U., 2003. Ozonation of drinking water: part I. Oxidation kinetics and product formation. Water Res. 37 (7), 1443–1467.

Wang, J.Z., Summers, R.S., Miltner, R.J., 1995. Biofiltration performance 1. relationship to biomass. J. AWWA 87 (12), 55–63.