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COMPARATIVE MEASUREMENTS OF MICROBIAL ACTIVITY IN DRINKING WATER BIOFILTERS

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Abstract—Tetrazolium reduction assays, phospholipid analysis, and 16S rRNA (rDNA) sequence analysis were applied to assess the distribution, composition and activity of microbial communities developing in biofilters treating non-ozonated and ozonated drinking water. The response of media-attached biomass to both operating temperature (3°C vs. > 12°C) and ozone application point was assessed. As judged by 2-(*p*-iodo-phenyl)-3-(*p*-nitrophenyl)-*s*-phenyl tetrazolium chloride (INT) reduction, the dehydrogenase activity in biofilter systems that were operated with non-ozonated water was 55% lower than in identical filters operating with ozonated water. There was no significant difference between the microbiological activity measured in a biofilter series treating ozonated water and an identical series where ozonated water was introduced at an intermediate point. The biomass levels in biofilter systems that were operated with ozonated water were 47% higher on average than identical systems operated with non-ozonated water. Operating temperature had no significant impact on total biomass levels; however, specific dehydrogenase activity was 70% higher in systems operated at ambient temperatures (> 12°C) than in systems held at 3°C. Phospholipid and rDNA analysis suggests that there was a community structure response to ozone application and operating temperature, but no response to different ozone application points. © 2001 Elsevier Science Ltd. All rights reserved

Key words—biofiltration, biomass, INT reduction, phospholipid analysis, denaturing gradient gel electrophoresis (DGGE), drinking water

INTRODUCTION

Biological filtration (biofiltration) has gained increasing attention in the United States, especially in cases where ozone is used to inactivate the cysts and oocyst of pathogenic protozoa, *Cryptosporidium parvum* and *Giardia lamblia*. In addition to its biocidal properties, ozone partially oxidizes complex organic molecules in natural and reclaimed waters, and makes them more readily biodegradable (Langlais *et al.*, 1991). Biological filtration relies on indigenous microorganisms to colonize filter media and use natural organic matter (NOM) for substrate; this reduces microbial regrowth potential and formation of carcinogenic disinfection by-products (DBP) in distribution systems (Langlais *et al.*, 1991). Due to its low maintenance costs and effective NOM removal, biofiltration is becoming an attractive unit process, and efforts have been put toward better understanding the relationship between the microbial biomass enriched in these systems, and effective, controllable organic matter removal.

Dugan (1998) and Wang *et al.* (1995) have developed a model that accurately predicted assimilable NOM removal through a biofiltration system, based on pH, ozone application, empty bed contact time (EBCT), influent biodegradable dissolved organic carbon (BDOC) and biofilter biomass, in systems operated at 20°C. However, in identical biofilters operated at different temperatures, similar performance was observed in systems containing different amounts of biomass (Moll *et al.*, 1999a, b), suggesting that biomass quantity is not the best surrogate for metabolic activity. In biofilters, some microbiological activity results from NOM degradation. The biodegradation of NOM within a biofilter includes a series of cellular oxidation/reduction reactions, dehydrogenation, and a final transfer of electrons to a soluble electron acceptor (Trevors, 1984; Madigan *et al.*, 1997). Tetrazolium salts, such as 5-cyano-2, 3-ditolyl tetrazolium chloride (CTC) and 2-(*p*-iodo-phenyl)-3-(*p*-nitrophenyl)-*s*-phenyl tetrazolium chloride (INT), have been used to measure dehydrogenase activity in different environments, including pure cultures (Rodriguez *et al.*, 1992; Yu *et al.*, 1995), activated sludge (Chung and Neethling, 1989; Kim *et al.*, 1994), soil (Yu *et al.*, 1995), air (Hernandez *et al.*, 1999), drinking water

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(Servais *et al.*, 1992), groundwater and seawater (Rodriguez *et al.*, 1992) using direct microscopy and extraction techniques. Tetrazolium compounds compete with the enzymes involved with microbiological respiration, acting as a reduced hydrogen acceptor, and provide for the measurement of intra- and extracellular enzyme activity (Trevors, 1984). Good correlation between tetrazolium reduction, oxygen uptake rate, and ATP measurements (Chung and Neethling, 1989; Kim *et al.*, 1994) were reported in pure cultures and wastewater experiments assessing microbiological activity, clearly indicating that tetrazolium reduction accurately responds to metabolic activity changes when aerobic bacterial cells respond to substrate limitations in their immediate environment. Tetrazolium reduction tests on drinking water biofilters may allow a better understanding of the relation between microbial community activity and filter performance.

A main goal of this study was to assess microbiological activity in drinking water biofilters using INT, and relate it with operating conditions, performance, and microbial community structure. Microbiological activity was defined herein as NOM-associated intercellular dehydrogenase activity and viable biomass was defined as the amount of active cells present in a biofilter. A third parameter, specific activity, was defined as the INT reduction activity normalized by the biomass, and accounted for the relative activity of a given biofilter population. It was important to develop this assay-defined metric because cells in different growth stages experience different activity levels (Madigan *et al.*, 1997) and, as recent biofiltration research has suggested, the existence of large viable biomass levels does not necessarily imply higher activity or NOM removal levels (Moll *et al.*, 1999).

Recent biofiltration studies have assessed changes in biomass and microbial community structure, due to ozone application, for a surface water (Harsha Lake, OH) and a groundwater isolated NOM, (Hanover, Germany) (Moll, 1998). The temperature effect on filters treating a surface water (Manatee Lake, FL) was also examined (Moll *et al.*, 1999).

During this study, biomass grown in pilot-scale biofilters was used to assess the impact of temperature, ozone application and application point under controlled operating conditions. The microbial community structure of these biofilters was concurrently assessed by lipid phosphate/fatty acid analysis (PLFA) (Findlay and Dobbs, 1993) and denaturing gradient gel electrophoresis (DGGE) of 16sRNA (the area of the DNA that codes for the 16S ribosome) (Muyzer *et al.*, 1993). The purpose of the DGGE analysis was to compliment and enhance community structure information provided by PLFA analysis. PLFA analysis has been applied to different types of sediments, varying from marine intertidal to freshwater streams (Dobbs and Findlay, 1993), domestic wastewater (Victorio *et al.*, 1996), and a

wastewater application which identified the presence of *Gordona* spp. (formerly *Nocardia* spp.) cells in activated sludge tanks (Cha *et al.*, 1999). PLFA methods have rarely been applied to characterize drinking water biofilters (Moll, 1998; Fonseca *et al.*, 1999; Moll *et al.*, 1999).

MATERIALS AND METHODS

Experimental design

Indigenous biofilm cultures were established with Barker Lake (Boulder County, CO) water in four pilot-scale biofilters. The four biofilters were operated in parallel and were maintained from January to September 1999, at the Betasso Water Treatment Plant in Boulder, CO, assessing the impact of temperature, ozone application, and ozone application point (Fig. 1). The filters were acclimated from January until April. At the end of April, based on DOC removals (less than 5% variance in DOC removal, for similar influent DOC concentrations), the filters were considered acclimated. During the months of April until September, Barker Lake water was characterized by: DOC ranging between 1.7 and 5.1 mg/L, low alkalinity (13–34 mg CaCO₃/L), low turbidity (0.6 to 4 NTU), neutral pH (7 ± 0.3), and temperature ranging between 12 and 22°C. Each biofilter system consisted of a three column series of 2.54 cm (i.d.) by 60 cm length glass columns (ACE GLASS, Knoxville, KY) filled with sand (effective size 0.44 mm) and was operated in a down flow mode at a rate of 35 mL/min (hydraulic loading rate of 4.2 m/h), yielding a total EBCT of 13 or 17 min depending on the system (Fig. 1). The ozone doses applied for intermediate filter ozonation (IFO) and pre-filter ozonation (PFO) were, 1.3 ± 0.52 and 1.3 ± 0.35 mg O₃/mg DOC (transfer efficiency ranged between 70 and 90%), respectively. The ozone remaining in the liquid phase was allowed to dissipate for 60 min before reaching the filters, to ensure that no ozone was allowed to contact the filter media. Temperature effects were monitored only in the PFO biofilters. One PFO biofilter was operated at ambient temperature (> 12°C), while the other was maintained at 3°C (PFO-3°C). Each biofilter system consisted of three individual filter segments in series and were numbered #1, #2 and #3 with increasing EBCT. The first segment of all biofilters, and the second segment of the IFO, were backwashed twice a week. The other segments were backwashed when the total pressure in the filter reached 40 psi or the flow rate dropped below 30 mL/min. Backwashing was performed in an up-flow mode, for approximately 3 min, using effluent water from the respective filters.

Ozone measurements

Ozone in the gas (ozone contactor influent and effluent gas) and liquid (ozone contactor and dissipator effluents) phases was measured daily (APHA, 1995).

Optimization of microbial activity measurement with INT

A method was developed to measure microbiological activity in drinking water biofilters modified from tetrazolium reduction assays used for activated sludge (Chung and Neethling, 1989; Kim *et al.*, 1994). The effects of INT concentration, incubation time, and extraction solvents were assessed on biofilter biomass, and these factors were optimized to ensure maximum sensitivity of the tetrazolium assay as applied to operating biofilters (similar to the methods of Rodriguez *et al.* (1992)). Due to the limited amount of sand media that could be collected from the pilot-scale biofilters, these optimization tests were developed with media obtained from a bench-scale sand column fed

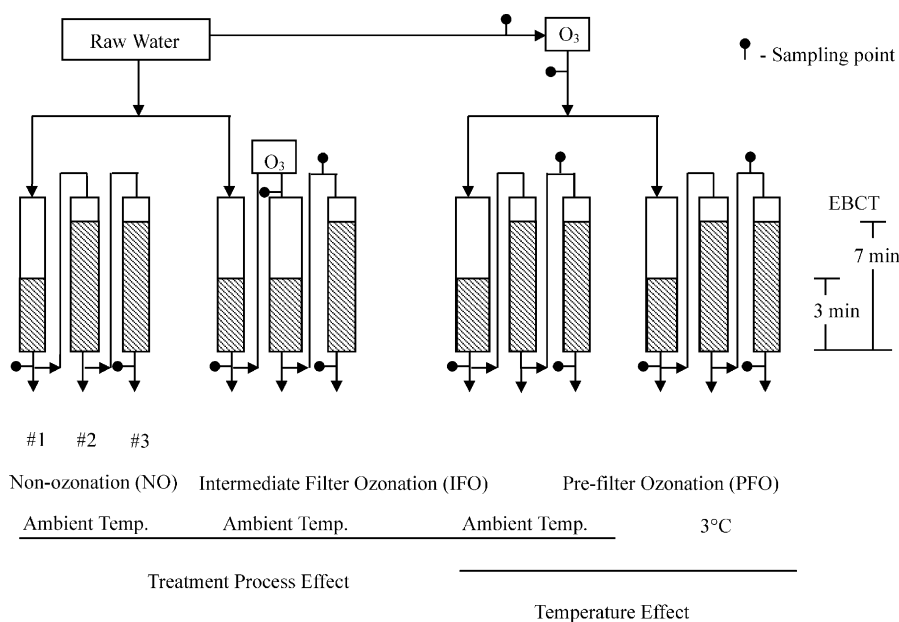


Fig. 1. Biofiltration apparatus.

with Barker Lake, Boulder Reservoir and Boulder Creek sediments.

An assessment for INT's potential toxicity to biofilter biomass was executed because drinking water biofilters do not support high microorganism densities (Dugan, 1998; Moll, 1998). To establish the toxicity threshold and useful range of INT concentrations, the tetrazolium reduction activity of biomass grown on sand media, and on Boulder Creek sediment, was assessed with solutions containing 0.025, 0.05, 0.1, 0.2 and 0.3% INT. Boulder Creek sediment biomass levels were approximately the same as those measured in the pilot-plant biofilters (ca. 90 nmol PO₄/g dry weight), and therefore, this media was used to assess the effect of incubation time in the INT reduction test. Two and four hour INT incubation times were chosen for comparison, based on successful INT assays previously reported in the literature (Chung and Neethling, 1989; Yu *et al.*, 1995). Boulder Reservoir sediment was used to assess the effect of extraction solvent on the INT assay sensitivity. Two different extraction solvents, methanol and ethyl acetate, were compared.

Biological filters activity assays

The first segment of all four biofilter systems and segments NO-#2 and IFO-#2 were assayed for INT reduction activity on a weekly basis. In addition, the first two columns of all systems were sampled on four separate occasions (during the months of July and August). Triplicate media samples (0.1–0.2 g dry weight) were collected weekly from the top of the biofilters with a clean metal spatula, and placed in sterile centrifuge tubes. The samples were incubated in 2 mL of 0.2% INT (prepared fresh and protected from light) and incubated at their operating temperatures for 2 h. The tubes were vigorously shaken on a vortex device every 15 min to ensure that all biomass present in the sample was well mixed with the INT solution. After 2 h, the reaction was stopped with 37% formalin (final concentration of 2%), and centrifuged at 1200 × *g* for 15 min. The supernatant was discarded, and the pellet was resuspended in 4 mL of HPLC grade ethyl acetate for 30 min. The solution was then centrifuged at 600 × *g* for

5 min, and the absorbance of the supernatant measured at 490 nm against an ethyl acetate blank.

Phospholipid and fatty acid analysis

Total biomass (attached and suspended) associated with the filter media was measured using lipid phosphate analysis modified from the method of Findlay *et al.* (1989). During biofilter operation, triplicate filter media samples (0.2–0.5 g wet weight) were drawn concurrently with those samples for INT reduction. At the end of these experiments, biofilters segments NO-#1, PFO-#1, PFO-3°C-#1 and IFO-#2 were destructively sampled and profiles were developed symmetrically over their depth.

Community structure was assessed by phospholipid fatty acid analysis (PLFA), according to the method of Findlay and Dobbs (1993). Duplicate media samples (2 g of dry weight) were collected from the first segment of all filters, and the second segment of the IFO system at the end of the experiment (August). The fatty acids were grouped as monoenoic fatty acids (markers for Gram-negative bacteria), normal saturated fatty acids (general biomass markers; markers common to all microorganisms), terminally branched saturated fatty acids (indicating Gram-positive bacteria and related anaerobic prokaryotes), polyenoic fatty acids (markers for microeukaryotes), branched monoenoics (markers for *Actinomyces*) and mid-branched saturates (markers for sulfate-reducing bacteria) (Dobbs and Findlay, 1993; Findlay and Dobbs, 1993).

Denaturing gradient gel electrophoresis of 16S rDNA

Composite samples (0.5 g) were collected from the top of each filter for 16S rDNA (chromosomal DNA genes that code for the 16S ribosome) extraction, PCR amplification and analysis by denaturing gradient gel electrophoresis (DGGE) (Microbial Insights, Knoxville, KY) (Muyzer *et al.*, 1993). The information provided by this method is qualitative—it provides information on the presence or absence of different populations.

Dissolved organic carbon

Dissolved organic carbon (DOC) samples were filtered through a HVLP 0.45 µm pore size membrane (Millipore, Bedford, MA), acidified to pH 2 and stored at 4°C for no longer than 14 days. The samples were measured by Persulfate-Ultraviolet Oxidation Method (APHA, 1995) with a TOC analyzer (SIEVERS 800, Boulder, CO).

Statistical analysis

The variability and errors associated with analytical values shown in tables, or plotted in graphs, correspond to one standard deviation, and were calculated using propagation of standard error (Ang and Tang, 1975). The relationships among the effluent DOC concentrations and removal percentages were calculated using ANOVA (Microsoft EXCEL 5) and the Bonferroni test, available in the commercial software package SYSTAT 7. The relationships between the PLFA profiles were determined using the widely accepted principal component analysis (PCA) (Brereton, 1990; Jackson, 1991) module in SYSTAT.

RESULTS

For all media tested, the activity results (INT reduction) did not change through the concentration range assessed; similar activity results were obtained for 0.025, 0.05, 0.1, 0.2 and 0.3% solutions. An INT concentration of 0.2% was chosen because it corresponds to an extra-cellular concentration of approximately 4 mM, which is commonly reported for other successful tetrazolium reduction assays in the literature (Chung and Neethling, 1989; Kim *et al.*, 1994). No differences were observed for a 2 or 4 hour incubation time and therefore the shorter time was used. Different formazan extraction solvents provided very different extraction efficiencies and thus analytical sensitivity. Ethyl acetate was chosen because it provided more sensitive results in a lower linear absorbance range, comparable to those previously reported (Chung and Neethling, 1989; Rodriguez *et al.*, 1992).

Absolute activity, viable biomass levels, and specific activity (absolute activity normalized by the viable biomass) profiles were determined for selected segments from each biofilter (Fig. 2). DOC removal was determined through all biofilter segments (Fig. 3). Figure 4 shows the relationship between DOC removal, viable biomass and activity for all ozonated biofilters. Biomass profiles (media taken from the top of each segment, as well as through the entire dept of the first segment) were obtained in all biofilters. The profiles were comparable for all biofilters and therefore only the data observed from PFO-3°C biofilter is presented (Fig. 5).

Figure 6 shows the distribution of the different fatty acids extracted from the pilot-plant biofilter samples. The differences between the microbial communities of all filters was assessed using PCA to compare the relative abundance of 57 fatty acids present in the four biofilter systems. PC1 and PC2 (set of artificial axes created by PCA analysis that allows the comparison of the different samples based

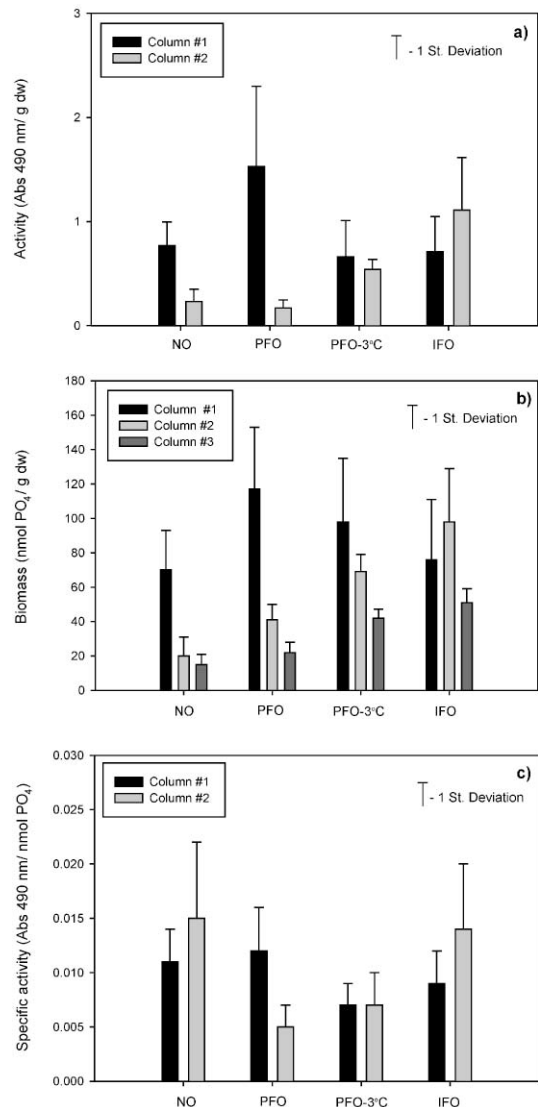


Fig. 2. INT reduction activity (a), biomass levels (b) and specific activity (c) observed in the top media layer of biofilters treating non-ozonated water (NO), water ozonated at an intermediate state (IFO), and pre-ozonated water (PFO) at ambient temperature (> 12°C) and 3°C. [$n = 12$ for NO-#1, PFO-#1, PFO-3°C-#1, IFO-#1 and IFO-#2, and 4 for the remaining columns. Ozone doses for IFO and PFO were, 1.3 ± 0.52 and 1.3 ± 0.35 mg O₃/mg DOC, respectively].

on their fatty acid profile) respectively accounted for 56 and 29% of all variance within the data set (Fig. 7). The microbial communities that were grown in the biofilters were also assessed by DGGE of PCR amplified extracted 16S rDNA modified from the method of Muyzer *et al.* (1993) (Fig. 8).

DISCUSSION

As judged by INT reduction, higher activities were observed in the filter segments treating ozonated

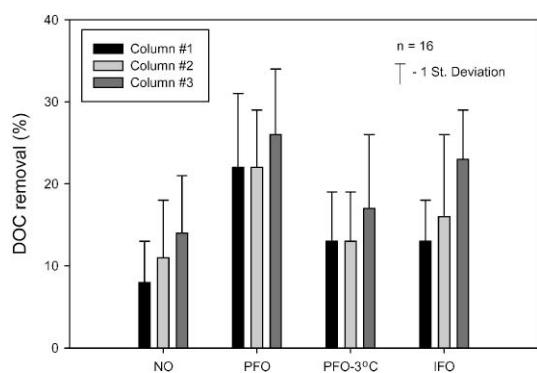


Fig. 3. Cumulative DOC removal by biofilters treating ozonated and non-ozonated (NO) water (Ozone doses for intermediate filter ozonation (IFO) and pre-filter ozonation (PFO) were, 1.3 ± 0.52 and 1.3 ± 0.35 mg O_3 /mg DOC, respectively).

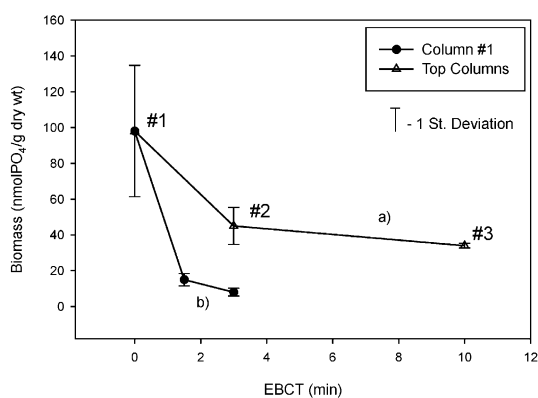


Fig. 5. Biomass level of the: (a) top media layer from each of the three biofilter segments [$n = 12$ for column #1 and 4 for columns #2 and #3], and (b) media throughout the depth of the first column ($n = 3$), in a biofilter treating pre-ozonated water and operated at 3°C .

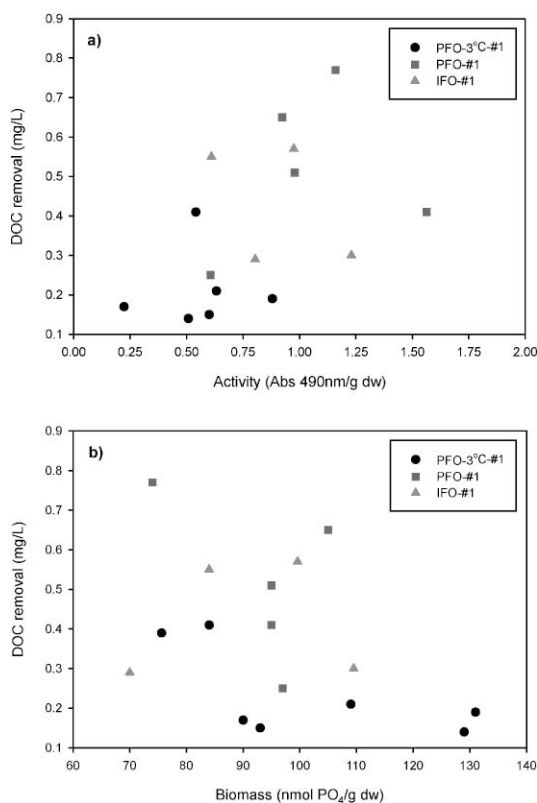


Fig. 4. DOC removal as a function of activity ($R = 0.5$) and biomass ($R = 0$) levels in the ozonated biofilters (IFO-#1, PFO-#1 and PFO-3°C-#1). [R —Pearson correlation coefficient].

waters than those treating non-ozonated water (Fig. 2). The columns immediately after ozonation, IFO-#2 and PFO-#1, showed statistically ($P < 0.05$) higher activities than those receiving non-ozonated water, IFO-#1 and NO-#1. There was no significant statistical difference ($P < 0.05$) between the activity levels of IFO-#2 and PFO-#1, and between NO-#1 and IFO-#1, as determined by ANOVA and the

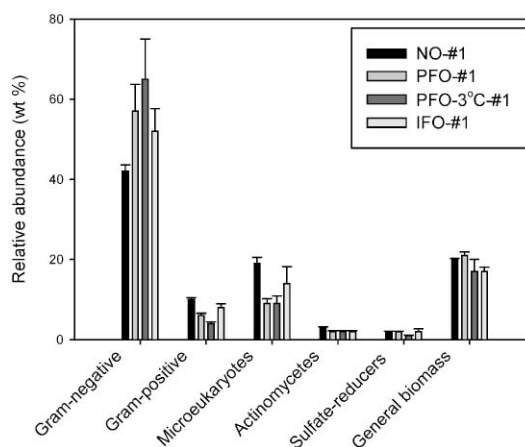


Fig. 6. Relative abundance (wt%) of fatty acids extracted from membrane phospholipids in microorganisms present in the top media layer of biofilters treating ozonated and non-ozonated (NO) water (Ozone doses for intermediate filter ozonation (IFO) and pre-filter ozonation (PFO) were, 1.3 ± 0.52 and 1.3 ± 0.35 mg O_3 /mg DOC, respectively) [$n = 2$].

Bonferroni test. This demonstrated that the activity measurement developed in this study was reproducible in biofilters operated under identical conditions. The higher activity levels observed in the biofilters treating ozonated water were due to the higher levels of available substrate present in the water; ozone application was responsible for increasing biodegradable dissolved organic carbon (BDOC) in the water by approximately 30% compared to the raw water (results not shown), regardless of ozone application point. This BDOC increase is in accordance with other studies (Moll, 1999; Dugan, 1998). The higher substrate levels present in the ozonated feed water promoted higher activity levels within the biofilters, as confirmed by INT reduction. Despite the similar activities levels measured in the NO and PFO-3°C

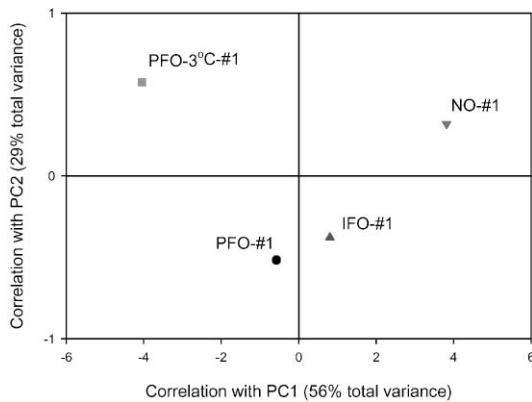


Fig. 7. Principal component analysis of phospholipid fatty acids of biofilters treating non-ozonated water (NO), water ozonated at an intermediate stage (IFO) and pre-ozonated water (PFO) at ambient temperature ($>12^{\circ}\text{C}$) and 3°C (PFO- 3°C).

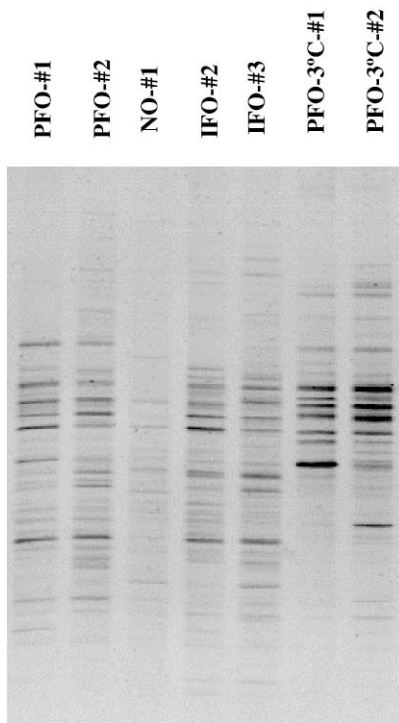


Fig. 8. Denaturing Gradient Gel Electrophoresis (DGGE) analysis of PCR amplified 16S-rDNA extracted from biofilter media.

($P < 0.05$) biofilters, the DOC removal performance of the latter was superior. The results obtained suggest that the community at 3°C , although respiring at lower rates, had more readily degradable substrate available due to ozonation, and therefore higher overall removal efficiency. In this experiment, the removal efficiency gained from ozonating the influent DOC was greater than the efficiency lost due to lowered reaction rates associated with lower operating temperatures.

One of the motivations for including an intermediate ozonation step was to assess if a first biofiltration stage would benefit the ozonation process (by removing turbidity, for example) and allow more ozone available to react with refractory humic substances present in the water, increasing BDOC production and biodegradation potential (and consequently microbial activity). However, this was not verified. DOC removal was similar among biofilters with pre-ozonation and intermediate ozonation (Fig. 3). This may have been due to the extremely low turbidity of the source water, giving no advantage to a pre-filtration step before ozonation, or because the DOC removed through IFO-#1 did not contribute to the water ozone demand (species that can react with ozone and decrease ozonation efficiency) and therefore its removal produced no overall ozone transfer advantage.

The biomass levels measured in the biofilters are within the range previously reported for drinking water biofilters (Wang *et al.*, 1995; Dugan, 1998; Moll, 1998). The patterns of biomass levels observed in the biofilters assessing ozone application and ozone application point were similar to the relationships observed for activity levels (i.e. higher biomass levels were observed in biofilters treating freshly ozonated water). However, contrary to what was observed for activity levels, the temperature did not affect the biomass levels measured in PFO-#1 and PFO- 3°C -#1. The biomass levels for PFO-#1 and PFO- 3°C -#1 were not statistically different ($P < 0.05$), despite the larger fraction of DOC removed in the former. This effect may be due to the fact that organisms present in natural water sources may be able to grow to comparable levels of biomass, but metabolize at lower rates. This lack of correlation between viable biomass and substrate removal was also observed by Moll *et al.* (1999), who reported that biofilters running at 20 and 35°C had different biomass levels, yet similar DOC removal rates.

Figure 4 shows the relationship between activity (a), and biomass (b) with DOC removal, in the first segment of each ozonated biofilter. Data shown corresponds to the last two months of operation. This period was chosen because it corresponded to a steady-state operation and consistency in the influent raw water DOC, which varied only by 10% (2.6 ± 0.26). In this study, microbial activity showed a better correlation with DOC removal than biomass (Pearson correlation coefficients were 0.5 and 0, respectively, $P < 0.5$). Surprisingly, no correlation was observed for the biofilter treating non-ozonated water for activity and biomass.

The impact of backwashing frequency on viable biomass was assessed for all biofilters. In a study assessing seasonal impact on full-scale biofilters, backwashing frequency proved to be one of the parameters that most affected biomass biofilter levels (Fonseca *et al.*, 1999). Figure 5 shows how

backwashing affected biomass levels in PFO-3°C filter system. In the first column, the depletion of readily assimilable compounds through the column depth (EBCT 0–3 min) apparently resulted in a concurrent decrease in biomass levels. However, microorganism levels colonizing the top of the second column, also with EBCT of 3 min (which was only sporadically backwashed) were 100% higher. There was no difference between the assimilable compounds available to the communities colonizing the bottom of the first and the top of the second column, and therefore, the differences observed in the viable biomass levels can be attributed to the different backwashing regimes.

Specific activity (Fig. 2) did not vary with depth for most biofilters, except for the PFO, suggesting a relationship between activity and biomass under the same operational conditions. However, specific activity varied across filters operated under different conditions, regardless of biomass levels. NO-#2 had a much higher specific activity than PFO-3°C-#1 despite the lower substrate removed, and the lower amounts of biomass levels. On the other hand, comparison of specific activity values for PFO and PFO-3°C confirmed different metabolic rates observed in these two biofilters (with similar biomass levels). Caution should, therefore, be exercised when comparing this normalized parameter between systems operating under different physiological conditions.

Concerning microbial community structure (Fig. 6), fatty acids associated with gram-negative bacteria were the predominant phospholipid biomarker in all biofilters. These phospholipid distributions were similar to PLFA analysis previously reported (Moll, 1998; Fonseca *et al.*, 1999; Moll *et al.*, 1999). Ozone application slightly increased the abundance of Gram-negative type fatty acids and decreased those associated with microeukaryotes. Biofilters operated at ambient temperature contained less PLFA markers associated with gram-negative bacteria (monoenoic fatty acids) and greater amounts of gram-positive markers, with respect to those filters operating at 3°C.

PCA successfully differentiated biofilter population and response to ozone application and operational temperature (Fig. 7). The biofilters fed ozone at ambient temperature (PFO and IFO) showed similar correlation with PC1 and PC2 (artificial axes resulting from PCA analysis that explain most variability within the data set). The other two filters, NO and PFO-3°C, correlated differently with both axes, suggesting the existence of a different microbial community structure. The fatty acids that correlated with both PC1 and PC2 are markers for a large range of microorganisms (results not shown) and therefore it was not possible to relate each biofilter to the presence of specific classes of microorganisms.

Based on PCR and subsequent DGGE analysis, no obvious response to depth was observed for the

communities colonizing PFO-#1 and #2, PFO-3°C-#1 and 2, IFO-#2 and #3 (Fig. 8), suggesting that the ecology of the population did not change with increasing EBCT. However, the DGGE band pattern changed significantly with ozone application and operating temperature. There is a great similarity in band patterns between IFO-#2 and #3, PFO-#1 and #2. PFO-3°C-#1 and #2 have unique bands that distinguish them from the former two, and NO-#1 was markedly different from all other biofilters. Based on DGGE analysis, ozone application point did not affect the microbial community developed within these filters. These results are in agreement with those obtained by PLFA analysis. Agreement between these two techniques was observed in a study assessing microbial population shifts during the bioremediation of an oil spill (McNaughton *et al.*, 1999).

Moll (1998) reported differences in fatty acid profile (with increasing EBCT), using PLFA analysis, which differs from the results obtained in this study. There are two possible explanations for the different results obtained in these studies; the first lies on the different backwashing regimes experienced by the two filter segments. In the study conducted by Moll (1999), only one filter segment was used and therefore the different depths had been exposed to the same backwashing conditions. The second may be due to the fact that the DGGE band pattern does not provide quantitative information about the individual activity of each population in the overall community, and therefore the relative importance of those microorganisms for the different depths cannot be assessed.

CONCLUSIONS

A microbiological activity assay (INT reduction) applied to drinking water biofilters showed better correlation with DOC removal through the biofilters treating ozonated waters than did viable biomass (Fig. 4). However similar dehydrogenase activities were measured in the biofilters processing raw water and ozonated water at 3°C, despite the higher amount of DOC removed in the latter. Both these effects should be evaluated separately. Since the INT assay is much easier, faster and cheaper than its phospholipid counterpart, this is a promising technique which can be used to obtain *in situ* information by plant operators and/or laboratories, to measure biofilter activity. Similar activity levels were observed within ozonated biofilters, regardless of ozone application point, as well as for biofilters not treating ozonated water or operated at reduced temperatures (Fig. 2). These results correlated with DOC removal (Fig. 4). In biofilters operating under otherwise identical conditions, lower temperature slowed the metabolism of the microbial community, resulting in lower dehydrogenase activities (Fig. 2) and less DOC

removal (Fig. 3). Backwashing affected the biomass levels present in the filters, with lower levels observed in the filter segments subject to a higher backwashing frequency. There is some indication that the DOC present in the influent water may influence the levels of activity present in the filter media (Fig. 4). This information would be of fundamental importance in future modeling efforts.

PCA analysis successfully differentiated the microbial community present in biofilters based on ozone application and operational temperature, and 16S rDNA analysis by DGGE confirmed the microbial community shifts observed by PLFA.

Viable biomass is currently used as an input parameter in models predicting DOC removal. However, for filters operating under different temperature regimes viable biomass did not correlate with DOC removal performance. The results from this research suggest that a more careful understanding of the relationship between influent DOC removal, and activity may be useful in modeling efforts.

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