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Impact of Temperature on Drinking Water Biofilter Performance and Microbial Community Structure

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Seasonal changes in removal of natural organic matter (NOM) by drinking water biofilters are often attributed to temperature differences. Bench-scale sand biofilters treating NOM isolated from a surface water source were operated in parallel at 5, 20, and 35 °C to isolate the effect of temperature from other water quality and operational parameters, which also vary seasonally. The biofilter operated at 5 °C achieved significantly lower removal of NOM and the NOM fraction that reacts with disinfectants (disinfection byproduct precursors) compared to the filters operated at 20 and 35 °C, which had similar performance levels. Viable biomass, measured as lipid phosphate, was significantly higher at the top and bottom of the filter operated at 20 °C. Phospholipid fatty acid (PLFA) profiles indicated an increasing gradient in markers for Gram-negative bacteria and microeukaryotes as biofilter operation temperature decreased, replacing general fatty acids and markers for Gram-positive bacteria and sulfate-reducing bacteria, which were observed in greatest abundance in the filter operated at 35 °C. Principal components analysis differentiated the microbial PLFA profiles based on biofilter operation temperature and filter depth. These results were corroborated by identifications of the dominant microbial colonies isolated on R2A agar.

Introduction

Filtration with attached biomass can be an effective drinking water treatment process for the control of biodegradable organic matter (BOM). However, biofilter performance is impacted by factors such as water quality (e.g., BOM concentration and characteristics, pH, turbidity), temperature, backwash chemistry, and design parameters. Optimization of this treatment step requires continuing refinement of our knowledge about the relative importance and impact of these factors on biofilter performance.

Recent experiments have assessed biofilter performance under different conditions such as varying contact time (*1*−*5*), biomass concentration in the filter bed (*6, 7*), preozonation dose (*8, 9*), filter media (*10, 11*), and backwash chemistry (*10*−*13*). The removal of total organic carbon (TOC), biodegradable dissolved organic carbon (BDOC), assimilable organic carbon (AOC), natural organic matter (NOM) constituents that react with chlorine or other disinfectants to form chlorinated or oxidized products [disinfection byproduct (DBP) precursors], ozonation byproducts, and turbidity have been used to quantify biofilter performance (*7, 11, 14*).

Several studies have demonstrated that filter designs that support a greater biomass provide better removal of BOM in drinking water biofilters (*2, 6, 7*). Biomass growth and activity vary significantly with temperature (*15, 16*), leading to differences in the removal of BOM, which is a key factor for limiting growth of heterotrophic plate count (HPC) bacteria and coliforms in filter effluents and distribution systems (*2, 17*).

Many drinking water sources experience seasonal temperature variations of 20−30 °C. This variability in source water temperature may impact removal of BOM and particulates by biofilters, as well as impact the microbial community structure present in biofilters. Previous research assessing the impact of temperature on biofiltration has been limited to long-term studies on full- and pilot-scale biofilters, where the filters were run at ambient temperature, and seasonal fluctuations were monitored. These studies showed that TOC or dissolved organic carbon (DOC), AOC, and chlorine demand removal efficiencies decline during the winter months (*2, 18*−*20*). Decreased removal of specific ozonation byproducts such as formate (*21*), methyl glyoxal (*22*), and glyoxal (*23*) in biofilters has also been observed during the winter when compared to the summer.

A problem with directly relating temperature to biofilter performance in these studies is that conditions besides temperature, such as BOM concentration and nature, particulates, and microbial community structure, fluctuate seasonally due to normal seasonal cycles in surface water bodies (*24*). These water quality changes often require adjustments in treatment plant operation, such as coagulant and ozone doses, and pH. During these pilot- and full-scale studies of biofiltration, such operational adjustments were required and the stability of the BOM characteristics was not addressed; thus, the impact of temperature could not be isolated. This study was designed to examine the impact of temperature on biofilter performance and community structure under controlled conditions, such that the effects of seasonal water quality and microbial variability are eliminated.

Materials and Methods

Filter Operation and Sampling. Biofilter sand media were acclimated in the upflow mode with settled Ohio River water for 1 month prior to packing 40 cm of the acclimated media into 2.54 cm × 60 cm glass chromatography columns (Ace Glass, Louisville, KY). Sand was the chosen biofilter medium for these experiments because it is the most commonly used filter medium in full-scale rapid media filters. While high microbial densities can be achievable with granular activated carbon (GAC), it is difficult to separate BOM removal by adsorption from microbial utilization. During the 45−73 days of filter operation, solutions of NOM, isolated by nanofiltration (NF) from Manatee Lake (FL) water (MLW), were used as the feedwater. The NOM was isolated in one batch in October 1997 with a Filmtec NF90 membrane filter (Hiniker, Mankato, MN) using a modification of the method of Serkiz and Perdue (*25*). DOC analysis and fractionation into hydrophobic and hydrophilic fractions by using XAD-8 resin were performed immediately following concentration of the NOM and 2 months later, during the study. The DOC

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concentration and the fractions were the same within 6.0% and 1.2%, respectively, indicating that the NOM stock stayed constant in its chemical makeup over time. The solutions were prepared by diluting the stock MLW NOM solution (230 mg/L DOC) to a DOC concentration of 4.2 mg/L with tap water that had been passed through a GAC filter to remove chlorine and organic matter. The solution was ozonated continuously at an average ozone-to-TOC ratio of 1.3 mg of O_3/mg of TOC. The ozone was allowed to dissipate from the water in a reservoir with a hydraulic retention time of approximately 2 h, before the water was pumped to three parallel filters at a hydraulic loading rate (HLR) of 3.6 m/h, for 5−8 weeks before sampling. This HLR was maintained throughout the experiment for the three filters which had design bed depths of 0.4 m of sand media with an effective size (10th percentile of the media size distribution) of 0.4 mm, yielding an empty bed contact time **(**EBCT**)** of 7.0 min. The biofilters were backwashed once each week to a bed expansion of 50% for 5 min with freshly collected filter effluent water. Two of the filters were water-jacketed, and the temperatures of the filters were regulated to 5.2 ± 1.4 and 35 ± 1.4 °C using recirculating water baths. The third filter was allowed to remain at ambient temperature (20 ± 0.9 °C). The temperature of the influent and effluent water was monitored continuously using electronic temperature probes and recorded twice daily.

Samples of filter media (0.3−0.6 g wet weight) were taken in triplicate from the top of each biofilter once a week throughout the study for biomass analyses as extractable lipid phosphate. Samples were collected once a week from the feed (following the ozone dissipator) and the effluent of each biofilter for DOC. After the biomass at the top of the filters had reached steady-state, intensive liquid-phase sampling was performed to determine steady-state operation of the filters. A filter was considered to be operating at steady-state when the biomass concentration at the top of the filter no longer increased with respect to time (three subsequent samples did not vary by more than 20%). During the 2 weeks of steady-state operation, additional samples were taken for BDOC, AOC, chlorine demand, and the formation of the DBPs total organic halogen (TOX), trihalomethanes (THM), and six haloacetic acids (HAA6) under uniform formation conditions (UFC). For UFC, chlorination of samples was carried out under the following conditions: 24 ± 1 h holding time, 20 ± 1 °C temperature, pH of 8.0 ± 0.2, and a 24 h chlorine residual of 1.0 ± 0.4 mg/L (*26*). Following the 2 week liquid-phase sampling campaign, the filters were shut down, and triplicate samples of filter media were taken from the top, middle, and bottom of each biofilter for biomass and community structure analyses by determining phospholipid fatty acid (PLFA) profiles and identifying culturable isolates.

Substrate Utilization Analyses. DOC analyses were performed using an organic carbon analyzer (Rosemount Dohrmann DC-180) by the UV/persulfate oxidation method (*27*). BDOC was quantified by the method of Allgeier et al. (*28*). AOC samples were analyzed according to Standard Method 9712 (27). Chlorine demand was determined by measuring the chlorine dose and the 24 h chlorine residual of samples by the DPD colorometric method (*27*). TOX samples were analyzed according to the Adsorption−Pyrolysis−Titrimetric Method (*27*) using an adsorption module (Dohrmann AD-3) followed by injection in an organic halide analyzer (Rosemount Dohrmann DX-20). THM analysis was performed by U.S. EPA Method 502.2, Revision 2.1 (*29*), using a purge and trap (Tekmar LSC 2000), and GC (Varian 3400). The column was a fused silica capillary megabore column, 30 m × 0.53 mm i.d., with a 3 mm film thickness (J & W Scientific). The compounds were detected using a Hall detector, and quantified by comparison with a standard calibration curve. HAA analysis was performed by U.S. EPA Method 552.2, Revision 1 (*30*). The analysis was conducted on a GC with a fused silica capillary column, 30 m × 0.25 mm i.d., 0.25 mm thickness (J & W Scientific), and an electron capture detector. Six HAAs were quantified by this method: chloroacetic acid, dichloroacetic acid, trichloroacetic acid, bromoacetic acid, dibromoacetic acid, and bromochloroacetic acid.

Biomass and Community Structure Analyses. Total viable biomass was quantified as extractable lipid phosphate (*31*). PLFA profiles were determined on 10 g (wet weight) media samples by the method of Findlay and Dobbs (*32*). Solvent-only controls were treated identically to samples. The lipids were fractionated by silicic acid column chromatography, and polar lipid fatty acids were released as fatty acid methyl esters by mild alkaline transesterification. PLFAs were quantified using a GC (Hewlett-Packard 5890) with a flame ionization detector linked to a mass spectrometry (MS) detector (Hewlett-Packard 5972). Fatty acid standards (14-carbon to 20-carbon) (Sigma Chemical Co., St. Louis, MO) were run with each set of samples for tentative peak identifications, prior to MS. Peak areas were quantified by adding arachadic acid ethyl ester and capric acid ethyl ester as internal standards before GC injections. Fatty acids are designated by the number of carbon atoms, number of double bonds, and position relative to the aliphatic (ω) end of the molecule; prefixes i and a refer to iso and anteiso branching, respectively; prefix cy indicates cyclopropyl fatty acids; suffixes c and t refer to cis or trans configuration of the unsaturation nearest the aliphatic end of the molecule; me indicates midchain branching. The relative abundance of the individual fatty acids was expressed as the percent of the total mass concentration of fatty acids in each sample.

Heterotrophic plate counts were performed to quantify the culturable bacteria attached to filter media samples using Standard Method 9215 C (*27*). The microbial community attached to the sand particles was removed by shaking the samples for 30 min in 100 mL of 0.3% sodium pyrophosphate solution in 250 mL plastic bottles followed by sonicating the suspension in a bath sonicator for 30 s. The supernatant liquid was decanted into a 250 mL plastic centrifuge bottle, and the procedure was repeated. The two supernatant fractions were pooled. Samples were taken from the pooled supernatant fractions for heterotrophic plate counts. The samples were diluted in sterile saline (0.85% NaCl), and 0.1 mL of appropriate dilutions was spread onto R2A agar plates. The plates were counted following a 7 day incubation at 28 °C. Differential counts of each colony morphology were also performed, and dominant colony types were isolated, Gram-stained, and identified using the MIDI identification system (Microbial ID, Inc., Newark, DE). The limitations of subculturing bacteria in the laboratory are well documented (*33*). The efficiency of the separation procedure of the microbial community from the filter media was not determined, but was likely less than optimum. Additionally, selective growth of bacteria occurs on laboratory growth media, and many microorganisms are unculturable. The temperatures of biofilter operation were different from the incubation temperature of the HPC test, which could lead to isolation of different microorganisms with higher growth rates at the temperature of the assay.

Statistical Analyses. Plotted data that have error bars associated with them are shown as the mean ± one standard deviation of triplicate samples. The relationships among the effluent concentrations and removal percentages by the biofilters of selected liquid-phase parameters were determined by ANOVA using Microsoft Excel (version 5.0) and SYSTAT (version 5.03 for Windows). The relationships among the PLFA profiles of the biofilters were assessed using the General Linear Model option of the MGLH module and the Principal Components Analysis (PCA) option of the Factor

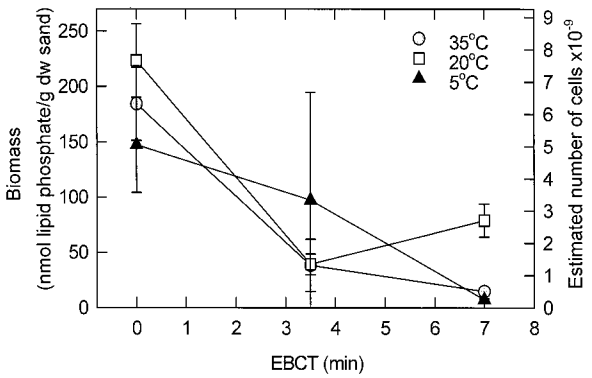

FIGURE 1. Steady-state biomass and cell numbers in biofilters as a function of depth (EBCT) and temperature.

module in SYSTAT. Data used for PCA were row-centered ($x_i - x$) and standardized to give a constant variance of 1. PCA expresses a large number of observed variables in terms of a smaller number of hypothetical new variables [principal components (PCs)] which reflect any intrinsic pattern in the multidimensional data set, and allows the maximum variance of the original data set to be evaluated (*34*). Relationships among samples are readily observed by plotting samples in two dimensions on the basis of their scores for the first two PCs (which represent the greatest amount of variance).

Results and Discussion

Biomass. Figure 1 shows the total viable biomass concentration, expressed as nanomoles of lipid phosphate per gram dry weight of sand and as the estimated number of cells (*31*) associated with the filter media for the three biofilters treating the MLW solution. The value reported for the top of the filters is the average steady-state biomass taken over 4 weeks ($n = 4$), while the values for the middle (EBCT 3.5 min) and bottom (EBCT 7.0 min) are the result of a single sampling taken in triplicate at the end of the filter run. The steady-state total viable biomass concentrations at the top and bottom of the filter operated at 20 °C were statistically significantly greater than the biomass at the top and bottom of the filters at 35 and 5 °C ($P = 0.009$ and $P < 0.001$, respectively). The lower steady-state biomass in the biofilter operated at 5 °C may be attributable to the slower metabolism at low temperature, which would decrease the rate of nutrient utilization and incorporation into cell material. The lethal temperature for an organism is not far above the optimum (*35*), which may account for the reduced growth at 35 °C. As shown in previous studies of drinking water biofilters (*6, 7*), biomass generally decreases as a function of filter depth, due to a decrease in labile substrates through the biofilter depth.

Seasonal biomass variations in a drinking water reservoir have been correlated to temperature variations (*36*), just as bacterial populations in a natural stream have been shown to be stressed by low temperatures during winter months, leading to a decrease in growth rate (*36*). Likewise, stream bacterial isolates have been observed to consistently have optimal temperatures 5−20 °C above environmental water temperatures (*37, 38*), further indicating reduced growth at low temperatures. During the current study, however, the stable thermal regime suggests that the microbial populations might adapt to the environmental temperature, as was observed by Zeikus and Brock (*39*) in bacterial populations in a river where the seasonal temperature regime was relatively stable.

Substrate Utilization. Steady-state mean influent concentrations and percent removal of NOM constituents by the biofilters treating MLW NOM solutions at the three temperatures are shown in Table 1. The 5 °C biofilter yielded the lowest removal for all parameters, and those for DOC, BDOC, UFC TOX, UFC HAA6, and chlorine demand were statistically significantly different ($P \leq 0.05$) from the removal extents yielded by the other two filters. No significant differences were observed between the effluent concentrations and removal of all parameters for the filters operated at 20 and 35 °C. Operating the biofilter at 5 °C relative to 20 and 35 °C decreased the removal performance by an average of 42%, with AOC the least effected (23%) and UFC HAA6, UFC TOX, and chlorine demand the most effected (57, 54, and 52%, respectively). The same trends were seen for removal of precursors for specific HAAs and THMs.

These results, with the biofilters operated under tightly controlled temperature conditions, agree with previous studies, performed on full- and pilot-scale biofilters, which reported less removal of TOC, THM precursors, AOC, and chlorine demand during the cold water conditions of the winter months compared to warmer water conditions. A 34% decrease in TOC removal, and a 59% decrease in removal of THM precursors, was observed in a full-scale biofilter when operated at temperatures less than 14 °C compared to when it was operated at temperatures greater than 14 °C (*19*). Likewise, Price et al. (*18*) reported no removal of AOC in dual media filters with an EBCT of 4.5 min treating an ozonated surface water at temperatures less than 16 °C, while at temperatures greater than 16 °C, the average AOC removal was 44%. Prévost et al. (*120*) demonstrated that higher EBCTs, up to 20 min, were required in biological activated carbon (BAC) filters operated at 1 °C to obtain maximum removal of chlorine demand. This indicates that in order to meet treatment goals at cold temperatures, higher EBCTs may be needed than is necessary for biofilters operated at warmer temperatures.

Microbial Community Structure Shifts. PLFA profile analysis was performed on sand samples taken from the top, middle, and bottom of the three biofilters at the end of the run. PLFAs with known phylogenetic affinities have been used to define functional groups of microorganisms (*40*), and, thus, PLFA profiles have been used extensively to characterize the structure and physiological stress of microbial communities from marine and freshwater sediments (*41, 42*), soil rhizospheres, and compost (*43, 44*) and, more recently, in engineered systems (*45*). PLFA analysis identified 68 fatty acids; of these, 34 fatty acids accounted for at least 94% of the total PLFAs present in each sample. The remaining 34 fatty acids each represented less than 1% of the total PLFAs present. The 34 most abundant fatty acids were assigned to functional groups of microorganisms, and their weight-percent abundance was compared by PCA.

The relative abundance of the different types of fatty acids is shown in Figure 2. Monoenoic fatty acids, which indicate the presence of Gram-negative bacteria, were dominant in all biofilters (48−69 wt %), followed by normal saturated fatty acids which are general biomass markers (16−30 wt %), terminally branched saturated fatty acids which indicate Gram-positive bacteria and related anaerobic prokaryotes (6.3−11 wt %), polyenoic fatty acids which are markers for microeukaryotes (2.6−6.7 wt %), branched monoenoics which are markers for *Actinomycetes* (1.9−3.4 wt %), and mid-branched saturates which indicate the presence of sulfate-reducing bacteria (0.62−5.6 wt %).

An increasing gradient in the abundance of monoenoic fatty acids, which are common to Gram-negative bacteria, was observed as the temperature decreased from 35 to 5 °C. The relative abundance of normal saturated fatty acids which are not used as markers for particular types of microorganisms, and, to a lesser extent, mid-branched saturates and terminally branched saturates, which are indicative of sulfate-reducing bacteria and Gram-positive bacteria, respectively, decreased as the operation temperature decreased. These changes in membrane fatty acids may be partially interpreted

TABLE 1. Impact of Temperature on Steady-State Performance of Biofilters

parameter		concn of influent	removal (percent) 5 °C filter	20 °C filter	35 °C filter
DOC (mg/L)[a,b]	mean	4.0	15[A]	24[B]	24[B]
	SD[c]	(0.3)	(3)	(3)	(2)
BDOC (mg/L)[a,b]	mean	1.6	38[A]	60[B]	60[B]
	SD	(0.2)	(7)	(7)	(6)
AOC (μg/L)	mean	1400	43	55	57
	RD[d]	(180)	(1)	(19)	(14)
UFC TOX (μg OF Cl^-/L)[a,b]	mean	470	10[A]	22[B]	22[B]
	SD	(60)	(6)	(2)	(4)
UFC THM (μg/L)	mean	180	11	15	18
	SD	(20)	(14)	(14)	(26)
UFC HAA6 (μg/L)[a,b]	mean	120	14[A]	31[B]	34[B]
	SD	(10)	(3)	(3)	(2)
Cl_2 demand (mg/L)[a,b]	mean	4.3	14[A]	27[B]	31[B]
	SD	(0.1)	(4)	(3)	(3)

[a] Indicates significant differences between treatment mean effluent concentrations by ANOVA ($P \leq 0.05$). [b] Letters indicate significantly different mean removal percentages (A ≠ B) by Bonferroni post hoc test. [c] Standard deviation of quadruplicate samples. [d] Relative deviation of duplicate samples.

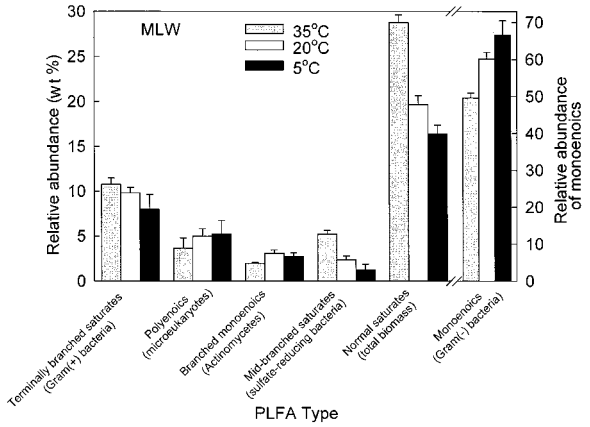

FIGURE 2. Impact of temperature on relative abundance of PLFAs in biofilter samples

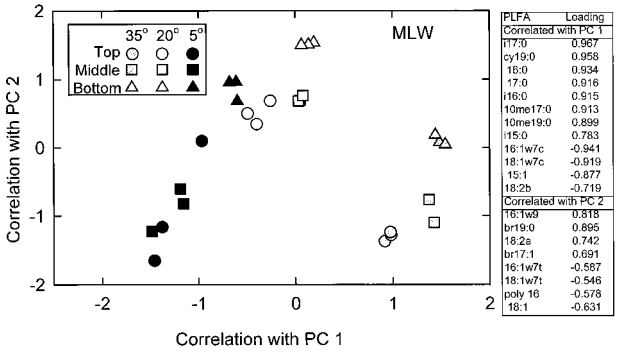

FIGURE 3. Impact of temperature and depth on relationships among PLFA profiles.

with respect to maintenance of membrane fluidity. The monunsaturated fatty acids are more fluid, while saturated and branched fatty acids are more stable and rigid. As the water temperature decreases, the relative amount of monounsaturated fatty acids may increase to maintain membrane fluidity.

PCA indicated differences in the microbial communities present in the three biofilters at the three depths (EBCTs) sampled (Figure 3). Principal components 1 and 2 explained 42% and 22% of the total variance, respectively. PC 1 differentiated samples based on operation temperature at all three depths, and contained 19 of the 34 PLFAs (component loadings ≥0.5). The 10 fatty acids carrying positive loadings with respect to PC 1 were present in greatest abundance in the filter operated at 35 °C. These fatty acids included high loadings for three terminally branched saturates (i17:0, i16:0, and i15:0), which are Gram-positive markers; two mid-branched saturates (10me17:0 and 10me19:0), markers for sulfate-reducing bacteria; and two normal saturates (16:0 and 17:0). The nine fatty acids with negative loadings were present in greatest abundance in the filter operated at 5 °C, and included two of the most abundant fatty acids, the monoenoics 16:1ω7c and 18:1ω7c.

PC 2 differentiated samples with respect to filter depth at all three temperatures of operation. For a filter operated at a given temperature, samples from the top had more negative scores on PC 2, while samples from the middle and bottom had more positive scores. PLFAs characteristic of Gram-positive bacteria and other anaerobic bacteria (br19:0, a17:0), and microeukaryotes (18:2a, 20:4ω6) which carried high positive loadings on PC 2, were present in greater relative abundance at the bottom of the filters. The difference in community structure as a function of filter depth may be due to the depletion of labile substrates in the top of the filter, leaving the more recalcitrant compounds as the only available substrates present at lower biofilter depths (*1*−*4*). This may select for slower growing microorganisms, such as Gram-positive bacteria and fungi, which are able to degrade an array of complex molecules, and thus may have a selective advantage in nutrient-poor environments, whereas Gram-negative bacteria as a group are faster growing and better adapted to relatively high-nutrient environments.

Relatively little work has been done previously to characterize microbial populations in biological filters used to treat drinking water. A few studies have been performed to examine microbial communities associated with drinking water treatment plants by characterizing isolates. Beck and Bonde (*46*) studied changes in bacterial community structure through a pilot water treatment plant, which included ozonation followed by BAC filtration. Upon exposure to ozonation byproducts, the bacterial flora isolated from the BAC filters changed in character from predominantly fluorescent pseudomonads to a dominance of other *Pseudomonas* species (*Ps. alcaligenes*, *Ps. pseudoalcaligenes*) which are characteristically able to utilize highly oxidized organic compounds such as oxalate. In a study of full-scale BAC filters and their effluents (*47*), the predominant bacterial genera isolated from the filters were *Pseudomonas*, *Flavobacterium*, *Bacillus*, and *Arthrobacter*. This is consistent with findings reported here that markers for Gram-negative bacteria were

TABLE 2. Identities of Bacteria Isolated from Biofilter Media

isolate (filter temp, depth)[a]	MIDI identification	characteristics[b]
#1 (20 °C, T)	did not subculture	same colony morphology as #10, #13
#2 (20 °C, T)	*Staphylococcus aureus*	Gram (+); animal cutaneous tissue major habitat, found sporadically in water
#3 (35 °C, T)	*Acidovorax delafieldii*	Gram (−), β-subclass of proteobacteria, aerobic facultatively chemolithoautotrophic hydrogen-oxidizing bacteria; widespread in soils, freshwaters; wide substrate range: oa, fa, aa, alc, carb
#4 (20 °C, B)	*Azospirillum brasilense*	Gram (−), α-subclass of proteobacteria; microaerophilic N-fixing soil bacteria; favored substrates oa. aerotactic movement
#5 (35 °C, B)	*Methylobacterium mesophilicum*	Gram (−), α-subclass of proteobacteria; obligately aerobic facultatively methylotrophic bacteria; ubiquitous in nature: soils, dust, fresh water; grow on variety of C-1 compounds, as well as complex organic substrates oa, carb, aa, alc)
#6 (20 °C, B)	did not subculture	Gram (−)
#7 (20 °C, B)	no match	Gram (−)
#8 (20 °C, T)	did not subculture	Gram (−)
#9 (35 °C, T)	*Enterococcus faecium*	Gram (+), microaerophilic bacteria; animal intestine major habitat, may be associated with plants; complex nutritional requirements
#10 (35 °C, M)	did not subculture	same colony morphology as #1, #13
#11 (35 °C, M)	did not subculture	Gram (−)
#12 (5 °C, M)	*Vibrio cholerae* non-O1	Gram (−), γ-subclass of proteobacteria; human intestinal tract natural reservoir, free-living forms found mainly in marine environment; positive sucrose fermentation, and lysine and ornithine decarboxylation
#13 (20 °C, T)	did not subculture	same colony morphology as #1, #10
#14 (20 °C, M)	*Rhodobacter capsulatus*	Gram (−), purple nonsulfur bacteria (α-subclass of proteobacteria); anoxygenic photoheterotrophs, widely distributed in aquatic habitats; facultative chemoheterotrophs in the presence of O_2 in dark; wide substrate range: oa, fa, aa, alc, carb
#15 (5 °C, T)	no match	Gram (−)
#16 (20 °C, T)	*Bacillus sphaericus*	Gram (+), aerobic sporeforming bacteria; soil, water sediments are major habitats

[a] Filter operational temperature and depth from which the bacterium was isolated. [b] oa, organic acids; fa, fatty acids; aa, amino acids; alc, alcohols; carb, carbohydrates.

predominant in biological filters, and that Gram-positive bacterial markers were also present.

Heterotrophic Plate Counts and Isolate Identification. A decreasing gradient in HPC as a function of filter depth was seen, consistent with the lipid phosphate gradients observed (Figure 1). As expected, the number of colony forming units (CFU) counted on the R2A agar plates, 4.7×10^6 to 5.1×10^7 CFU/g dw media, was 2−3 orders of magnitude lower than the number of bacteria estimated from the measured lipid phosphate concentrations using a conversion factor of 100 nmol of lipid phosphate = 3.43×10^9 cells based on sediment communities (*31*). This is likely due to selective growth of bacteria on laboratory growth media, and the unculturability of many microorganisms (*48*).

Two colony types (isolates #1 and #2), distinguished by colony morphology, were dominant in most of the filter samples (sum of the two types was 47−85%), except for samples from the bottom of the filter operated at 5 °C (37%), and from the middle and bottom of the filter operated at 35 °C (0%). Two other colony types (isolates #3 and #4) were observed at relatively lower percentages of the total CFU on most plates (0−15%). Isolate #3 was present in greater numbers in the samples from the bottom of the filters operated at 5 and 20 °C (35 and 26%, respectively) whereas isolate #4 was more common in samples from the filter operated at 5 °C (5 to 22%). A fifth colony type was dominant in samples from the middle and bottom of the filter operated at 35 °C (67 and 95%, respectively). Sixteen of the most prevalent colony types (including the five dominant colony types) were picked and isolated by streaking on R2A agar. Of these 16 isolates, 5 were not amenable to subculturing, including the most common colony type (isolate #1). Positive identifications were made for 8 of the 16 isolates using the MIDI system. Table 2 shows the isolates, operational temperature, and depth of filter from which they were isolated, identifications, and major characteristics of each organism.

Of the bacteria isolated from the filters, 70% were Gram-negative, and 30% were Gram-positive. This is consistent with the distribution of the bacterial PLFA markers identified in the filters (Figure 2). The bacteria which were identified include organisms found in natural waters and soils (*Acidovorax delafieldii*, *Rhodobacter capsulatus*, *Methylobacterium mesophilicum*, *Bacillus sphaericus*, and *Azospirillum brasilense*), as well as organisms which may be transient members of natural water populations due to dissemination by human and animal hosts (*Vibrio cholerae* non-O1, *Enterococcus faecium*, and *Staphylococcus aureus*). Of the five most prevelant bacterial isolates, only isolate #2, which was identified as *Staphylococcus aureus* and was common to filters operated at all temperatures, is commonly found in human and animal hosts. The data gathered in this study suggest that presence of potential pathogens in the biofilters may not be temperature dependent, as the three bacteria identified as organisms with predominantly animal hosts were isolated from filters operated at three different temperatures (Table 2).

Results from this study indicate that low operation temperature decreases the biomass and biofilter perfor-

mance, suggesting that optimized drinking water treatment design and operation must account for seasonal temperature variations. Low temperature not only decreases the rate of substrate metabolism, but also changes the microbial community structure. These changes are not the effect of seasonal variation in BOM and influent microbial community, but that of temperature alone. To fully evaluate the consequences of these population shifts, future studies must isolate the impact of these changes in community structure on substrate removal efficiency.

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