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Effects of Carbon Source, Carbon Concentration, and Chlorination on Growth Related Parameters of Heterotrophic Biofilm Bacteria

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ABSTRACT

To investigate growth of heterotrophic biofilm bacteria, a model biofilm reactor was developed to simulate a drinking water distribution system. Controlled addition of three different carbon sources (amino acids, carbohydrates, and humics) at three different concentrations (500, 1,000, and 2,000 ppb carbon) in the presence and absence of chlorine were used in separate experiments. An additional experiment was run with a 1:1:2 mixture of the above carbon sources. Biofilm and effluent total and culturable cells in addition to total and dissolved organic carbon were measured in order to estimate specific growth rates (SGRs), observed yields, population densities, and bacterial carbon production rates. Bacterial carbon production rates (μg C/L day) were extremely high in the control biofilm communities (range = 295–1,738). Both growth rate and yield decreased with increasing carbon concentrations. Therefore, biofilm growth rates were zero-order with respect to the carbon concentrations used in these experiments. There was no correlation between growth rate and carbon concentration, but there was a significant negative correlation between growth rate and biofilm cell density ($r = -0.637$, $p = 0.001$ control and $r = -0.57$, $p = 0.021$ chlorinated biofilms). Growth efficiency was highest at the lowest carbon concentration (range = 12–4.5%, amino acids and humics respectively). Doubling times ranged from 2.3–15.4 days in the control biofilms and 1–12.3 days in the chlorinated biofilms. Growth rates were significantly higher in the presence of chlorine for the carbohydrates, humics, and mixed carbon sources ($p = 0.004$, < 0.0005, 0.013, respectively). The concept of *r*/*K* selection theory was used to explain the results with respect to specific growth rates and yields. Humic removal by the biofilm bacteria (78% and 56% for the control and chlorinated biofilms, respectively) was higher than previously reported literature values for planktonic bacteria. A number of control experiments indicated that filtration of drinking water was as effective as chlorination in controlling bacterial biofilm growth.

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Introduction

Microbial utilization of dissolved organic carbon (DOC) in natural freshwater ecosystems has mainly been studied in terms of planktonic bacteria [2, 8, 9,10, 17]. Traditionally, low molecular weight organics such as amino acids and carbohydrates have been estimated to be responsible for a significant fraction of net carbon production in planktonic bacterial communities from a variety of lakes and rivers [18, 23, 24]. Conversely, in recent years, high utilization of DOC material other than the simple compounds mentioned above has been estimated to constitute a large fraction of net bacterial carbon production in freshwater and marine ecosystems [1, 2, 60, 61, 67]. Amon and Benner [1, 2] and, more recently, Volk et al. [67] have demonstrated that the high molecular weight fraction of dissolved organic matter (DOM) is less recalcitrant than previously thought.

Although most studies have mainly focused on the planktonic component of the food web, a number of investigations have implicated surface associated communities as being responsible for significant carbon turnover rates [5, 27, 47, 66, 67, 69]. These bacterial communities have been shown to have a disproportionately high contribution to nutrient turnover rates as compared to their planktonic counterparts. Nitrogen, carbon, and sulfur cycles can be compressed into linear zones that are millimeters thick in microbial mats, trickling filters, and sediments as compared to meters and tens of meters in lakes and oceans. Kaplan and Newbold [27] (and references therein) estimated that the benthic populations in streams and rivers can be significant sites of DOC utilization and are the primary sites for DOC diagenesis. This pool of natural organic matter (NOM) contains the reduced organic carbon required for the production of energy and biomass during heterotrophic bacterial metabolism. Utilization of this NOM can result in the growth of bacteria in both natural and engineered aquatic environments.

An area of applied microbiology that is significantly affected by the presence of utilizable carbon is drinking water. Excess organic material in drinking water is associated with growth of bacteria in the distribution system, increased chlorine demand in finished waters, and the formation of disinfection by-products [15, 31, 32, 33, 38, 49, 56]. Although the measurement of specific utilizable components of NOM has not been the focus of drinking water research, measurement of the total utilizable fraction has encompassed a significant amount of effort in the past 10 years [14, 20, 26, 35, 51, 63, 64]. A number of methods have been applied to measure the readily utilizable fraction of NOM present in aquatic systems, which can be divided into two basic groups: (1) assimilable organic carbon (AOC) and (2) biodegradable organic carbon (BDOC).

In this study, we utilized a biofilm reactor system (annular reactors) to investigate community response to three of the commonly accepted primary DOC components in aquatic environments. Three different organic substrates constituting amino acids, carbohydrates, and humics were separately tested for their ability to support heterotrophic bacterial communities under varying trophic conditions. AOC-free drinking water was generated by use of granular activated carbon/biologically activated carbon (GAC/BAC) filtration and used as dilution water for the reactors. Chlorinated and control reactors were operated in parallel to examine the effects of oxidative stress and nutrient limitation on growth parameters. Operationally, the annular reactors could be viewed as biological filters similar to BDOC columns, but they are much easier to model as continuously stirred tank reactors (CSTRs). This allowed us to measure specific growth rates (SGRs), observed yields/growth efficiency, bacterial carbon production (BCP), and biomass/cell density at different substrate loading rates. BDOC columns and a carbon unsupplemented (negative) control annular reactor were used to measure the BDOC content of the AOC-free water.

Materials and Methods

Experimental System

The generation of mixed heterotrophic bacterial populations was accomplished with the use of a model biofilm reactor system consisting of rotating annular reactors [65]. Polycarbonate based, the reactors consist of a stationary outer cylinder with a rotating inner drum forming an annular space between the inner drum and the outer cylinder. Twelve removable polycarbonate slides are located in machined grooves in the inside wall of the outer cylinder, allowing for biofilm sampling. The total submerged surface area in the reactors is 0.18 m^2 and the liquid volume is 630 mL. The rotational speed of the inner drum can be varied to create the desired shear stress on the wall of the outside cylinder. In our case a rotational speed of 30 revolutions per minute was used in all work, simulating the shear stress at the wall of a 4-inch-diameter (102-mm-diameter) pipe with a flow velocity of 1.0 foot per second (0.3 meters per second). These reactors can be modeled as continuously stirred tank reactors (CSTRs). The total flow rate to the reactor was set to provide a hydraulic detention time of 2.1 h for all experiments. The lower colonized areas of the annular reactors were immersed in a water bath maintained at 20±1°C utilizing an external cooling device. The influent solution to each reactor con-

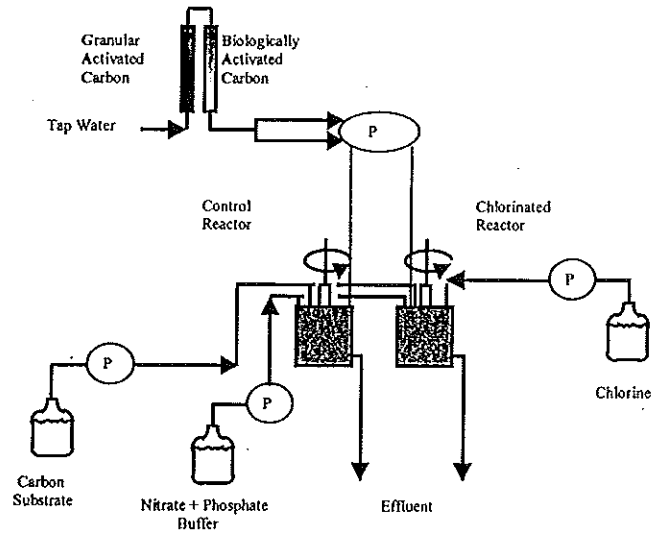

Fig. 1. Schematic of experimental setup. The circles with *P* inside refer to pumps. See text for further details.

sisted of dilution water from the BAC filter, plus the substrate and buffer solution (see Fig. 1).

City of Bozeman, Montana, USA, drinking water was treated in two columns containing granular activated carbon media to provide the dilution water at a flow rate of 4.5 mL min^{-1}. The first column removed chlorine from the water and contained granular activated carbon (GAC, 12′ 40 mesh Nuchar, Westvaco). The second column contained biologically activated carbon (BAC) originally obtained from a full-scale water treatment plant (City of Laval, Quebec, Canada) and had been receiving Bozeman drinking water for more than 3 years prior to the experiments. BAC filters can be used in the treatment of drinking water to decrease the amount of utilizable carbon that passes into the distribution system [7, 21, 22]. The assimilable organic carbon (AOC) level of the effluent from the BAC column was typically less than 25 micrograms per liter (mg acetate equivalents L^{-1}) as determined by the method of van der Kooij [64] as modified by LeChevallier et al. [34]. The BAC column effluent contained a mixed population of heterotrophic bacteria (ca. 10^4 to 10^5 total cells mL^{-1} by epifluorescence microscopy) that colonized the reactor surface and provided a continuous supply of nutrient limited bacteria to the reactor. Substrate and nitrate/phosphate feed solutions were made up in glass carboys using Nanopure reagent-grade water that had been autoclaved prior to use. All glass carboys were combusted at 500°C for 4 h prior to adding Nanopure water and autoclaving. Sterile substrate or nutrient solutions were added by syringe to the carboys after the water had been autoclaved and cooled to room temperature.

Prior to each experiment the annular reactor top assembly/inner drum and all tubing (Masterflex L/S silicone) were autoclaved prior to assembly and chlorine disinfection. A solution of 50 mg L^{-1} free chlorine was then added to the reactors for at least 24 h. After 24 h with the inner drum rotating, the chlorine solution was drained and a sterile feed of Nanopure water was initiated for 24 h. A sterile sodium thiosulfate solution was added to the system to remove any remaining free chlorine prior to inoculation.

Media

Three different carbon source mixtures used in separate experiments were composed of amino acids, carbohydrates, or humic material and were supplied at a rate of 0.25 mL min^{-1} from concentrated stock solutions. The amino acid mixture was composed of L-glutamic acid, L-aspartic acid, L-alanine, and L-serine. The carbohydrate mixture was composed of D(+)-glucose, D-galacturonic acid, D(+)-galactose, and D(−)-arabinose. All substrates were HPLC grade purchased from Sigma. The amino acids and carbohydrates experiments utilized equimolar concentrations as carbon of the compounds in each group. The humic substrates were extracted from Elliot Silt Loam Soil, BS102M, purchased from the International Humic Substances Society (IHSS). The humics concentration was based on total organic carbon (non-purgeable) analysis of a concentrated stock solution. A concentrated stock solution was prepared by adding IHSS humic material (100 g) to 1 L of 0.1 N NaOH, mixed for 24 h, and then centrifuged at 10,000′ g at 4°C to remove particulates. The concentrated humic stock solution (approx. 1,000 mg C L^{-1}) was stored in the dark at 4°C. After addition of the humic stock solution to the sterile Nanopure water, the pH was adjusted to approximately 7 (±0.2) using 2 N HCl. Nitrate (as KNO_3) and phosphate (as KH_2PO_4 + K_2HPO_4) were supplied at a rate of 0.25mL min^{-1} at concentrations of 612 and 591 mg L^{-1} (9.9 and 6.2 mM), respectively. Carbon feed was controlled in order to provide nominal influent concentrations at three different levels: (1) 500 ppb, (2) 1,000 ppb, and (3) 2,000 ppb.

Experimental Overview

A total of nine experiments were run, three with amino acids as the substrate, three with carbohydrates as the substrates, two with humics as the substrates, and one with a mixture of the three substrates. Five samples (n = 5 samples × 3 experiments = 15 measurements) were used for substrate evaluation with respect to total cells (TDC) and culturable cells (cfu) in effluent at the three carbon feed levels (i.e., 500, 1,000, and 2,000 ppb C). These same samples were used for calculating specific growth rates (SGRs), bacterial carbon production (BCP), and observed yields. The reactors were designated as control (no chlorination), or test (chlorination at 1,000 and 2,000 ppb C) on a random basis. Since both reactors received the same treatment at the 500 ppb C level, evaluation of bacterial density in the effluent and biofilm was used to assess the reproducibility of the annular reactors.

The mixture experiment was designed to complement the other eight experiments, but with a few changes in protocol. Carbon feed was initiated at a concentration of 250 ppb (equimolar as carbon, 2:1:1 mixture of humics, amino acids, and carbohydrates, respectively) and chlorination was started at this stage.

An additional annular reactor was operated as a negative control without supplemental carbon feed. Buffer was supplied to this reactor (as nitrate and phosphate, 612 and 591 mg L^{-1}, respectively)

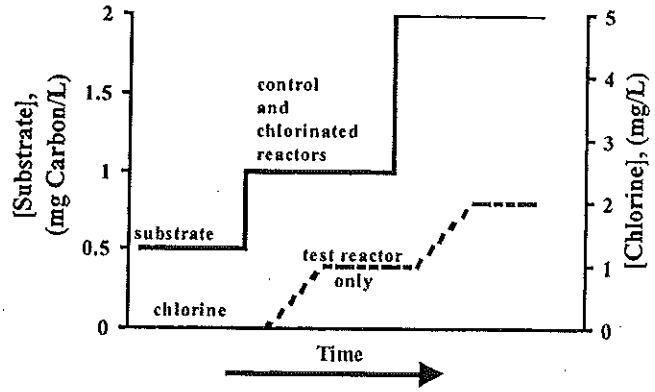

Fig. 2. Carbon and chlorine dosing protocol for all experiments.

and sampling was performed periodically over the course of 12 months.

Since the effluent from the GAC/BAC columns was used for colonization of the annular reactors as well as a supply of dilution water, background carbon in the drinking water could have been a confounding factor in substrate evaluations. Therefore, tap water influent and effluent of the GAC/BAC columns was sampled for DOC removal in order to ascertain the amount of carbon removed across these biological filters.

As an additional control, BDOC columns were operated according to Ribas et al. [51]. Briefly, SIRAN glass bead (Schott Glassworks) filled chromatography columns (Kontes) were operated (in the dark) for the measurement of the BDOC present in the AOC "free" water. These columns (in series) were maintained on the influent water used for the annular reactors for 3 months prior to initiation of sampling (sampling period of 261 days). Prior to this the columns had been operating separately (i.e., not in series) for 1 year on City of Bozeman tap water. Hydraulic detention time for the BDOC columns was set to be equivalent to the annular reactors (2.1 h) while the hydraulic detention time in the GAC/BAC columns was 15 min.

Reactor Operation

Figure 2 shows the substrate and chlorine feed protocols used during each experiment. Initially each reactor was fed a substrate concentration of 500 mg C L^{-1} until a pseudo steady state based on effluent total and culturable cell counts (14–30 days) was reached. The substrate concentration was then increased to 1,000 mg C L^{-1}, the reactors operated until a new steady state was attained, and then chlorine addition to one of the two reactors was started. The chlorine dose was slowly increased until a free chlorine residual of 0.15 to 0.2 mg L^{-1} was measured in the effluent. The reactors were operated until another pseudo steady state was reached, then the substrate concentration was again increased to 2,000 mg C L^{-1}, until another pseudo steady state was reached, and then the chlorine feed dose was slowly adjusted until the effluent residual of the chlorinated reactor was 0.15 to 0.2 mg L^{-1}. Triplicate (duplicate for the humics) experiments for each of the three substrate groups were performed. Experiments were typically run for 90 to 150 days.

At steady state operation the reactors were sampled for total and culturable cells in the effluent and biofilm. TOC measurements in addition to biovolume determinations were also obtained under these operating conditions.

TOC Measurements

NPOC Determination. Samples for total organic carbon (TOC) were processed immediately. Filters used for TOC analysis were presterilized 0.2-mm pore-size nylon syringe filters (Fisher Scientific, Poretics, Livermore, CA), prewashed with 35 ml of 0.1 N HCl followed by 4 × 35 mL of Nanopure reagent-grade water using a 30 ml sterile, disposable syringe that was also used for the sample. The first 10 to 20 ml of the filtered sample was discarded. All TOC samples were acidified prior to analysis using 0.2 ml of 2 N HCl in 40 ml of sample. All samples were analyzed for non-purgeable organic carbon (NPOC) using a Shimadzu TOC 5000A with high-sensitivity platinum catalyst by the high temperature combustion method and an autosampler. Standard curves for the TOC 5000A were developed using four concentrations of potassium hydrogen phthalate (Shimadzu), and the resulting linear calibration curve typically had a coefficient of determination (r^2) of 1.00 and never less than 0.98. A standard (from the standards used to develop the standard curve) was always run with each group of samples to correct for any variations in carrier gas flow rate. All glassware was precombusted at 500°C for 4 h, minimum, whenever possible. Autosample vials were acid washed for at least 12 h in concentrated sulfuric acid containing NoChromix (Godax Laboratories, Inc., Fisher Scientific), rinsed six times with Nanopure reagent grade water, and precombusted at 300 to 400°C for 6 hours, minimum. Glass volumetric flasks and analytical pipettes were acid washed and rinsed six times with Nanopure water. Parallel filtered and unfiltered samples from the influent and effluent were used to determine the amount of carbon (as NPOC) in the particulate and fluid phase. System blanks comprising the reagent grade Nanopure water were run at the beginning and end of each sampling period. A minimum of four measurements were made on all samples. The coefficient of variation (C.V.) for all samples averaged 1.8% (range, 0.07 to 7.4%, $n = 215$).

Carbon Balance across Reactors. NPOC was determined as described above for all input parameters (AOC-"free" dilution water, buffer and carbon supplement) and reactor output. The following designations are utilized:

AOC_f is the filtered AOC dilution water (DOC)
AOC_{nf} is the unfiltered AOC dilution water (TOC)
B_{nf} is the unfiltered buffer solution (TOC)
C_f is the filtered carbon solution (DOC)
C_{nf} is the unfiltered carbon solution (TOC)
C-out_f is the filtered carbon in the reactor effluent (DOC)
C-out_{nf} is the unfiltered carbon in the reactor effluent TOC)

The total amount of soluble carbon entering the reactors was determined from the relationship

$$\text{Carbon in} = (4.5/5.0) \times (AOC_f) + (0.25/5.0) \times (B_{nf}) + (0.25/5.0) \times (C_{nf}) \quad (1)$$

where the numbers refer to the flow rates for each solution. Carbon out = C-out$_f$. Both the buffer and the AOC dilution water (AOC$_f$) were assumed to be unavailable for bacterial utilization and are summed to give the non-utilizable fraction as shown below:

$$\text{Nonutilizable} = (0.25/5.0) \times (B_{nf}) + (4.5/5.0) \times (AOC_f) \quad (2)$$

C-out$_f$ represents the total concentration of DOC that was not removed across the annular reactor. If the nonutilizable fraction was wholly represented in both the influent and effluent carbon measurements, it could be subtracted from the total amount of dissolved carbon in the reactor effluent (i.e., C-out$_f$). This difference represented the amount of the supplemental dissolved organic carbon substrate feed that was not removed across the reactors. Subtracting this difference from the substrate feed concentration in the influent provided the amount of the feed carbon that was removed across the reactors. The substrate feed concentration was determined directly from the carboy. As well, the difference between any of the unfiltered (TOC) and filtered (DOC) solutions provided the amount of particulate organic carbon (POC). This POC contribution arises from the bacterial cells in the water and was used to determine an average value for carbon/cell when divided by the appropriate total cell count for that sample. One to three carbon determinations were made at the pseudo-steady-state stage of duplicate experiments and average carbon substrate removal amounts were computed from these samples. Similar measurements were used to determine the amount of carbon removed across the BDOC columns, GAC/BAC filter, and negative control reactor.

Influent and Effluent Cell Sampling

Grab samples of the dilution water (i.e., reactor influent from the GAC/BAC filter) and effluent from each reactor were routinely collected and sampled for total and culturable cells. Samples for total cell counts were fixed using formalin (0.7% final concentration) for 24 h at 4°C. Fixed samples were filtered onto 0.2 μm pore size black polycarbonate filters (Poretics), stained on the filter using 4',6-diamidino-2-phenylindole (DAPI) (0.1 μg/ml) for 30 min and rinsed with 5 ml of filter sterilized Nanopure water [29]. Culturable cell counts were determined by spread plating dilutions in triplicate on R2A agar medium [50] and incubating at room temperature for 7 days. Chlorine concentrations were determined using the N,N-diethyl-p-phenylenediamine (DPD) colorimetric method and a digital colorimeter (LaMotte, Chestertown, MD). Both total free residual and combined residuals were determined in the effluent as well as chlorine dosage levels applied to the reactors.

Specific Growth Rates, Yield, and Bacterial Carbon Production

In order to determine SGRs, observed yields, and BCP the reactors were modeled as CSTRs such that mass balances across the reactors can be solved for the particular parameters, assuming steady state operating conditions [65]. Bacterial carbon production (BCP, μg Cell carbon/cm^2 h^{-1}) was calculated using biovolume and carbon μm^{-3} determined from microscopy and the physical parameters of the reactors and operating protocols. Assuming that the difference between influent and effluent total cell counts at steady state is due to biofilm growth in the reactor, a simple subtraction (X_1–X_0) gives the number of bacteria produced from the biofilm and conversion to biomass provides BCP. Observed yield was calculated as the ratio of biomass produced divided by substrate utilized.

Samples stained for direct counts were also used to determine the biovolumes. Image analysis was performed with the use of a charge coupled device (CCD) camera (Optronics, 470T) connected to Image Pro (V. 3.0, Media Cybernetics) computer software for archiving of images on CD-ROM. A minimum of 20–30 random images were collected and analyzed for morphological parameters (length, width, area, perimeter, feret box, roundness, etc.) with the use of the freeware image analysis program Image Tool (V. 1.27), from the University of Texas Health Sciences School at San Antonio (UTHSCSA, available at http://ddsdx.uthscsa.edu/dig/itdesc.html). Color images were converted to grayscale and thresholding performed manually. Images were analyzed at a final magnification of 2,000–6,000× depending on cell sizes. Microscopic magnifications of 1,000–1,500× resulted in approximately 10–14 pixels μm^{-1}. Bacteria predominantly rods and cocci. Therefore, biovolume was calculated as follows: Vol = $(\pi/4) \times \omega^2 \times (l-\omega/3)$, where l is length and ω is width [4, 45]. Biovolume determinations were performed on 2–5 separate samples from the reactors during pseudo-steady-state operation at the 1,000 and 2,000 ppb carbon feed levels. Calibration for morphometric analysis was performed with a calibration slide etched with 10 μm wide lines. Comparison of calibration lines to the measurement of 1 μm latex spheres (Polysciences, Ltd., Warrington, PA) showed good agreement (measured diameter = 1.0 ± 0.1 μm S.D.).

Biofilm Sampling

The biofilm in the reactors was sampled by removal of a slide (area of approximately 30 cm^2) and aseptically scraping the biofilm from the slide into a sterile buffer solution. The biofilm/buffer solution was then homogenized for 1 min using a Tekmar Tissuemizer with UT-dispersing tool T25type S25N-8G (Cincinnati,OH) at the maximum setting. This suspension was then analyzed for total and culturable cell counts as described in previous paragraphs.

Statistical Analyses

A general linear model (GLM) analysis of variance (ANOVA) was used to analyze all data with nested treatments designated as chlorination/control, substrate type, and carbon feed level [11, 41, 57]. In addition, Tukey's pairwise comparisons were used when one factor had more than two levels and data analysis was within one treatment factor. A one-sample t-test was used where specific means were being compared. Acceptance or rejection of the null hypothesis was based on an α-level of 0.05 in all cases. The statistical program Minitab [41] was used for all analyses. Model II

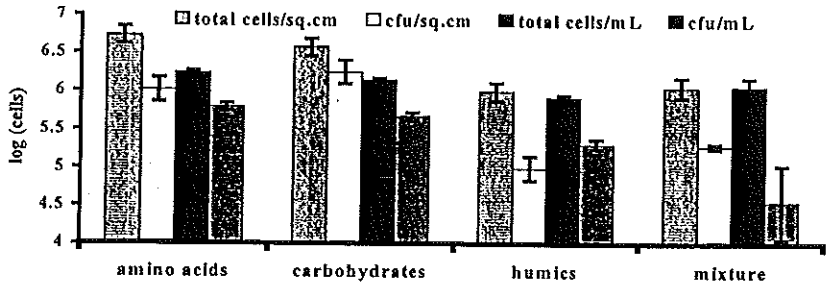

Fig. 3. Cell counts in the effluent and biofilm from the control stage of the experiments separated by substrate type. Total cells mL^{-1} are significantly different for all substrates while the amino acids and carbohydrates are equivalent for culturable cells mL^{-1} (Tukey's pairwise comparisons). n = 30, 30, 20, and 5 for the amino acids, carbohydrates, humics, and mixture, respectively, for the cells mL^{-1} data. n for the biofilm data is 6 for all substrates except the mixture (n = 3). See text for further details.

regressions were carried out by the method of geometric means as detailed in Sokal and Rohlf [57].

Results

GAC/BAC Filter and BDOC Column Performance

The BDOC columns were operated as separate units (not in series) on unsupplemented tap water for over 1 year. Carbon removal across the two columns was generally lower than for the GAC/BAC filter. There was no significant difference between the two BDOC columns, the first column removed 9.2 ± 4.8 % (n = 11, ± S.D.) while the second column removed 10.0 ± 4.7 % (n = 10) of the influent native DOC. Both were significantly different from the GAC/BAC filter, where carbon removal averaged 18 ± 4.7 % (n = 19) of the influent native DOC. In terms of absolute carbon removal the BDOC columns averaged 93 ± 51 μg C L^{-1} while the GAC/BAC filter removed an average of 157 ± 86 μg C L^{-1} over the same sampling period. These differences are magnified when one considers that the hydraulic residence time in the GAC/BAC filter was 15 min compared to 2.1 h for the BDOC columns. Over the course of all samples (773 days) the GAC/BAC filter removed an average of 190 ± 178 μg C L^{-1} (17 ± 10%). When the BDOC columns were run in series and receiving the AOC "free" water, the percentage removal of utilizable carbon was 2.4 ± 5.4% and equated to 32 ± 38 μg C L^{-1}. This compares to the negative control reactor operated with the AOC "free" water where supplementation with nitrate and phosphate produced no net (average) removal across the reactors (−3.3 ± 7.4% or −11 ± 38 μg C L^{-1}, n = 7). Neither of these values was significantly different from zero (p = 0.28 and 0.67, respectively) by a one-sample t-test where the null hypothesis mean was zero. The GAC/BAC filter released bacteria at a steady rate with the total cell counts producing less variability than the culturable cells. Over the course of the experiments, cell numbers in the influent (i.e., GAC/BAC effluent) to the annular reactors averaged 3.8×10^5 total cells mL^{-1} (95% C.I., 3.64×10^5–4.15×10^5, n = 247) and 5.66×10^4 cfu mL^{-1} (4.19×10^4–6.53×10^4, n = 260).

Control Stage (500 ppb Carbon Feed Level), Annular Reactors

Effluent. Frequent sampling was performed at this stage of the experiments in order to evaluate the reproducibility of the supplemented annular reactors within any one experiment. Figure 3 shows the results from biofilm and effluent sampling at the control stage of the experiments. A GLM ANOVA was used to analyze the data from the amino acids, carbohydrates, and humics experiments where n values were 15, 15, and 10, respectively, for the effluent data. The mixture experiment was not included in this analysis because of the different protocols used (initial feed concentration was 250 ppb carbon) and the lack of true replication. However, the means and standard deviations (n = 5) for the unchlorinated control reactor are shown for comparison. Determination of steady state at this stage (500 ppb C) was made by the equivalency of total and culturable cells in the reactor effluent with respect to time (i.e., no significant difference among the five measurements). The GLM ANOVA based on five separate measurements (effluent averages) from the low carbon feed level of each experiment (three experiments gives n = 15, or two experiments gives n = 10) and subsequent analysis showed that the individual reactors were equivalent within substrates for both total cells (p = 0.581) and culturable cells (p = 0.178). This also indicated that the reactors were at steady state and based on these results, the within substrate data was pooled and both the total cell counts [log(tdc)] and culturable cell counts [log(cfu)] were significantly different between substrates (p < 0.0005 for both measures; n = 30, 30, and 20). However, Tukey's pairwise comparisons showed that the culturable cells were equivalent for the amino acids and carbohydrates. The percentage variance in the data for the unexplained error com-

Table 1. Pearson Product Moment Correlation Coefficients (r) between total cell counts and culturable cells and the percentage of culturable cells normalized to total cells in reactor effluents at the two stages of analysis

Substrate	Measure	500 ppb C	Control Test Level	Chlorine 1000 ppb C	2000 pub C
Ammo acids	r	0.593 (71)	0.763 (100)	0.667 (72)	0.424[1] (46)
	cfu/tdc	49 (26)	42 (21)	23 (16)	21 (15)
Carbohydrates	r	0.511 (47)	0.675 (60)	0.684 (42)	0.508[2] (16)
	cfu/tdc	41 (22)	42 (23)	8 (12)	5 (14)
Humics	r	0.424[1] (63)	0.366 (90)	0.565 (51)	0.365[3] (48)
	cfu/tdc	35 (31)	36 (16)	8 (14)	1.5 (2.5)

500 ppb C refers to the control level of the experiments and is the combined results for both reactors. Test level is the 1,000 and 2,000 ppb C loading levels. $p < 0.0005$ for all correlations except 1 ($p = 0.005$), 2 ($p = 0.037$), and 3 ($p = 0.011$). Numbers in brackets for r are n-values and numbers in brackets for ratio are standard deviations in percent. n was the same for both r and ratio. Influent cells had a percentage culturability of 25 (21) and $r = 0.349$ (185). Control effluents were not significantly different at the mid and high carbon loading levels.

ponents was 1.5% for total cells and 3.2% for culturable cells and the between experiments variance component was 10.7% for total cells and 8.8% for culturable cells, indicating good replication. As a measure of the culturability for individual substrates we normalized the culturable cells to total cells (i.e., cfu/TDC) in the reactor effluents. This ratio and the correlation between culturable and total cells are reported in Table 1 for paired samples from the control and test stage of the experiments. All three supplements produced a significant increase in the ratio of cfu/TDC ($p < 0.0005$).

Biofilm. Biofilm sampling was performed once at each feed level for the amino acids and carbohydrates experiments ($n = 3$). The humic experiment was performed twice and two samples were obtained from one of the experiments giving a pseudo replication n value of 3. The mixture experiment was performed once and the results are based on three separate samples for the total cells and culturable cells. Biofilm sampling at this stage also indicated that the reactors within any one treatment (i.e., substrate source) were equivalent for total and culturable cells ($p = 0.088$ and 0.868, respectively) prior to increasing the carbon concentration and initiation of chlorination.

Test Stage (1,000 and 2,000 ppb Carbon Feed Levels), Annular Reactors

Biofilm. Figure 4, panels A and B, shows the total cells and cfu per square centimeter in the biofilms for the control and chlorinated treatments at the carbon feed levels of 1,000 and 2,000 ppb. Reported values are means and standard deviations and p-values result from a GLM ANOVA with carbon levels nested within substrates, performed on the separate reactors. Response to the substrates in the control reactor was compared at the three carbon levels. In the presence of chlorine, comparison between substrates was analyzed at the mid and high carbon feed levels due to the added treatment of chlorination.

Total and culturable cell populations in the biofilm were significantly impacted by chlorination in the presence of carbohydrates ($p = 0.001$ and $p < 0.0005$) and humics ($p = 0.001$ and $p < 0.0005$) resulting in decreased cell numbers compared to the low carbon feed level. Increased carbon concentration (1,000 to 2,000 ppb) had a relatively minor effect on the total cell density in the biofilm. Cell numbers stabilized in the presence of chlorine for humics and carbohydrates, whereas the mixture and amino acids showed increased population densities. In the presence of amino acids chlorination did not affect the culturable biofilm cell density. Comparing substrates within the control reactors indicated that total cell counts in the biofilm were equivalent ($p = 0.246$) while the culturable cells were significantly different ($p = 0.005$). This difference was due to the humics, which supported a significantly lower population of culturable cells. Culturable cells in the biofilm were significantly different ($p < 0.0005$) for all substrates once chlorination was initiated. While the mixture experiment was performed only once, it was the only carbon source that produced significantly higher total cell densities at each carbon level ($p < 0.0005$). Similar to the amino acids, chlorination had no effect on the number of total cells ($p = 0.080$) when the mixture carbon concentration was increased (i.e., 1,000 to 2,000 ppb). The negative control reactor had 2.45×10^6 total cells/cm^2 (95% C.I. 1.97×10^6–3.01×10^6) and 4.36×10^4 cfu/cm^2 (1.87×10^4–1.02×10^5) in the biofilm determined on two separate sampling dates. This can be compared to the

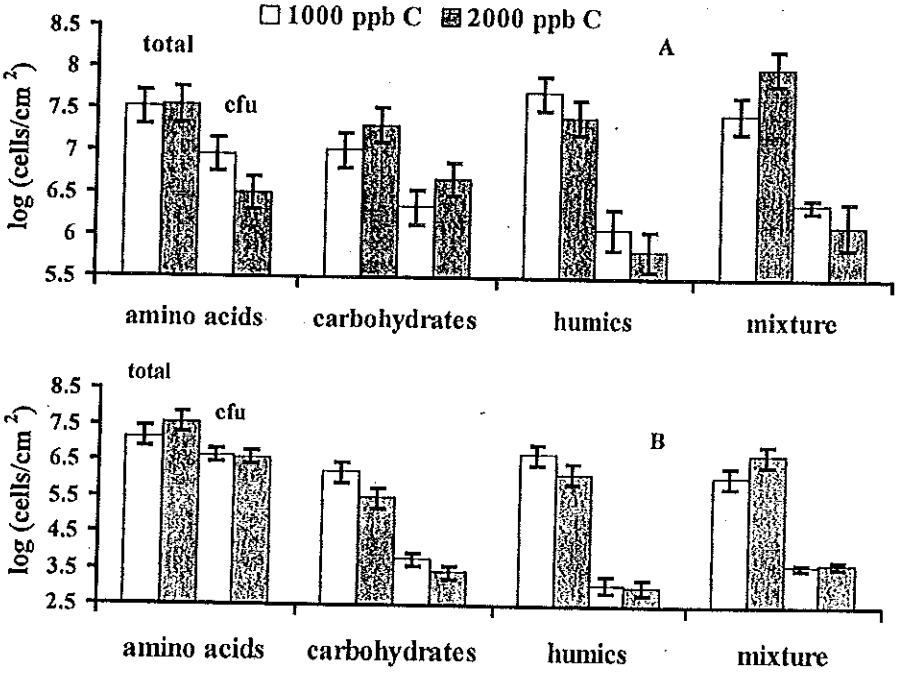

Fig. 4. Total and culturable cells per square cm in the biofilm for the four substrates and two higher carbon feed levels. Reported values are means and standard deviations resulting from a GLM ANOVA on the separate reactors. Panel A is the control reactor and panel B is the chlorinated reactor. Error bars are standard deviations. Note the different y-axis for panels A and B. See text for further details.

maximum cell density in the supplemented reactors of 1.06 $\times$ 10^8 total cells/cm^2 (4.36 $\times$ 10^7–2.57 $\times$ 10^8) in the mixture experiment at 2,000 ppb C and 9.12 $\times$ 10^6 cfu/cm^2 (6.8 $\times$ 10^6–1.22 $\times$ 10^7) in the amino acids experiments at 1,000 ppb C.

Effluent. In addition to the biofilm cell densities shown above, effluent cell measurements were required for the determination of growth rates, yields, and BCP. Figure 5, panels A and B, shows results for effluent culturable and total cells from the replicated experiments, and Table 1 provides data for percent culturable cells and correlations between total cells and culturable cells. There was no significant difference in percent culturable cells between the two higher carbon feed levels in the control reactors. Although culturability tended to decrease as chlorination time proceeded, the decrease was not significant. The substrates and carbon levels within substrates were significantly different for total and culturable cells ($p < 0.002$ for all four) in both the control and chlorinated reactor effluents. Total and culturable cell populations in the control reactor effluents generally increased over the course of the experiment; however, significant increases were only noted for the 1,000 to 2,000 ppb C levels for the amino acids and carbohydrates ($p < 0.002$). Humics did not support a significantly different number of effluent cells at any of the carbon feed levels. Increased carbon in the amino acids chlorinated reactor (from 1,000 to 2,000 ppb C) did not have a significant effect on the total or culturable cells in the effluent, whereas the carbohydrate cell counts decreased upon increased carbon concentration in the presence of chlorine. The humics were the only substrate that produced significantly higher cell counts (two-sample t-test, $p = 0.022$ and $p = 0.0001$ for total and culturable cells, respectively) in the presence of chlorine when the carbon feed was increased between the mid and high levels.

As an indication that the responses we were measuring were due to the added carbon supply and not utilization of organic carbon in the AOC "free" water from the GAC/BAC columns, we compared the influent and effluent cell numbers in the negative control reactor. There was no significant difference in either the total or culturable effluent cell counts in the negative control over the course of 250 days when compared to the influent cell numbers ($p = 0.223$ and 0.237, respectively; $n = 16$). Effluent total cells were 2.90 $\times$ 10^5 cells/ml (95% C I., 2.18 $\times$ 10^5–3.86 $\times$ 10^5) while culturable cells were 1.03 $\times$ 10^5 cfu/ml (6.00 $\times$ 10^4–1.79 $\times$ 10^5).

Carbon Substrate Removal

Carbon removal across the reactors using filtered nonpurgeable organic carbon (NPOC) data was used for determination of the substrate removal rates. Table 2 shows the fraction of substrate removed, regression coefficients, and 95% confidence intervals for the slopes, while Table 3 shows

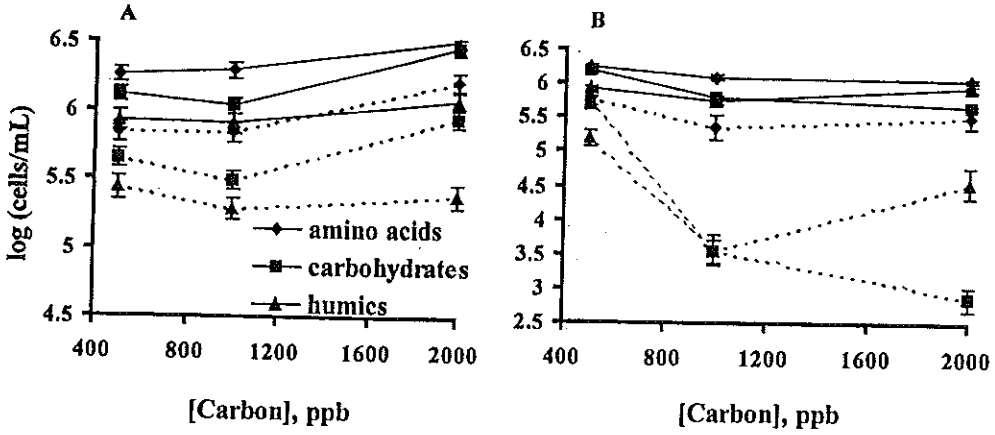

Fig. 5. Total and culturable cells per milliliter in reactor effluents. Panel A is control reactor effluent and Panel B is the chlorinated reactor effluent. Solid lines are total cells mL^{-1} and dashed lines are cfu mL^{-1}. Note the different *y*-axis scales for panels A and B.

the normalized (to biofilm biomass) specific removal rate. When carbon removal flux (μg C used cm^{-2} h^{-1}) was plotted against the influent loading rate (μg C cm^{-2} h^{-1}) a linear relationship was seen in the data where the slope represents the fraction of carbon removed across the reactors. All intercepts were negative (range −0.069 to −0.0024 μg C cm^{-2} h^{-1}), except for the carbohydrate control reactor, where the intercept was 0.0081 μg C cm^{-2} h^{-1}. This indicates that in most cases there was a positive *x*-intercept or a minimum substrate loading rate that would support a steady-state biofilm community.

Since this was a Model II regression, the method of geometric means was used for the regression analysis. The linear relationship noted above did not always occur for the normalized carbon removal rate plotted in the same manner. Using a GLM ANOVA, there was a significant difference in normalized carbon removal rates between substrate groups ($p = 0.005$) and between carbon levels within substrate groups ($p < 0.0005$) for the control reactor. In the presence of chlorine, specific removal rates were not significantly different between substrates ($p = 0.123$) but were significantly different between carbon levels within substrates ($p < 0.0005$). Removal rates were only calculated for the chlorinated reactor after chlorination had been initiated.

Growth Rates, Yields, and Bacterial Carbon Production

Data collected during operation of the annular reactors was used to obtain the information necessary to determine specific growth rates (SGR), substrate flux, observed yield, and bacterial carbon production (BCP) for the biofilm organisms. These results provided in Table 4. In general yield, BCP, and SGRs were negatively affected by chlorination, with the exception being the mixture as carbon source. In the unchlorinated control reactors, yield was fairly steady but tended to decrease with increasing carbon concentration, while BCP increased significantly for all substrates except the humics. All three parameters measured in the negative control reactor were significantly lower than the values from the supplemented reactors. The overall response for the data from all substrate groups (combined data) was first analyzed to determine general trends. Control reactors were compared at 500, 1,000, and 2,000 ppb C while the chlorinated reactors were compared at 1,000 and 2,000 ppb C. There were significant differences for all three parameters between substrate groups ($p < 0.0005$) and carbon levels within substrate groups ($p < 0.0005$). The only exceptions were the chlorinated reactor SGRs, where the carbon levels within the substrates were not significantly different ($p =$

Table 2. Linear regression data for carbon flux (μg carbon used cm^{-2} h^{-1}) as a function of influent substrate loading rate (μg carbon cm^{-2} h^{-1})

Substrate	Reactor	Slope (n_1, n_2)	r^2	95% C.I.
Amino Acids	Control	0.98 (2, 15)	0.992	0.89–1.07
	Chlorinated	1.02 (2, 15)	0.981	0.91–1.14
Carbohydrates	Control	0.90 (2, 11)	0.980	0.80–1.0
	Chlorinated	0.08 (2, 11)	0.932	0.008–0.14
Humics	Control	0.78 (2, 10)	0.952	0.63–0.92
	Chlorinated	0.58 (2, 10)	0.818	0.37–0.79
Mixture	Control	0.64 (1, 6)	0.991	0.54–0.74
	Chlorinated	0.50 (1, 6)	0.993	0.43–0.57
Control	Control	0.05 (1, 7)	0.904	0.03–0.07

The slope represents the fraction of the influent carbon loading removed across the reactors in the 2.1-h detention time of reactor operation. n^1 is the number of experiments for which TOC data was obtained and n^2 is the number of data points used in the regression. Model II regressions were performed by the method of geometric means. 95% confidence interval is for the slope of the regression. Results for the mean normalized specific carbon removal rates are reported at the level of substrate and reactor only.

Table 3. Mean substrate specific removal rate normalized to biofilm bomass

Substrate	Reactor	Mean normal carbon removal rate (h^{-1})	95% C.I. for mean
Amino acids	Control	0.080	0.075–0.083
	Chlorinated	0.234	0.204–0.269
Carbohydrates	Control	0.129	0.121–0.137
	Chlorinated	0.243	0.198–0.299
Humics	Control	0.118	0.110–0.127
	Chlorinated	0.200	0.165–0.243
Mixture	Control	0.078	0.042–0.146
	Chlorinated	0.538	0.341–0.849
Control	Control	0.048	0.029–0.081

Values are backtransformed from the log transformed data. Analysis was performed separately on the chlorinated and control reactors. The substrate control was analyzed separately. Data used for the chlorinated reactor was two levels, 1,000 and 2,000 ppb C, after chlorination was initiated.

0.409). A one-way ANOVA indicated there was a significant difference between reactor biofilm growth rates within substrate groups ($p < 0.0005$) and that there was no significant difference between amino acids and carbohydrates. However, there was a significant difference between SGRs on the humics and the other two substrates (Tukey's pairwise comparisons). In general, SGRs for the humics reactors were less than those from the carbohydrates and amino acids reactors. SGRs in the presence of chlorine were significantly different ($p = 0.006$) and greater than those in the absence of chlorine. There was a significant difference between carbon levels ($p < 0.0005$), with the low level significantly different from the mid and high levels (Tukey's pairwise comparisons) but no significant difference between the mid and high level. Reactor biofilm SGRs at the 500 ppb C level were greater than both the 1,000 and 2,000 levels (the 500 level was) always prior to the commencement of chlorination).

Discussion

In these experiments, we have focused on the role of surface associated biofilm communities in the turnover of the most commonly detected carbon substrates in aquatic systems [18, 23, 27, 46, 67]. While most studies have focused on planktonic organisms, a few exceptions have attempted to utilize biofilm bacteria in order to determine kinetic constants or substrate utilization profiles [14, 28, 30, 39, 67]. These studies have utilized BDOC columns or BAC filters, both of which were monitored in these experiments. Drinking water has been the focus of most of these studies involved with bacterial biofilms because of the potential for detrimental growth within the distribution system as well as the positive aspects of biofilm formation in BAC filters. Regrowth within the distribution system is a cause of concern due to the potential health hazards, deterioration of the water's aesthetic qualities (e.g. taste, odor, and color), and the persistence of indicator bacteria (e.g., coliforms) within the biofilm. Conversely, biofilm growth in BAC filters is encouraged for a number of reasons, including (1) decreased chlorine demand in finished waters, (2) decreased regrowth potential due to BDOC removal, and (3) decreased disinfection by-product formation. In addition to these three positive aspects, it is advantageous to encourage bacterial growth at a point where some control can be applied and not in the distribution system where the only real control is to increase disinfection levels. Chlorine tends to be the disinfectant of choice, especially in North America, and no attempts have been made to measure bacterial growth-related parameters in its presence.

Control Stage Carbon Level

Analysis of the negative control reactor data for total and culturable cells as well as DOC removal showed that the AOC "free" dilution water was in fact relatively free of utilizable carbon. Thus, the GAC/BAC filter was very effective in removing utilizable carbon from the finished drinking water and the responses measured in the supplemented control reactors were due to the added carbon substrates. The negative control reactor had a net removal of carbon not significantly different from zero, while the BDOC columns removed an extra 32 ± 38 μg C L^{-1} over and above what was removed by the GAC/BAC filter. As a flux this was equivalent to 15.2 μg C L^{-1} h^{-1}, or approximately 2% of the removal flux for the GAC/BAC filters (average was 760 μg C L^{-1} h^{-1}). As a percentage of the excess substrate supplied to the reactors this amounted to 0–14% at the lower carbon feed level (500 ppb C). GAC/BAC filtration removed an average of 18 ± 4.7% of the influent TOC, typical for percentage removals in raw freshwater and marine systems [2, 28, 60, 67]. GAC/BAC filtration is usually performed in the treatment train, and not on the finished, distributed water as was the case for this study. This likely indicates the formation of a bacterial community on the filters that is uniquely suited to the degradation of the organics in the source water, which is not surprising when one considers that this filter has been operating for approximately 7 years.

Based on the results from the control level of the experi-

Table 4. Specific growth rates, bacterial carbon production, and observed yield according to substrate type, carbon concentration, and control versus chlorinated biofilm communities

Substrate	Carbon (ppb)	SGR[a]		BCP[b]		Yield[c]	
		Control	Cl_2	Control	Cl_2	Control	Cl_2
Negative control	n/a	0.0034 (0.0003, 0.0066)	n/a	0.00084 (0.0019, −0.0002)	n/a	0.0032 (0.0085, −0.002)	n/a
Amino acids	500	0.019 (0.017, 0.022)	0.066 (0.060, 0.073)	0.0093 (0.009, 0.010)	0.0092 (0.0086, 0.0099)	0.12 (0.11, 0.14)	0.15 (0.14, 0.16)
	1,000	0.008 (0.007, 0.009)	0.008 (0.007, 0.010)	0.013 (0.012, 0.014)	0.0053 (0.0044, 0.0065)	0.08 (0.07, 0.09)	0.041** (0.037, 0.045)
	2,000	0.017 (0.015, 0.020)	0.0035 (0.0028, 0.0042)	0.023** (0.021, 0.025)	0.005 (0.004, 0.006)	0.084 (0.077, 0.092)	0.020 (0.18, 0.022)
CHO[d]	500	0.042** (0.036, 0.048)	0.035 (0 032, 0.039)	0.0051 (0.0048, 0.0056)	0.0065 (0.0061, 0.0070)	0.089** (0.082, 0.097)	0.121 (0.114, 0.128)
	1,000	0.012 (0.010, 0.014)	0.031 (0.025, 0.037)	0.0043 (0.0040, 0.0047)	0.002 (0.0016, 0.0024)	0.038 (0.035, 0.042)	0.025 (0.023, 0.028)
	2,000	0.018 (0.016, 0.021)	0.039 (0.032, 0.047)	0.0117** (0.011, 0.013)	0.00004 (0.00003, 0.00005)	0.046 (0.042, 0.050)	0.019 (0.017, 0.021)
Humics	500	0.063** (0.050, 0.079)	0.066 (0.057, 0.077)	0.0031 (0.0027, 0.0035)	0.0028 (0.0025, 0;0031)	0.045 (0.040, 0.0052)	0.069 (0.062, 0.075)
	1,000	0.0016 (0.0013, 0.0021)	0.009 (0.007, 0.012)	0.0037 (0.0033, 0.0042)	0.0016** (0.0011, 0.0022)	0.034 (0.029, 0.038)	0.042 (0.036, 0.048)
	2,000	0.0027 (0.0022, 0.0034)	0.01 (0.007, 0.013)	0.0049 (0.0044, 0.0056)	0.005 (0.004, 0.007)	0.035 (0.030, 0.040)	0.036 (0.031, 0.041)
Mixture	250	0.04 (0.024, 0.067)	0.014 (0.008, 0.025)	0.0067 (0.005, 0.009)	0.0004 (0.0001, 0.0018)	0.25 (0.18, 0.34)	0.0 (0.0, 0.0)
	1,000	0.022 (0.013, 0.037)	0.03 (0.015, 0.061)	0.014 (0.010, 0.018)	0.0017** (0.0008, 0.0034)	0.14 (0.11, 0.20)	0.044* (0.031, 0.062)
	2,000	0.005** (0.003, 0.008)	0.042 (0.020, 0.0085)	0.025** (0.019, 0.034)	0.014 (0.007, 0.029)	0.13 (0.09, 0.17)	0.11 (0.079, 0.16)

n/a, not applicable.

[a] specific growth rate (SGR, h^{-1}).

[b] bacterial carbon production (BCP, μg C/cm^{-2} h^{-1}).

[c] observed yield (μg cell C/μg C).

[d] CHO = carbohydrates.

95% confidence intervals are in brackets. Values are backtransformed means from the log transformed data. *n*-values are 15, 15, 10, and 5 for the amino acids, carbohydrates, humics, and mixture, respectively.

* $p = 0.042$ and refers to the significant difference between the chlorinated carbon feed levels of 1,000 and 2,000 ppb, carbon, all other values are not significantly different.

** $p < 0.005$ and refers to a significant difference between the control carbon feed levels of 500, 1,000, and 2,000 ppb carbon, ** indicates where the difference arises according to results from a one-way ANOVA (Tukey's pairwise comparisons). Stars indicate which level contributes to the significant difference.

ments, the substrates that provided the most favorable growth conditions were the amino acids, followed by the carbohydrates and humics. Carbohydrates supported a total effluent population of cells that was 80% of the amino acids, whereas humics maintained a population that was 47% that of the amino acids. The same trend was seen for the culturable cells, where the carbohydrates and humics supported 75% and 32% of the amino acids cell density. Biofilm total cell density is similar, with the carbohydrates and humics supporting 75% and 15% of the amino acid population, respectively. The only parameter that did not follow this trend was the culturable biofilm cells from the carbohydrates experiments. In this case a 27% increase in culturable cells were seen in the carbohydrate biofilms compared to the amino acid biofilms (humics support 5% of the amino acid cell density).

Test Stage Carbon Levels

Once chlorination began, the differences in the substrates were magnified to a larger degree than for the control stage of the experiments. Again, overall, the amino acids were the best substrate for the control reactors. The amino acids produced higher culturable cell counts than the amount of total cells arising from the humic experiments. All of the control reactors exhibited an increase in effluent total cell counts from the lower to higher carbon feed levels, with only the humic's increase being insignificant. The chlorinated communities followed a different trend, with total cells equivalent across carbon levels for the amino acids and carbohydrates, while the significant decrease seen in the carbohydrate culturable cells was not observed in the amino acid experiments. The humics were the only substrate that showed a significant increase in effluent total cells and culturable cells after chlorination was initiated (Fig. 5).

Total and culturable cells were significantly correlated in all cases, but the correlation was particularly weak for both the influent cells and the humic effluent cells. Culturability (i.e., cfu/TDC) was significantly impacted in the presence of chlorine for all of the substrates tested, especially in the presence of humics and carbohydrates. This implies that an attempt to use culturable cells as an indicator of activity in the reactors would produce nonsensical results with respect to yield, BCP, and SGRs. The range of culturability (5–50%) is considerably above the range of literature values in natural environments (0.01–10%). This could be due to the (presumed) lower community diversity in drinking water systems as compared to most other environments. Drinking water bacterial isolates tend to be Proteobacteria and consist of a number of organisms, most of which show the ability to grow on agar plates under laboratory conditions [7, 25, 37, 40, 55, 70].

The biofilm sampling data does not show the same trend as that seen for the effluent data. All substrates produced significantly higher counts in control biofilm culturable cells when going from low to high level carbon concentrations. As a fraction of the total biofilm cells, culturability decreased at the high carbon levels to 25–50% of the culturability seen at the low carbon level. Total cell counts indicated that there was an overall increase in the number of cells with increasing carbon concentration for all substrates. At the middle carbon level the humics were significantly higher than the carbohydrates and amino acids for total biofilm cells (control), whereas at the higher carbon level the humics were intermediate to the amino acids (high) and carbohydrates (low). For the chlorinated reactor, amino acids were the better substrate for biofilm cells at both carbon levels while the humics were significantly higher than the carbohydrates. For the culturable biofilm (and effluent) cells, amino acids seemed to provide some protection from the effects of chlorination since there was no difference between the number of cells that grew on R2A agar between the chlorinated and control reactors at both of the chlorinated carbon levels. This is also apparent when considering the percentage of biofilm (and effluent) culturable cells with respect to total biofilm (and effluent) cells where the amino acids show 10% (21%) culturability while the humics and carbohydrates showed 0.9% (1.5%) and 0.07% (5.4%) at the highest carbon level. Overall, the only difference in culturable biofilm cells for the control reactors was between the mid-level amino acids and high-level humics, again, an indication of the poor relationship between culturable cells and potential activity in the biofilms.

Whereas the amino acids and carbohydrates were almost totally removed in the control reactors (98 and 90%, respectively), humics removal amounted to 78% of the influent feed. This value is 2–10× published literature values, which range from 1 to 45% [1, 59, 60, 61, 62, 67]. Although most of these studies have been performed in batch planktonic cultures, the values closest to ours, from Volk et al. [67] (25%) and Merlet et al. [39] (45%) have used methods that entail the utilization of biofilm bacteria. This compares to the steady-state biofilm removal of 78% for the control and 58% for the chlorinated biofilms over the course of 2 h as measured in the reactors.

It is not surprising that amino acids provided for the highest population densities in the biofilms. Selection of the amino acids for this study was based on both environmental availability and relationship to central metabolism. Energetically, amino acids provide an easily utilizable carbon, energy, and nitrogen source, whereas carbohydrates only satisfy carbon and energy requirements. In this case the communities are forced to assimilate nitrate as the nitrogen source. The need to derepress nitrate assimilatory pathways, as well as the relative energetic cost required to reduce nitrate to ammonia, is high compared to the assimilation of glutamate in the amino acids mixture. Conversely, the carbohydrate communities would be expected to expend a significant amount of energy in both the assimilation of nitrate and the anabolic reactions required for the synthesis of all precursor metabo-

lites such as amino acids and nucleotides. The humics were a highly heterogeneous source of carbon, nitrogen, and trace elements. Trace-level concentrations of a variety of amino acids and carbohydrates bound within the polyaromatic backbone of the humic structure would be expected to provide a relatively diverse nutritional supply contingent on the ability of the communities to gain access. In this regard, the function of the biofilm could aid to a considerable degree. Chlorination provided for some level of humic oxidation, likely leading to a consequent release of nutrients, while the biofilm itself certainly confers an increase in degradative capacity [13, 36, 68, 69]. A variety of explanations are plausible for this increased degradative capability. The increased surface area to volume ratio in the biofilm provides for a larger reactive surface area. Through forming biofilms, cells can alter their local environment to allow for more favorable growth conditions and potential interactions between species. Sequestration of the humic material within the biofilm could result in enhanced degradative capacity, such that the organisms are capable of utilizing a larger fraction of the influent material.

However the increased degradative capability arises, the consequence is that the BDOC fraction in water is potentially much higher than previously thought. Utilization of the high molecular weight fraction has been shown for marine, streamwater, and drinking water bacteria [1, 2, 12, 32, 67]. This study and others have shown that biofilm communities have a much higher capacity to extract energy and carbon from recalcitrant sources. Whether one is interested in the regrowth potential (AOC) of drinking water or the removal of chlorine demand and the potential formation of disinfection by-products (BDOC), utilization of suspended planktonic organisms to derive results for systems where most of the activity occurs on surfaces does not provide realistic results.

Growth Rates, Production, and Yield

Most investigators would accept that treated drinking water is an oligotrophic aquatic environment. Operationally, however, oligotrophic systems have generally been accepted to be those in which the rate of food supply is very low and when carbon is limiting, in the range of fractions of milligrams C L^{-1} day^{-1} [3, 53]. From this viewpoint the dilution water entered the reactors at a maximum carbon loading rate of 1.0 mg C L^{-1} day^{-1}, whereas the reactors themselves have carbon loading rates of approximately 6, 12, and 24 mg C L^{-1} day^{-1}. These values are comparable to the mean BDOC loading rate entering (i.e., finished drinking water) the GAC/BAC filters, which was 18 mg C L^{-1} day^{-1}. This high carbon loading rate entering the GAC/BAC filters is due to the very low residence time (15 min) and by the definition given above it would not be oligotrophic. Given the values above, the supplemented reactors were not receiving an amount of carbon that was excessively above what was in the drinking water itself. The quality of the substrate was better in the supplemented amino acids and carbohydrates experiments but likely of similar quality for the humics and mixture experiments. Since equilibrium within the reactors was indicated by the previously mentioned measured parameters, reactor operation was at pseudo steady-state prior to increasing the carbon concentrations.

The concept of *r*- and *K*-selection theory in relation to microbial ecology can explain several of the findings with respect to SGRs, production, and observed yields. Andrews and Harris [3] introduced the concept of *r*- and *K*-selection theory to microbial ecology in 1986. Within the context of *r*/*K* selection theory, population density is of paramount importance with respect to the environmental niche that an organism occupies. Maximization of survival fitness occurs in response to either uncrowded (*r*-selection) or crowded (*K*-selection) environments. *K* refers to the density of individuals that a given environment can support while *r* refers to the maximum specific rate of increase of an organism. Thus, either the organism or the environment can be defined in terms of *r*/*K* selection theory. *r*-environments tend to be transitory and arise because of increased food supply and/or a severe reduction in the population density. A *K*-environment exists when the population is in equilibrium with its surroundings such that specific rates of increase are close to zero and food supply is maximally utilized.

Initiation of our experiments resulted in an *r*-environment situation because of the conditions in the reactors. The microorganisms, which colonize the annular reactors, originated from the oligotrophic and uncrowded environment (mid 10^5 total cells mL^{-1}) of the GAC/BAC filters. As soon as they entered the reactors, they were faced with a relative surfeit of carbon and colonized the reactors at a low population density. Substrate concentrations were relatively low and in the range of 2.1–3.5 μM (amino acids), 1.7–2.1 μM (carbohydrates), and approximately 25 μM C (humics). These concentrations were insufficient to support high cell densities, and low cell densities tend to allow for higher growth rates given a constant supply of food. Once steady-

state was reached, cell densities were still relatively low at the lowest feed concentration as compared to the higher cell densities found in the reactors at the higher feed concentrations (see Figs. 3, 4, and 5). This paradigm of population density, crowding, and rate of food supply can be used to explain the trend in growth rates seen in our experiments, as described below.

Control Communities. Specific growth rates (SGRs) for all substrates were significantly higher at the low carbon feed level as compared to the higher feed levels. The correlation between biofilm biomass and SGR was significantly negative for both the control and chlorinated communities (r = -0.637, p = 0.001 and r = -0.570, p = 0.021, respectively), while there was no correlation between SGR and substrate concentration for either of the communities. In addition to *r/K*-selection theory predicting this consequence, a number of other investigators have found the same correlation for planktonic bacteria, whereby higher standing stocks of biomass are significantly correlated with a lower SGR [3, 43, 44, 48]. SGRs on carbohydrates and amino acids were equivalent at the higher carbon feed levels and both were significantly higher than for the humic grown communities. Although the higher substrate concentrations support higher cell densities, SGR could be limited because of the lower per capita food supply. Table 3 shows that utilization rates normalized to biofilm biomass are 1 7–7.0× lower for the control reactors as compared to the chlorinated reactors. This indicated that the amount of substrate per unit biomass was significantly lower in the control, compared to the chlorinated communities. Biofilm cell density increased by approximately an order of magnitude or more for the control reactors while carbon supply was only doubled. The control reactors removed most of the amino acids and carbohydrates and a significant portion of the humics in order to support higher population densities growing at lower rates. Doubling times at the highest carbon feed level for the control reactors were 8.3 days for the mixture, 2.3 days for the carbohydrates, 15.4 days for the humics, and 2.5 days for the amino acids. As a comparison, the negative control reactor had a doubling time of 12.3 days, which is close to the value obtained by Van Der Wende et al. [65] (16.7 days) using annular reactors in unamended drinking water.

Yield decreased in the order amino acids (0.08–0.12 μg cell C/μg C) > carbohydrates (0.038–0.089) > humics (0.034–0.045) with the highest yield values obtained at the lowest feed concentration. Decreased per capita food supply in conjunction with high cell density could have led to increased maintenance requirements and a concomitant decrease in the efficiency with which the individual organisms converted substrate to biomass. Higher biomass yield due to utilization of small molecules has been shown by Amon and Benner [2] and Schweitzer and Simon [54]. Although the yield measured in our experiments tended to be at the low end, they are in the range of values reported in the literature [6, 18, 62]. Low cell yields would be preferred in biological filters, since the organisms arising from the filter would have to be removed prior to releasing the water into the distribution system. Therefore, encouraging high biomass in a biological filter would tend to decrease cell yield because of the rather inefficient conversion of substrate to biomass (however, see BCP below). The mixture provided for the highest yield, likely due to the diversity of nutrients present, but again the highest yield (range, 0.13–0.25) was at the lowest carbon feed level. Yield was significantly positively correlated with BCP (r = 0.555, p = 0.005) with respect to all of the substrates in the control reactors.

BCP in the control communities tended to increase as carbon concentration increased, although not significantly in the case of the humics. A number of studies on BCP rates on epiphytic surfaces are within the range of our BCP estimates when normalized to surface area. Thomaz and Wetzel [58] measured rates in the range of 0.93–1.85 μg C cm^{-2} day^{-1} (at 20°C) on the surface of the aquatic macrophyte Juncus effusus detritus by ^{3}H-leucine incorporation rates and showed that BCP increased with increasing biomass whereas growth rate decreased. They also estimated turnover times of 4.8–15.2 days for their samples, very similar to the turnover times measured in the annular reactors.

Although the SGR and yields decreased as carbon concentration increased in our experiments, increased BCP was likely due to the higher biomass density present at the higher feed concentrations. Since the growth rates were independent of the external substrate concentration, the biofilm communities were not growing according to the conventional Monod kinetic model used for free-living bacteria. These results are similar to those found by Moller et al. [42], who used a direct microscopic image analysis technique to determine that growth rates of attached Pseudomonas putida in chemostat studies were independent of the dilution rate. This has implications for the modeling of bacterial growth in distribution systems. Most of the models assume that growth is first order (i.e., Monod kinetics) in the biofilm, whereas this study indicates that this is not correct.

This also implies that knowledge of the growth rates of the organisms in a distribution system or biological filter is unpredictable and requires direct measurements as were done in this study.

Chlorinated Communities. SGRs were greater under conditions of chlorination than those measured in the control reactors at the same carbon feed level, except for the amino acids, which were lower. The correlation between biomass and SGR was significant and negative (see above). Although the SGRs for reactors within any one substrate were significantly different, for SGR the carbon levels within the reactors were not significantly different. The amino-acids-grown chlorinated biofilm communities were seemingly unaffected by the presence of a free chlorine residual, biofilm biomass was not significantly affected by chlorination, and substrate utilization was the same as for the control reactors. As such, one would expect the growth rates to be equivalent for the chlorinated and control communities, but the SGR in the chlorinated reactor was significantly lower than that in the control reactor. This is likely due to the small but significant impact of chlorination on the effluent cells. The effluent cell concentration in the chlorinated amino acid communities was 63% and 35% of the control effluent population at the mid and high carbon feed level, respectively. SGRs were equivalent at the mid-level feed concentration, but the amino acid chlorinated reactor SGR was 20% of the control reactor at the high carbon feed level. Another possible explanation is an interaction between the dual stress provided by nutrient limitation and chlorination. Biofilm and effluent cell concentrations for the carbohydrates and humics are significantly affected by chlorination (Figs. 4 and 5) and substrate is relatively more plentiful (see Tables 2 and 3), with the consequent result that SGRs are higher (Table 4). The carbohydrate-fed reactors, with the highest concentration of substrate in the effluent as well as the lowest biomass in both the effluent and biofilm, showed the highest SGRs under chlorination, with the exception of the mixture reactor at the highest carbon feed level. Doubling times at the highest carbon feed level for the chlorinated reactors were 1 day for the mixture, 1.1 days for the carbohydrates, 4.3 days for the humics, and 12.1 days for the amino acids.

Observed yields in the chlorinated communities were negatively correlated with SGR (r = -0.525, p = 0.037) and positively correlated with biomass (r = 0.566, p = 0.022). No correlations were detected for the control reactors. Thus, as SGR increased and biomass decreased, the communities became progressively less efficient at converting substrate into increased amounts of biomass. This could be due to the injury caused by the oxidative stress involved with chlorination and the need to increase the amount of substrate used for maintenance vs growth. The trend seen for the control reactors, where yield decreased as carbon concentration increased, was also seen for the chlorinated reactors. Cell yield for the humics was equivalent in the chlorinated reactor vs the control reactor at the high carbon feed level. Given that both of the humic-fed populations were stressed (one by chlorination and the other by nutrient limitation), maintenance energy requirements would have a larger effect on the more slowly growing communities, potentially resulting in a lower yield in the control communities as compared to the chlorine-stressed organisms [52]. Increased yield could also be due to an interaction between chlorine and humics, whereby humic oxidation results in a subsequent release of more readily utilizable substrates and a change in the character of the humic material. This phenomenon has been shown to occur when water is ozonated or chlorinated [16, 19, 21, 22]. A highly significant and positive correlation between yield and BCP was noted for the chlorinated reactors (r = 0.899, $p < 0.0005$).

BCP in the chlorinated reactors was also correlated with biomass (r = 0.619, p = 0.011). BCP was 366 and 347 μg C L^{-1} day^{-1} for amino acids, 136 and 3 for the carbohydrates, 110 and 326 for the humics, and 113 and 969 μg C L^{-1} day^{-1} for the mixture at the two chlorinated carbon feed levels. The high SGR in the chlorinated carbohydrate community was insufficient to offset the low biomass and yield such that BCP was the lowest measured in these experiments. The next lowest BCP value of 58 μg C L^{-1} day^{-1} was measured in the negative control reactor. This indicates that biological filtration is as effective as chlorination in controlling bacterial growth and production. BCP in the humics-grown (chlorinated) communities was low at the 1000 ppb C feed level and increased to a value not significantly different from the control reactor BCP at the 2,000 ppb C level. This could be attributed to the higher SGRs in the chlorinated humics (3.6× the control) communities since the yields were similar (0.036-chlorinated and 0.035-control) but biomass was lower in the chlorinated system. The mixture was the only substrate that showed a progressive increase in BCP for both the control and chlorinated reactors. A combination of high yield (0.13 μg cell C/μg C), high biofilm biomass (4.95×10^6 cells cm^{-2}), and high growth rate (doubling time = 1 day) in the chlorinated reactor was insufficient to offset the higher

biomass (1.11×10^8 cells cm^{-2}), equivalent yield (0.11 μg cell C/μg C), and low growth rate (doubling time 8.3 days) of the control reactor. BCP under chlorination with the mixture was 56% of that measured in the control reactor.

Replication between (ca. 10%) and within (<5%) experiments in the annular reactors was reproducible when sufficient time for equilibrium was allowed between carbon feed levels. Substrate preference in the control communities was amino acids > carbohydrates > humics, when considering only the cell densities in the effluent and biofilm. Specific growth rates and observed yields decreased with increasing biofilm biomass. Accordingly, the highest growth rates and yields were observed at the lowest feed concentrations for all substrates tested. This implies that biofilm growth rates were independent of bulk fluid substrate concentration (zero-order kinetics). With respect to cell densities, free chlorine residuals in the range of 0.15–0.20 mg chlorine L^{-1} had a relatively insignificant impact in the presence of amino acids, a significant negative impact in the presence of carbohydrates, and a relatively positive impact in the presence of humic substances. Humic utilization in chlorinated and control biofilm communities was significantly higher than previously reported values. Chlorination had a positive impact on the specific growth rate when compared to control communities at the same carbon feed level. Bacterial carbon production rates were equivalent to or exceeded the highest values previously reported for natural environments. Considering SGRs, BCP, and yield, biological filtration of the drinking water was as effective as (or better than) chlorination in controlling growth within the annular reactors.

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References

1. Amon RMW, Benner R (1994) Rapid cycling of high-molecular-weight dissolved organic matter in the ocean. Nature 369:549–551
2. Amon RMW, Benner R (1996) Bacterial utilization of different size classes of dissolved organic matter. Limnol Oceanogr 41:41–51
3. Andrews JH, Harris RF (1986) *r*-and *K*-selection and microbial ecology. Marshall KC, (ed) Advances in Microbial Ecology, Vol 9. Plenum Press, New York, pp 99–147
4. Bjornsen PK (1986) Automatic determination of bacterioplankton biomass by image analysis. Appl Environ Microbiol 51:1199–1204
5. Bryers JD (1987) Biologically active surfaces: processes governing the formation and persistence of biofilms. Biotech Prog 3:57–68
6. Button DK (1985) Kinetics of nutrient-limited transport and microbial growth. Microbiol Rev 49:270–297
7. Camper AK, LeChevallier MW, Broadaway SC, McFeters GA (1986) Bacteria associated with granular activated carbon particles in drinking water. Appl Environ Microbiol 52:434–438
8. Chrzanowski TH, Sterner RW, Elser JJ (1995) Nutrient enrichment and nutrient regeneration stimulate bacterioplankton growth. Microb Ecol 29:221–230
9. Coveney MF, Wetzel RG (1992) Effects of nutrients on specific growth rate of bacterioplankton in oligotrophic lake water cultures. Appl Environ Microbiol 58:150–156
10. Currie DJ (1990) Large-scale variability and interactions among phytoplankton, bacterioplankton, and phosphorus. Limnol Oceanogr 35:1437–1455
11. Dunn OJ, Clark VA (1974) Applied Statistics: Analysis of Variance and Regression John Wiley and Sons, New York
12. Freeman C, Lock MA (1992) Recalcitrant high-molecular-weight material, an inhibitor of microbial metabolism in river biofilms. Appl Environ Microbiol 58:2030–2033
13. Freeman C, Lock MA (1995) The biofilm polysaccharide matrix: A buffer against changing organic carbon supply? Limnol Oceanogr 40:273–278
14. Frias J, Ribas F, Lucena F (1994) Substrate affinity from bacterial strains and distribution water biofilms. J Appl Microbiol 76:182–189
15. Glaze WH, Andelman JB, Bull RJ, Conolly RB, Hertz CD, Hood RD, Pegram RA (1993) Determining health risks associated with disinfectants and disinfection by-products: research needs. J AWWA 85:53–56
16. Goel S, Hozalski RM, Bouwer EJ (1995) Biodegradation of NOM: Effect of NOM source and ozone dose. J AWWA 87:90–105
17. Gremm TJ, Kaplan LA (1997) Dissolved carbohydrates in streamwater determined by HPLC and pulsed amperometric detection. Limnol Oceanogr 42:385–393
18. Hanisch K, Schweitzer B, Simon M (1996) Use of dissolved carbohydrates by planktonic bacteria in a mesotrophic lake. Microb Ecol 31:41–55
19. Hanna JV, Johnson WD, Quezda RA, Wilson MA, Xiao-Qiao

L (1991) Characterization of aqueous humic substances before and after chlorination. Environ Sci Technol 25:1160–1164
20. Huck PM (1990) Measurement of biodegradable organic matter and bacterial growth potential in drinking water. J AWWA 82:78–86
21. Huck PM, Fedorak PM, Anderson WB (1991) Formation and removal of assimilable organic carbon during biological treatment. J AWWA 83:69–80
22. Huck PM, Zhang S, Price ML (1994) BOM removal during biological treatment: a first-order model. J AWWA 86:61–71
23. Jorgensen NOG, Jensen RE (1994) Microbial fluxes of free monosaccharides and total carbohydrates in freshwater determined by pad-hplc. FEMS Microbiol Ecol 14:79–94
24. Jorgensen NOG, Tranvik L, Edling H, Graneli W, Lindell M (1998) Effects of sunlight on occurrence and bacterial turnover of specific carbon and nitrogen compounds in lake water. FEMS Microbiol Ecol 25:217–227
25. Kalmbach S, Manz W, Szewzyk U (1997) Dynamics of biofilm formation in drinking water: Phylogenetic affiliation and metabolic potential of single cells assessed by formazan reduction and in situ hybridization. FEMS Microbiol Ecol 22:265–279
26. Kaplan LA, Bott TL (1990) Nutrients for bacterial growth in drinking water: Bioassay evaluation PB 89-213 995/AS, 1–7. Springfield, VA, National Technical Information Service
27. Kaplan LA, Newbold JD (1993) Biogeochemistry of dissolved organic carbon entering streams. Ford TE (ed) Aquatic Microbiology, an Ecological Approach. Blackwell Scientific Publications. pp 139–165
28. Kaplan LA, Newbold JD (1995) Measurement of streamwater biodegradable dissolved organic carbon with a plug-flow bioreactor. Wat Res 29:2696–2706
29. Kjell AH (1993) Total and specific bacterial counts by simultaneous staining with DAPI and fluorochrom-labeled antibodies. Kemp PF, Sherr BF, Sherr EB (eds) Handbook of Methods in Aquatic Microbial Ecology. Lewis Publishers, Boca Raton, FL, pp
30. Kott Y, Ribas F, Lucena F (1997) Comparison between the evaluation of bacterial regrowth capability in a turbidimeter and biodegradable dissolved organic carbon bioreactor measurements in water. Comparison between the evaluation of bacterial regrowth capability in a turbidimeter and biodegradable dissolved organic carbon bioreactor measurements in water. J Appl Microbiol 83:347–352
31. LeChevallier MW, Babcock TM, Lee RG (1987) Examination and characterization of distribution system biofilms. Appl Environ Microbiol 53:2714–2724
32. LeChevallier MW, Becker WC, Schorr P, Lee RG (1992) Evaluating the performance of biologically active rapid filters. JAWWA 84:136–146
33. LeChevallier MW, Schulz W, Lee RG (1991) Bacterial nutrients in drinking water. Appl Environ Microbiol 57:857–862
34. LeChevallier MW, Shaw NE, Kaplan LA, Bott TL (1993) Development of a rapid assimilable organic carbon method for water. Appl Environ Microbiol 59:1526–1531
35. Lucena F, Frias J, Ribas F (1990) A new dynamic approach to the determination of biodegradable dissolved organic carbon in water. Environ Tech 12:343–347
36. Manem JA, Rittmann BE (1992) Removing trace-level organic pollutants in a biological filter. J AWWA 84:152–157
37. Manz E, Szewzyk U, Ericsson P, Amann R, Schleifer K-H, Stenstrom T-A (1993) In situ identification of bacteria in drinking water and adjoining biofilms by hybridization with 16S and 23S rRNA-directed fluorescent oligonucleotide probes. Appl Environ Microbiol 59:2293–2298
38. McFeters GA (1990) Enumeration, occurrence, and significance of injured indicator bacteria in drinking water. McFeters GA (ed) Drinking Water Microbiology. Springer-Verlag, New York, pp 478–492
39. Merlet N, Merlet Y, Prèvost M, Desjardins R, Bablon G (1991) Removal of organic matter in BAC filters: The link between BDOC and chlorine demand (WQTC), 1111–1127 Denver, CO, American Water Works Association Research Foundation WQTC Annual Meeting, Orlando, FL
40. Mikell AT Jr, Smith CL, Richardson JC (1996) Evaluation of media and techniques to enumerate heterotrophic microbes from karst and sand aquifer springs. Microb Ecol 31:115–124
41. Minitab Release 12(12.1) (1998) USA, Minitab Inc
42. Moller S, Kristensen CS, Poulsen LK, Carstensen JM, Moli S (1995) Bacterial growth on surfaces: Automated image analysis for quantification of growth rate-related parameters. Appl Environ Microbiol 61:741–748
43. Moriarty DJW, Bell RT (1993) Bacterial growth and starvation in aquatic environments. Kjelleberg S (ed) Starvation in Bacteria. Plenum Press, New York, pp 25–53
44. Morris DP, Lewis Jr, WM (1992) Nutrient limitation of bacterioplankton growth in Lake Dillon, Colorado. Limnol Oceanogr 37:1179–1192
45. Norland S (1993) The relationship between biomass and volume of bacteria. Kemp PF, Sherr BF, Sherr EB, Cole JJ (eds) Handbook of Methods in Aquatic Microbial Ecology. Lewis Publishers, Boca Raton, FL, pp 303–307
46. Paerl HW (1991) Ecophysioiogical and trophic implications of light-stimulated amino acid utilization in marine picoplankton. Appl Environ Microbiol 57:473–479
47. Paerl HW, Pinckney JL (1996) A mini-review of microbial consortia: Their roles in aquatic production and biogeochemical cycling. Microb Ecol 31:225–247
48. Pollard PC, Greenfield PF (1997) Measuring in situ bacterial specific growth rates and population dynamics in wastewater. Wat Res 31:1074–1082
49. Putnam SW, Graham JD (1993) Chemicals versus microbials in drinking water: a decision sciences perspective. J AWWA 85:57–61
50. Reasoner DJ, Geldreich EE (1985) A new medium for the enumeration and subculture of bacteria from potable water. Appl Environ Microbiol 49:1–7

51. Ribas F, Frias J, Ventura F, Mohedano L, Lucena F (1995) Assessment of biological activity and fate of organic compounds in a reactor for the measurement of biodegradable organic carbon in water. J Appl Bact 79:558–568
52. Russell JB, Cook GM (1995) Energetics of bacterial growth: Balance of anabolic and catabolic reactions. Microbiol Rev 59:48–62
53. Schut F, Prins RA, Gottschal JC (1997) Oligotrophy and pelagic marine bacteria: facts and fiction. Aquat Microb Ecol 12:177–202
54. Schweitzer B, Simon M (1995) Growth limitation of planktonic bacteria in a large mesotrophic lake. Microb Ecol 30:89–104
55. Shirey JJ, Bissonnette GK (1991) Detection and identification of groundwater bacteria capable of escaping entrapment on 0.45-µm-pore size membrane filters. Appl Environ Microbiol 57: 2251–2254
56. Sobsey MD, Dufour AP, Gerba CP, LeChevallier MW, Payment P (1993) Using a conceptual framework for assessing risks to health from microbes in drinking water. J AWWA 85:44–48
57. Sokal RR, Rohlf FJ (1995) Biometry, the Principles and Practice of Statistics in Biological Research. WH Freeman and Company, New York
58. Thomaz SM, Wetzel RG (1995) [3H]Leucine incorporation methodology to estimate epiphytic bacterial biomass production. Microb Ecol 29: 63–70
59. Tranvik L, Kokalj S (1998) Decreased biodegradability of algal DOC due to interactive effects of UV radiation and humic matter. Aquat Microb Ecol 14:301–307
60. Tranvik LJ (1988) Availability of dissolved organic carbon for planktonic bacteria in oligotrophic lakes of differing humic content. Microb Ecol 16:311–322
61. Tranvik LJ (1993) Microbial transformations of labile dissolved organic matter into humic-like matter in seawater. FEMS Microbiol Ecol 12:177–183
62. Tranvik LJ, Hofle MG (1987) Bacterial growth in mixed cultures on dissolved organic carbon from humic and clear waters. Appl Environ Microbiol 53:482–488
63. Van Der Kooij D (1992) Assimilable organic carbon as an indicator of bacterial regrowth. J AWWA 84:57–65
64. Van Der Kooij D, Visser A, Hijnen WAM (1982) Determining the concentration of easily assimilable organic carbon in drinking water. J AWWA 74:540–545
65. Van Der Wende E, Characklis WG, Smith DB (1989) Biofilms and bacterial drinking water quality. Wat Res 23:1313–1322
66. van Loosdrecht MCM, Lyklema J, Norde W, Zehnder AJB (1990) Influences of interfaces on microbial activity. Microbiol Rev 54:75–87
67. Volk CJ, Volk CB, Kaplan LA (1997) Chemical composition of biodegradable dissolved organic matter in streamwater. Limnol Oceanogr 42:39–44
68. Wolfaardt GM, Lawrence JR, Robarts RD, Caldwell DE (1994) The role of interactions, sessile growth, and nutrient amendments on the degradative efficiency of a microbial consortium. Can J Microb 50:331–340
69. Wolfaardt GM, Lawrence JR, Robarts RD, Caldwell SJ, Caldwell DE (1994) Multicellular organization in a degredative biofilm community. Appl Environ Microbiol 60:434–446
70. Ye RW, Averill BA, Tiedje JM (1994) Denitrification: production and consumption of nitric oxide. Appl Environ Microbiol 60:1053–1058