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Involvement of humic substances in regrowth

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Abstract

There appear to be interactions in the distribution system that complicate the ability to use AOC/BDOC as an independent assessment of regrowth potential. Two such complications are the limitation of the assays themselves and the potential interaction between the organic carbon concentration with the presence of disinfectants and pipe materials. To address these interactions, a series of experiments spanning several years have been conducted in model distribution systems at the Center for Biofilm Engineering (CBE) using soil-derived humics. When compared to easily utilized organics, humic substances supported the same order of magnitude of biofilm organisms. As carbon concentration was increased from 500 to 1000 to 2000 ppb, there was no increase in growth rate of the organisms, suggesting zero-order kinetics. If the system was chlorinated, there was less biomass, but growth rates were higher. In the presence of corrosion products, humic-fed systems supported more organisms than a control system fed biologically treated water. When free chlorine was maintained at a residual of about 0.2 mg/l, biofilm numbers on the surfaces were reduced. Phosphate alone did not result in fewer bacteria, while a combination of chorine and phosphate had the best results (lowest biofilm numbers). Adjustment to pH 9 was not effective. Recently completed work compared increasing levels of humic substances in the presence of free chlorine and monochloramine on biofilm growth on a number of surfaces (PVC, epoxy, cement, ductile iron). As the concentration of humic substances was increased from 0, 0.5 to 2 mg/l, there was an increase in biofilm numbers on all surfaces. This effect was the most pronounced on iron surfaces. These results illustrate that carbon compounds not measured by the BDOC or AOC tests may profoundly influence biofilm numbers. In addition, iron surfaces are at much higher risk for elevated biofilm counts in the presence of humic substances, even if disinfection is practiced. However, corrosion control may mitigate this interaction.

Keywords: Humic substances; Biofilm; Heterotrophic plate count bacteria; Corrosion; Disinfection

1. Introduction

Intuitively, one of the most logical factors that should be associated with biofilm growth and heterotroph proliferation is the amount of organic carbon in the water available for bacterial metabolism. Rittmann (1989) has suggested that this fraction be called biodegradable organic matter (BOM), since it is independent of any measurement method. Two measurement-specific BOM subsets have been defined. Biodegradable organic carbon (BDOC) is the portion of the organic carbon mineralized by heterotrophic organisms; it is determined by measuring the difference in the initial and final concentration of dissolved organic carbon after incubation with a mixed microbial population. Assimilable organic carbon (AOC) is that portion of the biodegradable organic carbon that can be converted to cell mass by

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either a single organism or a defined suite of bacteria and is expressed as a carbon concentration by means of a conversion factor or calibration. These methods have evolved over time, which is evident by the literature summarized by Huck (1990) and subsequent publications by Lucena et al. (1990), Jolis et al. (1992), Kaplan et al. (1993), and LeChevallier et al. (1993).

Knowledge of threshold levels for AOC and/or BDOC where microbial growth is limited would be helpful in designing water treatment methods to prevent downstream heterotrophic growth or as a monitoring tool to predict when a system may be at risk for heterotroph proliferation. van der Kooij et al. (1989) and van der Kooij and Hijnen (1990) showed a correlation between AOC and regrowth in a non-disinfected distribution system, and provided evidence for biological stability in the Netherlands when the AOC concentration (P17 + NOX) is reduced to 10 μg acetate C eq/l (van der Kooij, 1992). LeChevallier et al. (1991) has suggested that coliform regrowth may be controlled by influent AOC levels (P17 + NOX) below 50 μg acetate C eq/l. Servais et al. (1991) have associated biological stability with a BDOC level of 0.2 mg/l, but Joret et al. (1994) have stated that the value is 0.15 mg/l at 20 °C and 0.30 mg/l at 15 °C. In pilot distribution system studies, Camper (1996) reported no correlation of AOC with coliform and heterotroph regrowth in well-mixed systems, but regrowth events were associated with elevated influent AOC concentration. A field study of 31 utilities performed for the same study by LeChevallier et al. (1996) indicated that distribution systems receiving water with lower AOC concentrations tended to have fewer regrowth problems. However, other instances have demonstrated no correlation between distribution system BDOC or AOC measurements, suspended bacteria, and fixed biomass. Most of this information has been obtained in small projects or engineering design feasibility studies and disseminated by word of mouth by utilities and consultants.

The lack of consistent correlation of AOC and/or BDOC with regrowth and biological stability is due to several issues. These may include (1) an inadequate number of BOM measurements to truly represent the level of organic carbon available for growth, (2) a significant interaction of other factors (disinfectant, distribution system materials, etc.) that govern microbial growth, and/or (3) the presence of organic carbon promoting biological growth not measured by these tests. BOM tests can be difficult for the average utility to perform and high in cost, resulting in a limited number of data points for a particular system. Consequently, the ability to extrapolate the values in a system where water quality can change considerably with time is questionable. The growth of heterotrophic bacteria on the surfaces of water treatment and distribution system components is the net result of many factors specific to a particular system. Due to the complexity of the interactions of factors supporting this growth, clear correlation with specific individual components is often difficult to obtain. The third possibility, that there are components of the organic material recalcitrant to degradation in the tests but available to bacteria on surfaces, is the focus of this manuscript.

Humic substances are a ubiquitous constituent of the natural organic matter (NOM) found in the environment (Choudhry, 1984; Aiken et al., 1985). The general term ‘‘humic substances’’ encompasses a variety of fractions identified on the basis of their solubility in alkali and acid. Humic acids are soluble in dilute alkaline solution (e.g. 0.5 N NaOH) but precipitate from an acidified solution (pH < 2). Fulvic acids are the portion that remains in the aqueous acidified solution (soluble in both acid and base). The remainder is humin, which cannot be extracted by dilute base and acid (Choudhry, 1984). These humic substances, particularly humic and fulvic acids, typically account for the majority of the dissolved organic carbon (DOC) found in water (Aiken and Cotsaris, 1995). Aqueous DOC concentrations are highly variable; in surface water, values range from 1 to 60 mg/l DOC, with typical values from 2 to 10 mg/l (Beckett, 1990). The extreme values are found in highly colored waters containing predominantly humic substances (Stumm and Morgan, 1981). DOC levels in seawater, groundwater and rainwater are usually lower than in rivers and lakes and are generally less than 1 mg/l (Beckett, 1990).

Humic substances are thought to be formed during the microbial degradation of organic matter (Flaig, 1963; Beckett, 1990). These degradation products may be further modified through a variety of reactions including polymerization, condensation and oxidation (Beckett, 1990) to produce highly complex organic molecules. The end result is a suite of compounds that

vary in molecular weight from a few hundred up to several million Daltons (Choudhry, 1984). As summarized by Beckett et al. (1987), humic substance fulvic acids range from 800 to 1500 g and humic acids 1500 to 4000. In contrast, Dawson et al. (1981) suggest that humic acids are predominately Daltons molecular weights (MW) >1000. The MW for humic acids is origin dependent, with an order of <soil<-peat bog<coal reported (Beckett et al., 1987). Weight-average molecular weights of the order of 300 to >200,000 have been reported for soil humic acids (Choudhry, 1984) 700 to 2,000,000 for fulvic and humic acids from marine sediments (Rashid and King, 1969, 1971), and <700 to >26,000 for humic acids from natural waters (Gjessing, 1976).

Although the composition of humic substances is complex, there are some general characteristics. Humic acids consist of phenolic (Ar–OH) or quinone (Ar=O) aromatic rings bridged by –O–, $-CH_2-$, –NH–, –N=, –S–, and other groups (Stevenson, 1985). Attached to the aromatic backbone are functional groups that include carboxyls, enols, alcohols, ethers, ketones, aldehydes, esters, amines and amides. Amino acids, amino sugars, peptides and aliphatic compounds may also be present (Stevenson, 1982). The oxygen present in these functional groups is in large measure responsible for water solubility, acidity, metal complexing capacity, surface activity, and surface adsorption properties exhibited by humic substances (Beckett, 1990).

The conceptual model of humic substance conformation has evolved over the past decade. The traditional theory is that humic substances are large polymers and may occur in linear or coiled, cross-linked conformations, depending on the properties of the solution. According to this model, at high concentration, low pH, and high ionic strength these molecules exist in the coiled conformation, while they exist as flexible linear polymers at neutral pH, low ionic strength, and low concentration (Stevenson, 1994). Another theory suggests that humic substances in solution are a loosely bound self-association of relatively small molecules, which are dominated by intermolecular hydrophobic interactions as binding forces (Conte and Piccolo, 1999). The shape of humic substances is dependent on the environment. In aqueous environments, humic substances assume extended shapes due to intramolecular electrostatic repulsive interactions. The hydrophobic effect causes the exterior to be hydrophilic and the interior to be hydrophobic. When adsorbed at interfaces at low ionic strength, they assume flat configurations (Stumm and Morgan, 1981; Wershaw, 1999).

The general thought is that humic substances are recalcitrant organic molecules. In part, this is due to their ability to form complexes with extracellular enzymes and thus competitively and non-competitively inhibit their activity (Wetzel, 1992). There are exceptions to this thought, however. A unique situation is where bacteria can use quinone moieties of humic substances as electron acceptors and/or shuttles for anaerobic Fe(III)-reduction (Scott et al., 1999). Other instances exist where the humic substances serve as a carbon and energy source for heterotrophic bacteria. De Haan (1974) suggested that a *Pseudomonas* species could co-metabolize fulvic acids via the mechanism used for lactic acid oxidation. This same researcher used benzoic acid addition in water to stimulate bacterial growth, which in turn lead to the disappearance of color contributed by fulvic acids (De Haan, 1977). Co-metabolism of high MW fractions of DOC was enhanced in organisms, including *Caulobacter* sp., when glucose was added at low concentrations (Stabel et al., 1979). When the direct use of NOM as a carbon and energy source was examined, high molecular weight compounds supported more bacterial growth than low molecular compounds in both freshwater and marine environments. It was concluded that high molecular weight compounds are diagenetically younger and more biodegradable than smaller sized NOM (Amon and Benner, 1996). It is important to note that these studies were done using batch systems, usually with suspended bacteria. Only one reference exists where it has been suggested that biofilm bacteria are capable of using humic materials (Volk et al., 1997).

One property of humic substances that may alter their susceptibility to microbial degradation is their propensity of attaching to surfaces. The most widely studied phenomenon is the adsorption to iron oxides in the soil and aquatic environments (Parfitt et al., 1977; Tipping, 1981; Tipping et al., 1981; Davis, 1982; Gu et al., 1996). Gu et al. (1995) showed that different fractions of NOM are adsorbed by iron oxide with different affinities and capacities. On a carbon-weight basis, larger size hydrophobic NOM fractions

had higher adsorption affinities and capacities and were preferentially adsorbed over smaller size hydrophilic fractions. Due to the difficulty in quantitatively identifying NOM fractions from a mixture before and after adsorption, competitive adsorption has not yet been explored (Gu et al., 1996).

It has been hypothesized that humic substances undergo a conformational change in structure as they are absorbed to iron surfaces, which exposes their utilizable attached functional groups. First, the adsorption of humics on iron oxide surfaces forces the humic molecules to collapse, allowing for maximum points of interaction between their oxygen-containing functional groups and iron oxide surface sites. This may occur through ligand exchange or H-binding mechanisms (Stumm and Morgan, 1981). The collapse of the molecule may cause it to uncoil and expand, exposing the previously hidden usable functional groups (Gu et al., 1994), making them available for microbial attack.

Presented below are overviews of a series of experiments performed at the Center for Biofilm Engineering (CBE) over the past several years that document the influence of humic substances on biofilm proliferation. A common theme has been that soil-derived humics can act as the sole carbon and energy source for bacterial growth, and that this effect is most pronounced in the presence of iron oxides (corroding iron surfaces). The design of many of these research efforts has included other factors including disinfection, varying pipe materials, and corrosion control, and the importance of these interacting variables is also highlighted.

2. Materials and methods

The majority of the projects listed below had common materials and methods; exceptions are noted in the sections on the specific projects. More detailed information on each project may be obtained in the references included in the appropriate section. The laboratory setup typically consisted of a suite of annular reactors (model 920LJ manufactured by Biosurface Technologies) containing coupons of the pipe/surface material of choice. Each reactor contained only one coupon type. The annular reactor drum holds 20 coupons measuring roughly 1 cm in width and 15 cm in length. These reactors have a variable speed rotating drum, a volume of roughly 1 l, and a high surface area to fluid volume ratio. The rotational speed of the reactors was set at roughly 90 rpm to simulate the shear stress in a 4-in. pipe with a fluid velocity of 1 ft/s.

The reactors are fed with a constant stream of Bozeman tap water that has been treated via a granular activated carbon (GAC) column to remove background chlorine and then a biologically activated carbon (BAC) column to reduce the amount of biodegradable organic matter in the water. This process also produces a water of relatively stable chemical and biological characteristics and produces minimal biofilm growth. This water was pumped at a constant flow rate into the annular reactors to achieve a detention time of 2 h per reactor. This detention time was sufficient to allow biofilm growth while minimizing planktonic growth. The BAC-treated water also provides an undefined, mixed population inoculum to the reactors.

The humic solution was prepared using Elliot Silt Loam obtained from the International Humic Substances Society. One hundred grams of this soil was added to 1 l of 0.1 N sodium hydroxide in a baked glass bottle to minimize carbon contamination. This soil solution underwent constant mixing for 2–4 days after which it was centrifuged at a $\times g$ factor of 4 for 20 min. The supernatant was then poured into another baked glass bottle and a small sample was removed and diluted with nanopure water for determination of the carbon concentration of this stock solution. The dissolved organic carbon (DOC) concentration of the solution was measured with the Shimadzu TOC-5000A Total Organic Carbon Analyzer using the methods described below for the annular reactor samples. The final concentration usually measured between 1000 and 1500 mg/l DOC depending on the length of the mixing time. The volume of humic stock solution to add to the feed jug was calculated using the flow rate and volume in the annular reactors, the volume of autoclaved ultrapure water in the feed jug, and the desired concentration of humics to be added to the reactors. After the humics were added to the ultrapure water in the feed jug, the pH was adjusted with 2 N hydrochloric acid (HCl) to match the pH in the influent as closely as possible. Nitrogen and phosphorus were added in a separate feed so that

the reactors were carbon-limited. Typical influent DOC concentration from the humics, accounting for dilution from the BAC-treated water and nitrogen/phosphorus source varied from 0.5 to 2 mg/l, depending on the experiment.

If disinfectants were added, separate feeds of chlorine prepared from a sodium hypochlorite solution or preformed monochloramine was used. Influent concentrations and reactor effluent residuals were monitored routinely.

The number of heterotrophic plate count bacteria in the biofilm and in reactor influent and effluent were monitored routinely. Biofilms were scraped, dispersed by homogenization, diluted as needed, and spread plate in triplicate on R2A agar. Incubation was at room temperature for 7 days. Total cell counts were also performed routinely. Similar procedures were used for the influent and effluent samples.

3. Results and discussion

3.1. Humic utilization, biostability of drinking water

The first project that utilized humic substances involved the comparison of this carbon source with that of amino acids and carbohydrate as substrates for biofilm growth. The work was funded by the American Water Works Association Research Foundation under the auspices of a project entitled ''Investigation of the Biological Stability of Water in Treatment Plants and Distribution Systems''. Complete results can be found in Ellis et al. (2000), Camper et al. (2000a) and Butterfield et al. (2002b).

The amino acids, carbohydrates, and humics were added at three separate levels (500, 1000, 2000 ppb) to annular reactors with and without the presence of chlorine. Biofilm, influent and effluent total and culturable cells as well as dissolved organic carbon were measured to obtain the specific growth rates, populations, observed yields, and the accumulation of biofilm carbon on the polycarbonate surfaces of the reactors. Biofilms were allowed to develop for several months; one experiment was approximately a year in length.

At the lowest carbon concentration and in the absence of chlorine, the humic substances produced ca. 10^6 total cells and 10^5 culturable cells per square centimeter. When compared to the populations produced by the easily utilized amino acids (8×10^6 and $10^6/cm^2$), the number of biofilm cells was surprisingly high. Overall, amino acid counts were highest, with the humic total cells being 15% and culturable cells 32% of this maximum. As the substrate levels increased, there was a general trend towards more biofilm cells, and the humics produced cell numbers closer to those of the amino acids. Nearly all of the amino acids and carbohydrates were removed across the reactor in the 2-h residence time, and a surprising 78% humic removal was also seen. For all carbon substrates, the specific growth rates and yields were highest at the lower carbon levels, leading to zero-order kinetics. For humics in particular, we believe this may be caused by the large amount of substrate bound to the biofilm (8.3–11 μg C/cm^2), which would lead to growth rates independent of the bulk fluid concentration. As a point of reference, the doubling time on humics was 15.4 and 2.3 days with amino acids, and yields for these two substrates were 0.034–0.045 to 0.08–0.12 μg cell C/μg C, respectively.

When the humic carbon loading was increased to 1000 and 2000 ppb, there were comparisons of results from data obtained with and without the presence of chlorine. Chlorine was adjusted at the influent to provide an effluent residual of 0.15–0.2 mg/l. At both carbon levels, the presence of chlorine decreased biofilm numbers. At the 1000 ppb level, there was approximately a 1-log decline in total counts (7×10^8 to 7×10^7). Culturable counts declined by over 2.5 logs. Another interesting result was that growth rates in the presence of chlorine were higher than those in the equivalent control phase.

3.2. Humic/UV interactions and biofilm formation

Work funded by the U.S. EPA through the Montana Water Center was designed to examine the impact of UV treatment of humic substances on biofilm growth. The final report is available from the Montana Water Center (Camper et al., 2000b). Polycarbonate annular reactors were fed tap water that had been treated with a biological column to remove background concentrations of organic matter, this low-nutrient water treated with a high dose (18,500 mW s/cm^2) of UV, biologically treated water amended with 1.4 mg/l soil derived humic substances, or biologically treated water

amended with 1.4 mg/l UV-treated humics (doses listed below). Characterization of the humic substances before and after treatment with all three UV doses was carried out using XAD-8 resin columns to determine hydrophobicity/hydrophilicity, UV 254 measurements, and changes in biodegradability via BDOC sand columns.

Based on initial experiments, doses of 480, 4800 and 25,600 mW s/cm^2 were chosen for oxidation doses for the humic substances. There was little change in the measured characteristics of hydrophobicity/hydrophilicity, aromaticity as determined using UV 254, or biodegradable fraction at the two lower doses. Only at the highest dose of 25,600 mW s/cm^2, which is 53 times the lowest dose, was there a change observed. At this dose, the hydrophobicity decreased 38%, hydrophilicity increased 49%, aromaticity decreased 29%, and biodegradability increased 24% over that of non-UV-treated humic substances. Non-UV-treated humics were approximately 20% biodegradable as measured using the BDOC test.

Humic substances, either UV-treated or untreated, resulted in a biofilm that was at least 1 log higher than the same water before the addition of humic substances. The UV-treated humic substances resulted in only slightly higher biofilm numbers at the two lower doses. At the highest dose, there was nearly 10 times more biofilm in this reactor as compared to the non-UV-treated humic amended system. This same dose also produced the greatest changes in the characteristics of the humic substances. Although this dose is unrealistically high, the results do suggest that under certain circumstances UV treatment may enhance the availability of organic carbon found in the water.

3.3. Importance of surface/humic interactions

A recently completed project was designed to investigate the importance of relevant distribution system surfaces, humics, and disinfectants on HPC growth in biofilms. This work was part of a larger project entitled "Influence of Distribution System Infrastructure on Bacterial Regrowth" funded by the American Water Works Association Research Foundation. The data are being compiled in a final report that should be available in the near future from AWWARF. In addition, a manuscript is available (Camper et al., 2003).

The four materials tested were ductile iron, PVC, epoxy coating, and cement lining. Coupons of these materials were placed in duplicate annular reactors. The experimental design consisted of four phases. During phase 1, the reactors were fed with biologically treated water with no disinfectant present. In the second phase, biologically treated water was again used, but one set of reactors (each contained one type of material) was fed enough free chlorine to provide a residual of 0.2 mg/l and the second set received sufficient monochloramine to give the same residual. During phase 3, the biologically treated water was amended with 0.5 mg/l dissolved organic carbon from humic substances, and the disinfectant regime was the same as in phase 2. In the final phase, the dissolved organic carbon concentration was raised to 2 mg/l, and again the disinfectant residuals were retained at 0.2 mg/l.

Each phase lasted a minimum of 3–4 months to allow the processes in the annular reactors to approach equilibrium. When disinfectants were added, the dose was sufficient to maintain target effluent concentrations of 0.2 mg/l as free chlorine and total chlorine, respectively. Evidence from this study supports the phenomenon of the increased bioavailability of humic substances by adsorption to iron surfaces. The biofilms in the iron reactors were found to be significantly denser than the biofilms in reactors containing any of the other materials when the reactors were supplemented with humics at 0.5 and 2 mg/l C. In phase 1 (no disinfectant, no added carbon), the biofilm densities in the iron reactors were not significantly different from the densities in the cement reactors and there was very little difference between the densities on all four materials. However, in phases 3 and 4 where the supplementary humic substances were added, the densities in the iron reactors were significantly greater than in any other.

Of the materials other than iron there appeared to be very little difference in the impact of either disinfectant on the biofilm densities as a function of material. Based solely on the statistical results, no regular pattern could be discerned in the biofilm data regarding which reactors had the highest or the lowest densities in any of the phases. However, the trends in the data showed that the PVC always had the lowest densities or did not differ significantly from the material with the lowest densities. The iron reactors had the highest biofilm

densities in all phases. The cement and epoxy fell between the PVC and iron with no apparent order of ascendance.

The biofilms in the iron reactors in phase 4 (2.0 mg/l humics, added disinfectant) were significantly greater than the biofilm in the same reactors in the control phase. This indicates that the disinfectant level of 0.2 mg/l was insufficient to control the increased growth at the highest carbon level in the iron reactors when compared to the control phase. However, for the biofilms on the remaining materials, the biofilms in phase 4 did not significantly differ from the biofilms on the same materials in phase one where no carbon or disinfectant was added. This indicates that the disinfectant level of 0.2 mg/l was sufficient to control the increased growth at the highest carbon level in epoxy, cement, and PVC reactors when compared to the control phase, but was not effective on the iron surfaces. This effect was seen for both monochloramine and free chlorine.

3.4. Impact of corrosion products, humics, chlorine and corrosion control on biofilms

Because of the suspected interaction of humic substances with iron oxides, a project was specifically designed to determine if the presence of iron oxides enhanced biofilm growth in the presence of humic substances. This work was funded by the U.S. EPA and has resulted in two manuscripts (Butterfield et al., 2002a,c).

For this work, the biofilms were grown in small columns containing glass beads, glass beads covered with a synthetically created iron oxide, or crushed corrosion products taken from a cast iron drinking water distribution pipe. The surfaces were initially exposed to humic substances (termed "loading") with the exception of a control. After initial exposure, all but one column received influent humics at a concentration of ca. 3 mg/l. For glass beads, the humic addition was pH adjusted to 7.3, 8 or 9. For corrosion products, only pH 7.3 and 8 were used. For both corrosion products and glass beads, a set of columns at pH 7.3 received chlorine at an effluent residual of 0.15–0.2 mg/l, another was chlorinated plus phosphate added. A control with humics and phosphate at pH 7.3 was also run. The pH adjustment and phosphate amendment were selected to simulate commonly used corrosion control strategies in distribution systems. After the columns had reached steady state (approximately 2 months), the effluent was monitored extensively for total and culturable counts for 10–14 days. Columns were sacrificed at the end of the experiments and culturable counts and exoproteolytic enzyme activity determined. A weighted hierarchical process called the simple multiattribute rating technique was used to determine which columns resulted in the best performance, which was selected to be the least number of culturable cells in the biofilm and effluent and minimal enzyme activity on the surfaces of the particles.

The addition of humic substances was a major factor in biofilm formation. The absence of added humics was the second most important factor following chlorine for the minimization of biofilm as measured by both enzyme activity and culturable cells. The interaction between iron oxides and humics also was high for the corrosion products, suggesting that the interaction of humics with a reactive iron surface is favorable for biofilm formation. The interaction with the less reactive synthetic iron oxide coating on the beads was less important. Interestingly, the corrosion products removed from the distribution system had a much higher adsorption affinity for the humics. The corrosion products also retained sufficient humics during the initial loading that even if no other humics were added to the column influent, they supported far more biofilm than the equivalent iron oxide bead system. Of the corrosion control schemes tested, pH adjustment had little positive effect (in fact, the pH 9 system had higher counts than the column held at pH 7.3). Phosphate addition with chlorine was slightly more effective than chlorine alone when the corrosion products were used.

3.5. Extension of results to point-of-use devices

The demonstrated interaction between heterotrophs and humics may be extended to point of use devices that utilize granular activated carbon (GAC). GAC and powdered activated carbon (PAC) are widely used in the water industry to remove organic matter, including color, taste and odor compounds as well as micropollutants. Humic substances are also removed; Owen et al. (1995) showed that high molecular weight fractions of NOM are preferentially

removed by GAC binding. These organics may then act as a growth substrate for the attached bacteria. Davies and McFeters (1988) reported that the growth rate of attached *Klebsiella oxytoca* was 10 times higher than suspended cells in a low-nutrient medium containing glutamate as the carbon source. The GAC had been exposed to the glutamate prior to colonization by the bacteria. Others have noted that organic carbon adsorbed on GAC can desorb, be transported out of the micropores, and then be degraded by bacteria attached to GAC (Chang and Rittmann, 1987; Speitel and DiGiano, 1987). This mechanism may enhance the attachment of bacteria and subsequent growth of biofilms on carbon particles. Regardless of the mechanisms, it is common knowledge that GAC in water treatment systems is populated by bacteria. This property has been exploited in the process called biological filtration. In biological filtration, filter media, typically granular activated carbon, is used in a filtration process that is optimized for the growth of indigenous heterotrophic bacteria that degrade the available fraction of organic carbon in the water. The filter media used in the process is termed biologically active carbon (BAC).

It is also known that as water passes through GAC filters, a significant number of bacteria can be released. The increase in the numbers of organisms from filter influent to effluent can be two orders of magnitude (Ahmad and Amirtharajah, 1995). These organisms may be released as single cells, aggregates, or attached to filter media particles (filter fines). The released biofilm organisms then cause the elevated HPC counts seen downstream of GAC used in drinking-water filters and POU devices.

4. Conclusions

These experiments have illustrated that humic substances can act as the sole carbon and energy source for biofilm bacteria. The numbers of total and culturable bacteria produced by the humics are similar to that of an equivalent amount of easily used organic material such as amino acids or carbohydrates. These mixed population biofilms appear to be adapted to utilizing this complex carbon source. Even though doubling times are slower on humics, the yields are within the same order of magnitude as seen with carbon substrates generally perceived to be more degradable. The carbon in the humics is about 20% degradable as determined using the BDOC test over a 5-day period, but removals of over 75% at influent concentrations of up to 2 mg/l were seen in the 2-h annular reactor residence time. Growth rates did not increase as the carbon concentration increased (zero-order kinetics). There was also a noticeable accumulation of humics within the biofilm; with age, there was a change in color with a gradual darkening of the biofilm. These results demonstrate that the use of AOC or BDOC tests may greatly underestimate the amount of carbon substrate available for the growth of heterotrophs in biofilms. The latter point is even more pronounced in the presence of iron corrosion products. Pre-adsorbed humics on corrosion products can be a source of growth potential for heterotrophs even if there is no carbon in the feed water. Iron surfaces consistently supported more biofilm bacteria than other materials at low-humic carbon levels (0.5 mg/l), and the discrepancy between materials was more pronounced as the amount of humic substances in the reactor influent was increased to 2 mg/l.

Although there may be some reduction in numbers of organisms grown on humics in the presence of chlorine or monochloramine, there was an increase in the specific growth rate of those remaining on the surface. Disinfection efficacy is reduced if the surface is reactive (iron) over that of relatively inert polymers.

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