FOULING OF A HYDROPHOBIC MICROFILTRATION MEMBRANE BY ALGAE AND ALGAL ORGANIC MATTER: MECHANISMS AND PREVENTION

by

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Abstract

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Microfiltration is a membrane filtration process increasingly used in potable water treatment because it effectively removes turbidity and particles which include pathogens. However, the permeability of microfiltration membranes usually decreases during filtration as a result of the accumulation of aquatic material on or within the membrane, a phenomenon referred to as fouling.

While the fouling of microfiltration membranes by surface waters has largely been attributed to the presence of natural organic matter (NOM) in these waters, the fouling caused specifically by algae and their associated algal organic matter (AOM) has not been rigorously investigated. The fouling of a hydrophobic microfiltration membrane system typical of those used in potable water treatment was therefore tested with a series of model organic compounds, selected to represent AOM, and three genera of algae grown in the laboratory, *Scenedesmus*, *Asterionella,* and *Microcystis*. Finally, the use of coagulation/flocculation pre-treatment using alum to reduce fouling by algae and AOM was examined.

Model compounds including proteins and polysaccharides were capable of significant and irreversible fouling of the hydrophobic microfiltration membrane. Two fouling

mechanisms by these compounds were identified: (1) limited pore constriction caused by the adsorption of small organic molecules onto the membrane pore walls and (2) pore obstruction caused by large organic aggregates that form under specific solution conditions.

Fouling of the hydrophobic membrane by the different algae varied significantly. Algal cells accumulated on the membrane surface during filtration and caused no fouling or fouling which was mostly reversible. By contrast, AOM was capable of significant and mostly irreversible fouling. Because the AOM produced by each alga varied significantly in concentration and size, their fouling varied significantly as well. Similarities in fouling mechanisms were observed between AOM and the model compounds.

Coagulation/flocculation pre-treatment using alum may be an effective method to reduce the fouling caused by several types of organic matter, including AOM. Reductions in fouling are achieved when the alum dose exceeds the minimum effective alum dose (MEAD) established by jar test for removal of the organic matter by settling.

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1 INTRODUCTION

1.1 MICROFILTRATION IN POTABLE WATER TREATMENT

Microfiltration is a low pressure membrane filtration process that separates unwanted contaminants and particles from a solution using filtration through porous membranes with typical pore diameters on the order of 0.1-1 μm. Microfiltration is one of several membrane filtration processes used in potable water treatment which also include reverse osmosis, nanofiltration, and ultrafiltration. Reverse osmosis and nanofiltration are high pressure membrane filtration processes which are designed to retain very small solutes and have traditionally been used for desalination and softening. Ultrafilration is a membrane filtration process similar to microfiltration but uses membranes with smaller pore sizes, usually defined by their molecular weight cut-offs (typical range = 1-100 kD).

Microfiltration is increasingly used in drinking water treatment for several reasons including its small spatial requirement, its efficient removal of turbidity and, of particular importance, its complete removal of particles larger than the membrane pore size. It has been shown that microfiltration is capable of removing a number of pathogens from water, including the two protozoans *Cryptosporidium parvum* and *Giardia muris* [1] and the toxin releasing cyanobacteria *Microcystis aeruginosa* [2]. The complete removal of pathogens from water in the context of the Surface Water Treatment Rule (SWTR) has generated great interest in the application of microfiltration in potable water treatment [3].

Microfiltration and ultrafiltration are used in a number of different schemes in surface water treatment applications [3, 4]. They are sometimes used as the main treatment process in potable water treatment, "as is" for the removal of particles and microorganisms or in conjunction with a coagulation process. In the latter, the membrane replaces the media filters in the conventional potable water treatment design and the combination of coagulation and membrane filtration allows the removal of some organic matter in addition to particles and microorganisms. Microfiltration and ultrafiltration are also used as pretreatment to high pressure membrane filtration processes such as nanofiltration and reverse osmosis. In some of these applications, the microfiltration or ultrafiltration membrane is exposed to surface waters with little or no treatment before filtration.

While removal of turbidity, pathogens and some organic matter can be achieved by microfiltration membranes, these membranes are also susceptible to membrane fouling which limits their use. Membrane fouling consists in a loss of membrane permeability that occurs over time and which results from the accumulation of material called foulant on the surface and within the porous structure of the membrane during filtration.

1.2 MEMBRANE FOULING: A PHYSICAL DESCRIPTION

Microfiltration can be described by Darcy's law [5], which states that the flow through a porous medium is directly proportional to the pressure gradient across that medium and the permeability of the medium. In the case of a porous membrane, the permeate flux through the membrane, J ($m^3/m^2.s$), is proportional to the transmembrane pressure, ΔP

(kPa), and inversely proportional to the membrane resistance, R (1/m) and the viscosity of the fluid, μ (N.s/m^2):

$$J = \frac{Q}{A} = \frac{\Delta P}{\mu . R} \tag{1.1}$$

where Q (m^3/s) is the permeate flow rate and A (m^2) is the membrane surface area. Dividing the permeate flux by the transmembrane pressure yields the specific flux, SF:

$$SF = \frac{J}{\Delta P} = \frac{1}{\mu . R} \tag{1.2}$$

The specific flux, SF (m^3/m^2.s.kPa), is a measure of the volume of water that can be treated by a membrane over time given a certain transmembrane pressure and a certain surface area.

Fouling results in a decrease of the membrane permeability over time, i.e. increases in R and decreases in SF. This decrease in permeability over time means that progressively less water can be filtered at a given pressure or that higher pressures have to be generated to maintain the permeate flux. Either of these two effects is undesirable to the sustainable use of membrane filters to treat surface waters.

1.3 MEMBRANE FOULANTS

The fouling of a membrane is strongly influenced by the membrane characteristics, pore size and material composition, as well as by the characteristics of the fouling water, including the size and composition of foulants and the water chemistry. This is because fouling is a result of foulant rejection, which is influenced by the size of the foulants relative to the membrane pore size, as well as the interactions that exist between the

foulants and the membrane material and the interactions that exist between individual foulants.

In the context of surface water treatment, major membrane foulants include inorganic particles (iron oxides, alumino-silicates), microorganisms (bacteria, protozoa, algae), and natural organic matter (NOM). With respect to microfiltration (pore size ≈ 0.1-1μm), inorganic particles and microorganisms are most likely to foul a membrane by forming a cake on the external surface of the membrane. This fouling will be more or less severe depending on the properties of the cake (e.g., particle size, particle surface charge, compressibility). NOM, on the other hand, can foul a membrane both internally and externally. Of the three types of foulant, NOM is thought to play a critical role in membrane fouling because it interacts with and/or adsorbs onto many surfaces in an aqueous environment. NOM can adsorb onto a membrane material and block or constrict the membrane pores. It can also coat the surfaces of particles and influence their interaction with one another or with the membrane.

1.4 MEMBRANE FOULING BY ALGAE AND ALGAL ORGANIC MATTER

Photosynthetic microorganisms present in water include eukaryotes (algae) and prokaryotes (cyanobacteria). For the sake of clarity however, these organisms will all be referred to in this work as algae and the organic matter released by these organisms as algal organic matter (AOM).

The NOM found in surface waters can be divided into different fractions. Humic and fulvic acids are fractions of NOM that adsorb onto XAD-8 resin at pH 2 and humic acids

are distinguishable from fulvic acids by their precipitation at pH 1 (http://www.ihss.gatech.edu/). These two fractions of NOM are together commonly referred to as humic substances. These are generally characterized by high SUVA (specific ultra violet absorbance) values and are considered more hydrophobic than the NOM that remains in solution after their isolation. Humic substances account for 50% of the dissolved organic carbon (DOC) present in an average river water [6]. Because they can be so abundant and easily isolated, humic substances are sometimes used as a surrogate for NOM in surface waters and consequently received much attention in the original work investigating membrane fouling by surface waters. This work demonstrated that humic substances could indeed cause significant membrane fouling [7, 8] and suggested they might be a major source of fouling when filtering surface waters. Work that followed, however, showed that non-humic (hydrophilic) organic matter present in surface waters [9, 10] and in waste water effluents [11] can also cause significant membrane fouling. In fact, membrane fouling caused by the hydrophilic fractions of the NOM of a particular water can exceed that caused by its hydrophobic fractions [9, 10].

Studies have shown that freshwater algae can release considerable quantities of organic matter during growth and decline [12, 13]. The organic matter released by several of these organisms has been shown to contain compounds with saccharidic components [12]. Organic matter extracted from cells of blue-green algae (cyanobacteria) has also been shown to contain compounds with saccharide and protein-like signatures [14, 15]. These types of compounds have low SUVA values and are usually regarded as non-humic or hydrophilic. If hydrophilic organic matter is capable of significant membrane

fouling and algae can contribute significant amounts of this material to surface waters, it would be expected that algae and their AOM could cause significant fouling of membranes used to treat these waters. Yet their potential for microfiltration membrane fouling has not been rigorously examined. In fact, little is known of the potential for membrane fouling by different algae and their exudates, which may be important for the treatment of surface waters rich in nutrients and characterized by seasonal algal blooms.

In order to investigate the potential for membrane fouling by algae, two approaches were therefore taken. In the first approach, model compounds representative of AOM were filtered through a microfiltration membrane under different solution conditions and the resulting fouling measured. The model compounds tested included two proteins and two polysaccharides. In this approach, the aim was to test the physico-chemical factors that may influence the fouling of a membrane by organic compounds similar to AOM. The results of that part of the work are presented in chapter 3. In the second approach, three types of algae (bacillarophyceae, chlorophyceae, cyanobacteria) were cultured in the laboratory and their fouling of a microfiltration membrane tested. Fouling by the organisms was tested during growth and decline of the organisms since the organic matter they release may vary in quantity and characteristics depending on growth phase. That work is presented in chapter 4.

1.5 FOULING PREVENTION AND REDUCTION

A common first approach to controlling membrane fouling is the use of hydraulic backwash of the membrane. Backwash is a process in which the flow through a membrane is reversed in an attempt to dislodge fouling material and recover permeability

lost during filtration. Experience has shown, however, that hydraulic backwash is only partially effective in controlling fouling by organic matter. In a treatment application, the inability of hydraulic backwashes to maintain the membrane permeability over time eventually requires the interruption of operation and the cleaning of fouled membranes by chemical means, using alkaline solutions or oxidants for example [16]. While chemical cleaning can be effective, it requires time for membrane soaking and rinsing during which the membrane is unproductive. It also generates wastes which must be disposed of safely. Moreover, chemical cleaning should be regarded as a last barrier to fouling since fouling has already occurred when this form of cleaning is considered.

An alternative or complimentary approach to controlling fouling is coagulation pretreatment. Many studies have reported decreases in the fouling of various membranes by natural surface waters or model waters containing organic matter when coagulation was applied to the water prior to filtration [9, 17-22]. Pre-coagulation likely lessens fouling by capturing foulants into large flocs which remain suspended in the retentate or are retained on the external surface of the membrane where they cause relatively little resistance to flow during filtration. However, there is little information on the effect of pre-coagulation on membrane fouling caused specifically by hydrophilic organic matter such AOM.

The ability of pre-coagulation to reduce membrane fouling by hydrophilic organic matter was therefore tested using a combined coagulation/flocculation/microfiltration system with some of the model compounds mentioned earlier. The reduction in membrane

fouling caused by AOM was tested using the same system with one alga previously seen to cause significant irreversible membrane fouling. This work is presented in chapter 5.

1.6 HYPOTHESES AND OBJECTIVES

The research described in this thesis was undertaken to test three hypotheses relating to the fouling of microfiltration membranes by algae in water supplies. These hypotheses were:

- AOM associated with algal growth and decline can cause significant and hydraulically irreversible membrane fouling,
- Model proteins and polysaccharides, representative of a large fraction of algal organic matter, could be used to investigate the physico-chemical factors controlling the extent of algal fouling, and
- Coagulation pre-treatment might be used to reduce algal fouling.

The objectives of this work were to:

- Investigate the physico-chemical factors controlling the extent of algal fouling using model proteins and polysaccharides selected to represent the AOM released by algae as a result of cell growth or lysis,
- Determine the extent of fouling of a membrane by three genera of algae (a chlorophyceae, a bacillariophyceae and a cyanophyceae), and their AOM as a function of growth phase, and
- Investigate the prevention or reduction of algal fouling using coagulation as a pretreatment process.

1.7 THESIS STRUCTURE

This thesis consists of an introduction chapter (chapter 1), a background chapter (chapter 2), three results chapters (chapters 3 though 5) and one conclusion chapter (chapter 6). In chapter 1, the motivations, hypotheses and objectives for the work are presented. In chapter 2, the literature relevant to the work accomplished is reviewed. In chapters 3 and 4, the work examining the fouling of a hydrophobic microfiltration membrane by several model compounds and several algae (including a cyanobacterium) are presented, respectively. In chapter 5, the use of coagulation pre-treatment to reduce the microfiltration membrane fouling caused by AOM and several other types of organic matter is investigated. Finally, chapter 6 offers conclusions to the entire work and provides a discussion of the significance of this work as well as a discussion of possible future directions this work could be taken. Each results chapter includes the sections that are normally found in scientific publications (introduction, materials and methods, results, discussion, and conclusions).

2 BACKGROUND

In order to understand the fouling of microfiltration membranes that algae may cause, one has to consider that algae contributes several different types of foulants to a water. These foulants include large algal cells, cell fragments, and dissolved or colloidal algal organic matter (AOM). Each of these is likely to foul a microfiltration membrane by different mechanisms. Algal cells (> 1 μm), being greater than the pore size of a typical microfiltration membrane (0.1-1 μm), would be expected to foul the membrane by forming a cake on the membrane surface. Cells fragments and AOM could foul a microfiltration membrane internally or externally depending on their size relative to the membrane pores. Because these foulants cover a wide range of sizes, a review of what is currently known about the fouling of membranes, especially microfiltration membranes, by particles of varying sizes and organic matter may be instructive.

2.1 FOULING BY INORGANIC PARTICLES

Surface waters may contain significant concentrations of inorganic particles and their ability to cause fouling of various membranes has been the subject of a number of studies. These particles are usually composed of iron oxy/hydroxides, silicates, aluminosilicates (clays), and carbonates. They can cover a wide range of sizes from a few nanometers to hundreds of microns.

For large particles (micron size), the type of membrane system used, dead-end or cross-flow, may impact membrane fouling significantly. In a dead-end filtration design, these particles are rejected at the membrane surface and form a cake. The resistance caused by

such a cake will depend on the porosity of the cake, the thickness of the cake and the size of the colloids making up the cake. By contrast, these large particles can be transported away from the membrane surface in a cross-flow filtration design and the formation of a cake prevented [22]. In such a system, whether large particles accumulate in a cake depends on a balance between permeation rate and several back transport mechanisms (e.g., shear induced diffusion, lateral migration) [22]. Therefore whether large particles form a cake and foul a membrane may depend on the membrane system used.

Nonetheless, several studies have indicated that cakes composed of large particles or loose aggregates do not cause significant fouling of microfiltration and ultrafiltration membranes [23-26]. These studies used hematite, silver and latex particles. Another study, however, has reported significant fouling of an ultrafiltration membrane by kaolin clay [27]. In this case, the fouling was associated with the formation of a cake but this cake was easily removed by backwash.

Some studies have indicated that more severe flux decline occurs when the colloidal particles or aggregates being retained by a filtration membrane are of a size comparable to the membrane pores [23, 24, 26]. Under these conditions, pores are said to be completely or partially blocked by only a few colloidal particles in a short period of time after which a surface cake usually forms. This type of fouling causes high specific flux decline by severely impeding the flow through the membrane pores. The work conducted by Huang with latex particles and a hydrophobic PVDF microfiltration membrane indicated that this type of fouling is largely irreversible to hydraulic backwash [26].

Colloids significantly smaller than the pore size of a membrane which do not interact or interact weakly with the membrane material are likely to pass through the membrane. Under these conditions, little fouling is observed [23, 24, 26]. Small colloids may also adsorb onto a membrane material however. Under these conditions, their removal by the membrane can be important but the fouling they cause may be limited depending the extent of the constriction of the pores [23].

Ionic strength, pH and the presence of natural organic matter (NOM) can impact the flux decline that results from inorganic colloidal fouling. Aggregation of inorganic colloids is promoted by pH values close to the pH_{pzc} of the colloids and by high ionic strengths that compress diffuse double layers. Waite *et al.* [25] studied the impact of hematite aggregate structure on flux decline in ultrafiltration and found that diffusion limited aggregation (at $pH \cong pH_{pzc}$ and/or high I) yielded aggregates with an open structure and low filtration resistance whereas reaction limited aggregation (at $pH \neq pH_{pzc}$ and/or low I) produced more compact aggregates with higher filtration resistance. Schafer *et al.* [23] reported large differences in the fouling of a 0.22 μm modified hydrophilic PVDF membrane by 75 nm hematite colloids depending on the order in which the colloidal suspension was mixed with high concentrations of electrolyte and NOM. NOM adsorption prior to the addition of the electrolyte stabilized the hematite particles and the flux decline was limited unless calcium was added. This is consistent with the work of Tiller and O'Melia who showed that the adsorption of NOM can influence the stability of inorganic colloidal particles through electrostatic and/or steric repulsion [28]. Alternatively, adding the electrolyte first aggregated the hematite colloids and resulted in higher flux declines,

presumably because aggregates of a size comparable to the membrane pore size were formed. In this latter case, the presence of calcium lowered specific flux decline by producing seemingly more permeable aggregates. Kim *et al.* [24] also showed the effect of varying salt concentrations on the fouling of a polysulfone ultrafiltration membrane by colloidal silver sols. High salt concentrations limited flux decline by precipitating the sols at the membrane surface. Moderate salt concentrations were said to compress double layers within the membrane pores, thereby allowing particles to travel deeper into the membrane porous structure and lowering their rejection while slightly increasing the extent of flux decline.

2.2 FOULING BY NATURAL ORGANIC MATTER

Natural organic matter (NOM) is ubiquitous in natural environments and subject to great spatial (geography) and temporal (season) variability. The average concentration of dissolved organic carbon, DOC, in North American rivers ranges from 2 to 10 mg/L [6]. Surface water NOM is composed of aquagenic NOM, generated by aquatic organisms (bacteria, algae…) and pedogenic NOM, products of the decomposition of terrestrial plants and soil microorganisms leached through soils into rivers and streams [6, 29].

The NOM found in surface waters can be divided into different fractions. Humic and fulvic acids are fractions of NOM that adsorb onto XAD-8 resin at pH 2 (http://www.ihss.gatech.edu/). After elution from the resin, humic acids are separated from the fulvic acids by precipitation at pH 1. These two fractions of NOM are commonly referred to as humic substances and are considered hydrophobic. The remaining, hydrophilic, fractions of NOM found in surface waters are said to include

hydrophilic organic acids, amino acids, carbohydrates, and small fatty acids [6, 29]. Humic and fulvic acids typically account for 10 and 40 % respectively of the DOC of a river or stream [6].

Information on the nature of the chemical bonds that exist in the different fractions of NOM can be obtained by ^{13}C nuclear magnetic resonance or NMR [6, 30, 31]. NMR analysis reveals that the fractions of NOM isolated by XAD-8 resin are relatively rich in aliphatic and unsaturated carbon, as well as contain carbonyl and hydroxyl groups. The hydrophilic fractions tend not to contain significant amounts of aromatic nor aliphatic groups.

The molecular weights reported for aquatic NOM span a large range. A number of studies, using ultrafiltration and gel chromatography, have indicated that humic substances in water approach 5 kD and rarely exceed 10 kD in size [6]. However, a number of studies have also indicated the presence of some large molecular weight organic matter in surface water NOM [10] and humic acid isolates [7] using ultrafiltration. It should be noted that both gel chromatography and ultrafiltration suffer from experimental drawbacks and results may differ depending on the operating conditions used for each technique. Some molecular weight data are also obtained from isolated fractions of the NOM, not the original solution of NOM. It has been postulated that NOM might be present in much higher apparent molecular weight in its natural environment, possibly as a result of aggregation [29].

The fouling of microfiltration membranes by the NOM found in surface waters is complex. Some studies have investigated the membrane fouling caused specifically by humic acids and attributed this fouling to large aggregates that deposit on the membrane's upper surface and eventually lead to the formation of a cake [7, 8, 32]. Others have examined membrane fouling caused by natural surface waters and attributed fouling to dissolved or colloidal NOM [10, 14, 21, 33, 34] that constricts or obstructs the membrane pores. Increasingly however, experimental evidence suggests that organic matter of a relatively large size (organic colloids) present in surface waters has a significant impact on the fouling of microfiltration membranes.

Data on the reversibility of NOM fouling to hydraulic backwash is limited. Crozes *et al.* [27] noted that the fouling of two ultrafiltration hollow fiber membranes, one hydrophobic (acrylic polymer), the other hydrophilic (cellulose), caused by tannic acid (plant polyphenols) was significant and unaffected by hydraulic backwash. Yuan *et al.* [7] demonstrated that the fouling of a 0.16 μm polyethersulfone membrane by Aldrich humic acid was caused by the rejection of large humic aggregates on the membrane surface and that a large fraction of this fouling could be eliminated by physical washing. However, the physical washing employed in that study consisted in a rather unusual wiping of the fouled membranes with paper towels. Whether this fouling would have been reversible to a more conventional hydraulic backwash is unknown. Huang investigated the fouling of a hydrophobic PVDF microfiltration membrane by NOM from a water rich in fulvic acids and found that hydraulic backwash was largely ineffective in reducing fouling [26].

Experiments have shown that hydrophobic effects influence the interaction of NOM with a membrane and its subsequent fouling. One seemingly consistent trend in fouling by NOM is its ability to foul hydrophobic membranes to a higher extent than hydrophilic membranes. Fan *et al.* [10] saw much higher flux decline from a surface water NOM filtered through a hydrophobic PVDF microfiltration membrane than through a morphologically similar but surface-modified hydrophilic PVDF membrane. Howe and Clark demonstrated that the fouling of both a 20,000 DA polyethersulfone and a 0.2 μm polypropylene membrane by two surface waters could be significantly diminished by prefiltration through a 0.2 μm polypropylene membrane but not through a 0.2 μm polyethersulfone membrane [33]. The authors hypothesized that foulants adsorbed to a greater extent onto the polypropylene membrane during prefiltration because it was more hydrophobic than the polyethersulfone membrane.

As noted previously, the size of the NOM is a factor that may strongly influence the extent of fouling. For example, Fan *et al.* [10] attributed the fouling of a 0.22 μm PVDF membrane by an NOM rich surface water to two fractions of the NOM, the hydrophilic neutrals and the hydrophobic acids, both of which contained considerable amounts of high molecular weight (>30 kD) material. Likewise, Howe and Clark observed that a large fraction of the fouling of a 0.2 μm polypropylene membrane by two surface waters was likely caused by NOM greater than 10 kD, which accounted for only approximately 10 % of total DOC [33]. These researchers noted, however, that the foulants in one of the

two waters included some alumino-silicates and emphasized that both organic and inorganic foulants must be considered when studying surface waters[33, 35].

Electrostatic interactions are important as well. Humic and fulvic acids contain carboxylic and hydroxyl groups which confer a negative charge to these molecules at near neutral pH [6, 29]. This negative charge increases as pH is raised. A membrane material may also bear some charge that may be positive or negative depending on the types of functional groups present (amines, carboxylic, etc…) and pH. Jones and O'Melia studied the adsorption of Suwannee River humic acid (SRHA) onto a slightly negative regenerated cellulose ultrafiltration membrane as a function of pH and ionic strength and related adsorption density to irreversible fouling [36, 37]. A rise in pH lowers adsorption by ionizing the functional groups on the humic acid and increasing the electrostatic repulsion that exists amongst the organic macromolecules and between the macromolecules and the membrane material. High ionic strength increases adsorption by shielding charge and electrostatic repulsion. Jucker and Clark studied the adsorption of SRHA and Suwannee River fulvic acids onto ultrafiltration membranes and reported similar results [38]. Schafer *et al.* [23] saw significant increases in the fouling of a 0.22 μm surface-modified hydrophilic PVDF membrane by Suwannee River humic acid with the addition of calcium, likely due to the aggregation of the humic acid.

Ionic strength and pH also play major roles if fouling occurs through the formation of a cake on the outer surface of the membrane. Yuan and Zydney showed that low pH values, close to the isoelectric point, were conducive to the aggregation of soil-derived

NOM and high ionic strength caused the formation of denser surface cakes both leading to more extensive total fouling [8].

2.3 FOULING BY ALGAE AND ALGAL ORGANIC MATTER

In contrast to the amount of work that has been done on the fouling of microfiltration membranes by inorganic colloidal particles and humic substances, considerably less is known about algal fouling. Yet membrane fouling by algae during surface water treatment may be significant since: (1) algae are known to impact a number of other surface water treatment processes including coagulation, filtration, and DBP formation, and (2) algal organic matter can represent a considerable fraction of DOC in surface waters during algal blooms.

The production of organic substances by algae has been demonstrated, for marine diatom blooms [39-42] and for freshwater algae [12, 13]. Blooms of algae are generally caused by an input of nutrients (nitrogen, phosphorus…) into surface waters. Algae first grow rapidly using the available nutrients. As nutrients are slowly depleted, algae produce or release increasing amounts of organic matter (sometimes referred to as exocellular organic matter or EOM). In the case of some diatom blooms, the complete depletion of nutrients and abundant EOM eventually culminate in the aggregation of the EOM into a matrix which captures diatom cells and promotes their sedimentation. The impact of algal organic matter similar to EOM on membrane fouling is largely unknown.

While a number of studies have looked at membrane fouling by algae and algal organic matter, these studies have not considered the different phases of growth that occur during

a bloom, assessed the extent of fouling reversibility, or generalized their findings with different genera of algae. A study conducted by Babel *et al.* [43] showed fouling of a microfiltration membrane due to the surface accumulation of the alga *Chlorella* in a thick layer of organic matter that was favorably produced under growth-limiting conditions: high or cold temperatures, high solar radiation, or nutrient supression. Storage of the *Chlorella* culture in nutrient-free water lowered the levels of algal organic matter but increased fouling. Her *et al.* studied the fouling of a nanofiltation membrane with cell extracts of blue green algae [15]. In that study, it was found that the algal organic matter was characterized by a low value of SUVA (specific UV absorbance) and was composed mostly of protein- and polysaccharide-like substances. This organic matter showed lower rejection by the nanofiltration membrane than Suwannee River humic acid yet fouled the membrane to a higher extent. Lee *et al.* [14] studied the fouling of several microfiltration and ultrafiltration membranes by a similar extract and reported significant fouling of the microfiltration membranes. Fouling was attributed to macromolecules and colloids of NOM which were predominantly composed of protein and polysaccharide-like substances.

2.4 COAGULATION – MICROFILTRATION

The combination of coagulation pre-treatment with microfiltration in potable water treatment is appealing for two main reasons. The first is that a combination of these two processes is capable of removing both turbidity and DOC. The second is the potential reduction in membrane fouling that may result. While microfiltration is very effective at removing turbidity and particulate matter, it generally only removes small amounts of DOC on its own. Flocs formed during coagulation/flocculation, however, usually capture

significant amounts of DOC (including DBP precursors). These flocs can be settled before membrane filtration (by conventional settling) or directly filtered through a membrane. Increases in DOC removal obtained from the use of coagulation pre-treatment with membrane filtration have been documented [18-20, 22]. The reduction of at least some membrane fouling during the microfiltration or ultrafiltration of surface waters with different forms of coagulation pre-treatment has also been shown [9, 17-22].

In a coagulation/flocculation/filtration system (no settling), the pH and coagulant levels that achieve the most efficient floc growth usually result in the lowest extent of membrane fouling [22, 44, 45]. One explanation for this is that conditions leading to only partial coagulation result in relatively small flocs capable of blocking pores internally and/or accumulating at the membrane surface in relatively impermeable deposits. Conditions optimized for destabilization lead to flocs large enough to be completely retained at the membrane surface in porous cakes.

Coagulation/flocculation/microfiltration of a surface water using different types of coagulants, including aluminum sulfate, polyaluminum chloride, ferric sulphate and ferric chloride was tested by Pikkarainen *et al.* [46]. The resistance to filtration caused by the accumulation of the coagulants and the associated turbidity and DOC in surface cakes was similar for all coagulants and decreased with increasing coagulant dose. Furthermore, DOC removal through this process showed a 15% improvement in DOC removal over conventional clarification when aluminum sulfate, polyaluminum chloride or ferric chloride were used [46].

One significant limitation to the coagulation/flocculation/microfiltration process is that the efficiency of the coagulation process may vary between different types of DOC. Carroll *et al*. [9] noted that coagulation of NOM characterized as neutral hydrophylic was poor relative to other types of NOM. Incidentally, this neutral hydrophylic fraction of the NOM produced the most fouling. Lahoussine-Turcaud *et al.* [22] observed reductions in the fouling of an ultrafiltration membrane using pre-coagulation that were larger with model waters containing humic and tannic acids than with natural surface water. This indicates that pre-coagulation may reduce membrane fouling to different extents depending on the water source and its composition.

2.5 KNOWLEDGE GAPS

In order to understand the potential impact of algae and AOM on the fouling of microfiltration membranes used in potable water treatment, a number of important questions need to be examined. What is the impact on fouling of algal cells relative to AOM? The literature suggests that the fouling of microfiltration membranes caused by large particles is generally limited and that the fouling caused by NOM can be significant. Can more fouling therefore be expected from the AOM than from the algal cells? How variable might the fouling caused by different algae be? The literature on membrane fouling by algae includes work that used only one green alga, *Chlorella*, and extracts from cells of undefined blue green algae. How reversible is the fouling caused by algae and AOM to hydraulic backwash of the membrane? No information is available on this topic. What measures might be taken to control fouling by algae and AOM if this fouling is significant and irreversible to hydraulic backwash? In particular,

coagulation/flocculation pre-treatment is an approach that is increasingly used to reduce the fouling of microfiltration membranes by NOM. Would the same approach work with fouling caused by algae and AOM? The work presented here attempts to answer these questions.

3 FOULING OF A HYDROPHOBIC MICROFILTRATION MEMBRANE BY A SELECTION OF MODEL COMPOUNDS UNDER VARYING CHEMICAL CONDITIONS

3.1 INTRODUCTION

Much work has been done to show that NOM in surface waters fouls membranes. This work established that different fractions of the NOM foul membranes to different extents. The hydrophilic fraction of NOM, in particular, has been singled out as a major contributor to membrane fouling. This fraction of NOM is said to contain large amounts of protein and polysaccharide. Work conducted with waste water effluents has also demonstrated the importance of hydrophilic organic matter in membrane fouling. Waste water effluents are rich in protein and polysaccharide that are generated through biological activity.

Algae produce and release organic matter during growth and decline. Algal organic matter (AOM) contains significant quantities of carbohydrates and proteins, yet little is known of its potential for membrane fouling. By filtering two model proteins and two model polysaccharides through a bench scale microfiltration membrane system under varying solution conditions, the likely potential of AOM to foul microfiltration membranes used in potable water treatment was studied. While the fouling of membranes by model proteins [47-51] and polysaccharides [52, 53] has previously been studied, this work differed in several ways. The membrane system used was a submerged dead-end hollow fiber microfiltration membrane system of the type commonly used in large scale applications, concentrations of the model compounds were kept low, water

chemistry was systematically varied, and the removal of each compound by the membrane during filtration was measured.

The objectives of this work were as follows: (1) to determine the extent of membrane fouling that results from the microfiltration of low concentrations of proteins and polysaccharides under varying chemical conditions, including pH, ionic strength and calcium concentration, (2) to identify the likely mechanisms for the observed fouling and (3) to determine whether the fouling of the membrane by these model compounds is reversible by hydraulic backwash.

3.2 MATERIALS AND METHODS

3.2.1 Model compound selection, characteristics, and detection

Four model compounds were tested: two proteins and two polysaccharides. The proteins tested were selected on the basis of three criteria: (1) the protein had to be available in relatively large quantities (100 mg at least), (2) the protein had to be in a purified form, and (3) the protein had to be of a fairly large size (no less than 50 kD). The motivation behind the third criterion stemmed from a consideration that small proteins, even if they adsorbed to the membrane material, probably would not constrict pores significantly and therefore not cause significant fouling. On the basis of these criteria, two proteins were selected: bovine serum albumin (BSA) and bovine gamma globulin (γG), both obtained from Sigma (A3912 and G7516). BSA has a molecular weight of 67 kD, has overall dimensions of 14 x 4 x 4 nm and has an isoelectric pH, or pH_{iep}, of about 4.7 [50]. γG consists of proteins isolated from bovine blood serum and includes several classes of

antibodies. The most dominant class of antibodies in γG is immunoglobulin G (IgG), with a molecular weight of about 155 kD, overall dimensions of 23 x 4.5 x 4.5 nm, and a pH_{iep} of about 6.6 [50]. The two model polysaccharides selected were dextran and alginic acid (sodium form). Both were obtained from Sigma (D1537 and A2033). Molecular weights similar to that of the proteins were selected in order to offer some comparison with the proteins. The dextran selected had a molecular weight of about 70 kD, the alginic acid between 80 and 120 kD. Dextran is a neutral polysaccharide composed of glucose monomers. Alginic acid is composed of uronic acids and is negatively charged at pH > 4.

Concentration of the model compounds in raw waters and permeate samples were measured by means of two colorimetric assays. Protein concentration was measured using the bicinchoninic acid (or BCA) assay [54, 55] while polysaccharide concentration was measured using a periodic acid/Schiff (or PAS) assay [56].

A BCA assay kit commercially available from Pierce was used (model 23225). A ratio of 1:100 was used when mixing reagents A and B in the kit and 250 μL of this mixture was added to 500 μL of sample for measurement. The high sample to reagent ratio was necessary due to the low protein concentrations being measured. Incubation was conducted at room temperature for several hours (usually 3) and absorbance was measured at 565 nm using a square cuvette with a 1 cm path length.

Two solutions were required for the PAS assay: Schiff reagent and a periodic acid solution. Schiff reagent was made by adding 1 g of pararosanilin hydrochloride to 100

mL of boiling milliQ water. The solution was cooled to 50°C and 1.36 mL of concentrated hydrochloric acid was added to it. The solution was then mixed with 3 g of powdered activated carbon, shaken for a few minutes and passed through a GF/C disc filter. Just before use, 17 mg of sodium bisulfite was added per 1 mL of the solution and the mixture incubated at 37°C until the solution had turned from its original dark red color to a light yellow. The periodic acid solution was composed of 11 mg of sodium periodate, 20 mL of milliQ water, and 1.4 mL of concentrated acetic acid. For measurement of a sample containing dextran, 0.1 mL of periodic acid solution was added to 1 mL of sample and the mixture allowed to react at room temperature overnight (about 20 hours). After periodate oxidation, 0.1 mL of Schiff reagent was added and the colorimetric reaction allowed to develop for 2 hours. Absorbance was measured at 555 nm. Note that the assay was found to be more sensitive for dextran than for alginic acid. As result, a cuvette with a 1 cm path length was used for dextran and one with a 10 cm path length was used for alginic acid. Volumetric ratios of sample to reagent were however the same for both polysaccharides.

3.2.2 Microfiltration unit

Membrane fouling was tested on a bench scale microfiltration unit (Figure 3-1). This unit is a dead end microfiltration unit using submerged hydrophobic hollow fiber membranes provided by US Filter. The microfiltration unit included a continuously stirred raw water vessel, a permeate water vessel, and a column containing raw water into which a membrane module was submerged. A peristaltic pump (Master Flex L/S) was used to filter raw water through the membrane from the column to the permeate vessel and simultaneously refilled the column with raw water, preserving a constant level of raw

water in the column. The membrane unit was run in a constant flow regime and pressure was measured on the vacuum side of the membrane module using a digital pressure gauge (CECOMP Electronics DPG1000B ±15PSIG-5). The permeate flux was maintained at about 36 gfd (61 L m^{-2} h^{-1}) throughout the filtration.

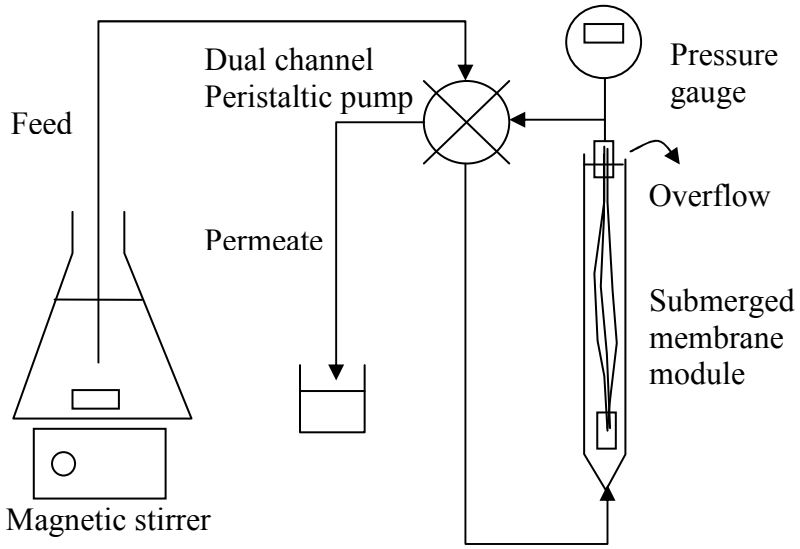


Figure 3-1. Microfiltration unit.

The microfiltration membranes tested were made of pure polyvinylidene fluoride (PVDF). Contact angle measurements of microfiltration membranes made with PVDF similar to the one used here are about 80º [34]. These membranes are considered hydrophobic. The membrane industry is currently using modified versions of these membranes which are hydrophilized through proprietary processes. These membranes are commonly referred to as hydrophilic PVDF membranes. Although less common in practice, pure hydrophobic PVDF membranes may be useful for the investigation of membrane fouling and fouling mechanisms. First, the surface composition of pure PVDF membranes is extremely well defined. In comparison, surface modifications sustained by hydrophilic PVDF membranes are often undisclosed and may affect fouling in specific

ways. Second, adsorption of organic matter from an aqueous solution to a hydrophobic surface is generally favorable such that the use of a hydrophobic membrane for studying fouling may offer a good representation of a hypothetical worst case scenario in regard to fouling by organic matter.

The membrane hollow fibers used had an approximate outer diameter of 0.66 mm, an inner diameter of 0.31 mm, a wall thickness of 0.17 mm, and a nominal pore size rating of 0.1 μm. X-ray photo electron spectroscopy (XPS) of the membrane material was conducted at the department of Materials Science & Engineering at the Johns Hopkins University. This analysis revealed that the membranes were made of essentially pure PVDF without significant functional groups on the surface. According to BET surface area analysis, the fibers had a specific surface area of 9.24 m^2/g equivalent to 86.8 cm^2 per cm of fiber. The membranes were provided by US Filter as long hollow fibers that were cut to desired length (~24 cm), assembled (5 fibers per module) and potted at each end into short pieces of polypropylene tubing (McMaster Carr 5392 K335) using an epoxy glue (McMaster Carr 66215 A21). One end of the modules was sealed, the other connected to a peristaltic vacuum pump drawing the raw water through the membranes in an 'outside-in' direction.

3.2.3 Microfiltration protocol

A fresh microfiltration membrane module was used in each experiment. Microfiltration runs followed a pre-established filtration protocol (Figure 3-2). In a 100 mL graduated cylinder, the membrane module was first wetted in a 1:2 isopropanol-water mixture for 60 minutes at a permeate flow rate of about 36 gfd (61 L m^{-2} h^{-1}), the permeate being

recycled as feed. The module was then briefly submerged in another graduated cylinder containing MilliQ water to remove large excesses of alcohol. The module was subsequently transferred to the 10 mL filtration column where it was rinsed with MilliQ water for 20 hours at a permeate flow rate of also 36 gfd (61 L m^{-2} h^{-1}) with the permeate going to waste. At the end of the 20 hours, permeate DOC was measured and rarely exceeded 0.3 mg/L. Specific flux was also measured throughout the MilliQ water rinse and usually declined by 10% or less over 20 hours. At this stage, the membrane was relatively clean of DOC, well equilibrated with water and had a well defined clean membrane specific flux of 50-60 gfd/psi (1230-1480 L m^{-2} h^{-1} bar^{-1}). The filtration of the water of interest could then be carried out. Filtration was conducted at a permeate flow rate of 36 gfd (61 L m^{-2} h^{-1}) for a total of 200 minutes. The filtration was interrupted by 2 backwashes, one at 120 minutes, another at 180 minutes. Backwashes consisted in a reversal of the flow through the membrane and were conducted at 36 gfd (61 L m^{-2} h^{-1}) for 1 minute using permeate previously produced by the membrane. The backwash established how much of the fouling was hydraulically reversible and irreversible. Permeate samples were collected throughout the filtration and the permeate concentration of the model compound determined using the appropriate assay.

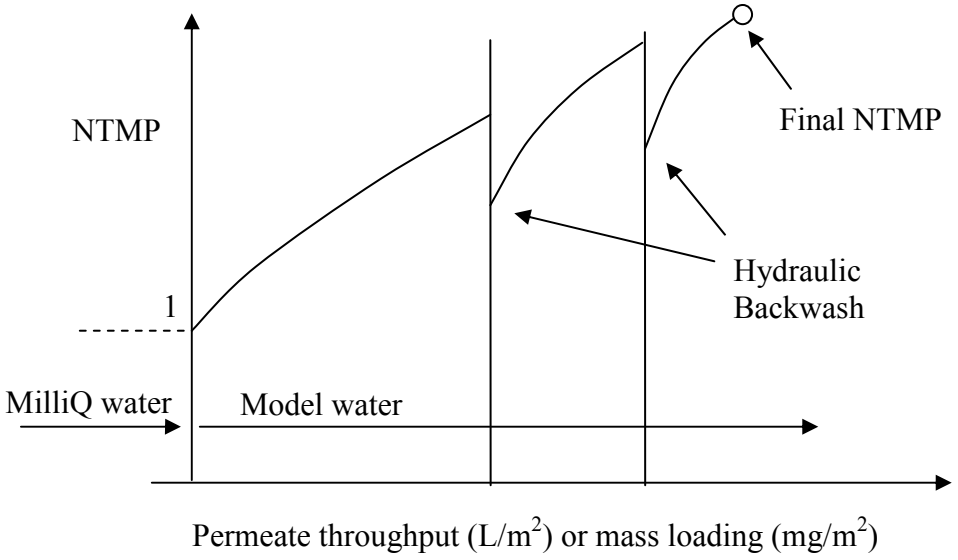


Figure 3-2. Microfiltration protocol used for the filtration of model compounds.

The membrane system was operated at a constant flow rate (2 mL/min); fouling was therefore measured as an increase of transmembrane pressure over the course of the filtration (Figure 3-2). Clean membrane transmembrane pressures were in the range of 0.6-0.7 (psi). Values of the transmembrane pressure were normalized to the clean membrane transmembrane pressure to yield the normalized transmembrane pressure, NTMP, calculated as follows:

$$NTMP = \frac{\Delta P}{\Delta P_o} \tag{3.1}$$

where ΔP_o represents the clean membrane transmembrane pressure at the end of the MilliQ water rinse and ΔP represents the transmembrane pressure during the filtration experiment. The use of NTMP allows the comparison of different filtration runs where the clean membrane transmembrane pressure varies slightly. Increases in NTMP during a filtration can be understood as increases in resistance. An experiment in which NTMP remains close to 1 is one in which little fouling occurs.

The model waters filtered consisted of 5 or 25 mg/L of the model compounds, 0.1 mM of buffer, and either sodium chloride (NaCl) or calcium chloride ($CaCl_2.2\ H_2O$) in varying amounts. Buffer and salt were added from concentrated stock solutions. The model waters ranged in pH from 4 to 10 and were buffered using three different buffers: sodium acetate, bicarbonate, and borate. The model waters were made the day before the filtration experiment and allowed to equilibrate for 20 hours. Turbidity of the model water was measured at the end of the equilibration period and used as a qualitative indication of destabilization/aggregation of the model compound. For each model compound, solution conditions were selected to range from conditions where the model compound would be expected to remain dissolved and stable [$pH > pH_{iep}$, low I] to conditions where the model compound might aggregate/precipitate [$pH \approx pH_{iep}$, high I, presence of calcium].

3.2.4 Electron microscopy of fouled membranes

Scanning electron microscopy (SEM) analysis of fouled membranes was conducted at the department of Material Sciences & Engineering at the Johns Hopkins University (http://www.jhu.edu/~matsci/facilities/). After a filtration run, membrane fibers were air dried overnight. Plane and cross sectional views of the fibers were imaged. Cross sectional views of the membrane fibers were produced by suspending a fiber in liquid nitrogen for a brief period of time and fracturing it. The fractured fiber was then mounted vertically onto the microscope stage.

TEM analysis was conducted at the Johns Hopkins Integrated Imaging center (http://www.jhu.edu/iic/). The following procedure was followed. Sections of the fibers

were fixed in 3% formaldehyde + 1.5% glutaraldehyde contained in 0.1M Na cacodylate, pH 7.4, 5 mM $CaCl_2$, 5 mM $MgCl_2$, and 2.5% sucrose for 1 hour at 22°C with gentle agitation and subsequently cut into small pieces (~2 mm in length). The pieces were then post-fixed in 1% OsO4/1% potassium ferrocyanide contained in 0.1M cacodylate, pH 7.4 for 1hr at room temperature, en bloc stained in Kellenberger's uranyl acetate (UA) overnight, dehydrated through a graded series of ethanol and subsequently embedded in Spurr resin. Sections were cut on a Reichert Ultracut UCT ultramicrotome, post stained with UA and lead citrate and observed on a Philips TEM 420 at 100kV. Images were recorded with a Soft Imaging System Megaview III digital camera and figures were assembled in Adobe Photoshop 7.0 with only linear adjustments in brightness and contrast.

3.3 RESULTS

Results of the microfiltration experiments are summarized in Table 3-1. The first six columns in the table identify, for each run, the model compound and its concentration as well as the solution condition tested. The next column reports the turbidity of the model water after the equilibration period. The last four columns describe the extent of membrane fouling by the model compound and the corresponding removal of the model compound by the membrane. Total fouling is represented by the value of the NTMP at the end of each filtration run (Final NTMP). Reversible fouling is expressed as the average percentage of total fouling that was reversed by the two hydraulic backwashes in each run. Finally, total removal of the model compound at the end of each run in units of mg/m^2 is reported and a qualitative indication given of the occurrence of a model compound breakthrough in the permeate (yes, no, partial).

With dextran [runs 18-20], removal was undetectable and negligible fouling occurred under all solution conditions tested. Turbidity measurements of the equilibrated dextran solutions remained low, indicating that no significant aggregation/precipitation of the dextran molecules occurred.

Alginic acid [runs 14-17] was removed completely in the presence of 3.3×10^{-3} M calcium [run 16]. In contrast, no removal of alginic acid was observed under the other solution conditions employed [runs 14, 15, 17]. Fouling was moderate in the absence of calcium and more substantial in the presence of 3.3×10^{-3} M calcium. Fouling was largely irreversible. Compared to the other solutions, increased turbidity in the presence of calcium suggests that precipitates or aggregates of alginic acid were formed in this case.

Removal of BSA was observed under all conditions tested [runs 1-6], increasing with increasing ionic strength and at the pH_{iep} of the protein [pH 4.7]. Nevertheless, fouling was modest under all conditions tested. Fouling that did occur was irreversible. Some experiments at BSA concentrations of 5 mg/L did not show a protein breakthrough over the duration of a run [runs 3 and 4]. Breakthrough was observed in runs conducted at 25 mg/L of BSA and the same solution conditions [runs 5 and 6], but fouling remained modest. Turbidity measurements of equilibrated BSA solutions suggest that no significant precipitation/aggregation of BSA occurred.

Substantial removal of gamma globulin was observed under all conditions tested [runs 7-13]. Fouling was modest for all runs at gamma globulin concentrations of 5 mg/L except at the pH_{iep} of the protein [run 10]. By contrast, fouling was substantial in the runs at 25 mg/L [runs 11-13], and particularly severe at the pH_{iep} of the protein or in the presence of 3.3×10^{-3} M calcium at pH 10 [run 12 and 13]. In addition, fouling was primarily irreversible. Turbidities of the equilibrated suspensions suggest varying degrees of precipitation/aggregation of the gamma globulin depending on the chemistry of the solution. Fouling showed similar results with the solution having the highest turbidity causing the most total fouling.

Increases in NTMP over the course of the filtrations followed two general patterns. Solution conditions under which the model compounds remained stable [i.e. low turbidity] displayed increases in pressure that remained steady or slowed down over time (Figure 3-3 and 3-5). In these cases, breakthrough of the model compounds occurred early during the filtration and removal was limited. In contrast, solution conditions under which the model compounds aggregated [i.e. elevated turbidity] displayed increases in pressure that worsened over time (Figure 3-4 and 3-6). In these cases, breakthrough of the model compounds occurred late in the filtration or not at all.

Table 3-1. Fouling of a hydrophobic PVDF microfiltration membrane by model compounds under varying solution conditions.

Run	Model compound	Concentration (mg/L)	pH	Ionic strength	[Ca] (mol/L	Turbidity (ntu) [1]	Final NTMP	Fouling reversible by BW (%)	Total removal (mg/m^2) [2]	Break-through
1	BSA	5.2	7	0.001	0	0.14	1.09	0	1.15	Y
2	BSA	5.2	7	0.01	0	0.13	1.10	0	1.76	Y
3	BSA	5.2	7	0.01	0.0033	0.13	1.08	0	2.37	N
4	BSA	5.2	4.7	0.001	0	0.17	1.07	0	2.19	N
5	BSA	25.0	7	0.01	0.0033	0.20	1.22	3	4.50	Y
6	BSA	26.0	4.7	0.001	0	0.19	1.34	0	3.67	Y
7	γ globulin	5.4	10	0.001	0	0.23	1.57	0	1.80	Partial
8	γ globulin	5.0	10	0.01	0	0.20	1.63	0	2.27	N
9	γ globulin	5.0	10	0.01	0.0033	0.18	1.58	0	2.38	N
10	γ globulin	5.2	7	0.001	0	0.39	2.57	9	2.48	N
11	γ globulin	24.6	10	0.01	0	0.40	3.90	2	4.64	Y
12	γ globulin	25.8	10	0.01	0.0033	0.51	11.79	9	6.39	Y
13	γ globulin	24.2	7	0.001	0	1.5	13.40 [3]	14	5.71	N
14	Alginic acid	4.8	7	0.001	0	0.16	1.85	7	0.00	Y
15	Alginic acid	5.6	7	0.01	0	0.17	1.48	2	0.00	Y
16	Alginic acid	5.0	7	0.01	0.0033	0.32	3.99	7	2.15	N
17	Alginic acid	5.2	4	0.001	0	0.17	2.12	3	0.00	Y
18	Dextran	4.8	7	0.001	0	0.13	1.05	0	0	Y
19	Dextran	5.2	7	0.01	0	0.13	1.04	0	0.04	Y
20	Dextran	5.0	7	0.01	0.0033	0.14	1.04	0	0.08	Y

(1) turbidity of the model water after 20 hr equilibration

(2) removal is computed from the measured permeate concentration and the membrane BET surface area

(3) filtration was stopped at 100 minutes because transmembrane pressure were exceeding the limitation of the filtration apparatus

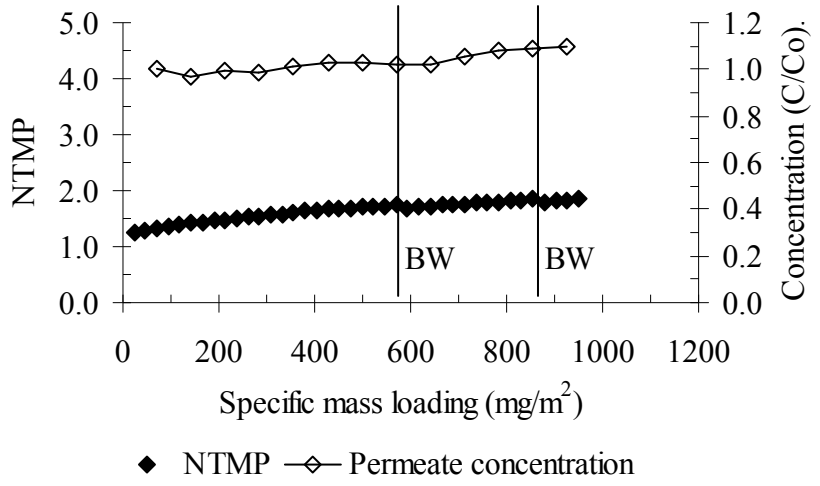


Figure 3-3. NTMP and permeate concentration as a function of mass loading during the filtration of 4.8 mg/L alginic acid at pH 7 and I = 0.001.

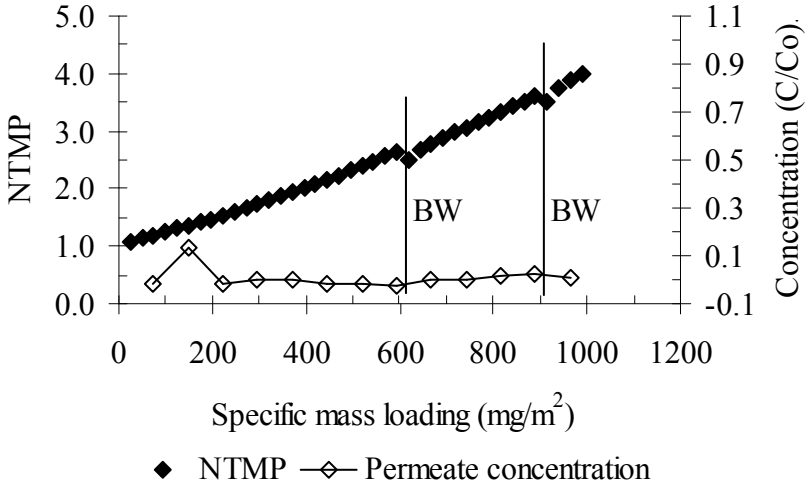


Figure 3-4. NTMP and permeate concentration as a function of mass loading during the filtration of 5 mg/L alginic acid at pH 7 in the presence of 3.3 x 10^{-3} M calcium (I = 0.01).

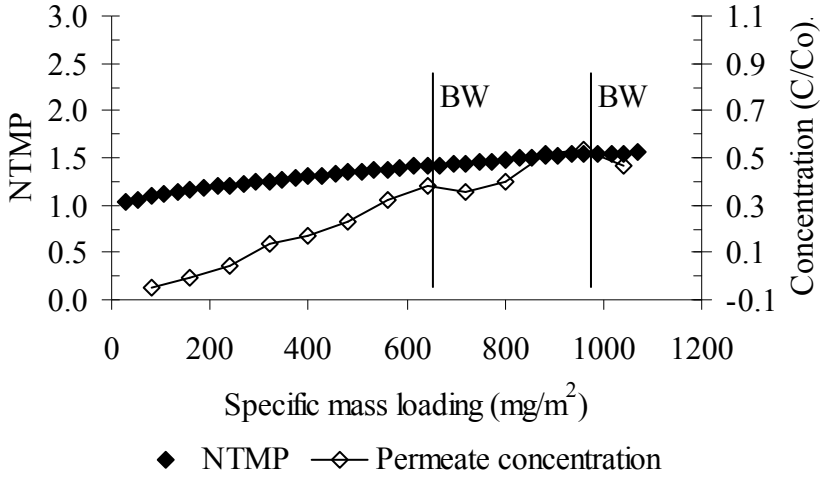


Figure 3-5. NTMP and permeate concentration as a function of mass loading during the filtration of 5.4 mg/L gamma globulin at pH 10 and I = 0.001.

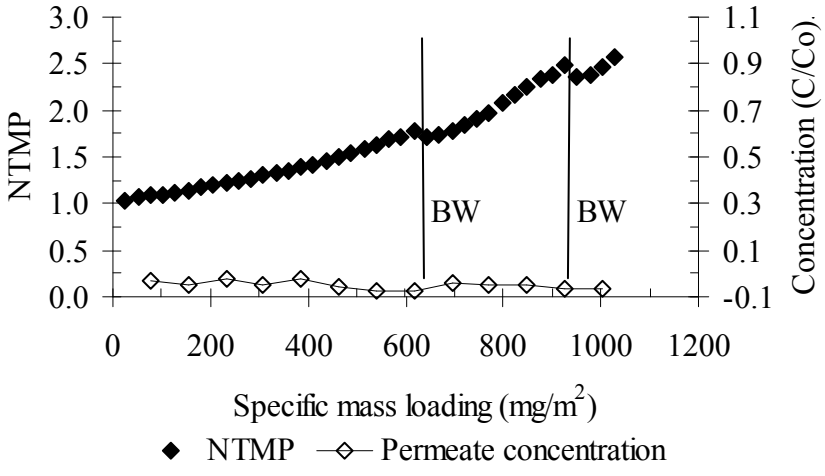


Figure 3-6. NTMP and permeate concentration as a function of mass loading during the filtration of 5.2 mg/L gamma globulin at pH 7 and I = 0.001.

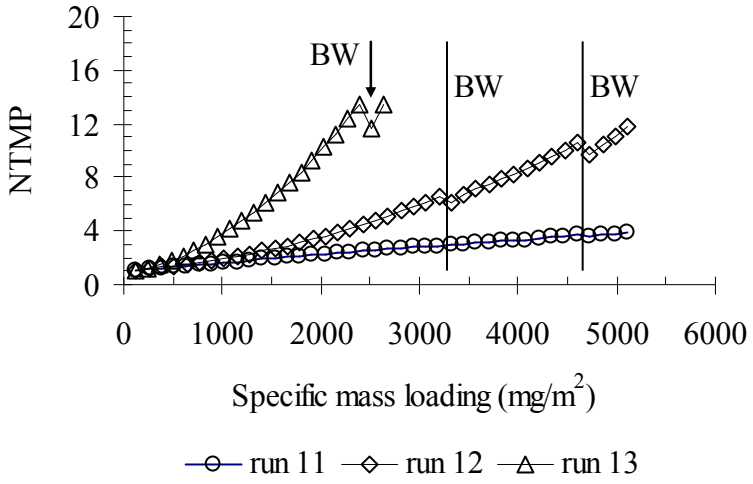


Figure 3-7. NTMP as a function of mass loading during the filtration of 25±0.8 mg/L gamma globulin at pH 10 and I = 0.01 (run 11), pH 10 and I = 0.01 as $CaCl_2$ (run 12), pH 7 and I = 0.001 (run 13).

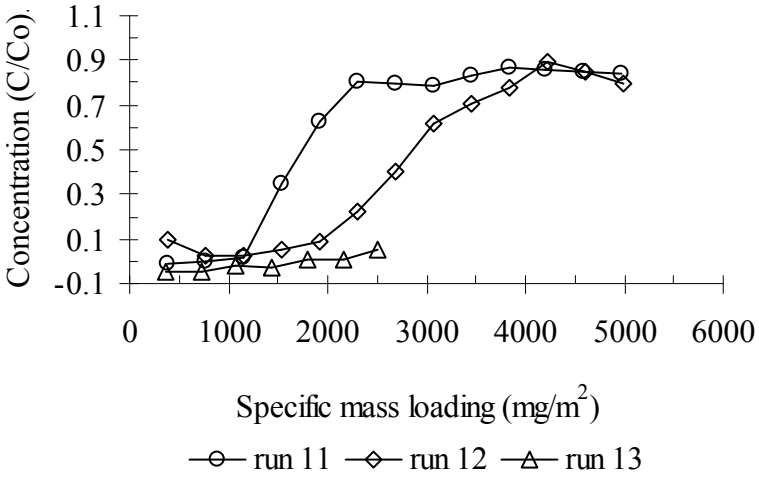


Figure 3-8. Permeate concentration as a function of mass loading during the filtration of 25±0.8 mg/L gamma globulin at pH 10 and I = 0.01 (run 11), pH 10 and I = 0.01 as $CaCl_2$ (run 12), pH 7 and I = 0.001 (run 13).

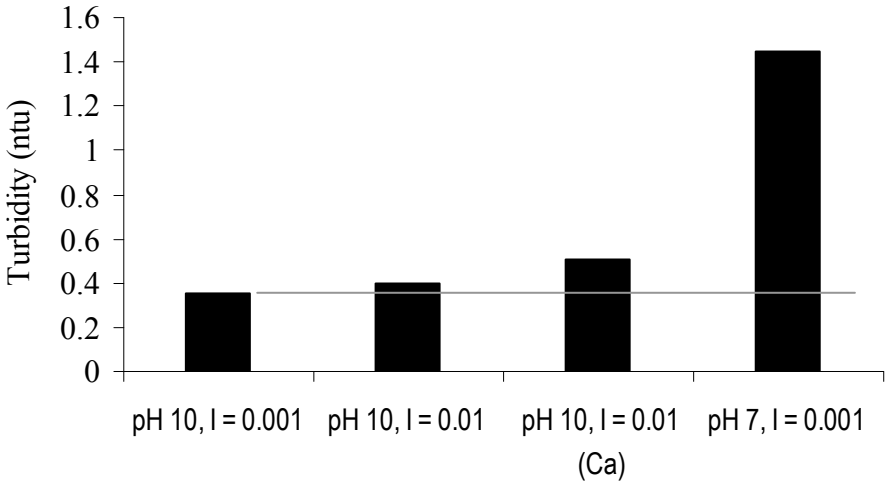


Figure 3-9. Turbidity of solutions containing 25 mg/L gamma globulin under varying solution conditions after 20 hrs of equilibration.

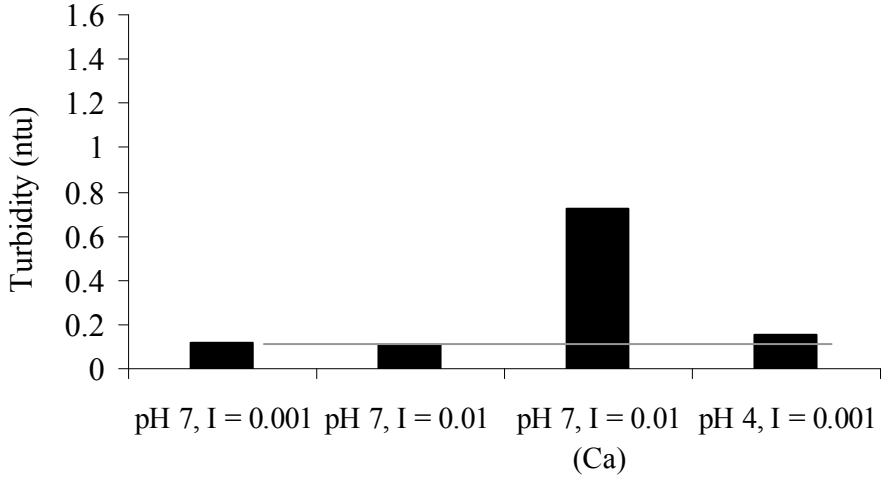


Figure 3-10. Turbidity of solutions containing 25 mg/L alginic acid under varying solution conditions after 20 hrs of equilibration.

Additional experiments were conducted in which the model compounds at a concentration of 25 mg/L were equilibrated for 20 hours under the solution conditions described in Table 3-1 and the turbidity of the solutions measured at the end of this equilibration period. BSA and dextran showed low turbidities for all conditions tested, indicating that these compounds remained stable, even at the higher concentrations. Alginic acid showed a substantial increase in turbidity in the presence of 3.3×10^{-3} M calcium (Figure 3-10). Gamma globulin displayed increasing turbidity in the order: pH = 10, I = 0.001 < pH = 10, I = 0.01 < pH = 10, I = 0.01 ($CaCl_2$) < pH = 7, I = 0.001 (Figure 3-9). Fouling caused by the gamma globulin under similar conditions followed the same order with the solution having the highest turbidity causing the most total fouling (Figure 3-7 and 3-8).

Observation of foulants on the membranes using electron microscopy was difficult. Moreover, clear observations were only possible when fouling was significant and aggregation had seemingly taken place. Electron microscopy images of two membrane fibers fouled under such conditions are shown in Figure 3-11. These fibers were severely fouled by solutions containing gamma globulin and alginic acid under the following conditions: 25 mg/L gamma globulin at pH 10 in the presence of 3.3×10^{-3} M calcium (Figure 3-11A) and 5 mg/L alginic acid at pH 7 in the presence of 3.3×10^{-3} M calcium (Figure 3-11B). A 0.5 μm thick layer of alginic acid is clearly visible on the external surface of the fiber in figure 3-11B. It was not apparent that alginic acid had accumulated within the porous structure of the membrane however. Gamma globulin aggregates are observed on the external surface of the fiber in figure 3-11A, and, although difficult to

see, there was some indication that the gamma globulin had penetrated the porous structure.

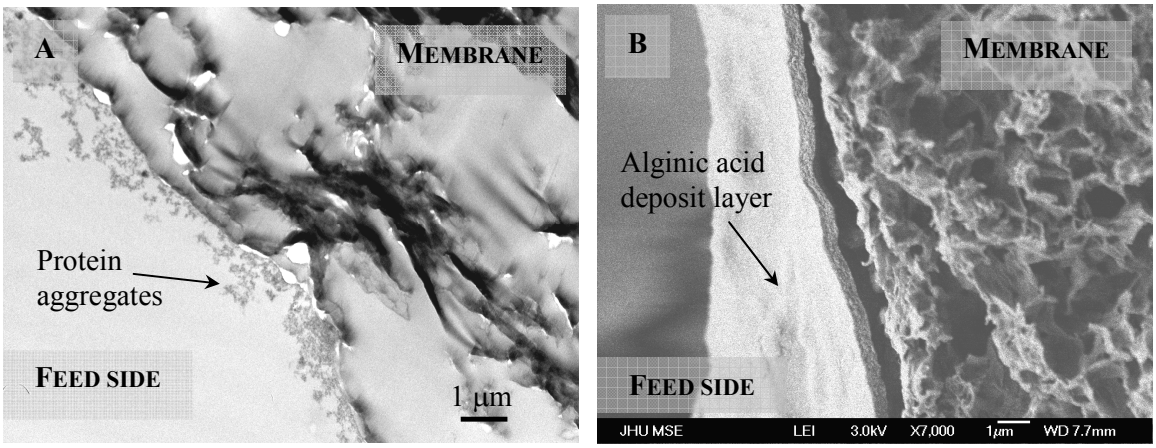


Figure 3-11. (A) TEM image of a 0.1 μm PVDF microfiltration membrane fouled by 25 mg/L of gamma globulin at pH 10 in the presence of 3.3×10^{-3} mol/L Ca. (B) SEM image of a 0.1 μm PVDF microfiltration membrane fouled by 5 mg/L of alginic acid at pH 7 in the presence of 3.3×10^{-3} mol/L Ca.

Backwashes operated at 61 (L m^{-2} h^{-1}) were ineffective in reducing the fouling caused by the various model compounds. However, one could argue that the flow rate used was too low. An additional experiment was therefore conducted in which a membrane fouled by 5 mg/L alginic acid in the presence of calcium was subjected to backwashes with increasing flow rates: 60, 120, and 180 (L m^{-2} h^{-1}). The results of this experiment are shown in Figure 3-12. In the figure, arrow 1 indicates where filtration was interrupted in order to sample the solution in the filtration column and test the concentration of alginic acid in this solution. Arrows 2 through 4 indicate where backwashes were conducted, in order of increasing flow rates. The concentration of alginic acid in the backwash water was also measured. Finally, arrows 5 and 6 indicate an attempt to chemically clean the

membrane with a solution containing sodium hypochlorite (200 mg/L as available chlorine). This cleaning consisted in filling the filtration column with the cleaning solution and letting the membrane soak in this solution for some time (1.5 hour for arrow 5 and 14 hours for arrow 6). After this time had elapsed, the column was emptied and refilled with MilliQ water. Filtration was then resumed with MilliQ water at 60 (L m^{-2} h^{-1}) for 30 minutes to establish how much of the fouling had successfully been reversed.

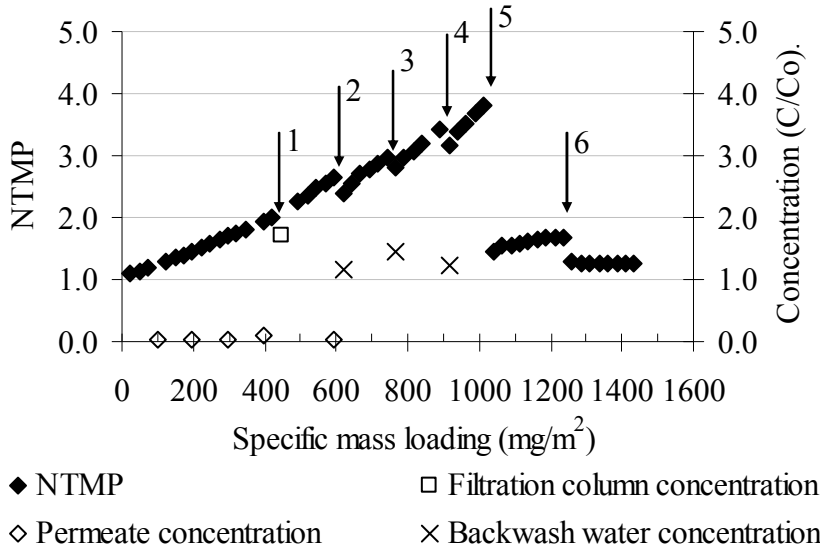


Figure 3-12. Physical and chemical cleaning of a hydrophobic PVDF microfiltration membrane fouled by a solution containing 5 mg/l alginic acid and $3.3x10^{-3}$ M calcium. Arrow 1: interruption of filtration to measure the concentration of alginic acid in the filtration column. Arrows 2-4: backwashes with increasing flow rates of 60, 120, and 180 (L m^{-2} h^{-1}). Arrows 5 and 6: soaking of fouled membrane for 1.5 and 14 hours in NaOCl (200 mg/L as free chlorine) solution.

As shown in Figure 3-12, increasing the flow rate of the backwash did not improve the reduction of fouling and the concentrations of alginic acid in the backwash waters were low. Under the given conditions, alginic acid fouled the membrane by building up a deposit layer on the membrane surface which could not be removed by hydraulic

backwash even at the high flow rates. By contrast, soaking in the NaOCl solutions appeared to reduce fouling significantly. NTMP had decreased to about 1.5 after a soak of 1.5 hours and to about 1.25 after a soak of 14 hours.

3.4 DISCUSSION

3.4.1 Fouling mechanisms

The combination of the model compound removal data and the patterns of increases in NTMP gives some indication of what the acting fouling mechanisms were for each compound under the different solution conditions tested. Dextran was simply not removed by the membrane and therefore did not foul. Alginic acid severely fouled the membrane when it aggregated in the presence of 3.3×10^{-3} M of calcium. In this case the fouling mechanism involved the obstruction of pores by the aggregates which led to the formation of a cake or gel layer on top of the membrane. Under the other solution conditions, removal was undetectable yet fouling was moderate. As alginic acid is notoriously difficult to dissolve, it is possible that an undetectable fraction of aggregates were present in these solutions which caused the observed fouling.

Fouling by BSA was limited. Its removal was high but culminated in a breakthrough for all the conditions tested. Total removal approached or slightly exceeded that of mono layer coverage [57]. It therefore appears that BSA fouled the PVDF membrane predominantly by a process of adsorption throughout the membrane pore space which caused a slight constriction of pores. As adsorption approached mono-layer coverage, fouling slowed down and total fouling was limited.

The removal of gamma globulin was high but gamma globulin was also susceptible to varying degrees of aggregation as the solution conditions were changed. Under stable condition [pH = 10, I = 0.001], gamma globulin behaved in a similar way to BSA and removal approached that of mono layer coverage [58]. It is likely that in this case, gamma globulin fouled the membrane via a process of pore constriction driven by internal adsorption. When aggregation was important [pH = pH_{iep} = 7], fouling was extremely severe and removal was total. In this case, the fouling mechanism likely involved the rapid obstruction of membrane pores by protein aggregates. Whether the initial pore obstruction occurred internally or externally is unknown; it may have started with a combination of the two and evolved into the external buildup of a cake layer. When aggregation occurred to a moderate extent [pH = 10, I = 0.01] and concentrations were not too low [25 mg/L], fouling was still relatively severe but breakthroughs were observed (Figure 3-7 and 3-8). Under these conditions, a combination of two simultaneous processes may have occurred: the adsorption of native proteins onto the membrane and the obstruction of pores by protein aggregates. The breakthrough was delayed when ionic strength was increased, more with calcium than sodium, probably as a result of the shielding of electrostatic repulsion between the proteins which allowed closer packing of the proteins on the membrane. In these experiments, the severe fouling was probably caused by the obstruction of pores by protein aggregates more than by the adsorption of native proteins.

The findings in this study with BSA are contradictory to a number of previous reports that attributed fouling by BSA to the deposition of BSA aggregates on membranes [47,

51]. These previous studies used BSA at high concentrations (≥100 mg/L and usually in the g/L range) however. If aggregates are naturally present in all BSA suspensions, the use of high BSA concentrations during filtration might have exposed the membranes to large amounts of them in a short period of time and caused rapid significant fouling. In addition, the aggregation of BSA might be promoted when BSA concentration is high. Low concentrations (≤ 25 mg/L) were used here because they are more relevant to natural surface waters. Operating conditions such as temperature, cross-flow velocity, and concentration have been shown to affect the aggregation of BSA during ultrafiltration [59].

On the other hand, the present finding that the occurrence of aggregates (gamma globulin or alginic acid) in a solution can control the extent of a microfiltration membrane fouling is consistent with many other studies that investigated membrane fouling by model compounds, including proteins at high concentrations (≥ 100 mg/L and usually in the g/L range) [47-51] and alginic acid at medium concentrations (≥ 25 mg/L) [52, 53]. The use of low concentrations (≤ 25 mg/L) in the present work highlights the influence of solution condition on the aggregation of the model compounds and its corresponding impact on membrane fouling.

3.4.2 Relationship between removal and fouling

On the basis of the total removal of the compound during filtration, it appears that proteins (runs 1-13) interacted much more favorably with the hydrophobic PVDF membrane than polysaccharides (runs 14-20) did. The only notable exception was alginic

acid at pH 7 in the presence of calcium where aggregation of the alginic acid took place and large amounts of the alginic acid deposited on the membrane surface.

The reason proteins were removed to a higher extent than polysaccharides may be related to the structure of proteins and the types of interactions that exist within them. Proteins are made up of amino acids which can be divided into two groups: amino acids with non polar "hydrophobic" groups and amino acids with "hydrophilic" polar groups [60]. The amino acids within a protein interact with one another as well as the surrounding environment to determine the shape of the protein. The conformation of a protein is controlled by its primary structure (amino acid sequence), which impacts its secondary structure (α helix, β sheet, and random coil arrangements) and its tertiary structure (folding of the whole peptide chain) [60, 61]. Upon contact, hydrophobic groups exposed on the external surface of a protein favorably interact with a sorbent surface and promote adsorption. [62] Adsorption also causes the environment of a protein to change which may alter the balance of interactions that hold its shape. [62] As a result, the protein may unfold and allow exposure of normally shielded hydrophobic groups to the sorbent surface. These types of interactions may explain why proteins were removed more than polysaccharides.

Because proteins interacted more favorably with the membrane surface, one would have expected that proteins fouled the membrane more than polysaccharides. This was not always the case. For example, alginic acid showed poor removal during filtration in the absence of calcium but exhibited moderate fouling. BSA, on the other hand, was

generally removed to a greater extent than alginic acid was but fouled the membrane slightly. Even within a class of compounds such as proteins, slight differences in the extent of removal were associated with significant changes in the extent of fouling; gamma globulin removal was slightly higher than for BSA but the fouling it caused was significantly greater. This lack of correlation between removal and the extent of fouling may arise from differences in the fouling mechanisms in which the compounds participated. Under the conditions tested, aggregation affected both alginic acid and gamma globulin but not BSA. Constriction or obstruction of a pore is likely more severe with an aggregate than it is with the adsorption of native macromolecules onto the pore walls. As a result, the removal by the membrane of a few aggregates may cause more fouling than comparable quantities of the native macromolecule.

3.4.3 Implications for microfiltration of natural surface waters

In the context of surface water treatment, concentrations of dissolved organic matter are typically in the mg/L range [6]. This work shows that proteins and polysaccharides, even at these low concentrations, can cause significant irreversible fouling of a hydrophobic PVDF microfiltration membrane, especially if the solution condition is such that the compounds aggregate. Note that by the end of run 12 in Figure 3-8 (25 mg/L gamma globulin, pH = 10, [Ca] = 3.3 x 10^{-3} mol/L), protein removal had decreased to approximately 10% but pressures were still rising sharply. This removal is equivalent to about 2.5 mg/L of gamma globulin or 1.25 mg C / L if one assumes that the protein contained 50 % carbon. Bearing in mind that the filtration experiments conducted here lasted only 5 hours and that a treatment plant has to operate continuously, the implication of these results is that the bulk of the fouling in a full scale plant of a microfiltration

membrane similar to the one used here may be caused by a very small fraction of the feed organic matter. Of course, these results apply to a hydrophobic PVDF microfiltration membrane. Results with other membranes may differ.

An important finding is that fouling seen with the model proteins and polysaccharides was almost exclusively irreversible to hydraulic backwash, regardless of solution condition and fouling mechanism. The experiment conducted with alginic acid in the presence of calcium furthermore suggests that the use of a high backwash flow rate would not improve the reversal of fouling appreciably. It is inferred that the interactions that exist between the foulants and the membrane are much stronger than the forces that can be generated by flowing backwash water. By contrast, the use of a cleaning solution such as NaOCl may be more effective. NaOCl can oxidize organic foulants but may also encourage their desorption from the membrane owing to the elevated pH of the solution. As previously noted, changes in chemical condition during backwash to alter the foulants or lower their interaction with the membrane may be desirable and improve the efficiency of backwash [16]. Another option to reduce fouling might involve pre-treatment processes such as coagulation that intercept or alter foulants before they reach the membrane.

While the important contribution to membrane fouling by hydrophilic organic matter found in surface waters was previously recognized, this work is the first of its kind to reproduce these findings with model compounds at relatively low concentrations ($\leq$ 25 mg/L) and recognize the importance of the solution chemistry in this process. Solutions

containing some of these model compounds under unstable solution conditions (e.g., alginic acid in the presence of calcium, gamma globulin at pH 7) might therefore represent a good surrogate for hydrophilic organic matter foulants naturally found in surface waters and consequently be used to test different approaches that could be taken to reduce the fouling they cause. Such approaches might include coagulation pre-treatment, various forms of chemical cleaning and the use of different membrane material (hydrophilic vs. hydrophobic) or membrane surface modifications (e.g., hydrophilic PVDF). The membranes used in this work were made of hydrophobic PVDF. Under similar conditions, hydrophilic membranes may not foul as much as the hydrophobic PVDF membrane tested here because the attachment of organic matter might be expected to be less on hydrophilic membranes compared to hydrophobic ones.

3.5 CONCLUSIONS

Solutions containing low concentrations of proteins and polysaccharides (5-25 mg/L) can foul a hydrophobic PVDF microfiltration membrane (pore size = 0.1 μm) significantly but the extent of fouling is strongly dependent on the identity of the protein or polysaccharide and the chemistry of the solution (e.g., pH, Ca). Under chemical conditions maintaining the stability of the organic compounds in solution, the compounds may either pass through the membrane (polysaccharides) or adsorb onto the membrane pore walls (proteins). The stable compounds that pass through the membrane do not foul while those that adsorb within the membrane porous structure only cause limited fouling by pore constriction. Fouling by the latter phenomenon is limited because the molecules are quite small (≤150 kD) relative to the membrane pore size (0.1 μm). Chemical conditions that destabilize the organic compounds in solution, on the other hand, result in

significant fouling likely due to the obstruction or severe constriction of pores by aggregates that form in solution. This fouling may occur internally within the membrane porous structure or externally on the membrane surface.

When fouling by proteins and polysaccharides occurs, this fouling is largely irreversible to hydraulic backwash. The use of chemical cleaning agents to alter the interaction between foulants and the membrane and/or degrade the foulants may therefore be necessary to restore the membrane permeability after such fouling.

On the basis of these experiments, it is likely that algal exudates containing proteins and polysaccharides hold the potential to cause significant fouling of a hydrophobic PVDF microfiltration membrane.

4 FOULING OF A HYDROPHOBIC MICROFILTRATION MEMBRANE BY THREE DIFFERENT ALGAE AND THEIR ASSOCIATED ALGAL ORGANIC MATTER

4.1 INTRODUCTION

Microfiltration is a process increasingly used in drinking water treatment for its ability to remove turbidity and pathogens. Fouling of microfiltration membranes, however, remains a problem in practice. As filtration progresses, foulants are removed by the membrane which lower its permeability. The natural organic matter (NOM) found in surface waters, in particular, has been identified as a major contributor to membrane fouling. Although algae have been shown to release considerable quantities of organic matter over growth and decline [13], the fouling caused specifically by organic matter of algal origin (algal organic matter or AOM), has received little attention.

Some studies have indicated that the fouling of microfiltration membranes by algae and AOM can be significant. A few studies have examined the fouling of several microfiltration membranes by the green alga, *Chlorella [43, 63, 64]*. These studies focused on the fouling caused by the accumulation of algal cell cakes on the membrane surface and indicated that the resistance of these cakes may be related to their compressibility and the presence of organic matter associated with the cells. Conditions unfavorable for algal growth (excessive light, cold or hot temperatures or nutrient limitations), in particular, are said to affect the quantity and composition of the organic matter associated with the cells and impact the resistance of the cakes [43]. Another study has investigated the fouling of microfiltration and ultrafiltration membranes by AOM

obtained by breaking down cells of blue-green algae (cyanobacteria) sampled from a lake [14]. This study indicated that the algal extract was rich in protein and polysaccharide-like substances and that fouling was likely caused by organic matter present as macromolecules and more importantly colloids. Nevertheless, a number of important issues related to fouling by algae and AOM have not been addressed. These issues include the relative impact on fouling of the algal cells compared with dissolved AOM, the variability in fouling which might be expected with different algal species, and the reversibility of algal fouling to hydraulic backwash.

As noted earlier in chapter 1, the term "algae" will be used throughout this work to refer to different photosynthetic microorganisms, including eukaryotes and prokaryotes (cyanobacteria). In this work, the fouling of a hollow fiber microfiltration membrane by three species of algae and their exudates as a function of growth phase was tested. The algae were obtained from two different culture collections and grown in defined media under controlled conditions. During growth and decline, the algal cultures were monitored for pH, algal counts, nitrate and phosphate concentrations, DOC concentration, protein concentration, carbohydrate concentration and bacterial counts. As each alga advanced through different stages of growth and decline, samples of the cultures were taken, diluted, and filtered through a hydrophobic hollow fiber microfiltration membrane whose fouling was measured.

The objectives of this work were as follows: (1) to determine the extent of membrane fouling that results from the microfiltration of three different algae at different phases of

growth and decline, (2) to determine whether fouling by algae is caused predominantly by AOM or by the algal cells, and (3) to determine the reversibility of fouling caused by algae and AOM to hydraulic backwash.

4.2 MATERIALS AND METHODS

4.2.1 Algal species

Three species were selected to represent three important classes of algae found in surface waters: *Scenedesmus quadricauda* (a chlorophyceae), *Asterionella formosa* (a bacillarophyceae) and *Microcystis aeruginosa* (a cyanophyceae). *Scenedesmus* (UTEX 76) was obtained from the Culture Collection of Algae at the University of Texas at Austin (www.utex.org) while *Asterionella* (UTCC 605) and *Microcystis* (UTCC 124) were obtained from the University of Toronto Culture Collection (www.botany.utoronto.ca/utcc/).

Microcystis and *Asterionella* were obtained as small samples of liquid cultures that contained the specified alga and some bacteria (only form available) and *Scenedesmus* was acquired as an axenic culture grown on a slant. However, bacteria were detected in the cultures of all three algal species grown in the laboratory, even the *Scenedesmus*. Either the slant of *Scenedesmus* provided was not completely free of bacteria on arrival or contamination occurred during one of the many manipulations that were required during culturing in the laboratory. In any case, the algae were grown in the presence of bacteria. Although bacteria were present with the algae, every attempt was still made to prevent the introduction of additional bacteria in the cultures grown in the laboratory (autoclaving of culture vessels, use of sterilized media, sterile technique used for inoculation…). For

reasons that will be discussed later, it is also believed that algae and AOM largely dominated the results presented here.

Images under a light microscope of the three different algae are shown in Figure 4-1. *Scenedesmus* has an elongated elliptical shape, is between 10 and 15 μm long and usually formed colonies of 4 adjacent cells. *Microcystis* has a spherical shape, a diameter of approximately 2 μm and did not tend to form large colonies under the conditions used. Colonies of two or three cells of *Microcystis* were the most that was ever observed. *Asterionella* has the shape of a rod, 30 to 40 μm long. It readily formed colonies with one end of the rods attaching to each other and the other end radiating outwards, thus assuming the form of a star.

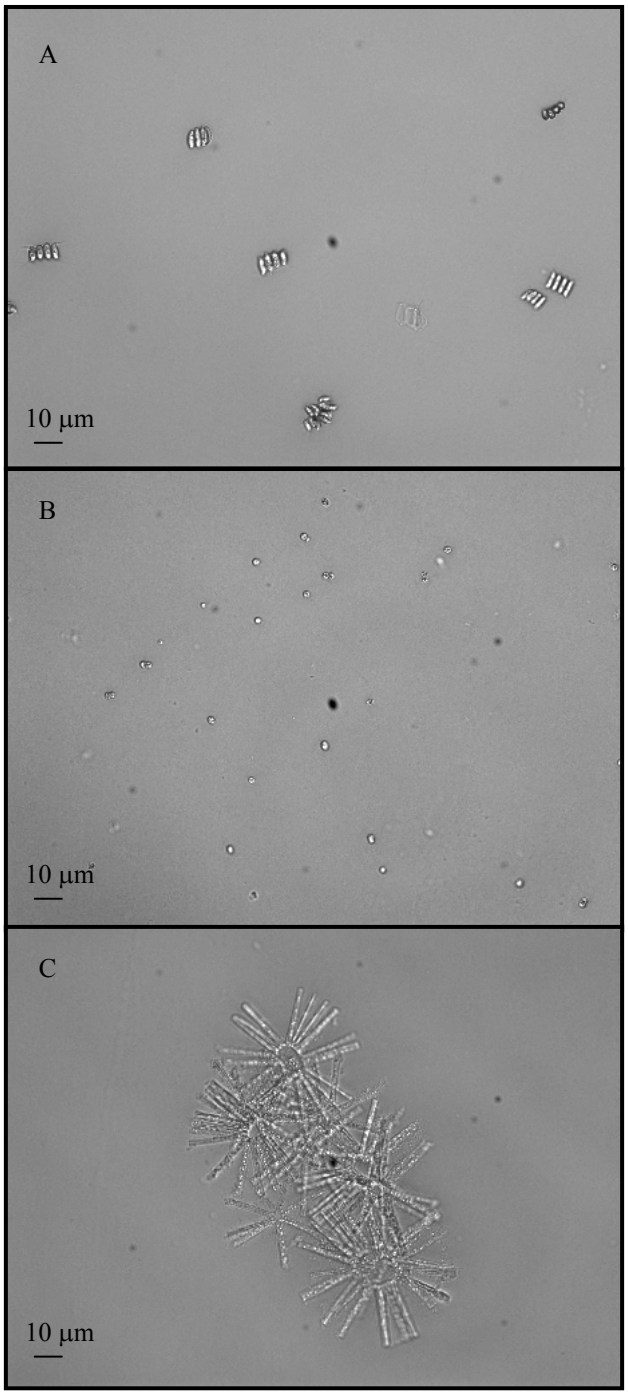


Figure 4-1. Light microscopy images of *Scenedesmus quadricauda* (A), *Microcystis aeruginosa* (B), and *Asterionella formosa* (C).

4.2.2 Growth media

Each algal species was grown in a different medium. *Scenedesmus* was grown on a form of the Woods Hole medium while *Asterionella* and *Microcystis* were grown on forms of the CHU-10 medium (CHU-10 for *Asterionella* and CHU-10* for *Microcystis*). These media are presented in the "Handbook of Phycological Methods" [65] and were only slightly modified for the purpose of this study. A description of the media and the procedures used to prepare them can be found in Appendix A. Common aspects of all three media are that they are completely defined media and that they contain moderate levels of inorganic nutrients (relative to other media) and low levels of dissolved organic carbon (from vitamins and EDTA). Calcium concentration was 2.5×10^{-4} (mol/L) for all three media. The ionic strength and pH of the three media ranged from 3 to12×10^{-3} and from 6.5 to 7.5, respectively. Moderate levels of major nutrients were desirable in order to observe nutrient depletion within a reasonable period of time and thus capture the different phases of growth of the algae. Maintaining low levels of dissolved organic carbon (DOC) in the original media was deemed essential since the focus of this work was on the relationship between AOM and membrane fouling and DOC was used as a measure of AOM production.

4.2.3 Culture

Small culture

Upon reception of the algae to the laboratory, small cultures of each alga were started in 30 mL test tubes. The test tubes (3 for each alga) were autoclaved, and then filled with approximately 7 ml of the appropriate sterile medium before being inoculated with a

small sample of the original cultures (loop from a slant or 0.5 mL from liquid culture). The algae were exposed to light supplied by two 40W fluorescent lamps (General Electric F40T12 PL/AQ ECO) mounted on a wall behind the tubes and four 20W fluorescent lamps (two General Electric F20T12 PL/AQ ECO and two Philips F20T12 / CW) mounted above. Full specifications for the lamps are provided in Appendix B. These lamps were turned on 14 hours and off 10 hours of everyday using a timer. While *Scenedesmus* and *Asterionella* grew well in the light, *Microcystis* grew better in low light conditions and was therefore placed further away from the light source. These small cultures were used as inocula to subsequent cultures. Test tubes (3 for each alga) containing sterile medium were inoculated about once a month using a small aliquot of a previously grown culture. Three tubes of each alga were inoculated in case contamination or die off in a tube occurred (contamination by a fungus occurred once). Each alga was grown through several cycles of these small cultures before a large culture was grown.

Large culture

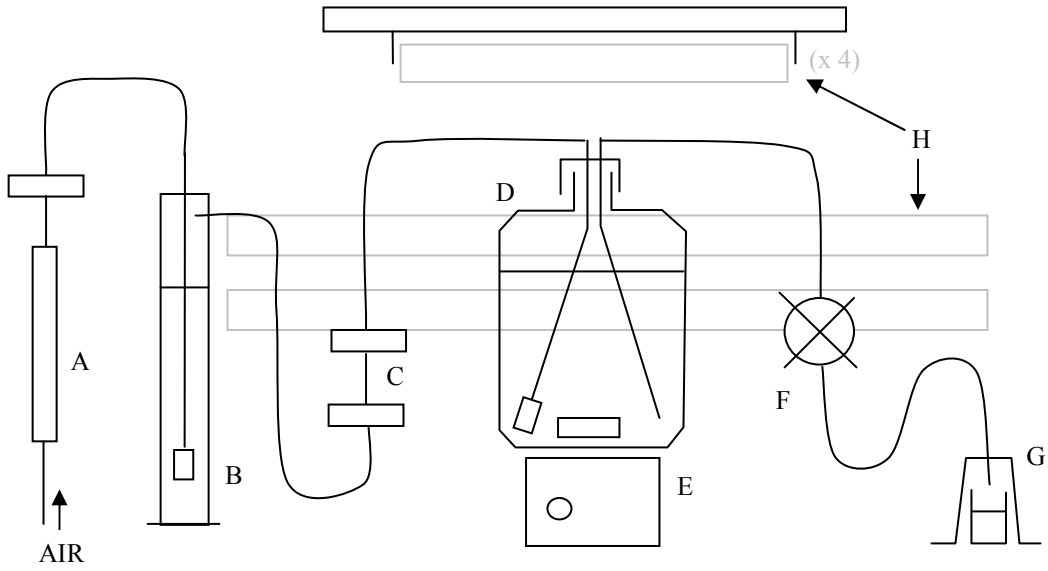
For the purpose of making microfiltration experiments, large cultures of the algae were necessary and therefore grown in reactors consisting of 2 L glass bottles. The reactor setup is shown in Figure 4-2. The setup comprised an aeration system (parts A through C) and a sampling system (parts F and G). All parts of the system past the air diffuser (part B) were autoclaved before sterile medium was added to the reactor. After the autoclaved reactor was filled with sterile medium, it was inoculated with a sample of alga from a small culture. The medium was continuously stirred using a magnetic stir bar and constant aeration was provided by an air diffuser connected to an air pump (of the kind

used for large aquariums). The air was passed through a carbon filter, saturated with water and filter-sterilized through two 0.2 μm polytetrafluoroethylene (PTFE) filters before reaching the reactor. Light was supplied using the same arrangement described previously for the small cultures. The reactor containing *Microcystis* was placed further away from the light source. Samples were withdrawn from the reactor using a sampling glass tube (2 mm ID, 4 mm OD) connected to a peristaltic pump via silicone tubing. Samples flowed from this pump to a sample bell under which a collecting vessel was placed. This method of sampling was designed to prevent contamination of the culture during the extraction of samples. Samples of 15 mL were collected in a 50 ml beaker for measurement of DOC, nitrate and phosphate concentrations, algal counts, protein concentration and carbohydrate concentration. Samples needed for bacterial counts were collected in sterilized 1.5 mL tubes.

4.2.4 Nutrient depletion and algal growth measurements

Concentrations of algal cells were measured using a microscope and hemacytometer counting slide. When the algae formed colonies, cell counts were made of the individual cells, not the colonies.

Nitrate and phosphate concentrations in the batch reactors were determined using a Dionex ion chromatograph (DX 120) with an injection loop of 250 μL and IonPac AS14 column (detection limit ≈ 0.05 mg/L for PO_4 and NO_3). Nutrient depletion was a useful indicator of the onset of the algal stationary phase.



A. Carbon filter

B. Air diffuser in water column

C. 0.22 μm PTFE filters

D. 2 liter glass bottle reactor

E. Magnetic stirrer

F. Peristaltic pump

G. Sample bell

H. Fluorescent lamps

Figure 4-2. Large culture reactor.

4.2.5 Bacterial counts

Since bacteria were present in the algal cultures, bacterial counts were performed. Two methods were used. The first consisted in measuring heterotrophic plate counts (HPC) using a spread plate method [66]. The procedure for this method was as follows. Small glass bottles containing 30-40 mL of R2A medium were autoclaved, allowed to cool and set aside at room temperature. These bottles were then used to prepare culture plates as the need arose. To prepare plates, one bottle was microwaved until the medium became liquid. Next to a lit Bunsen burner, medium was poured from the bottle into 6 sterile

polystyrene cell culture plates (60 x 15 mm – Corning 430166). Plates could be used directly after the medium had cooled and solidified or wrapped in plastic and set aside at room temperature for later use. To inoculate the plates, 100 μL of sample was dispensed using sterile technique onto the plate medium. Next, a bent glass Pasteur pipette was sterilized by dipping in 70% ethanol and passing through the flame of a Bunsen burner. The pipette was then used to evenly spread the sample onto the plate. Finally, plates were incubated at room temperature for 2 to 5 days. Counts were made after incubation using a microscope at 100x magnification. Extrapolation of the counts over the surface area of the ocular piece to the total surface area of the plate was made to obtain counts per mL. Dilution of the samples with autoclaved MilliQ water was often necessary to obtain reliable counts. These dilutions ranged from 0.1 to 0.004.

The second method was an acridine orange direct count (AODC) [66, 67]. Two solutions were needed in this method: a buffer solution containing 4.64 g/L NaH_2PO_4 and 12.3 g/L Na_2HPO_4 and a staining solution containing 5 ml of 25% glutaraldehyde and 12.5 mg of acridine orange (ACROS acridine orange ca 55%) in 7.5 ml of the buffer. The following procedure was used. The buffer and staining solutions were first filtered through a 0.2 μm polyethersulfone filter (Corning 431229) to remove particles. A bench top filtration unit (vacuum type with 25 mm filter holder and fritted glass support) was then thoroughly rinsed with MilliQ water and a 0.2 μm black polycarbonate membrane filter (Whatman Nuclepore track-etch membrane 110656) was placed in the filter holder. In the glass column above the filter, buffer was introduced followed by the sample. Volumes of buffer and sample varied depending on the sample volume desired (1000 to 10 μL) but

total volume was about 2 mL. Next, 5 drops of the staining solution were added with a Pasteur pipette and the filtration apparatus swirled to mix the solution. The solution was allowed to react for 3 minutes after which the vacuum to the filtration apparatus was turned on and the solution was pulled through the filter. With the vacuum still on, an additional 2 mL of buffer was inserted into the glass column and pulled through the filter. The vacuum was then turned off, the filter holder was taken apart and the filter gently set aside to dry in air for a few minutes. A microscope slide was wiped with 70 % ethanol and a drop of low-fluorescing oil placed on it. The dried filter was placed over the oil. Another drop of oil was placed on top of the filter. Finally a cover slip was placed over the filter. Counts of fluorescing bacteria were then made with a fluorescence microscope at 400x (Nikon LABOPHOT 2; super high pressure mercury lamp model HB-10101 AF; dichroic mirror DM 510, excitation filter B2A, and barrier filter BA 520).

4.2.6 Algal DOC, protein concentration and carbohydrate concentration

DOC, protein and carbohydrate concentrations were measured from samples of the culture that were first filtered through a Millipore HA 0.45 μm disc filter. DOC released by the algae into the medium during growth and decline was measured using a Dohrmann UV/persulfate carbon analyzer (Phoenix 8000).

Protein concentrations in the algal DOC were quantified using a form of the BCA assay [54, 55, 68]. A BCA assay kit commercially available from Pierce was used (model 23225). A ratio of 1:100 was used when combining reagents A and B in the kit and 250 μL of this mixture was added to 500 μL of sample for measurement. The high sample to reagent ratio was necessary due to the low protein concentrations being measured.

Reaction between the proteins and the copper / bicinchoninic acid reagents was allowed to take place at room temperature for 3 hours after which absorbance was measured at 565 nm using a square cuvette with a 1 cm path length. Bovine serum albumin (BSA) was used as a standard. While the assay gives similar responses to different proteins, changes in the intercept of the calibration curves were seen with the different media used (Figure 4-3). The reason for the interference is unknown but protein concentration in each medium was therefore estimated using its corresponding calibration curve. Dilution of the algal samples, when necessary, was also done with the appropriate medium, not MilliQ water. Protein concentrations are reported as mg/L BSA equivalents.

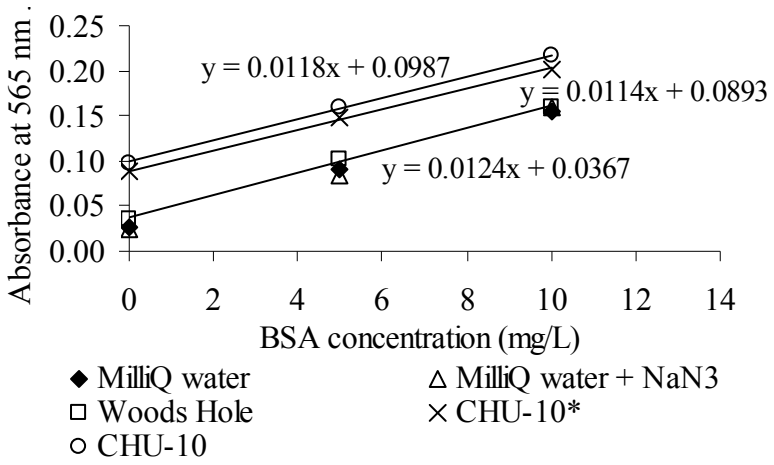


Figure 4-3. BCA assay for the determination of protein concentration (Standard = bovine serum albumin).

Carbohydrate concentrations were quantified using a form of the MBTH assay [69, 70]. The MBTH procedure for detection of monosaccharides was as follows. In a small test tube was added 0.3 mL of sample, 0.15 mL of 0.1 N NaOH and 0.15 mL of 0.5 % MBTH (5 mg MBTH in 1 mL of MilliQ water). The test tube was loosely covered and the mixture was then heated for 10 minutes in a boiling water bath. The mixture was taken

out of the water bath, allowed to cool and 0.15 mL of 1.2 % ferric chloride.$6H_2O$ (12 mg $FeCl_3.6H_2O$, 80 mL of concentrated HCl, and 1 mL of MilliQ water) was added. This last step caused the formation of a chromophore whose absorbance at 660 nm was measured after 15 minutes. Because the assay relies on a reaction that occurs with the aldehyde group of monosaccharides, detection of polysaccharides must be preceded by a hydrolysis of polysaccharides into monosaccharides. Hydrolysis of polysaccharides was conducted as follows. A glass ampule was filled with 1 mL of sample and 88.7 µL of concentrated hydrochloric acid (final concentration = 1.0 N). The ampule was sealed and heated for 6 hours in a boiling water bath. Next, the ampule was cooled and its top broken off. In a small test tube was placed 0.326 mL of the hydrolyzed sample. The test tube was put into a 50-60 ºC water bath where the sample was slowly and completely dried under a flow of air directed down the test tube using a Pasteur pipette. The sample was then reconstituted with 0.3 mL of MilliQ water and the detection with MBTH performed as outlined above.

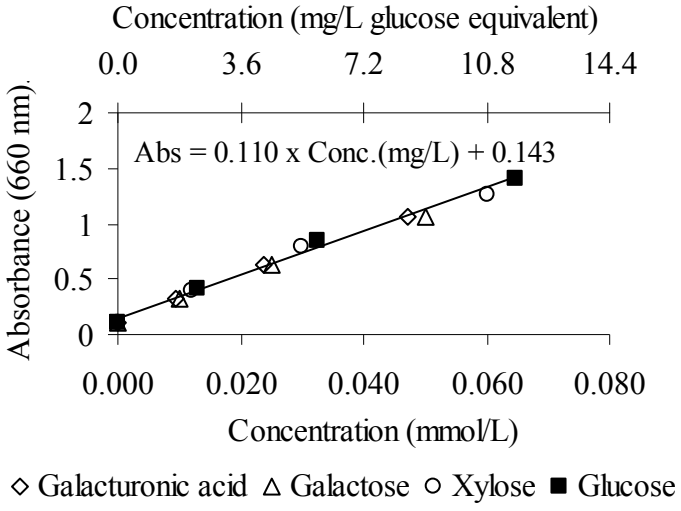


Figure 4-4. Analysis of monosaccharide using the MBTH assay.

The MBTH assay presented several advantages over other assays used for carbohydrate analysis: (i) the detection limit of the MBTH assay is quite low (about 1 mg/L glucose equivalent) which means that concentrating the sample prior to analysis was not necessary (Figure 4-4), (ii) very small sample volumes were required, (iii) the responses of different monosaccharides to the assay was comparable (Figure 4-4) with the molar absorptivity of the products of the reaction between MBTH and monosaccharides averaging 50 (cm^{-1} mol^{-1} L) (Table 4-1), (iv) measurements of monosaccharides and polysaccharides are both possible since a separate hydrolysis step is required for the detection of polysaccharides and (v) the chemical reaction behind the assay is specific enough that the assay would be expected to provide a fairly reliable measurement of carbohydrate concentrations with only modest interference.

One drawback to the assay, however, is that measurement of polysaccharides relies on the complete hydrolysis of polysaccharides to monosaccharides. As shown in Figure 4-5, complete hydrolysis of certain polysaccharides in 1 N HCl at 100ºC is not always achieved. Therefore, the values reported in this work should only be interpreted as approximate values. Unlike the BCA assay, MBTH standard calibration curves using glucose were similar for the three media used. Carbohydrate concentrations are reported as mg/L glucose equivalents.

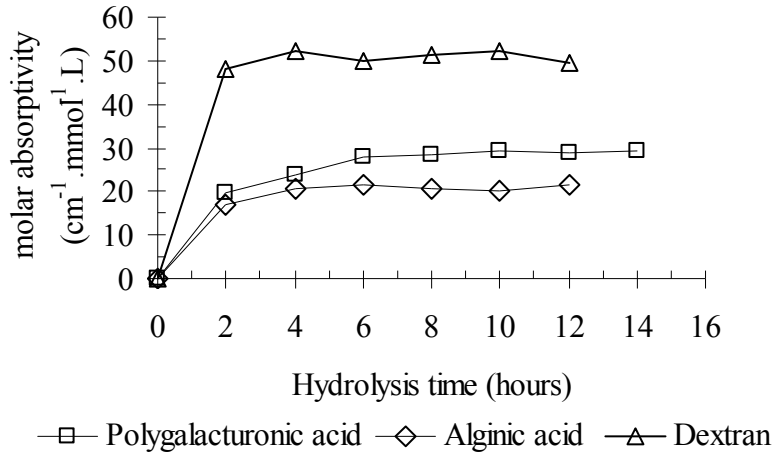


Figure 4-5. Polysaccharide hydrolysis over time at 100ºC in 1N HCl.

Table 4-1. Molar absorptivity of the products of the reaction between MBTH and different monosaccharides and polysaccharides.

Class	Compound	Molar absorbance (cm^{-1} $mmol^{-1}$ L)
Monosaccharides	Glucose	50
	Galactose	47
	Xylose	48
	Galacturonic acid	54
Polysaccharides	Polygalacturonic acid	28
	Dextran	50
	Alginic acid	22
	Carrageenan	49

4.2.7 Microfiltration unit and protocol

The same microfiltration unit and protocol as described in chapter 3 was used. The only difference was in the preparation of the model waters. The model waters used here consisted of algal culture samples diluted 1:5 with MilliQ water and the model waters were prepared immediately before the filtration. While most model waters contained both algae and AOM, samples of algal cultures were in some cases centrifuged and filtered

before being diluted with MilliQ water. Centrifugation was conducted at 4000 g for 3 minutes in 50 mL glass centrifuge tubes, under which conditions algal cells were spun down. After centrifugation, the supernatants containing the AOM were extracted using a glass pipette. The supernatants were then used to make up the model waters. In addition, supernatants were sometimes filtered before dilution. Filtration was performed with a vacuum apparatus (25 mm diameter disc filter holder) and 0.22 μm cellulose disc filters (Millipore GSWP). Cases where any such pre-treatment on the model water was performed are noted in the text.

4.2.8 Size fractionation of algal organic matter

Size fractionation using disc filters with decreasing pore sizes were performed on the organic matter released by each alga in the decline phase. The procedure was as follows. Four 30 mL aliquots of the algal culture were centrifuged in 50 mL glass tubes at 4000 g for 3 minutes to separate the algal cells from the solution. The supernatant of each tube was collected. Next, the supernatant solution was diluted with MilliQ water to yield solutions with TOC ≈ 5 mg C/L. A sample of the diluted supernatant was then filtered through one of two filters, a 0.45 μm (Millipore HA) or a 0.22 μm (Millipore GSWP) filter, using a vacuum filtration apparatus. A fraction of the GSWP filter permeate was subsequently filtered through an ultrafiltration filter YM100 (molecular weight cut-off = 100 kD) using a pressurized filtration setup made by Amicon. All filters were made of cellulose and were thoroughly soaked and/or rinsed with MilliQ water to remove any organic material that might leach from the filter before the filtration of the algal organic matter. In the end, four fractions were obtained: a centrifuged, an HA filtered, a GSWP filtered and a YM100 filtered fraction.

4.3 RESULTS

4.3.1 Scenedesmus

Growth

Figure 4-6 displays the growth of *Scenedesmus* in Woods Hole medium. Three phases of growth were observed: A log growth phase (from day 0 to 11), a stationary phase (from day 11 to 35) and a decline phase (from day 35 on). Nitrate and phosphate were taken up rapidly by the alga during the log growth phase. The depletion of nutrient coincided with the cessation of algal growth, as expected. Even though the Woods Hole medium has a high nitrogen to phosphorus molar ratio (N/P = 40) relative to Redfield stoichiometry (N/P = 16/1), both nutrients were completely used during the growth of the alga.

A slight increase in pH from 7 to about 8 was seen during the growth of the alga (Figure 4-7), as predicted by the overall photosynthetic reaction:

$$106\ CO_2 + 16\ NO_3^- + HPO_4^{2-} + 122\ H_2O + 18\ H^+ \rightarrow C_{106}H_{263}O_{110}N_{16}P + 138\ O_2$$

Measurements of DOC, protein and carbohydrate concentrations are shown in Figure 4-8. DOC increased slightly during the log growth phase (2 to 5 mg C/L) and significantly during the stationary (5 to 14 mg C/L) and decline (14 to 22 mg C/L) phases. Protein and carbohydrate concentrations were very low during the log growth phase. Polysaccharides were essentially absent throughout. Monosaccharides only appeared in the stationary phase and averaged 3 to 4 mg/L glucose equivalent. Protein concentrations increased from 2 to 8 mg/L BSA equivalent during the stationary phase and remained at this concentration throughout the decline phase. If one assumes that organic matter is roughly composed of 50% carbon, then a large fraction of the DOC was unaccounted for by

protein and carbohydrate. This difference could be due to the inability of the assays to measure some proteins and carbohydrates or the presence in the culture of different types of organic material, for example small molecules, fatty acids, lipids, and nucleic acids.

As shown in Figure 4-9, bacterial counts were detected by the AODC method in the culture of *Scenedesmus*. AODC counts were on the order of 1×10^6 cells/mL during the log growth and stationary phase and increased to 1×10^8 cells/mL during the decline phase. HPC were not detected however. It is believed that the large reactor system was well isolated from the surrounding environment and that the bacteria that grew in the large culture were introduced in the reactor along with the inoculum of *Scenedesmus* from the small culture. Supporting evidence for this is that HPC were undetected throughout the growth and decline of *Scenedesmus* in the large culture. In other words, no bacteria capable of growing on R2A medium were introduced into the large reactor during the course of these experiments. Apparently, the bacteria co-existing with the *Scenedesmus* were not able to grow until the beginning of the decline phase and also did not grow on the R2A medium used for the HPC.

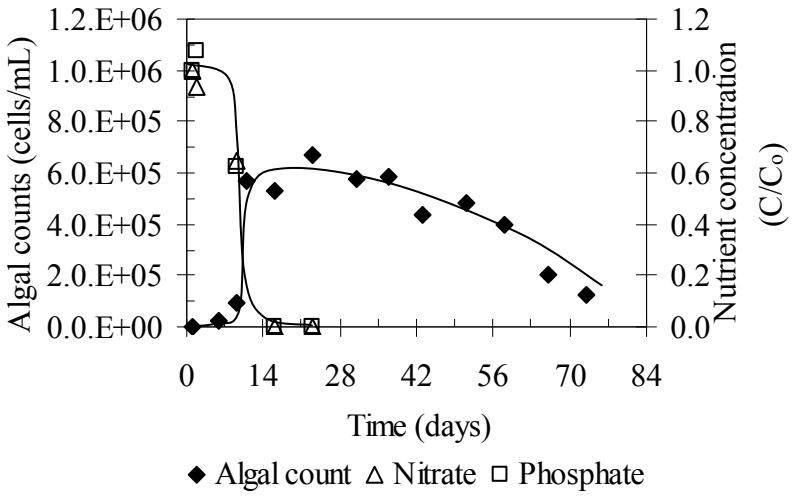


Figure 4-6. Algal counts, NO_3 and PO_4 concentrations in the culture of *Scenedesmus* in Woods Hole medium.

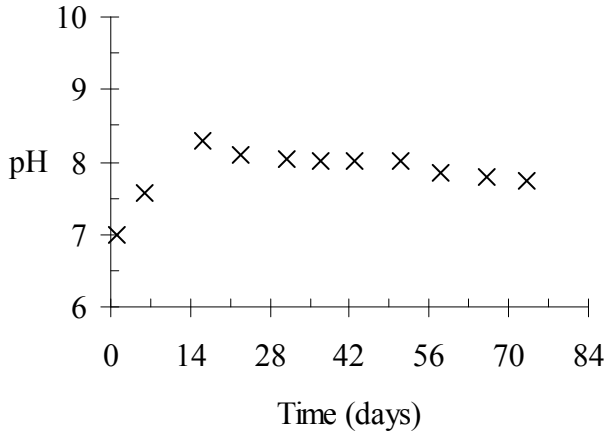


Figure 4-7. Changes in pH in the culture of *Scenedesmus.*

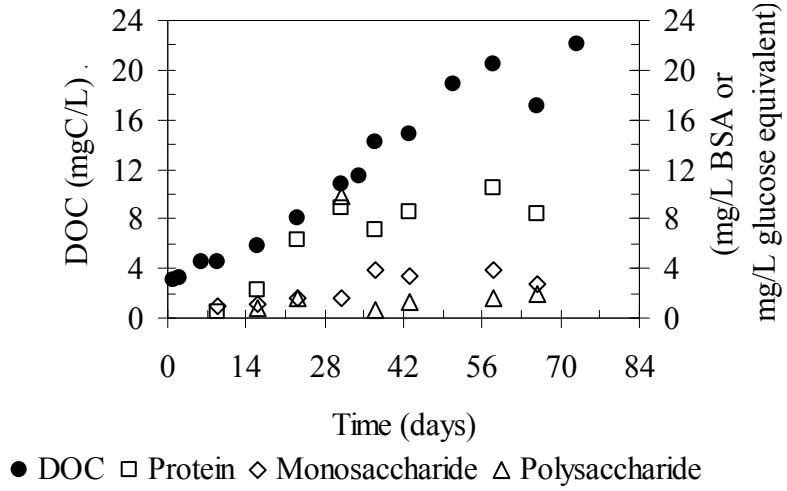


Figure 4-8. DOC, protein, monosaccharide and polysaccharide concentrations in the culture of *Scenedesmus.*

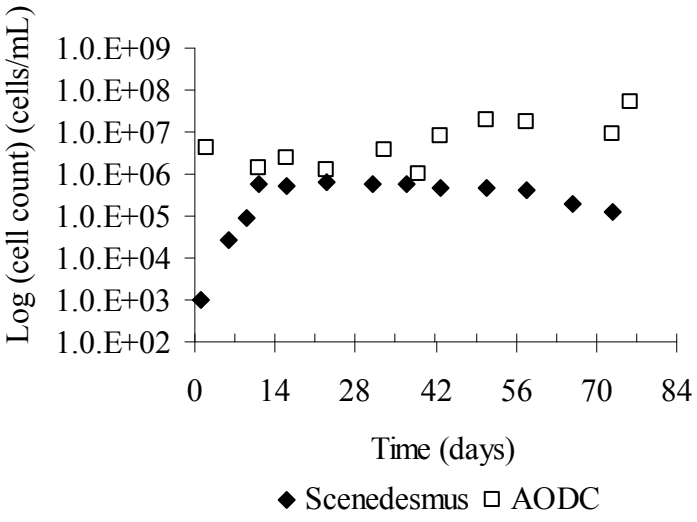


Figure 4-9. Algal and bacterial counts in the culture of *Scenedesmus*.

Membrane fouling

Microfiltration runs were performed at the different phases of growth and decline (Figure 4-10). The microfiltration conducted with water collected in the log growth phase (day 6) showed almost no membrane fouling. From there, fouling increased as the alga progressed through the stationary (day 17 and 34) and the decline phases (day 69). Increases in NTMP remained modest and reached 1.2 at the outcome of the filtration run conducted on day 69. Although fouling was modest, it was largely irreversible to hydraulic backwash. Cells of *Scenedesmus* were visibly pushed off the membrane surface and re-suspended into solution during backwash but little membrane permeability was recovered. This indicates that *Scenedesmus* cells probably did not contribute much to total fouling.

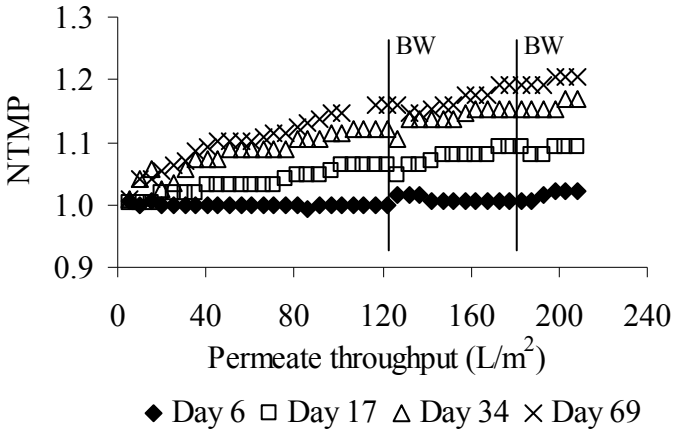


Figure 4-10. NTMP increase as a function of permeate throughput during the filtration of *Scenedesmus* at different stages of growth and decline.

Although DOC in the culture of Scenedesmus was not negligible, in particular during the stationary and decline phase, little DOC was removed by the membrane during the course of the filtrations. In both stationary and decline phases, 80 to 90 % of the DOC passed through the membrane (Figure 4-11). A general trend was seen, however, in which

slightly more DOC was removed in the early stages of filtration (0-60 L/m^2) compared to the later stages. Incidentally, rates of fouling also seemed higher in the early stages of filtration compared to the later stages (day 34 and day 69 in Figure 4-10).

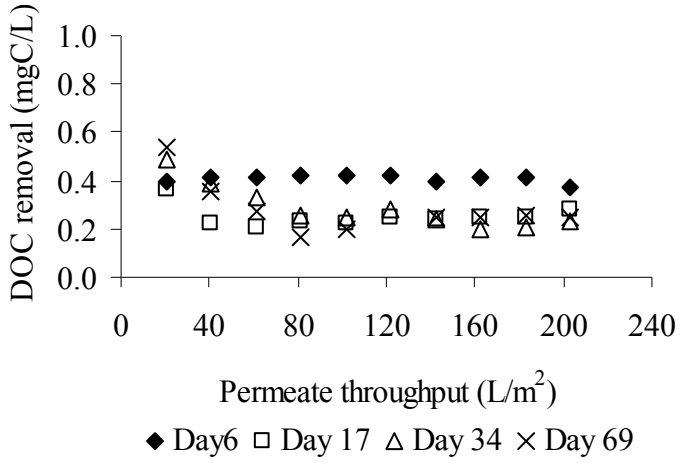


Figure 4-11. DOC removal as a function of permeate throughput during the filtration of *Scenedesmus* at different stages of growth and decline.

Size fractionation of the organic matter released by Scenedesmus

Size fractionation conducted on day 45 of the DOC released by *Scenedesmus* is shown in Figure 4-12. The alga was in its decline phase at this time and total DOC was approximately 16 mg C/L. The size fractionation revealed that the DOC was for the most part smaller than 100 kD in size. The fractionation with the cellulose based filters was in reasonable agreement with the DOC removal seen with the 0.1 μm PVDF hollow fibers.

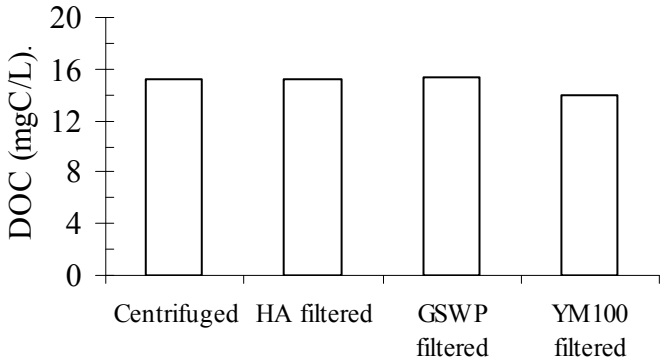


Figure 4-12. Size fractionation conducted on day 45 of DOC released by *Scenedesmus*.

4.3.2 Asterionella

Growth

Figure 4-13 displays the growth of *Asterionella* in CHU-10 medium. Two phases of growth were observed: a log growth phase (from day 0 to 14) and a decline phase (from day 19 on). Asterionella did not use up all the nitrogen and phosphorus available in the medium and did not reach as high a cell count as *Scenedesmus*. Several explanations may explain the modest cell counts observed. (1) Cells of *Asterionella* are larger than *Scenedesmus*; therefore the nutrient requirement for the generation of one cell of *Asterionella* would be expected to be larger than that for one cell of Scenedesmus. (2) *Asterionella* tended to aggregate and settle. As a result, at least some of the cell count decline observed after day 19 was a result of settling, not cell decay. (3) Since *Asterionella* needs silica for the formation of its outer shell, it is possible that silica limited its growth. Once this silica was exhausted, growth and uptake of nitrogen and phosphorus ceased.

An increase in pH was seen during the growth of the alga from 6.5 to 7.5 (Figure 4-14).

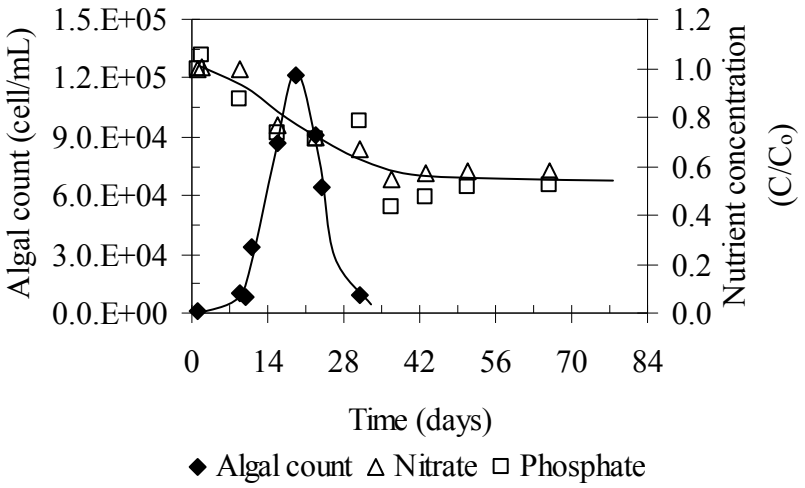


Figure 4-13. Algal counts, NO_3 and PO_4 concentrations in the culture of *Asterionella* in CHU-10 medium.

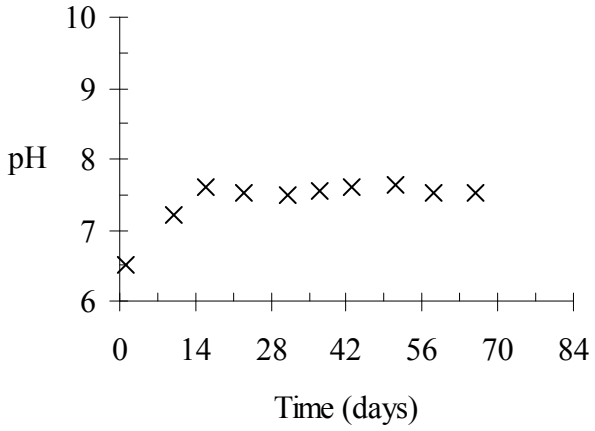


Figure 4-14. Changes in pH in the culture of *Asterionella.*

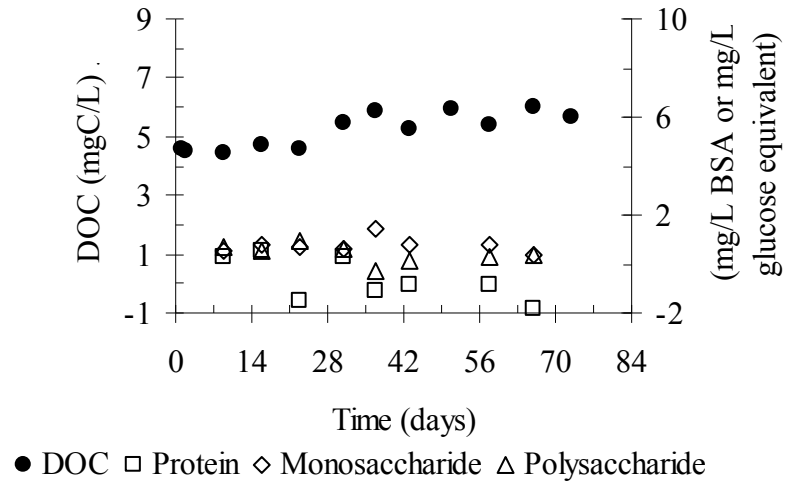


Figure 4-15. DOC, protein, monosaccharide and polysaccharide concentrations in the culture of *Asterionella.*

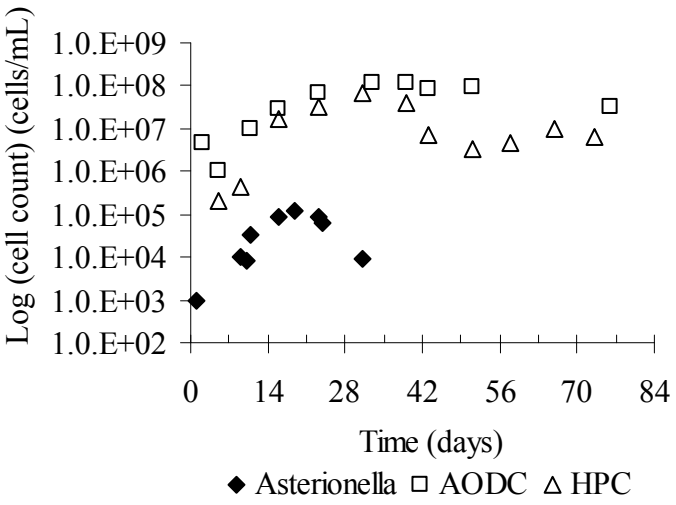


Figure 4-16. Algal and bacterial counts (AODC and HPC) in the culture of *Asterionella.*

Measurements of DOC, protein and carbohydrate concentrations are shown in Figure 4-15. *Asterionella* released very little DOC during growth and decline: a small increase of 1.5 mg C/L was seen from the moment of the inoculation of the medium to the last measurement at the end of the experiment. The limited release of DOC may have been the result of the modest growth achieved. However, roughly half of the nitrogen and phosphorus were consumed. If these nutrients were used completely, doubling of algal counts and DOC might be expected but DOC would still be only 3 mg C/L. Since DOC was very low, the measurements given by the protein and carbohydrate assays hovered around the baseline.

In the culture of *Asterionella*, bacterial counts were detected by the AODC and HPC methods. HPC measurements were less than the AODC, but both sets of measurements followed a similar trend. Bacterial counts increased from $1x10^5$ cells/mL (HPC) and $1x10^6$ cells/mL (AODC) to approximately $1x10^8$ cells/mL (HPC and AODC) by day 36 and then declined to $3x10^6$ cells/mL (HPC) and $3x10^7$ cells/mL (AODC) by the end of the experiment.

Membrane fouling

Results of the microfiltration runs are shown in Figure 4-17. No fouling was observed with the water collected in the log growth phase (day 10) but fouling was significant during the decline phase. The increase in NTMP was highest on day 24 and only slightly less severe in the remaining runs (days 38, 40, and 72). NTMP were in the 1.4 – 1.6 range at the conclusion of the filtration experiments in the decline phase. Fouling was largely

irreversible. Once again, algal cells were visibly re-suspended during backwash but backwash did not lower NTMP significantly. In order to determine the contribution of algal cells to total fouling, one filtration run was conducted with water sampled from the algal culture that was centrifuged (4000 g for 3 minutes) to remove the algal cells prior to dilution and filtration. This run conducted on day 40 was virtually identical to the run conducted on day 38, proving that cells of *Asterionella* did not cause significant fouling.

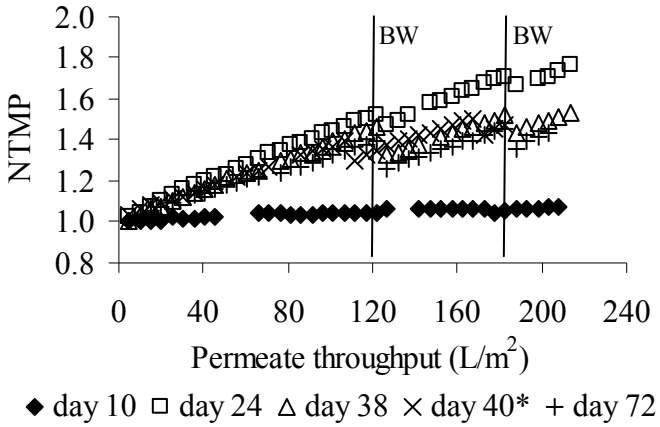


Figure 4-17. NTMP increase as a function of permeate throughput during the filtration of *Asterionella* at different stages of growth and decline. (* Algal culture sample was centrifuged prior to dilution and microfiltration).

Because DOC was low in the model water, DOC removals should be interpreted with some caution but it appears that small amounts of DOC were removed by the membrane during the microfiltration runs (Figure 4-18). Again, slightly more DOC was removed in the early stages of the filtration compared to the later ones.

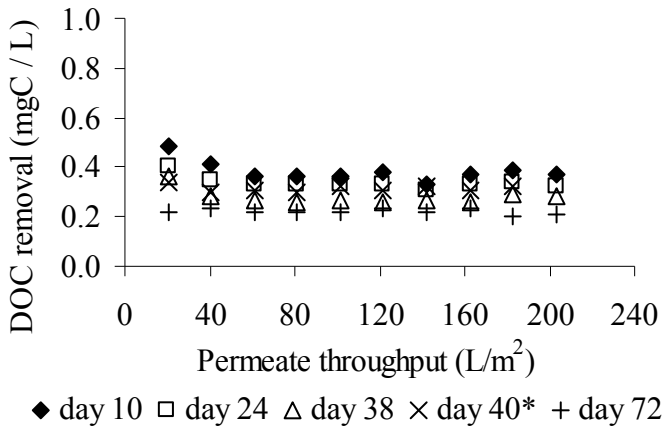


Figure 4-18. DOC removal as a function of permeate throughput during the filtration of *Asterionella* at different stages of growth and decline.

Size fractionation of the organic matter released by Asterionella

Size fractionation conducted on day 45 of the DOC released by *Asterionella* is shown in Figure 4-19. The alga was in its decline phase at this time and total DOC was approximately 6 mg C/L. The size fractionation revealed that a fraction of the DOC (33%) was larger than 0.45 μm and that the rest was smaller than 100 kD in size (about 0.01 μm). The fractionation with the cellulose based filters was in reasonable agreement with the DOC removal seen with the 0.1 μm PVDF hollow fiber microfiltration membrane. Recall that the model water used in the microfiltration runs consisted of algal water diluted 1:5 with MilliQ water. Therefore, DOC removal during microfiltration would be expected to be 33% of 1.2 mg C/L or 0.4 mg C/L.

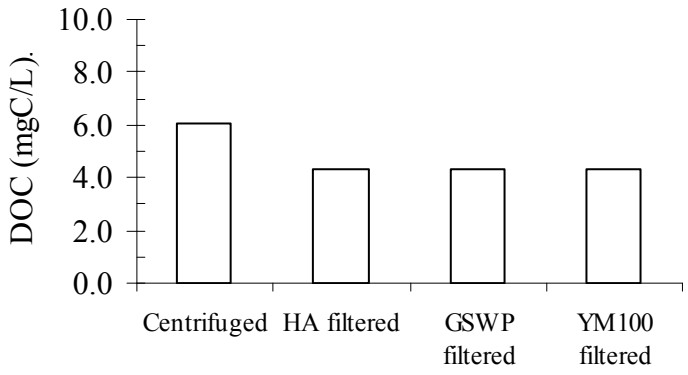


Figure 4-19. Size fractionation conducted on day 45 of DOC released by *Asterionella*.

4.3.3 Microcystis

Growth

Figure 4-20 displays the growth of *Microcystis* in CHU-10* medium. Three phases of growth were observed: a log growth phase (from day 0 to 23), a stationary phase (from day 31 to 51) and a decline phase (from day 51 on). Nitrogen and phosphorus were completely consumed during the growth of the *Microcystis* and depletion of nutrients coincided with the onset of the stationary phase. Of the three algae grown, *Microcystis* reached the highest cell counts ($8x10^6$ cells/mL). Nitrate and phosphate concentrations decreased at similar rates. Since the original media had an N/P = 16, it is concluded that uptake of nitrogen and phosphorus by the alga followed the same ratio.

An increase in pH was seen during the growth of the alga from 6.5 to about 8.5 (Figure 4-21). During the decline phase, pH decreased back down to 8.

Measurements of DOC, protein and carbohydrate concentrations are shown in Figure 4-22. *Microcystis* released considerable quantities of DOC. DOC increased from 4 to 14 mg C/L during the log growth phase (day 0 to 23), from 33 to 100 mg C/L during the stationary phase (day 31 to 51) and increased some more to 130 mg C/L before decreasing to 100 mg C/L during the decline phase. Monosaccharide concentration remained low throughout. Polysaccharides increased to 8 mg/L glucose equivalent during the log growth phase, from 8 to 40 mg/L glucose equivalent during the stationary phase, and remained at that concentration during the decline phase. Proteins increased to 5 mg/L BSA equivalent during the log growth phase, from 5 to 10-15 mg/L BSA equivalent during the stationary phase and remained at this level during the decline phase. As was the case for *Scenedesmus*, a large fraction of the DOC released by the *Microcystis* was not accounted for by proteins and carbohydrates.

In the culture of *Microcystis*, bacterial counts were detected by the AODC and HPC methods. HPC measurements were slightly less than the AODC, but both sets of measurements followed a similar trend. Bacterial counts increased from $1x10^5$ cells/mL (HPC) and $3x10^6$ cells/mL (AODC) to approximately $1x10^8$ cells/mL (HPC and AODC) by the end of the experiment. Since *Microcystis* is a photosynthetic bacterium, it is important to note that the bacterial counts given by AODC and HPC did not include *Microcystis*. Growth of the heterotrophic bacteria co-existing with *Microcystis* was steady but moderate.

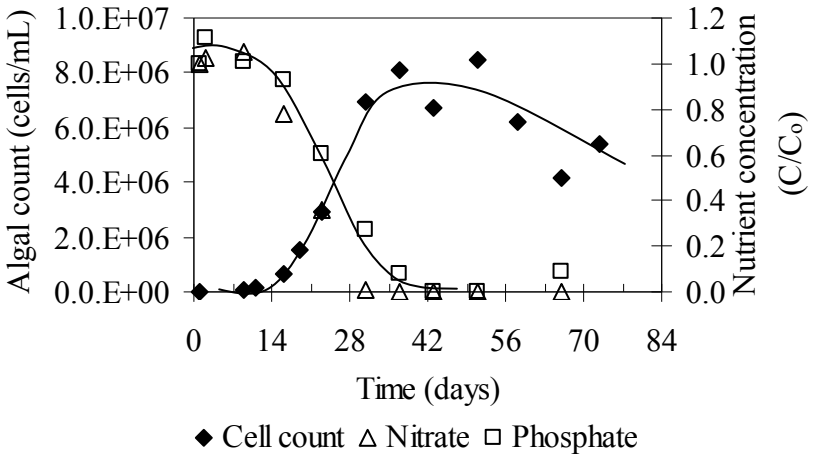


Figure 4-20. Algal counts, NO_3 and PO_4 concentrations in the culture of *Microcystis* in CHU-10* medium.

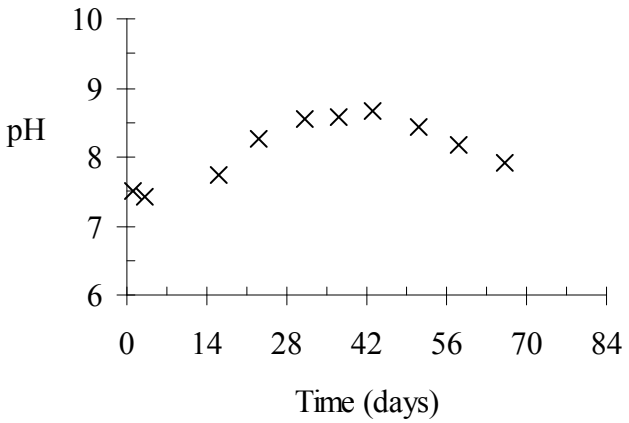


Figure 4-21. Changes in pH in the culture of *Microcystis*.

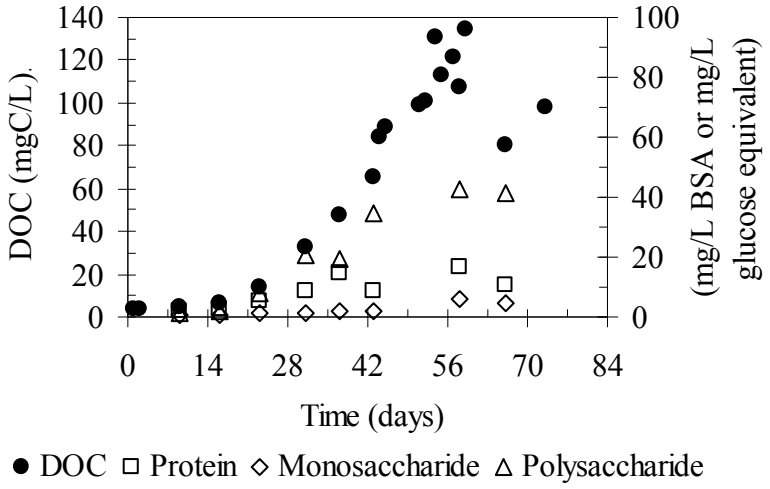


Figure 4-22. DOC, protein, monosaccharide and polysaccharide concentrations in the culture of *Microcystis*.

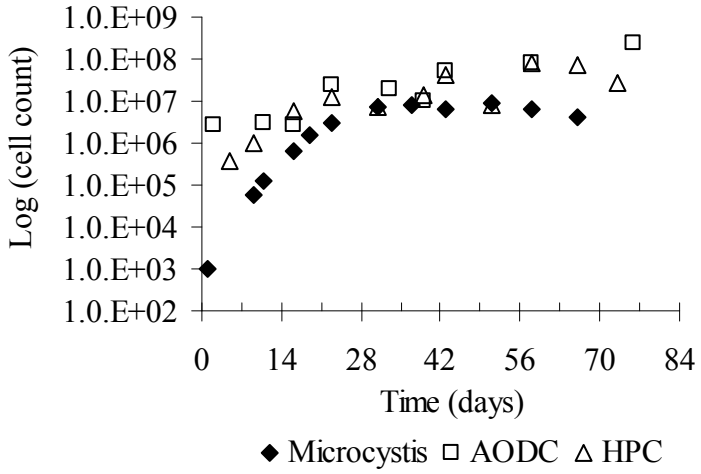


Figure 4-23. Algal and bacterial counts (AODC and HPC) in the culture of *Microcystis*.

Membrane fouling

Results of the microfiltration runs are shown in Figure 4-24 and 4-25. Fouling varied from slight (day 3) to significant (day 19) during the log growth phase but was extremely severe in the stationary (day 32 and 44) and decline (day 52) phases. So severe was the fouling that backwash had to be performed more frequently than usual (every 20 minutes) and runs eventually had to be stopped because the system could not handle the very low vacuum pressures required to maintain flow. Unlike *Scenedesmus* and *Asterionella*, some of the fouling observed with *Microcystis* was reversible by hydraulic backwash (e.g., day 19 and 32). During backwash, *Microcystis* cells were re-suspended in the solution.

In order to determine the impact of the *Microcystis* cells in the fouling process, a filtration run was performed with a sample of algal culture which was centrifuged prior to dilution and microfiltration. In this run conducted on day 44, fouling was more significant and less reversible than it had been on day 32. Since algal counts had not changed and DOC increased substantially between day 32 and 44, these results may indicate that both the *Microcystis* cells and the algal organic matter contribute to fouling. The cells of *Microcystis* may cause significant reversible fouling and the released AOM significant irreversible fouling.

The role of the heterotrophic bacteria co-existing with *Microcystis* was also investigated by performing a filtration run with a sample of algal culture which was centrifuged and pre-filtered (0.22 μm GSWP) prior to dilution and microfiltration. This run conducted on day 52 showed significant and mostly irreversible fouling. The pre-filtration of the model

water removed only small amounts of DOC. Since the *Microcystis* and most bacteria were excluded in this run, it is concluded that a significant fraction of the irreversible fouling observed was caused by the organic matter present in the algal culture. Furthermore, this organic matter was removed by a 0.1 μm PVDF membrane but not by a 0.22 μm cellulose membrane filter.

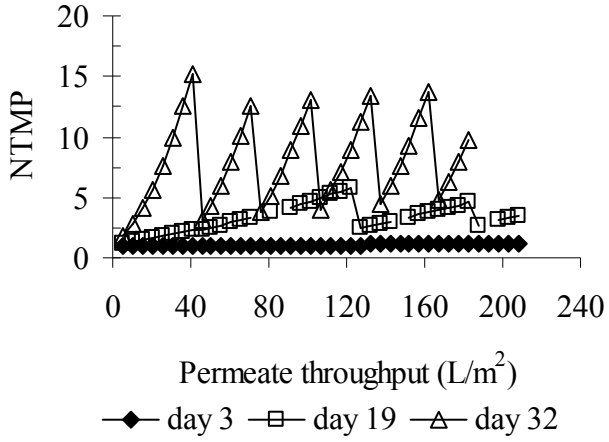


Figure 4-24. NTMP increase as a function of permeate throughput during the filtration of *Microcystis* at different stages of growth and decline.

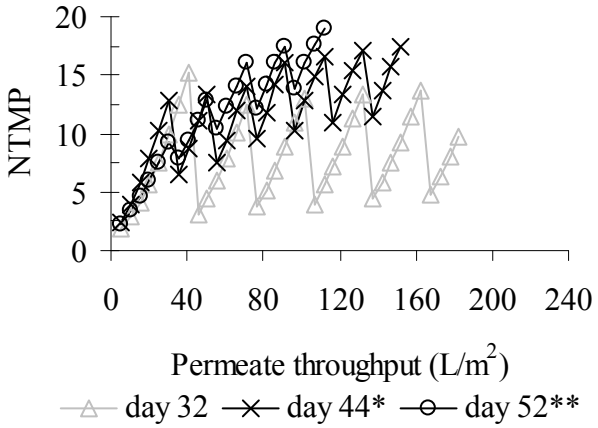


Figure 4-25. NTMP increase as a function of permeate throughput during the filtration of *Microcystis* at different stages of growth and decline. (*algal culture sample was centrifuged prior to dilution and microfiltration, ** algal culture sample was centrifuged and pre-filtered prior to dilution and microfiltration).

Removal of DOC released by *Microcystis* during microfiltration was substantial (Figure 4-26) and increased as the alga progressed through the various phases of growth and decline. Removal eventually amounted to 85 % of the DOC being loaded onto the membrane (day 44 and 52). A clear relationship was seen between increasing removal of DOC and an increasing extent of fouling.

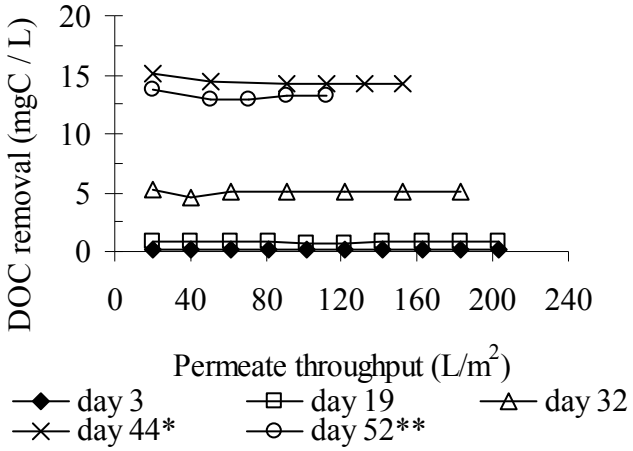


Figure 4-26. DOC removal as a function of permeate throughput during the filtration of *Microcystis* at different stages of growth and decline.

Size fractionation of the organic matter released by Microcystis

Size fractionation conducted on day 52 of the DOC released by *Microcystis* is shown in Figure 4-27. The alga was in its decline phase at this time and total DOC was approximately 110 mg C / L. Approximately 5 mg C / L was larger than 0.45 μm, 90 mg C / L was smaller than 0.22 μm but larger than 100 kD (about 0.01 μm), and the rest was smaller than 100 kD. Since the 0.1 μm microfiltration PVDF hollow fiber removed large amounts of DOC, it is inferred that a large fraction of the DOC released by *Microcystis* may have been between 0.1 and 0.22 μm.

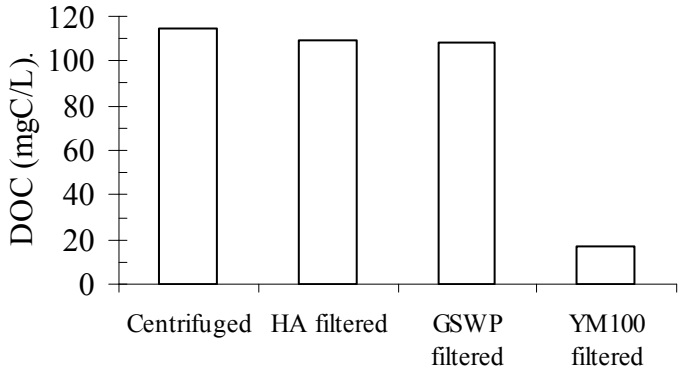


Figure 4-27. Size fractionation conducted on day 52 of DOC released by *Microcystis.*

4.4 DISCUSSION

4.4.1 Fouling by algal cells

Because the algal cells were significantly larger than the membrane pores, they formed a cake on the external surface of the membrane during filtration. However, it was observed that the cells of all three algae were re-suspended in solution during backwash. In addition, it was noted that cells of *Scenedesmus* and *Asterionella* did not appear to have caused fouling while that of *Microcystis* may have contributed some reversible fouling.

Whether cakes of cells might have been expected to cause fouling of the microfiltration membrane (i.e. increases in NTMP) can be crudely determined using a resistance in series approach and the Kozeny equation for flow through a granular medium [5]. According to this model, the flux is given by:

$$J = \frac{Q}{A} = \frac{\Delta P}{\mu.\left(R_m + R_c\right)} \tag{4.1}$$

where R_m and R_c refer to the resistance due to the membrane and the cake respectively. Q is the permeate flow rate (2 mL/min), A is the membrane surface area (20 cm^2) and μ is

the viscosity of the water ($1x10^{-3}$ N/s.m^2). The flow rate and the flux were kept constant in the system considered here. Therefore, the total transmembrane pressure at any time can be expressed as:

$$\Delta P = \mu.J.(R_m + R_c) \tag{4.2}$$

and the normalized transmembrane pressure be given by:

$$NTMP = \frac{\Delta P}{\Delta P_o} = \frac{\mu.J\left(R_m + R_c\right)}{\mu.J.R_m} = 1 + \frac{R_c}{R_m} \tag{4.3}$$

R_m can be calculated from the performance of the clean membrane:

$$R_m = \frac{A.\Delta P_o}{\mu.Q} = \frac{(2.0x10^{-3}m^2).(0.7psi)}{(1.0x10^{-3}\frac{N.s}{m^2}).(2\frac{mL}{\min})}.\frac{101000\frac{N}{m^2}}{14.7psi}.\frac{10^6 mL}{m^3}.\frac{60\sec}{\min} \tag{4.4}$$
$$= 2.89x10^{11}m^{-1}$$

and the resistance due to the cake can be estimated by [5]:

$$R_c = \frac{36K_K.(1-\varepsilon)^2\delta_c}{\varepsilon^3 d_p^2} \tag{4.5}$$

where K_k is the Kozeny coefficient (typically 5), ε is the cake porosity, d_p (m) is the diameter of the particles making up the cake and δ_c (m) is the thickness of the cake. The cake thickness can be obtained by assuming that all of the particles, i.e. the cells, were removed by the membrane (pore size = 0.1 μm) and is given by:

$$\delta_c = \frac{C.V.\left(\frac{\pi d_p^{\ 3}}{6}\right)}{A.(1-\varepsilon)} \tag{4.6}$$

where C (particles/mL) is the concentration of the particles in the raw water and V (mL) is the volume of water filtered through the membrane (V = 400 mL in this case). Porosity for the cell cakes is unknown but a relatively low value of 0.4 can be used to yield an upper estimate of the potential increase in NTMP.

Given the volume of water filtered (V = 400 mL) and the characteristics of the membrane used in this work ($R_m = 2.89x10^{11}$ m^{-1}, $A = 20x10^{-4}$ m^2), one can therefore easily calculate the increase in NTMP that would be expected from cakes formed by particles of different sizes present in varying concentrations in the raw water. By selecting ranges of particle sizes (0.3 – 30 μm) and concentrations ($1x10^5$ to $1x10^7$ particles/mL) that represent the different algae and model waters tested, the fouling due to the cell cakes can be estimated. The results of such an analysis are shown in Figure 4-28.

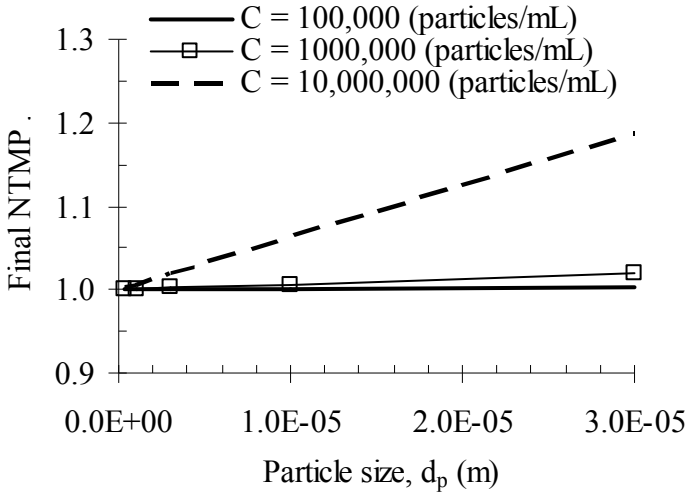


Figure 4-28. Increase in NTMP expected from a cake of particles formed during the filtration of 400 mL of water containing $1x10^5$-$1x10^7$ (particles/mL) through a hydrophobic microfiltration membrane ($R_m = 2.89x10^{11}$ m^{-1}, $A = 20x10^{-4}$ m^2) as a function of particle size.

As can be seen in Figure 4-28, the analysis reveals an increase in fouling with particle size. However, the values of NTMP do not exceed 1.2. The reason that fouling increases with particle sizes is because, for a fixed concentration of particles in the raw water, the thickness of the cake formed by the removal of these particles on the membrane surface increases with particle size. In fact, for the same cake thickness, a cake formed of small

particles would be expected to cause more headloss than one formed of large particles. In the model waters tested, concentrations of algal cells ranged from $2.0x10^4$ to $1.4x10^6$ (cells/mL). At these concentrations of particles, Figure 4-28 indicates that the predicted fouling caused by a cell cake of any particle size would be low (NTMP << 1.1). Modeling and experimental results are therefore in agreement in the cases of *Scenedesmus* and *Asterionella* where the algal cells did not appear to cause fouling. Experimental results with *Microcystis* displayed more reversible fouling than would be predicted by the model however.

The discrepancy between the modeling and experimental results in the case of *Microcystis* may have several explanations. (1) *Microcystis* released considerable amounts of AOM. It is possible that the cells of *Microcystis* deposited as a cake on the membrane surface and acted as a secondary filter towards the AOM which filled some of the void space within the cake. The result could have been a cake with considerable resistance to flow. As the cells of *Microcystis* were pushed off the membrane during backwash, so was the associated AOM and some of the fouling was thus reversible. (2) Cells of *Microcystis* may themselves have been coated with some AOM and led to the formation of a cake whose resistance to flow was higher than that predicted for a cake composed of solid incompressible spheres. (3) Fouling may have been caused by AOM and perhaps some *Microcystis* cells which attached irreversibly to the membrane surface to form fouling layers whose resistance to flow was influenced by the transmembrane pressure being applied. As the pressure increased, these fouling layers could have compressed thereby increasing the rate of fouling. During backwash, pressures were

lowered which could have allowed the fouling layers to relax and their apparent resistance to decrease. Cells which were only loosely deposited on the membrane surface would have been easily re-suspended by the backwash. Although the correct explanation is unknown, it is likely that AOM played a role. According to the simple analysis conducted here, cells of *Microcystis* alone could not have accounted for most of the reversible fouling observed.

Figure 4-28 also shows that the fouling caused by a cake of bacterial cells (d_p = 0.3-1 μm) present in the water at concentrations comparable to those in the model waters (C = $1x10^7$ cells/mL) and removed on the membrane surface during filtration would have been small. This is in general agreement with the results of the microfiltration experiments in which model waters containing fairly large amounts of bacteria showed only moderate fouling (e.g., experiments with *Scenedesmus* and *Asterionella*).

4.4.2 Fouling by algal organic matter

Algal organic matter (AOM) likely dominated the fouling observed in the microfiltration experiments. This was supported by several pieces of evidence. (1) The simple analysis of cake fouling presented above indicated that cells, either algal or bacterial, alone could not have caused the significant fouling observed. (2) Filtration experiments with *Asterionella* and *Microcystis* in which cells were excluded prior to filtration still showed significant and irreversible fouling. (3) Fouling of the membrane markedly increased as the organism generally approached the stationary phase at which time the DOC also increased. In fact, a correlation can be found between increasing removal of algal DOC

and increasing values of the final NTMP (irreversible fouling only) as shown in Figure 4-29.

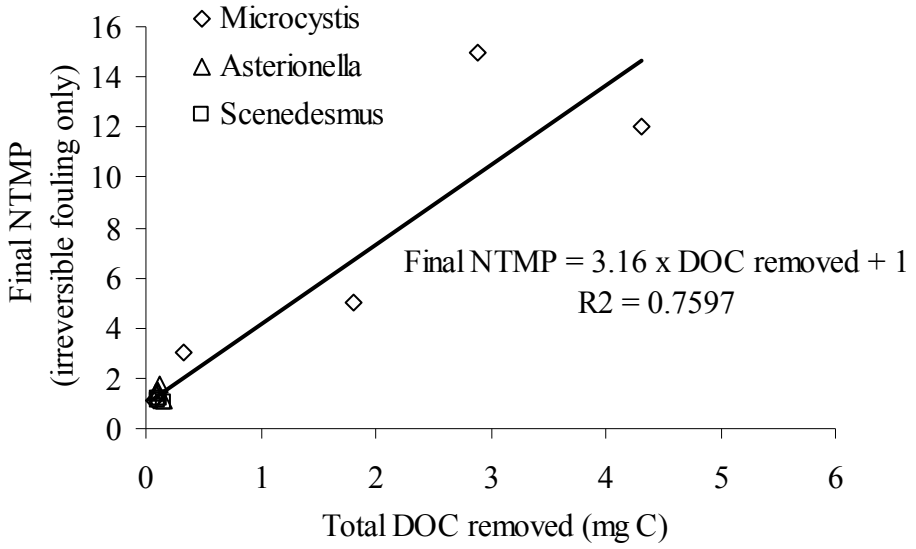


Figure 4-29. Relationship between total removal of algal DOC (mg C) and final NTMP (irreversible fouling only) during the filtration through a hydrophobic microfiltration membrane of waters containing three different algae and/or their AOM at various phases of growth and decline.

Membrane fouling by the AOM likely involved a mixture of several fouling mechanisms. On one hand, AOM could have contributed to fouling partially by interacting with the cakes of algal cells that formed on the membrane surface, thereby forming cakes that were relatively impermeable, although largely reversible (see discussion in 4.4.1 regarding fouling by *Microcystis*) . On the other hand, the AOM may have acted independently from the cakes of algal cells forming on the membrane surface. In the latter case, AOM which was significantly smaller than the membrane pore size could have entered and adsorbed within the membrane porous structure, thereby constricting pores internally and causing a moderate increase in transmembrane pressure. This fouling would be similar to that seen with BSA or gamma globulin when these were present in

stable solutions. By contrast, AOM close to or larger than the membrane pore size could have obstructed pores internally or at the surface of the membrane. This AOM could include cell fragments and organic matter aggregates which, owing to their large size, would be able to cause a severe constriction or obstruction of pores and consequently a significant increase in transmembrane pressures. This fouling would be similar to that seen with alginic acid or gamma globulin when these were present in solutions that promoted their aggregation or precipitation.

In the case of *Scenedesmus* and *Asterionella*, cell cakes were apparently not involved in the fouling process. Fouling seen with *Scenedesmus* resembled one involving slight pore constriction. Fouling was slightly more severe early on and slowed down as the filtration progressed but total fouling was limited (Final NTMP $\leq$ 1.2). Removal of DOC was generally low but slightly higher in the early stages (0.4-0.5 mg C/L) compared to the later stages (~ 0.2 mg C/L) of filtration, possibly indicating that adsorption sites became exhausted. Size fractionation with cellulose based filters confirmed that most of the DOC released by *Scenedesmus* was indeed significantly smaller than the microfiltration membrane pore size (100 nm). Fouling by *Asterionella*, on the other hand, may have included several mechanisms. With this alga, removal of DOC decreased in the early stages of filtration but rate of fouling did not taper off. Size fractionation indicated that a portion of the DOC released by Asterionella was larger than 0.45 μm, the rest smaller than 100 kD. It is possible that some of the organic matter in the smaller size range fouled the membrane by a process of internal adsorption and that the organic matter in the larger size range fouled the membrane by obstructing pores externally. Because DOC removal

was limited (~0.3 mg C/L), fouling by the waters containing the *Asterionella* was also limited (NTMP ≤ 1.6). Finally, fouling (total and irreversible) seen with *Microcystis* was more significant and complex. Experiments in which algal cells were present showed significant fouling (NTMP as high as 10-15) but this fouling was partially reversible. Possible explanations for this were discussed in section 4.4.1. Experiments in which the algal cells were absent, by comparison, showed significant but largely irreversible fouling (NTMP as high as 10-20). In this case, the increases in NTMP that occurred during filtration were reminiscent of a fouling mechanism involving organic aggregates: between backwashes, the increase in transmembrane pressure worsened as the filtration progressed and DOC removal was high (as high as 15 mg C/L). Removal of DOC was steady throughout the filtration and size fractionation revealed that a large portion of the DOC released by *Microcystis* was larger than 100 kD but smaller than 0.22 μm. Organic matter within this size range could quite conceivably include small cell fragments and organic matter aggregates.

Although the fouling behavior of the organic matter released by the algae can closely resemble that seen with some of the model compounds, it is likely that fouling by the algae involved a mixture of fouling mechanisms acting simultaneously. The organic matter released by the algae displayed a broad array of sizes and probably contained compounds other than proteins and carbohydrates.

4.4.3 Role of bacteria in fouling

Although the exact role that bacteria present within the algal culture is not known, there is reason to believe that it was secondary to that of the algae and the AOM. The fouling

observed with each culture did not correlate well with bacterial counts. For similar counts, much more fouling was seen with the culture of *Microcystis* than with the cultures of *Scenedesmus* or *Asterionella*. The analysis given in section 4.4.1 also indicates that cakes of bacterial cells in the amounts present in the model waters could have not have caused as much fouling as was observed. Indeed, one experiment conducted with pre-centrifuged and pre-filtered (0.22 μm) water from the *Microcystis* culture still showed significant fouling. This last experiment and the fact that fouling was generally correlated with the removal of DOC by the membrane support the hypothesis that fouling was caused by organic matter.

It is furthermore likely that the organic matter was produced or released by the algae since the increase in organic matter concentrations within the algal cultures coincided well with the onset of the stationary or decline phase for each alga. The bacteria, most of which were probably heterotrophic, would have had to rely on organic matter released by the algae to sustain their growth since the original media contained very little organic substrates. Under these conditions, it is likely that the bacteria consumed a fraction of the AOM released by each alga and unlikely that they contributed significant organic matter of their own to the solution.

4.4.4 Predicting fouling by algae and AOM

Algae are most likely to irreversibly foul a microfiltration membrane in a treatment plant through the release of algal organic matter. In the work presented here, the organic matter associated with the algae started to appear in significant amounts at the end of the log growth phase when nutrients became depleted. This suggests that the organisms produced

or released the organic matter as a response to nutrient depletion. It may have been excreted by the algae to achieve a certain objective, the nature of which is largely unknown, or it could have been released simply as a result of cell lysis. In any case, it would be expected that algae in a water source would be the most problematic to a microfiltration process when nutrient depletion is reached and organic matter is released.

The organic matter released by the algae was likely responsible for most of the irreversible fouling observed. Because the organic matter released by each organism varied greatly in concentration and size, the fouling it caused varied as well. The observed impact of the size of the AOM on fouling is in agreement with the results obtained with the model compounds presented in chapter 3, i.e. relatively large fragments or aggregates of organic matter cause significant fouling. The characteristics of the exudates certainly depend on the identity of the algae releasing it, as shown here, but could also depend on the concentration and composition of nutrients available to the organism. The influence of nutrients remains to be investigated.

If the results with the three algae (one actually being a cyanobacterium) studied here are any indications of the variations that can be expected in the organic matter associated with photosynthetic organisms in a natural setting, then predicting the extent of the microfiltration membrane fouling in a treatment plant by a water affected by a bloom of algae would be extremely difficult. If large amounts of organic matter were generated in the feed water, the potential would exist for significant membrane fouling. Prior knowledge of the typical concentrations and characteristics of the organic matter released

by an alga would almost certainly depend on previous experience by an operator, engineer or scientist with that particular organism however. The results presented here indicate light fouling of a hydrophobic microfiltration membrane by the AOM of *Scenedesmus* (a green alga), moderate fouling by the AOM of *Asterionella* (a diatom), and extremely severe fouling by the AOM of *Microcystis* (a cyanobacteria). This order followed the order of increasing DOC removal by the microfiltration membrane. Whether the order just described would remain valid under different growth conditions or with a different membrane system needs to be tested.

It should also be noted that all of the experiments presented here were conducted over short time scales (200 minutes). The irreversible fouling of a microfiltration membrane in a treatment plant could occur slowly over long time scales (days or weeks), even with algae that do not appear to release large concentrations of organic matter.

4.5 CONCLUSION

The fouling of a 0.1 μm hydrophobic PVDF microfiltration membrane by different algae and their AOM varies significantly. For the three organisms tested, a green alga (*Scenedemus*), a diatom (*Asterionella*) and a cyanobacteria (*Microcystis*), the extent of irreversible fouling generally followed the extent of DOC removal by the membrane; the greater the removal of algal DOC, the greater the fouling. *Microcystis* released particularly large amounts of organic matter under the conditions used. Most of this organic matter was removed (as much as 85% or 15 mg C/L) by the microfiltration membrane during filtration and was associated with severe irreversible fouling of this membrane (Final NTMP as high as 10-20). By comparison, much less DOC removal was

seen with the other two algae (0.2-0.3 mg C/L), causing moderate fouling (NTMP $\leq$ 1.6). On the basis of these results, it is therefore expected that hydrophobic microfiltration membranes similar to the one used here would also show different levels of fouling in a surface water treatment plant depending on the presence of varying algal populations in the source water.

Moreover, it was found that algal cells cause either little fouling or fouling which is likely to be reversible by hydraulic backwash. AOM, on the other hand, causes irreversible fouling. Simple correlations between the amounts of AOM released and the extent of fouling are not valid, however, because the nature of the AOM has a large impact on fouling. In this regard, the size of the organic matter may be particularly important. Organic matter that is significantly smaller than the membrane pore size adsorbs onto the membrane surface or passes through the membrane, causing limited fouling. Organic matter larger than or approaching the size of membrane pores may effectively obstruct pores, causing significant fouling.

Although the overall extent of fouling varies significantly from alga to alga, a significant fraction of the fouling observed with all three organisms was irreversible to backwash. Therefore, all algae and more particularly their AOM represent a concern to the long term operation of microfiltration processes in surface water treatment.

5 USE OF PRE-COAGULATION TO REDUCE THE FOULING OF A SUBMERGED HYDROPHOBIC HOLLOW FIBER MICROFILTRATION MEMBRANE BY VARIOUS TYPES OF ORGANIC MATTER

5.1 INTRODUCTION

The use of microfiltration in the treatment of surface waters is increasingly common. However, fouling of the membranes during filtration, in large part due to the removal of organic matter present in the water, remains a problem. Organic matter can foul a membrane through several mechanisms; by contributing to the formation of relatively impermeable fouling layers on a membrane surface, by obstructing pores either externally or internally, or by constricting pores via adsorption onto the membrane material.

Measures taken to control the fouling of microfiltration membranes in a surface water treatment plant include some that alleviate fouling after it has occurred and others taken to prevent fouling from occurring. The former encompass physical cleaning (backwash) and chemical cleaning (e.g., using NaOCl). The latter include the use of membranes which are hydrophilic, in hopes of limiting the attachment of foulants onto a membrane, and the use of pre-treatment such as coagulation/flocculation/settling or coagulation/flocculation. In coagulation/flocculation/settling, the addition of a metal coagulant (Fe^{III} or Al^{III}) to the water results in the coagulation/flocculation of some membrane foulants into large flocs which can be removed by settling prior to the membrane filtration. In coagulation/flocculation, the flocs are removed by the membrane directly but cause less fouling compared to the organic matter in its original state. It is

likely that the effective and sustainable use of microfiltration membrane processes in water treatment will rely on a combination of these measures in the future.

The use of coagulation/flocculation/settling or coagulation/flocculation as pre-treatment to a microfiltration process may be particularly interesting because: (1) the reaction of a metal coagulant with at least some of the membrane foulants, particularly organic matter, present in most surface waters can be expected and potentially used to reduce the fouling they cause, (2) the combination of some form of coagulation pre-treatment with microfiltration would be expected to remove more DOC (including disinfection byproducts precursors) than a microfiltration process alone, and (3) these pre-treatments could serve as a first barrier to fouling and be used in combination with other fouling reduction measures. The reduction of at least some membrane fouling during the microfiltration or ultrafiltration of surface waters with different forms of coagulation pre-treatment is well documented [9, 17-22]. So is the increase in DOC removal that results from the addition of the coagulation process prior to membrane filtration [18-20, 22].

The main limitation of coagulation pre-treatment, however, may be that it is only effective in reducing microfiltration membrane fouling caused by organic matter (dissolved or colloidal) which reacts with a metal coagulant. In regard to DOC, work examining removal in surface waters using coagulation/flocculation/settling have indicated the existence of a general correlation between high DOC removal and high specific ultraviolet absorbance (SUVA) at 254 nm of the water [71]. This correlation is believed to exist because waters with high SUVA usually contain large concentrations of

hydrophobic acids (humic and fulvic acids) which react strongly with metal coagulants. By contrast, waters with low SUVA are more likely to contain NOM which is relatively hydrophilic and reacts poorly with metal coagulants. Studies have also shown that the coagulation of hydrophobic acids relies on coordination reactions whereby the anionic functional groups on the organic matter are neutralized by cationic metal hydrolysis species and/or metal hydroxide precipitates [72, 73]. On the basis of these studies, one concludes that metal coagulants react most favorably with organic matter which has a high SUVA and is negatively charged. Therefore pre-treatment using some form of coagulation may be very effective at reducing the microfiltration membrane fouling caused by this type of organic matter but less effective with other types.

The fouling of a microfiltration membrane by algae and algal organic matter (AOM) can be significant. Yet the potential for reducing its fouling with coagulation pre-treatment has not been tested. AOM typically has low SUVA values but contains proteins and polysaccharides which may be negatively charged at near neutral pH. A study by Widrig *et al.* showed that the AOM produced by several algae in the log growth phase can be difficult to remove using coagulation/flocculation/settling with Fe^{III} and Al^{III} coagulants followed by filtration (0.2 μm nylon filters) [74]. Others have reported that the AOM produced by different algae reacts favorably with an Fe^{III} coagulant but that it can enhance or interfere the flocculation of quartz particles depending on its concentration relative to the particle concentration and the coagulant dose [75, 76]. If coagulation pre-treatment becomes a mainstream approach to reduce the fouling of microfiltration membranes caused by surface waters, then the ability or inability of pre-coagulation to

reduce membrane fouling caused by organic matter with low SUVA such as AOM, proteins and polysaccharides may be of interest.

In this work, the reaction of alum with organic matter and the ability of coagulation pre-treatment to reduce the fouling of a hydrophobic microfiltration hollow fiber membrane by organic matter was tested with four different types of organic matter: (1) natural organic matter (NOM) originating from the Great Dismal Swamp National Wildlife Refuge in southeastern Virginia and composed mostly of fulvic acids, (2) a model protein, γ globulin, (3) a model polysaccharide, alginic acid, in the presence of calcium and (4) AOM produced by a cyanobacteria, *Microcystis aeruginosa*, collected in the decline phase of the bacteria. The NOM from the Great Dismal Swamp is characterized by a high SUVA value (>3). In contrast, the model protein, the model polysaccharide and the AOM have relatively low SUVA values (<<1).

The objectives of this work were as follows: (1) Determine the extent of the reaction between aluminum sulfate (alum) and several types of organic matter with different SUVA values and charge densities using jar tests, and (2) provided that favorable interactions were observed between the alum and the different types of organic matter in (1), determine the ability of coagulation pre-treatment to reduce the membrane fouling caused by each type of organic matter using a coagulation/flocculation/microfiltration bench scale system.

5.2 MATERIALS AND METHODS

5.2.1 Model waters

Four model waters were prepared, each of which contained a different source of organic matter. In all model waters, sodium bicarbonate was used as a buffering agent at a concentration of $1x10^{-3}$ M. This concentration of buffer was used to establish a background ionic strength and also maintain pH between 8.3 and 6.8 with the addition of 0 to 50 mg/L of alum to the model waters.

The first model water contained NOM originating from the Great Dismal Swamp National Wildlife Refuge in Southeastern Virginia. Water from the Great Dismal Swamp (GDS) was collected in 2001and kept in the dark in a refrigerator until use. This water, rich in fulvic acids, had a dissolved organic carbon (organic carbon that passes through a 0.45 mm filter) or DOC concentration of 88 mg/L and a SUVA of 3.4 L/mg-m at the time that the experiments were conducted. Water from the GDS was pre-filtered with a 1.2 μm glass fiber disc filter (Whatman GF/C) and a 0.45 μm cellulose filter (Millipore HA) before dilution to make up the model waters (Final DOC = 4.5 ± 0.5 mg C/L). The GDS NOM carries a considerable net negative charge at the pH range considered here. The model waters containing GDS NOM were allowed to equilibrate for 20 hours before a jar test or a coagulation/flocculation/microfiltration experiment was conducted.

The second model water contained γ globulin at a concentration of 5 ± 0.4 mg/L (Total organic carbon, TOC ≈ 2.5 mg C/L). SUVA for this model water was approximately 0 L/mg-m (No UV absorbance was detected at 254 nm for this solution). Severe fouling of

a hydrophobic membrane was previously observed with γ globulin at pH 7 where aggregation of the protein took place (chapter 2). At the pH conditions tested here (pH > 7), the γ globulin is expected to hold a slight net negative charge and its aggregation may be limited. The model waters containing γ globulin were allowed to equilibrate for 20 hours before a jar test or coagulation/flocculation/microfiltration experiment was conducted.

The third model water contained alginic acid at a concentration of 5 ± 0.4 mg/L (TOC = 1.48 ± 0.3 mg C/L). SUVA for this model water was also 0 L/mg-m (No UV absorbance was detected at 254 nm for this solution). The model water containing the alginic acid also contained 3.3×10^{-3} M of calcium (from $CaCl_2$). In the presence of calcium, alginic acid was previously seen to aggregate and cause severe fouling of a hydrophobic microfiltration membrane (chapter 2). Under the pH conditions considered here, the alginic acid normally carries a large negative charge but part of this charge was neutralized by calcium. The model waters containing alginic acid were allowed to equilibrate for 20 hours before a jar test or a coagulation/flocculation/microfiltration experiment was conducted.

The fourth model water contained cells of the cyanobacteria, *Microcystis,* and its AOM. This model water was made by diluting (1:20) samples of the large *Microcystis* culture described in chapter 4 with MilliQ water. The samples of the culture necessary to make up the model waters were collected between day 54 and day 60, during the decline phase of the *Microcystis*, when DOC in the culture was high (DOC = 110-130 mg /L) and

shown to cause significant irreversible fouling of a hydrophobic microfiltration membrane (chapter 4). The model waters contained both *Microcystis* cells (cell count ≈ 2.4 x 10^5 cells/mL) and AOM (DOC = 6.4 ± 0.7 mg C/L). SUVA of the AOM was approximately 0.14 L/mg-m. The charge on the AOM is unknown. The model waters containing *Microcystis* and its AOM were prepared immediately before a jar test or a coagulation/flocculation/microfiltration experiment was conducted. Coagulation/ flocculation/microfiltration experiments and jar tests were conducted as closely in time as possible to minimize the impact of changes in the AOM on the results. In fact, concentrations of DOC, protein and carbohydrates changed little over the period of time considered (see chapter 4).

5.2.2 Jar tests

The reactions between alum ($Al_2(SO_4)_3.18H_2O$) and the different types of organic matter were tested using jar tests. The jar tests were conducted as follows. Six to twelve jars were tested for each model water. To each 250 mL jar, 150 mL of model water was added and the jars were placed in a jar test apparatus. The contents of the jars were rapidly mixed at 100 rpm and increasing amounts of alum were added to each jar. After the addition of alum to the last jar, rapid mixing was maintained for 2 minutes and followed by slow mixing at 25 rpm for 30 minutes. At the end of the flocculation, the jars were taken off the jar test apparatus and the contents of the jars were allowed to settle quiescently for 60 minutes. After sedimentation, a 50 mL sample of the settled water from each jar was gently withdrawn from just below the water surface using a wide mouth pipette. Half of the sample was used to determine settled turbidity and settled organic matter concentration (when appropriate). The other half was filtered through a

1.2 μm glass fiber filter (Whatman GF/C, prewashed with MilliQ water) and used to determine filtered turbidity and filtered organic matter concentration. The final pH of each jar was measured after sampling. Turbidities were measured with a Hach model 2100A turbidimeter. Organic matter concentrations were measured using organic carbon measurements (in the case of GDS NOM, alginic acid and AOM) or a protein assay (in the case of γ globulin). Minimum effective alum doses (MEADs) for organic matter removal by settling and filtration were qualitatively determined by plotting settled and filtered organic matter concentration as a function of alum dose. The alum doses where a significant fraction of the organic matter was removed by settling or settling/filtration were adopted as the settled and filtered MEADs, respectively.

5.2.3 Coagulation/flocculation/microfiltration unit

The ability of pre-coagulation to reduce membrane fouling caused by each type of organic matter was tested with the coagulation/flocculation/microfiltration system shown in Figure 5-1. It is similar to the microfiltration unit described in chapter 3 but includes an additional pump and a coagulation/flocculation process. Using a peristaltic pump, raw water was first pumped at a flow rate of 2 mL/min into a rapid mix vessel. This vessel consisted of a small sealed vial filled to a level of 4 mL (detention time, $\theta = 2$ min) whose content was rapidly mixed using a magnetic stir bar. Coagulant was added to the raw water using a syringe pump in the line leaving the raw water tank and entering the rapid mix vessel. Pressure build-up in the sealed rapid mix vessel caused water to flow out of this vessel and into the flocculation tank. The flocculation tank consisted of a 200 mL beaker filled with 80 mL ($\theta = 40$ min) of water placed in a jar test apparatus. In the flocculation tank, the water was flocculated by a paddle rotating at 25 rpm. Flocculated

water was then pumped to the filtration column, also at a flow rate of 2 mL/min using a dual channel peristaltic pump. The same pump was used to pump permeate through the membrane module, maintaining a constant water level in the filtration column. Detention time in the filtration column was approximately 5 minutes. The coagulation/flocculation process was started 40 minutes before the microfiltration process so that only flocculated water was allowed to reach the membrane. After these 40 minutes had elapsed, the filtration column was filled with flocculated water (a 5 minute process). The filtration was then started and the evolution of transmembrane pressures over time followed.

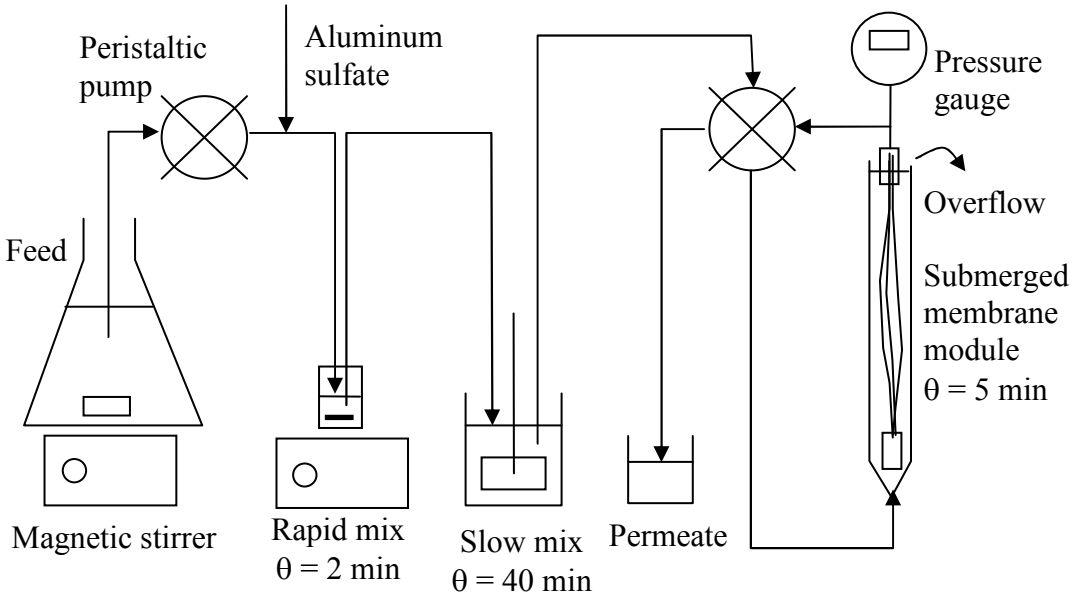


Figure 5-1. Coagulation/flocculation/microfiltration unit.

An effort was made to have similar flocculation conditions in both the jar tests and the coagulation/flocculation/microfiltration system in terms of mixing speeds and detention times. This was deemed important since the selection of the alum doses used in the coagulation/flocculation/microfiltration system with each type of organic matter was based on the results of the jar tests.

5.2.4 Coagulation/flocculation/microfiltration protocol

Filtration followed the same protocol previously described in chapter 3 (Figure 5-2). Filtration was conducted at a permeate flow rate of 36 gfd (61 $Lm^{-2}h^{-1}$) for a total of 200 minutes. The filtration was interrupted by 2 backwashes, one at 120 minutes, another at 180 minutes. Backwashes used a reverse flow rate of 36 gfd and were one minute long. During this time, mixing in the flocculation tank was maintained. The value of the NTMP at the end of each microfiltration run (final NTMP) was used to compare the fouling caused by the different model waters at the various coagulant doses tested (Figure 5-2). Organic matter concentrations in the permeate were measured throughout the filtration.

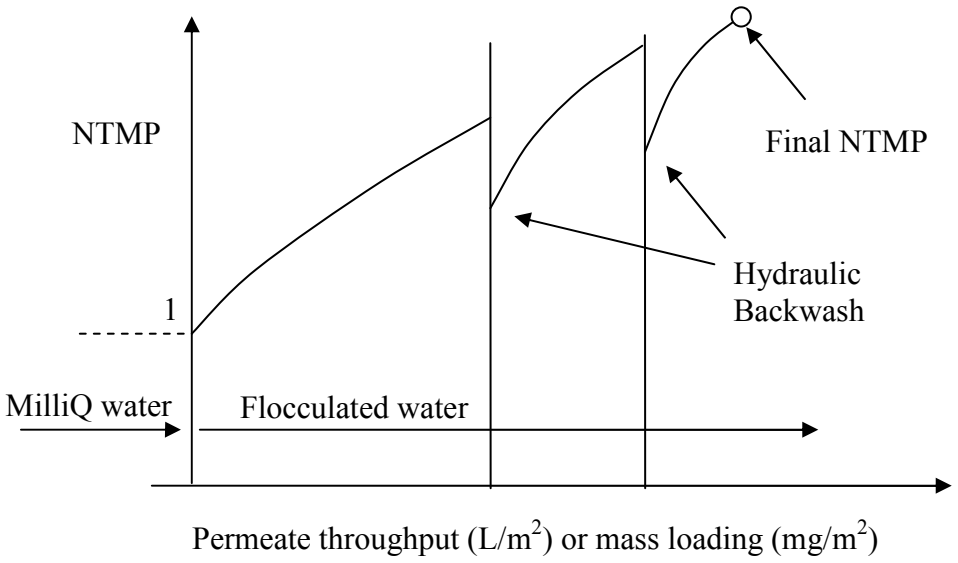


Figure 5-2. Filtration protocol with the coagulation/flocculation/microfiltration system.

5.2.5 Measurements of organic matter concentration

During jar tests and coagulation/flocculation/microfiltration runs, concentrations of GDS NOM, alginic acid and AOM were determined with a persulfate/UV carbon analyzer (Dorhmann 8000). Concentrations of γ globulin were measured with the BCA assay described in chapter 3.

5.3 RESULTS

5.3.1 Great Dismal Swamp natural organic matter

Jar test results for the model waters containing GDS NOM are presented in Figure 5-3 and 5-4. Alum doses are reported as mg of alum added per mg of DOC originally present in the model water. As shown in Figure 5-3, filtered and settled turbidities behaved similarly. Both increased as the alum dose increased from 0 to 0.58 (mg Al/mg DOC) and dropped suddenly around 0.58 (mg Al/mg DOC). The initial increase in the turbidities is the result of the formation of small flocs in the solution which could not be removed by settling or filtration (with a 1.2 μm filter). At alum doses below 0.58 (mg Al/ mg DOC), concentrations of aluminum species were probably too low to neutralize the charge on the GDS NOM. As a result, the flocs that initially formed bore a net negative charge which prevented them to grow into large flocs. Once the alum dose reached 0.58 (mg Al/mg DOC), the charge of the flocs formed became small enough to allow the rapid formation of large flocs that could be removed by settling or filtration. The results in Figure 5-4 are consistent with the picture presented above. The GDS NOM remained in solution as the alum dose increased from 0 to 0.58 (mg Al/mg DOC) and dropped abruptly at 0.58 (mg Al/mg DOC). Very little difference was seen between the concentration of the GDS NOM remaining in solution after settling or filtration. On the basis of the jar test results, the settled and filtered MEADs for the waters containing the GDS NOM were determined to be approximately 0.60 (mg Al/mg DOC). At this alum dose, flocs are expected to form that capture a large fraction of the GDS NOM.

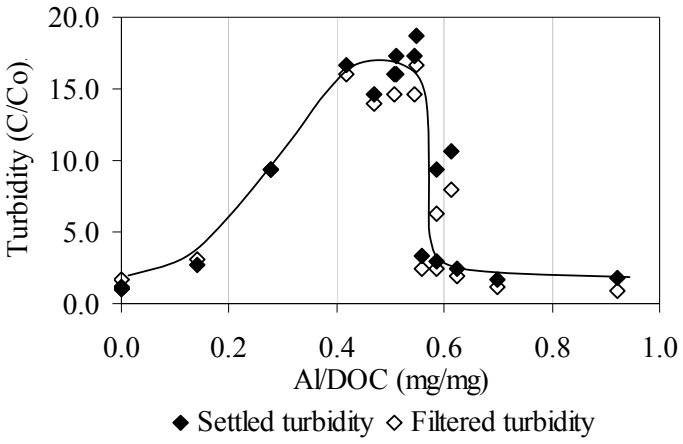


Figure 5-3. Settled and filtered turbidity during coagulation of water containing 4.5 mg C/L GDS NOM and $1x10^{-3}$ M $NaHCO_3$ (pH = 8.0-7.1).

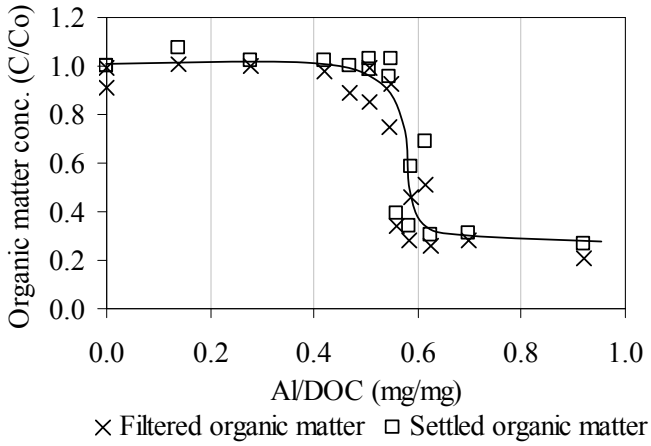


Figure 5-4. Settled and filtered organic matter concentration during coagulation of water containing 4.5 mg C/L GDS NOM and $1x10^{-3}$ M $NaHCO_3$ (pH = 8.0-7.1).

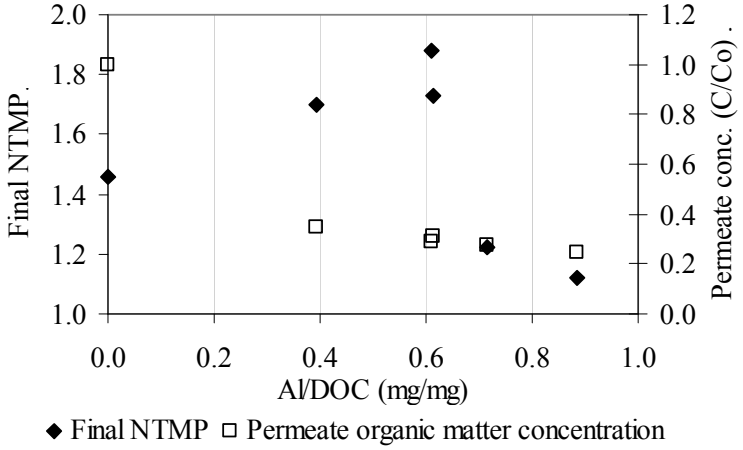


Figure 5-5. Performance of the coagulation/flocculation/microfiltration system as a function of alum dose for water containing 4.5 mg C/L GDS NOM and $1x10^{-3}$ M $NaHCO_3$ (pH = 8.0-7.1).

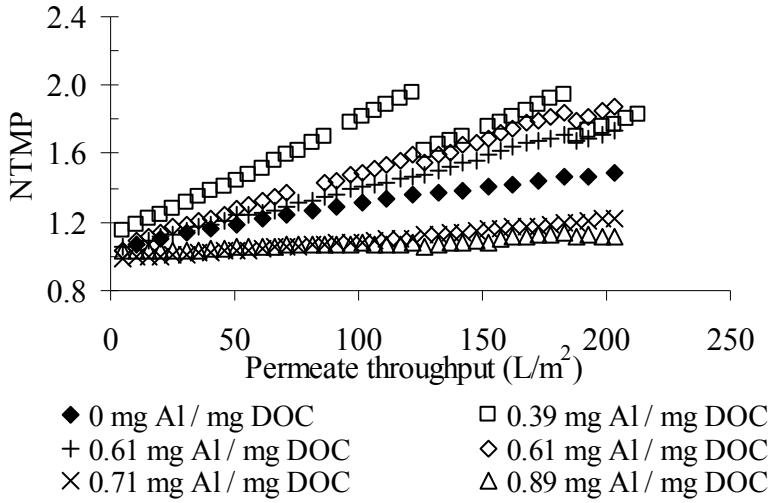


Figure 5-6. Membrane fouling during the coagulation/flocculation/microfiltration of water containing 4.5 mg C/L GDS NOM and $1x10^{-3}$ M $NaHCO_3$ at various alum doses (pH = 8.0-7.1).

The performance of the coagulation/flocculation/microfiltration system for waters containing GDS NOM as a function of alum dose is presented in Figure 5-5 and 5-6. Alum doses below and above the MEAD were tested. When no alum was added, some irreversible fouling was observed (Final NTMP ≈ 1.5) yet very little DOC was removed ($C/C_o \approx 1$). Most of the fouling of a hydrophobic microfiltration membrane caused by GDS NOM under similar conditions has previously been attributed to the obstruction of pores by very low concentrations of organic aggregates present in the GDS NOM [26]. At 0.47 (mg Al/mg DOC), the increase in NTMP over the course of the filtration was more severe than when alum was not added (Final NTMP ≈ 1.7), but a fraction of this fouling was reversible by backwash (Figure 5-6). In addition, significant amounts of DOC were removed ($C/C_o \approx 0.35$). At 0.61 (mg Al/mg DOC), fouling was still significant (Final NTMP ≈ 1.7) but became largely irreversible. At this alum dose, DOC removal was high ($C/C_o \approx 0.3$). At alum doses above 0.70 (mg Al/mg DOC), fouling was reduced (Final NTMP ≈ 1.2) and removal of DOC remained high ($C/C_o \approx 0.2$-0.3).

5.3.2 Gamma globulin

Jar test results for the model waters containing γ globulin are presented in Figure 5-7 and 5-8. Alum doses are reported as mg of alum added per mg of TOC originally present in the model water. As shown in Figure 5-7, settled and filtered turbidities behaved differently. In the case of settled turbidity, an increase in turbidity was observed as alum dose was increased from 0 to 0.16 (mg Al/mg TOC). Settled turbidity eventually reached a maximum at 0.16 (mg Al/mg TOC) and decreased rapidly at greater alum doses. In the case of filtered turbidity, a slight increase in turbidity was seen at 0.11 (mg Al/mg TOC) but filtered turbidity remained low otherwise. The results indicate that at alum doses between 0.15 and 0.25 (mg Al/mg TOC), flocs formed that were not removed by settling but were removed by filtration. This difference in alum dose necessary for the removal of the γ globulin by settling and filtration was confirmed by the protein removal results in Figure 5-8. For γ globulin, the MEAD based on settled protein concentration (0.25 mg Al/mg TOC) was higher than the MEAD based on filtered protein concentration (0.15 mg Al/mg TOC).

The settled MEAD was greater than the filtered MEAD and therefore represented a more conservative alum dose to use with the coagulation/flocculation/microfiltration system. An alum dose slightly greater than 0.25 (mg Al/mg TOC) was tested with the coagulation/flocculation/microfiltration system. At this dose, flocs that form are large enough to settle and should be sufficiently large to only cause limited fouling of the microfiltration membrane. The coagulation/flocculation/microfiltration system performance for waters containing γ globulin as a function of alum dose is presented in

Figure 5-9. When no alum was added, fouling of the hydrophobic microfiltration membrane was moderate (Final NTMP ≈ 1.7) and removal of the γ globulin by the membrane was high ($C/C_o \approx 0.1$) but diminished slightly as the filtration progressed. Under these solution conditions (pH = 8.3, I = 0.001), aggregation of the γ globulin was limited and fouling was probably dominated by a process of internal pore constriction due to the adsorption of the proteins onto the membrane pore walls (see chapter 3). At an alum dose of 0.32 (mg Al/mg TOC), fouling was significantly reduced (Final NTMP ≈ 1.1). Protein removal during the coagulation/flocculation/microfiltration with 0.32 (mg Al/mg TOC) was not measured but would be expected to be high ($C/C_o < 0.1$).

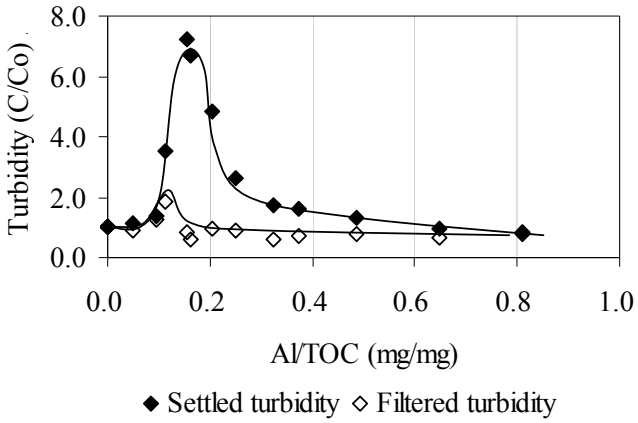


Figure 5-7. Settled and filtered turbidity during coagulation of water containing 5 mg/L γ globulin and $1x10^{-3}$ M $NaHCO_3$ (pH = 8.3-7.3).

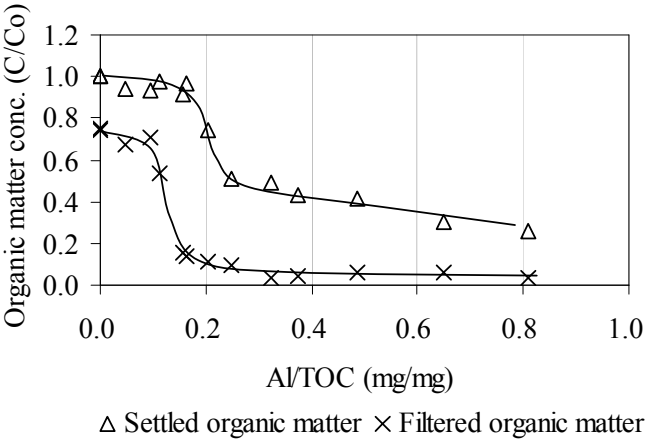


Figure 5-8. Settled and filtered protein concentration during coagulation of water containing 5 mg/L γ globulin and $1x10^{-3}$ M $NaHCO_3$ (pH = 8.3-7.3).

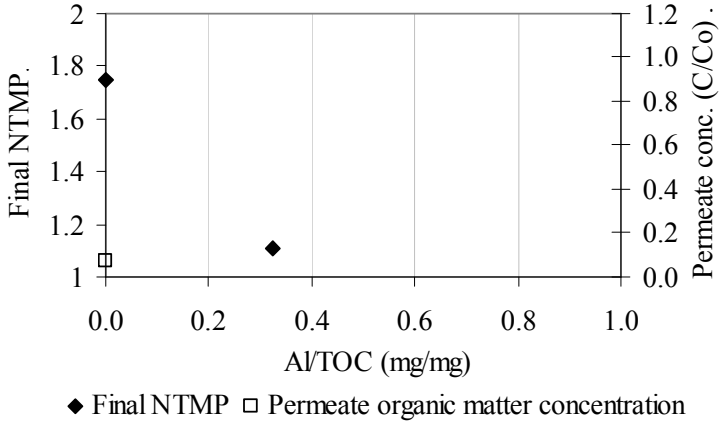


Figure 5-9. Performance of the coagulation/flocculation/microfiltration system as a function of alum dose for water containing 5 mg/L γ globulin and $1x10^{-3}$ M $NaHCO_3$ (pH = 8.3-7.7).

5.3.3 Alginic acid in the presence of calcium

Jar test results for the model waters containing alginic acid in the presence of calcium are presented in Figure 5-10 and 5-11. Alum doses are reported as mg of alum added per mg of TOC originally present in the model water. As shown in Figure 5-10, the turbidity data with alginic acid in the presence of calcium are more scattered than they were for γ globulin or GDS NOM. The reason for this is that turbidities of the waters containing the alginic acid in the presence of calcium did not vary greatly as a function of alum dose and were fairly low, ranging from 0.1 to 0.34. These measurements were therefore quite susceptible to experimental variations and artifacts. For example, the filtered turbidity of a sample was sometimes higher than its settled turbidity because the filtration step that distinguishes the two raised the turbidity of the filtered solutions slightly. Despite the scatter, however, it can be said that settled turbidity showed a notable decrease at 0.16 (mg Al/mg TOC) and that filtered turbidities remained low at all alum doses tested (Figure 5-10). The results of the organic carbon concentration remaining in solution after settling and filtration were more informative than the results with turbidity. As shown in Figure 5-11, concentration of the alginic acid after settling decreased significantly at 0.16 (mg Al/mg TOC). The concentration of the alginic acid after filtration, on the other hand, started out low and decreased as the alum dose increased up to 0.16 (mg Al/mg TOC). It was shown in chapter 3 that alginic acid under similar conditions (neutral pH, [Ca] = $3x10^{-3}$ M) formed aggregates. The occurrence of this aggregation was supported by the reduced concentration of alginic acid remaining in solution after filtration when alum dose was nil; over 70 % of the alginic acid was removed by filtration through the 1.2 μm glass fiber disc filter in the sample to which no alum was added (Figure 5-11). While

alginic acid aggregates clearly formed under these conditions, these aggregates apparently did not settle on their own. Alum doses of 0.16 (mg Al/mg TOC) were necessary to coagulate and flocculate the alginic acid aggregates into flocs that were large and/or dense enough to settle. For both settled and filtered alginic acid concentrations, a single MEAD of 0.16 (mg Al/mg TOC) was therefore identified.

The performance of the coagulation/flocculation/microfiltration system for waters containing alginic acid in the presence of calcium as a function of alum dose is presented in Figure 5-12. When no alum was added, significant irreversible fouling of the membrane was observed (Final NTMP ≈ 4) and removal of the alginic acid was complete ($C/C_o \approx 0$). The fouling mechanism under these conditions was shown to involve the removal by the membrane of alginic acid aggregates and the build-up of an impermeable cake on the membrane surface that obstructs the membrane pores (see chapter 3). When alum was added at doses greater than or equal to 0.25 (mg Al/mg TOC) with the coagulation/flocculation/microfiltration system, significant reductions in fouling were observed (Final NTMP ≈ 1.1–1.3) and the removal of the alginic acid was complete.

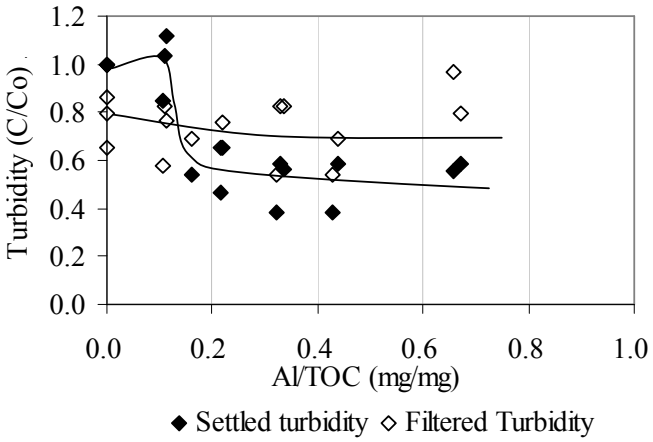


Figure 5-10. Settled and filtered turbidity during coagulation of water containing 5 mg/L alginic acid, $1x10^{-3}$ M $NaHCO_3$, and $3.3x10^{-3}$ M Ca (pH = 8.0-7.6).

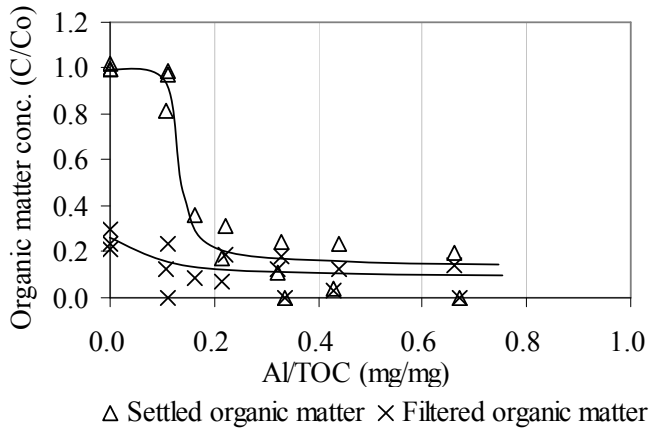


Figure 5-11. Settled and filtered organic carbon concentration during coagulation of water containing 5 mg/L alginic acid, $1x10^{-3}$ M $NaHCO_3$, and $3.3x10^{-3}$ M Ca (pH = 8.0-7.6).

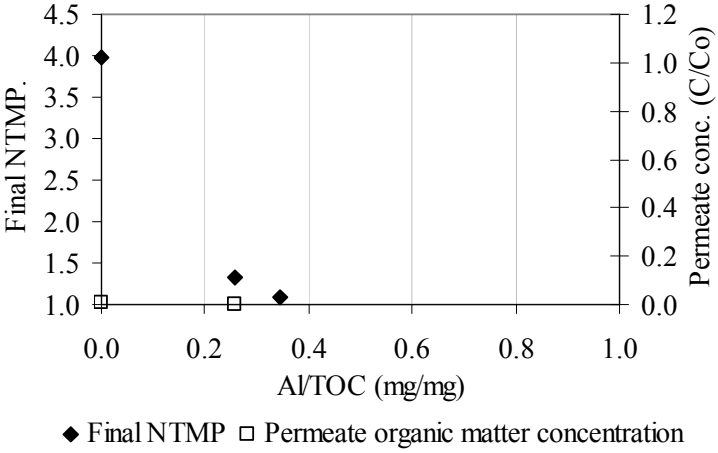


Figure 5-12. Performance of the coagulation/flocculation/microfiltration system as a function of alum dose for water containing 5 mg/L alginic acid, $1x10^{-3}$ M $NaHCO_3$, and $3.3x10^{-3}$ M Ca (pH = 8.0-7.8).

5.3.4 Microcystis and associated AOM

Jar test results for the model waters containing *Microcystis* and its AOM collected in the decline phase of the bacteria are presented in Figure 5-13 and 5-14. Alum doses are reported as mg of alum added per mg of DOC originally present in the model water. As shown in Figure 5-13, settled turbidities started to decrease at an alum dose of 0.20 (mg Al/mg DOC) and were very low by 0.37 (mg Al/mg DOC). Filtered turbidities were low at all alum doses tested. The results with turbidity reflect the behavior of the *Microcystis* cells as the alum dose was increased. Flocs of *Microcystis* cells started to form but remained small at 0.12 (mg Al/mg DOC). Cell flocs grew larger and some started to settle at 0.20 (mg Al/mg DOC). Essentially all of the cells flocculated and settled at 0.37 (mg Al/mg DOC). All the cells were removed by filtration through the 1.2 μm disc filters so the filtered turbidities were low at all alum doses. The results presented in Figure 5-14, on the other hand, reflect the behavior of the AOM as alum dose was increased. Concentrations of organic carbon remaining after filtration started to decrease at 0.12 (mg Al/mg DOC) and were significantly reduced as the alum dose reached 0.37 (mg Al/mg DOC). Concentrations of organic carbon remaining after settling were only measured when the alum doses were sufficiently high to remove the *Microcystis* cells from the solution. The limited data indicate that the organic carbon remaining after settling mirrored the behavior of the *Microcystis* cells. The concentrations of both were significantly reduced by settling once alum reached 0.37 (mg Al/mg DOC). A MEAD of 0.4 (mg Al/mg DOC) for effective coagulation and flocculation of the *Microcystis* cells and its AOM was adopted.

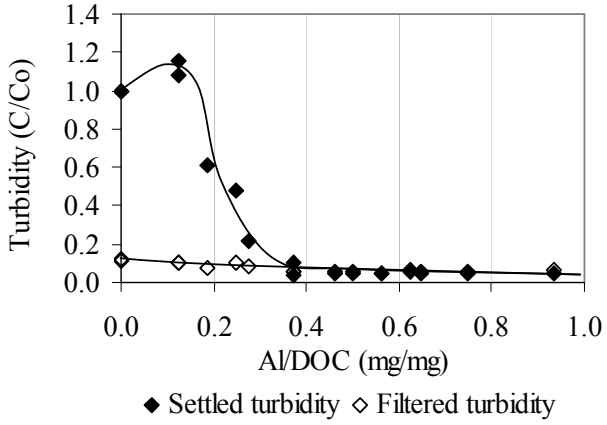


Figure 5-13. Settled and filtered turbidity during coagulation of water containing *Microcystis* and its AOM (cell count ≈ $2.4x10^5$ cells/mL, DOC ≈ 6.4 mg/L, [$NaHCO_3$] = $1x10^{-3}$ M) (pH = 8.0-6.5).

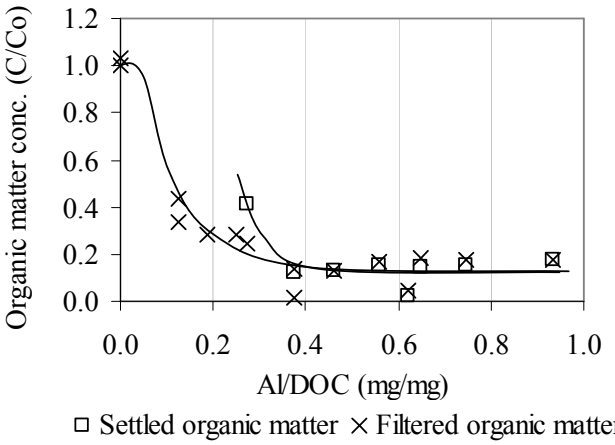


Figure 5-14. Settled and filtered organic carbon concentration during coagulation of water containing *Microcystis* and its AOM (cell count ≈ $2.4x10^5$ cells/mL, DOC ≈ 6.4 mg/L, [$NaHCO_3$] = $1x10^{-3}$ M) (pH = 8.0-6.5).

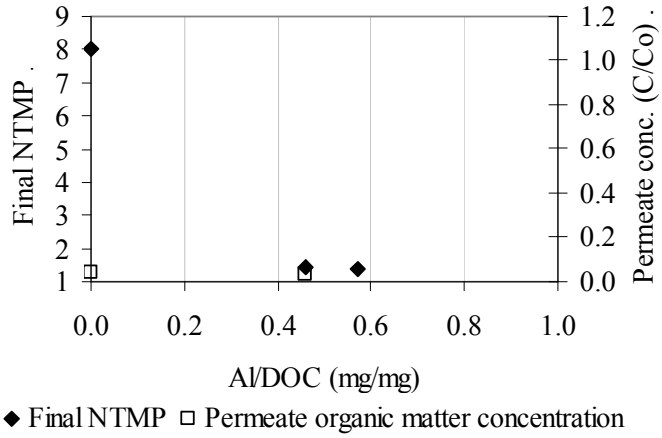


Figure 5-15. Performance of the coagulation/flocculation/microfiltration system as a function of alum dose for water containing *Microcystis* and its AOM (cell count ≈ $2.4x10^5$ cells/mL, DOC ≈ 6.4 mg/L, [$NaHCO_3$] = $1x10^{-3}$ M) (pH = 8.0-7.0).

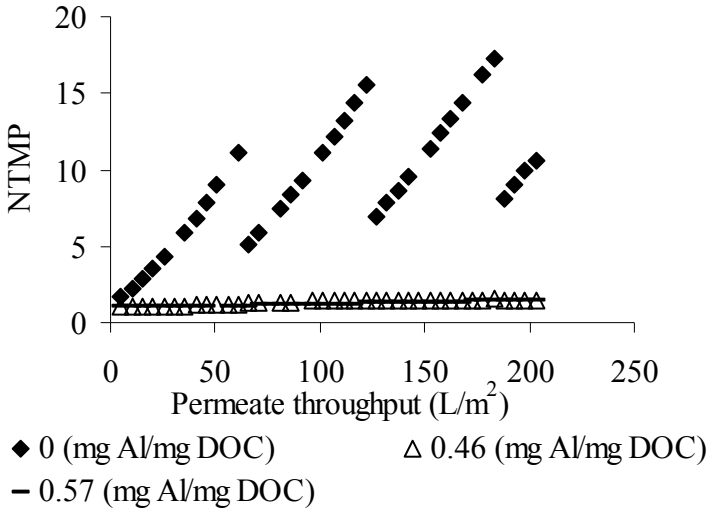


Figure 5-16. Membrane fouling during the coagulation/flocculation/microfiltration of water containing *Microcystis* and its AOM (cell count ≈ $2.4x10^5$ cells/mL, DOC ≈ 6.4 mg/L, $[NaHCO_3] = 1x10^{-3}$ M) at various alum doses (pH = 8.0-7.0).

The performance of the coagulation/flocculation/microfiltration system for waters containing *Microcystis* and its AOM as a function of alum dose is presented in Figure 5-15 and 5-16. When no alum was added, fouling was significant and a large fraction of it was irreversible (Final NTMP ≈ 8). Removal of the AOM under these conditions was high ($C/C_o \approx 0.2$). These results are similar to the results presented in chapter 4. When alum in the coagulation/flocculation/microfiltration system was added at concentrations greater than or equal to 0.46 (mg Al/mg DOC), membrane fouling was significantly reduced (Final NTMP ≈ 1.4) and DOC removal remained high ($C/C_o < 0.1$).

5.4 DISCUSSION

5.4.1 Reaction between alum and organic matter

The jar test results presented here demonstrate that organic matter characterized by low SUVA can react with a metal coagulant such as alum. The organic matter removals obtained here with γ globulin, alginic acid and the *Microcystis* AOM during the jar tests

were much greater than those that would be expected for surface waters with similar SUVA [73]. Experiments with hydrophobic acids [72] have identified two main mechanisms whereby organic matter reacts with a metal salt coagulant: (1) the reaction of cationic metal hydrolysis species with anionic functional groups on the organic matter to form complexes which may remain soluble (at least temporarily) or flocculate into solids and (2) the adsorption of the above mentioned soluble complexes onto metal hydroxide precipitates. Since both gamma globulin and alginic acid carry a net negative charge at the pH of coagulation used (pH > 7), it is likely that the anionic functional groups present on those molecules reacted with alum using similar mechanisms. During the jar tests, more alum was required for settling and filtration of the GDS NOM than the γ globulin and alginic acid. This is qualitatively consistent with charge neutralization; the net negative charge on the GDS NOM is expected to be higher than that on γ globulin (coagulation pH relatively close to pH_{iep} of the protein) and alginic acid (partial charge neutralization already accomplished by calcium) under the conditions tested. Because AOM reacted favorably with the alum, it is speculated that the AOM also carried a net negative charge and that its anionic functional groups allowed it to react with alum.

To confirm that the presence of anionic functional groups and a net negative charge on the organic matter played a dominant role on the reaction with alum, a series of jar tests were conducted with dextran and lysozyme (model water: 5 ± 0.4 mg/L model compound, $1x10^{-3}$ M $NaHCO_3$). Dextran is a neutral polysaccharide and lysozyme is a protein with a pH_{iep} of 10.3. Alum doses ranging from 0.12 to 1.28 (mg Al/mg TOC) were tested, resulting in coagulation pH that varied from 8.06 to 7.21. In this pH range,

the two compounds were either neutral or carried a net positive charge. During the jar tests, neither compound reacted with alum. These results indicate that a net negative charge on the organic molecules was indeed necessary for coagulation with alum to occur.

On the basis of these results, it appears that SUVA may not always be a good basis for prediction of the extent of the reaction between organic matter and a metal coagulant. Proteins and polysaccharides which have relatively low SUVA may or may not react with a metal coagulant such as alum; whether this reaction occurs depends on the presence of a net negative charge on these organic molecules.

5.4.2 Impact of alum dose on membrane fouling

The impact of alum dose on the reduction of membrane fouling caused by organic matter can be important as shown by the experiments with the GDS NOM. For this organic matter, addition of alum at doses below or approaching the MEAD (0.47 – 0.62 mg Al/ mg DOC) caused fouling which was more severe than when alum was absent. This increase in fouling is believed to be the result of the formation of small flocs during the coagulation and flocculation process which were removed by the membrane in the coagulation/flocculation/microfiltration system. These small flocs may have caused additional fouling of the membrane by forming a tight cake layer on the membrane surface and/or by obstructing pores. Evidence for the formation of flocs at these alum doses can be found in the elevated settled turbidity measurements in the jar tests (Figure 5-3). Removal of these small flocs by the coagulation/flocculation/microfiltration system is proven by the lower organic matter concentrations measured in the permeate water to

this system compared to the settled or filtered samples in the jar test (compare Figure 5-3 and Figure 5-5). At alum doses above 0.62 (mg Al/mg DOC), increases in alum doses decreased the extent of fouling. At these doses, large flocs were formed that captured a significant fraction of the GDS NOM (Figure 5-3). These large flocs appeared to deposit as very loose and permeable layers on the membrane surface during filtration that did not cause significant fouling.

As shown with GDS NOM, membrane fouling can increase or decrease with increases in coagulant dose depending on the value of the dose relative to the settled MEAD. A similar behavior has been reported for the fouling of an ultrafiltration membrane by water containing Aldrich humic acids [17]: at a pH of about 7, a low coagulant dose (under-dose) increased fouling whereas a high dose (for proper coagulation) reduced fouling. Increases in the fouling of a cross-flow ultrafiltration membrane by Fluka humic acids when coagulant addition resulted in the formation of small flocs has also been shown [22]. With surface waters, some results have indicated that fouling worsens before being reduced with increases in coagulant doses [18, 20, 44] while others have shown a general reduction in membrane fouling with increasing coagulant doses [17, 19]. In many studies, the value of the settled MEAD for the tested waters or the basis for the selection of the tested coagulant dose(s) tested are unfortunately not given or not clearly stated. The results presented here suggest that, for organic matter that reacts favorably with a coagulant, increases in alum doses above the settled MEAD are expected to reduce microfiltration membrane fouling. Doses below the settled MEAD can increase fouling.

For the four types of organic matter tested with the coagulation/flocculation/ microfiltration system, the use of alum doses above (by about 20-30 %) the MEAD based on organic matter removal by settling determined using jar tests achieved a significant reduction in membrane fouling. Provided the flocculation conditions are similar for the two systems, the use of a jar test may therefore be an effective way to determine the appropriate coagulant dose to employ with a coagulation/flocculation/microfiltration system. A procedure similar to the one that follows might be taken to determine the appropriate alum dose to use with a coagulation/flocculation/microfiltration system and an unknown water: (1) determine the DOC concentration in the raw water, (2) Conduct a jar test with alum doses ranging from 0 to 1 (mg Al/mg DOC), (3) measure the concentration of total organic carbon in the supernatant of the settled jars (starting with the jar having received the most coagulant and stopping when carbon concentrations exceed the limitations of the organic carbon analyzer), (4) define the point where organic carbon concentration begins to rise in the supernatants as the MEAD for organic matter removal by settling, and (5) multiply that dose by 1.3 (for an overdose of 30 %). Such a procedure could work for many waters but needs to be tested.

5.4.3 Limitations of pre-coagulation to reduce membrane fouling

A significant limitation to pre-coagulation is that its effectiveness likely depends on the extent of the reaction between the coagulant and the foulants. A coagulant may not react with all types of organic matter that can act as foulants towards a microfiltration membrane. The fouling caused by GDS NOM, alginic acid, γ globulin and the AOM produced by *Microcystis* was effectively reduced using pre-coagulation with alum because these compounds reacted strongly with alum. Organic matter which is neutral or

positively charged does not appear to react with alum and pre-coagulation may not reduce the microfiltration membrane fouling it causes effectively. Even with the organic matter tested here which reacted strongly with alum, some irreversible fouling was still observed. This residual fouling may be caused by flocculated matter that deposited on the membrane surface and was not removed by backwash or by a small fraction of organic matter which did not react with the alum. Consequently for most natural surface waters, pre-coagulation may be able to reduce fouling of a microfiltration membrane by organic matter significantly but not completely.

These variations in the extent of the reaction between a coagulant and organic matter could be particularly important to the prospect of reducing microfiltration membrane fouling caused by AOM using coagulation/flocculation pre-treatment. As shown in chapter 4, the characteristics of AOM vary from alga to alga. They could also vary depending on the environment in which an alga grows (nutrient levels, predation, light limitation, temperature variations…). Such variations in AOM affected the fouling of a hydrophobic microfiltration membrane (chapter 4) and may very well affect the effectiveness of coagulation/flocculation pre-treatment to reduce it.

5.4.4 Coagulation/flocculation pre-treatment to reduce the fouling of microfiltration membranes by surface waters

Because surface waters with a high SUVA (SUVA > 3) are likely to contain large amounts of hydrophobic acids which react strongly with metal coagulants, the microfiltration membrane fouling caused by such waters may be particularly suitable to reduction using coagulation/flocculation pre-treatment. With these waters, the use of

coagulation/flocculation pre-treatment with microfiltration would also be expected to improve organic matter removal significantly. By contrast, waters with a low SUVA (<< 3) are likely to contain a mix of compounds, some of which react with a metal coagulant, some of which do not. Therefore, the microfiltration membrane fouling caused by waters with low SUVA may be reduced to varying extents with coagulation/flocculation pre-treatment. More work is needed to evaluate the ability of coagulation/flocculation pre-treatment to reduce the microfiltration membrane fouling caused by organic matter and surface waters that have a low SUVA.

5.5 CONCLUSIONS

Organic matter with a wide range of SUVA values including fulvic acids, proteins, polysaccharides, and AOM can react with alum. Whether the reaction between the organic matter and the alum occurs depends heavily on the abundance of anionic functional groups on the organic matter and its resulting net charge.

Microfiltration membrane fouling caused by organic matter with various SUVAs can be significantly reduced although not completely eliminated with pre-coagulation. Significant reductions in microfiltration membrane fouling are achieved when organic foulants are captured into large flocs which deposit as loose, permeable layers on the membrane surface. Consequently, the reduction in microfiltration membrane fouling achieved with pre-coagulation will depend on the selection of (1) proper flocculation conditions and (2) an appropriate coagulant dose. Flocculation conditions must provide enough collision opportunities through a combination of shear rate and flocculation time. As for the coagulant dose, it may be determined using jar tests. At a coagulation pH

slightly above neutral (the conditions tested here), a slight overdose relative to the MEAD based on organic matter removal by settling is recommended.

Fouling by the AOM produced by *Microcystis* in the decline phase was significantly reduced with pre-coagulation. However, reduction of the fouling caused by AOM using pre-coagulation may be a function of the alga producing the AOM and the conditions under which it is produced (environmental stresses). The ability of pre-coagulation to reduce microfiltration membrane fouling by AOM looks promising but needs to be tested further.

6 CONCLUSIONS, SIGNIFICANCE AND FUTURE WORK

6.1 CONCLUSIONS

6.1.1 Fouling of a hydrophobic microfiltration membrane by a selection of model compounds under varying chemical conditions

The use of model compounds (bovine serum albumin, gamma globulin, alginic acid, and dextran) to evaluate the fouling of a hydrophobic hollow fiber microfiltration membrane by proteins and polysaccharides yielded valuable information. The systematic testing of the fouling caused by the model compounds under varying chemical conditions demonstrated the occurrence of two different fouling mechanisms. The first of these mechanisms dominates when a compound is stable in solution but interacts favorably with the membrane. Under these conditions, fouling occurs through a mechanism of pore constriction brought about by the adsorption of the organic matter onto the pore walls. This mechanism was observed with both proteins, bovine serum albumin and gamma globulin. The second mechanism dominates when a compound is unstable in solution and forms aggregates. Under these conditions, fouling likely results from the obstruction of pores by these aggregates. This mechanism of fouling was observed with the polysaccharide, alginic acid (only when calcium was present), and the protein, gamma globulin (when pH was close to the pH_{iep} of the protein or when ionic strengh was raised, especially with salt containing calcium). The second of the two mechanisms causes significantly more fouling, total and irreversible, than the first for the same amount of matter removed by the membrane. Based on these results, it is concluded that significant irreversible fouling of a hydrophobic microfiltration membrane is possible with organic

matter that includes proteins and polysaccharides and that the chemical environment in which these compounds are present exert an important influence on the extent of fouling. Furthermore, the fouling caused by the model compounds was almost completely irreversible to hydraulic backwash. Therefore, the fouling of microfiltration membranes in treatment plants caused by proteins and polysaccharides present in the source waters may build up over time.

6.1.2 Fouling of a hydrophobic microfiltration membrane by three different algae and their associated algal organic matter

Bench-scale experiments showed that waters containing different algae (*Scenedesmus*, *Asterionella*, and *Microcystis*) and algal organic matter (AOM) can foul a hydrophobic hollow fiber microfiltration membrane. This type of fouling can be expected to vary significantly from alga to alga but is largely controlled by AOM. *Scenedesmus* reached $6x10^5$ cells/mL and released up to 20 mg DOC/L. *Asterionella* reached $1.2x10^5$ cells/mL and released up to 2 mg DOC/L. *Microcystis* reached $8x10^6$ cells/mL and released up to 130 mg DOC/L. Fouling by waters containing *Microcystis* and its AOM was significantly greater than that seen with the other two algae. Algal cells accumulate on the membrane surface to form a cake during filtration but these cakes are easily re-suspended in solution during hydraulic backwash. Experiments showed that the cell cakes either did not cause fouling (*Scenedesmus* and *Asterionella*) or caused fouling which was largely reversible (*Microcystis*). Cakes of *Microcystis* may have caused more fouling than that of the other two algae because *Microcystis* produced large quantities of AOM which may have contributed to the fouling caused by the cell cakes. More generally however, it was found that AOM can foul a microfiltration membrane by acting independently from the algal

cells (*Scenedesmus, Asterionella, Microcystis*) and that this fouling is largely irreversible. This fouling process likely includes a combination of pore constriction via the adsorption of small (≤100 kD) organic matter onto the membrane pore walls and pore obstruction caused by large organic aggregates. More fouling, total and irreversible, can be expected when fractions of AOM approach the membrane pore size and fouling by pore obstruction takes place. This is in agreement with the experiments using the model compounds and the findings of Haiou Huang who observed more significant irreversible fouling of the same membranes by solutions containing latex particles when these particles approached the size of the membrane pores and attached to the membrane material [26]. Concentrations of AOM generally increase as algae reach the stationary and decline phase. For a given alga, fouling, total and irreversible, therefore increases as the alga progresses through these phases. AOM has a low SUVA (specific ultra violet absorbance), much less than 1, and contains proteins as well as polysaccharides.

6.1.3 Use of pre-coagulation to reduce the fouling of a submerged hydrophobic hollow fiber microfiltration membrane by various types of organic matter

A number of jar tests were conducted to test the reaction of several types of organic matter with alum. A limited number of experiments were then conducted to test the ability of coagulation/flocculation pre-treatment with alum to reduce the fouling of a submerged hydrophobic hollow fiber microfiltration membrane by these types of organic matter. The different organic matters tested included one with high SUVA (GDS NOM, SUVA = 3.4 L/mg.m), and three with low SUVAs (alginic acid, SUVA ≈ 0 L/mg.m; γ globulin, SUVA ≈ 0 L/mg.m; and *Microcystis* AOM, SUVA = 0.14 L/mg.m). Using jar tests, favorable reaction between alum and all of the organic matters tested was observed.

It was determined that this reaction largely depends on the presence of a net negative charge on the organic matters. Coagulation/flocculation pre-treatment was able to reduce the fouling of a hydrophobic microfiltration membrane by the different organic matters when the proper coagulant dose was used. Significant reductions in fouling (55-93%) occurred when the alum dose exceeded by 20-30% the minimum effective alum dose (MEAD) for organic matter removal by settling determined using jar tests. At this dose, the alum was able to capture the organic matter into large flocs which appeared to deposit as loose and permeable layers on the membrane surface and cause less fouling than the original organic matter. Doses that were equal to or below this MEAD could cause the formation of small flocs which may increase the extent of fouling compared to the organic matter in its original state. A procedure similar to the one that follows might therefore be taken to determine the appropriate alum dose to use with a coagulation/ flocculation/microfiltration system and an unknown water: (1) determine the DOC concentration in the raw water, (2) Conduct a jar test with alum doses ranging from 0 to 1 (mg Al/mg DOC), (3) measure the concentration of total organic carbon in the supernatant of the settled jars (starting with the jar having received the most coagulant and stopping when carbon concentrations exceed the limitations of the organic carbon analyzer), (4) define the point where organic carbon concentration begins to rise in the supernatants as the MEAD for organic matter removal by settling, and (5) multiply that dose by 1.3 (for an overdose of 30 %).

6.2 SIGNIFICANCE

The work conducted here at the bench-scale showed that the AOM of two algae and one cyanobacterium can cause significant and irreversible fouling of a hydrophobic

microfiltration membrane. From this, it is concluded that the presence of these organisms and their AOM in surface waters treated by similar membranes in full-scale water treatment plants could be problematic. The fouling of a microfiltration membrane by one green alga, *Chlorella*, and its AOM [43, 63, 64] as well as the fouling of a microfiltration membrane by AOM extracted from blue-green algae (i.e. cyanobacteria) in a lake had previously been shown [14]. The work presented here extends these findings to three new organisms, including a green alga, a diatom, and a cyanobacterium.

In addition, the present work offers valuable insights into the mechanisms of fouling by algae and their AOM. It was found that the AOM dominated the fouling process and that the fouling it caused was mostly irreversible. AOM of a relatively large size (> 100 kD) may be particularly apt at fouling microfiltration membranes through a mechanism of pore obstruction. This was supported by the microfiltration experiments conducted with the model compounds where unstable solution conditions leading to the formation of organic aggregates increased fouling. Therefore, algae and cyanobacteria that produce copious amounts of relatively large AOM may be expected to cause severe fouling of microfiltration membranes similar to the one used here.

The fouling caused by AOM could be particularly challenging to a microfiltration process in a surface water treatment plant because AOM is likely to occur seasonally. Its concentration can increase substantially and rapidly as an algal or cyanobacterial bloom develops. The work presented here demonstrates that predicting the extent of the fouling caused by an alga and its AOM would be difficult because the fouling caused by AOM

depends on the organism producing it and the conditions under which it is produced. Consequently, most algae and their AOM may have to be regarded as major potential foulants. Managing the potential fouling that this type of organic matter represents to a microfiltration process may therefore have to rely on control measures that include coagulation/flocculation pre-treatment, the use of hydrophilic membranes, and chemical cleaning (e.g., NaOCl).

Significant reductions in the fouling of a hydrophobic microfiltration membrane caused by various types of organic matter, including AOM, were shown using coagulation/flocculation pre-treatment. The results suggest that this approach might successfully be used to reduce the fouling of similar membranes by surface waters in full-scale plants. The present work indicates, however, that careful attention must be given to the selection of a coagulant dose to ensure that large flocs are formed. With proper flocculation conditions and coagulant doses, coagulation/flocculation pre-treatment may be one of several approaches taken to reduce the fouling of a microfiltration membrane by surface waters.

6.3 FUTURE WORK

6.3.1 Reduction of the fouling of a microfiltration membrane caused by organic aggregates

A significant body of work now exists that indicates that organic matter of a relatively large size (sometimes referred to as organic colloids) can have a significant impact on the fouling of microfiltration membranes. This has been shown for the microfiltration of surface waters [14, 21, 33, 34], wastewater effluents [11] and sludge supernatants in

membrane bioreactors [77]. Furthermore, a significant fraction of these organic colloids are believed to be non-humic and either contain proteins and polysaccharides or possess characteristics similar to these types of compounds [10, 11, 14, 34, 77]. In the work presented here, similar observations regarding the important role of organic aggregates in the fouling of a hydrophobic microfiltration membrane were made with well defined model compounds under specific solution conditions (alginic acid with calcium, gamma globulin at pH 7). The ability to use model compounds to emulate the fouling caused by naturally occurring organic colloids could open the way for several routes of investigation. In particular, the use of different approaches to control the fouling caused by these colloids during surface water treatment could now easily be tested. These approaches might include chemical cleaning and the use of hydrophilic or "hydrophilized" membranes.

Solutions containing NaOCl (an oxidant) or NaOH (a base) are commonly used to clean membranes fouled by organic matter. Experiments could be conducted to test the effectiveness of these two chemical agents to reverse the fouling caused by solutions containing model compound aggregates. Cleaning parameters such as cleaning agent concentrations and contact times may be examined.

The use of hydrophilic or "hydrophilized" membranes has gained popularity in practice. While the use of these hydrophilic membranes has increased, the effect of organic colloids on these membranes is unclear. On one hand, foulants may not attach on hydrophilic membranes as much as onto hydrophobic membranes. On the other hand, the

fouling of a microfiltration membrane by organic colloids may depend as much on the interaction that exists among the organic matter entities than on the interaction that exists between the organic matter and the membrane. In a study that tested the fouling of morphologically similar hydrophobic and hydrophilic membranes by surface waters, the hydrophilic membrane did not always foul less than its hydrophobic counterpart [34]. Similar experiments conducted with solutions containing model compound aggregates could provide some useful information regarding the removal of these types of foulants by hydrophilic membranes compared to hydrophobic ones.

6.3.2 Coagulation/flocculation pre-treatment to reduce fouling

The work presented here demonstrated that coagulation/flocculation pre-treatment might be used to reduce the fouling of a hydrophobic microfiltration membrane by different types of negatively charged organic matter. However, these experiments were conducted at a pH between 7 and 8. Coagulation is strongly influenced by coagulation pH; coagulation of organic matter at a lower pH (pH = 6) typically results in lower coagulant demand but is more susceptible to floc restabilization [78]. Experiments could be conducted to test the overall impact of a low coagulation pH on the potential reduction of microfiltration membrane fouling caused by different foulants using coagulation/ flocculation pre-treatment. Foulants of interest might include hydrophobic acids, AOM, proteins, and polysaccharides. Since restabilization can occur at a low coagulation pH, the selection of the coagulant dose may be more complex at a low pH compared to a high pH. Some studies have reported better performance of coagulation/flocculation/

microfiltration systems when the coagulation pH is low [17, 79]. Experiments with model compounds may support these findings and offer additional explanations for why this would be the case.

In the present work, it was also noted that the reduction in fouling observed with the different organic foulants using coagulation/flocculation relied on the favorable reaction of the organic foulants with alum. The question thus arises of whether neutral or positively charged foulants could be identified and tested that foul a microfiltration membrane but whose fouling could not be reduced using coagulation/flocculation. Subjecting a coagulation/flocculation/microfiltration system to organic matter which is large but unreactive towards a metal coagulant would constitute a good test. Dextran with very large molecular weights (e.g., 2000 kD) might be used in this regard.

Finally, several studies have reported that the settling of flocs formed during coagulation/flocculation prior to membrane filtration can decrease the overall reduction of fouling [18, 19]. These studies speculated that the flocculated material that deposits on a membrane may act as a secondary barrier that removes additional foulants during filtration. If flocculated material indeed acts as a secondary barrier to foulants, then the use of coagulation/flocculation might also reduce the fouling of organic matter which does not react or reacts only weakly with metal coagulants. Whether flocculated material in a coagulation/flocculation/microfiltration system can behave as a secondary barrier towards organic matter could be tested with a solution containing both a compound which reacts with a metal coagulant (e.g., gamma globulin) and a compound which does

not (e.g., dextran). If the compound which is normally unreactive to the metal coagulant were removed, it would be concluded that the flocculated material can indeed act as a secondary barrier.

6.3.3 Impact of nutrient concentrations on algal fouling

It was noted that the fouling caused by different algae (green alga, diatom, cyanobacteria) can vary significantly. This is believed to be the case because different algae produce AOM in different concentrations and with different properties (e.g., size). Another factor that may influence the properties of the AOM produced by algae, however, may be the levels of nutrients available to the algae. In the present work, each alga was grown in its own particular media. Would the AOM produced by each alga change if different media were used? More particularly, would changes in the nitrogen to phosphorus ratio in the media change the resulting AOM? Whether the media can impact the resulting AOM could easily be tested by growing one alga in several media with similar compositions but different N/P ratios and characterizing the AOM produced in each case (total concentrations, protein concentrations, carbohydrate concentrations, size distribution). The fouling of a microfiltration membrane caused by the different cultures could then be determined and compared to one another.

Appendices

APPENDIX A: PREPARATION OF ALGAL MEDIA

INTRODUCTION

This appendix contains a description of the media used to grow all three algae tested. *Scenedesmus* was grown on Woods Hole, *Asterionella* on CHU-10 and *Microcystis* on CHU-10* medium.

MAIN STOCK SOLUTIONS

Stock solutions of the following main compounds are prepared and subsequently used for making all three media. The solutions are prepared by adding the given mass of each compound to the corresponding volume of MilliQ water. Main stock solutions 1 and 5 are filter sterilized using sterile 0.22 μm filters (Millex GS Millipore) after they are made.

Table A-1. Main stock solutions.

Stock No	Compound	MW (g/mol)	Mass (g)	Volume (L)	Concentration (g/L)	Concentration (M)
1	$CaCl_2.2H_20$	147.02	1.104	0.030	37	0.25
2	$MgSO_4.7H_20$	246.36	1.850	0.050	37	0.15
3	H_3BO_3	61.83	0.050	0.050	1.0	0.016
4	$NaNO_3$	85.00	5.525	0.050	1.1×10^2	1.3
5	K_2HPO_4	174.18	0.278	0.050	5.6	0.032

TRACE METALS

Primary stock solutions of the following trace metals are prepared by adding the given mass of each compound to the corresponding volume of MilliQ water.

Table A-2. Trace metal primary stock solutions.

Stock No	Compound	MW (g/mol)	Mass (g)	Volume (L)	Concentration (g/L)	Concentration (M)
6	$CuSO_4.5H_20$	249.68	0.025	0.100	0.25	0.0010
7	$ZnSO_4.7H_20$	287.54	0.115	0.100	1.15	0.00400
8	$CoCl_2.6H_2O$	237.93	0.060	0.100	0.60	0.0025
9	$MnSO_4.H_2O$	169.01	0.388	0.100	3.88	0.0230
10	$Na_2MoO_4.2H_2O$	241.96	0.036	0.100	0.36	0.0015

Two different trace metal working stock solutions are then made: one for the Woods Hole media (Table A-3), the other for both forms of the CHU-10 media (Table A-4). The following procedure was used for making the trace metal working stock / Woods Hole:

1) Add approximately 50 mL of milliQ water to a small beaker and mix continuously
2) To this solution, add 0.110 g of $Na_2EDTA.2H_2O$ and approximately 0.25 mL of 1 N NaOH (freshly prepared from pellets) to adjust pH to 7
3) Add 0.040 g $FeCl_3.6H_2O$ to the solution and another 0.25 mL of 1 N NaOH to maintain the pH at 7
4) Add the appropriate volume of each trace metal primary stock (Table A-3)
5) Adjust total volume to 0.100 L with MilliQ water
6) Filter sterilize this solution through a sterile 0.22 μm filter (Millex GS Millipore)

Table A-3. Trace metal working stock / Woods Hole (Total volume = 0.100 L).

Compound	Stock No	Mass (g)	Volume (mL)	Concentration (g/L)	Concentration (M)
$CuSO_4.5H_20$	6		0.800	0.0020	8.0×10^{-6}
$ZnSO_4.7H_20$	7		0.400	0.0046	1.60×10^{-5}
$CoCl_2.6H_2O$	8		0.400	0.0024	1.0×10^{-5}
$MnSO_4.H_2O$	9		0.800	0.031	1.84×10^{-4}
$Na_2MoO_4.2H_2O$	10		0.400	0.0014	6.0×10^{-6}
$Na_2EDTA.2H_2O$		0.110		1.10	2.96×10^{-3}
$FeCl_3.6H_2O$		0.040		0.40	1.5×10^{-3}

A similar procedure was used for making the trace metal working stock / CHU-10:

Table A-4. Trace metal working stock / CHU-10 (Total volume = 0.100 L).

Compound	Stock No	Mass (g)	Volume (mL)	Concentration (g/L)	Concentration (M)
$CuSO_4.5H_20$	6		6.400	0.016	6.4×10^{-5}
$ZnSO_4.7H_20$	7		4.000	0.046	1.60×10^{-4}
$CoCl_2.6H_2O$	8		1.360	0.0082	3.4×10^{-5}
$MnSO_4.H_2O$	9		8.000	0.31	1.84×10^{-3}
$Na_2MoO_4.2H_2O$	10		17.640	0.063	2.6×10^{-4}
$Na_2EDTA.2H_2O$		0.110		1.10	2.96×10^{-3}
$FeCl_3.6H_2O$		0.060		0.60	2.3×10^{-3}

VITAMINS

Primary stock solutions of the following vitamins were prepared by adding the given mass of each vitamin into the corresponding volume of MilliQ water. Biotin is more difficult to dissolve than the other two vitamins. To prepare the biotin primary stock, place 50 mL of milliQ water in a beaker, add 3 drops of 0.1 N NaOH (freshly prepared) to raise pH, and heat on a hot plate (50 ºC), then add 2.5 mg biotin and mix for 10-15 minutes.

Table A-5. Vitamin primary stock solutions.

Vitamin	Mass (g)	Volume (L)	Concentration (g/L)
B12	0.0025	0.050	0.050
Biotin	0.0025	0.050	0.050

The following procedure was used to make the vitamin working stock solution (Table A-6):

1) Add approximately 25 mL of milliQ water to a small beaker and mix continuously
2) To this solution, add 6 mg of thiamine and 0.6 mL of the B12 and Biotin primary stock solutions
3) Adjust the total volume to 30 mL and filter sterilize through a sterile 0.22 μm filter (Millex GS Millipore). Keep refrigerated.

Table A-6. Vitamin working stock solution.

Vitamin	Mass (g)	Volume (mL)	Concentration (g/L)
B12		0.600	1.0×10^{-3}
Biotin		0.600	1.0×10^{-3}
Thiamine	0.006		0.2

PREPARATION OF WOODS HOLE

1) To slightly less than 1.0 L of MilliQ water, add 12.6 mg of $NaHCO_3$
2) To this solution, add 1 mL each of main stock 2,3 and 4
3) Adjust pH to 7 using 1 N HCl
4) Adjust the total volume to 1.0 L
5) Autoclave at 121º C for 45 minutes and let cool to room temperature overnight
6) Using sterile technique, add 1 mL each of main stock 1 ($CaCl_2.2H_2O$) and 5 (K_2HPO_4), 5 mL of trace metal working stock, and 1 mL of vitamin working stock

PREPARATION OF CHU-10

1) To slightly less than 1.0 L of MilliQ water, add 12.6 mg of $NaHCO_3$ and 57 mg of $Na_2SiO_3.9H_2O$
2) To this solution, add 1 mL of main stock 2, 3 mL of main stock 3, and 0.641 mL of main stock 4
3) Adjust pH to 6.5 using 1 N HCl
4) Adjust the total volume to 1.0 L

5) Autoclave at 121º C for 45 minutes and let cool to room temperature overnight
6) Using sterile technique, add 1 mL of main stock 1 and 1.628 mL of main stock 5, 5 mL of trace metal working stock, and 1 mL of vitamin working stock

PREPARATION OF CHU-10*

1) To slightly less than 1.0 L of MilliQ water, add 12.6 mg of $NaHCO_3$ and 585 mg of NaCl
2) To this solution, add 1 mL of main stock 2, 3 mL of main stock 3, and 0.641 mL of main stock 4
3) Adjust pH to 7.5 using 1 N HCl
4) Adjust the total volume to 1.0 L
5) Autoclave at 121º C for 45 minutes and let cool to room temperature overnight
6) Using sterile technique, add 1 mL of main stock 1 and 1.628 mL of main stock 5, 5 mL of trace metal working stock, and 1 mL of vitamin working stock

FINAL COMPOSITION OF MEDIA

A summary of the final composition of all three media is presented in Table A-7.

Table A-7. Final composition of media.

Compounds	**Woods Hole**	**CHU-10**	**CHU-10***
$NaHCO_3$	1.5×10^{-4} M	1.5×10^{-4} M	1.5×10^{-4} M
$CaCl_2.2H_20$	2.5×10^{-4} M	2.5×10^{-4} M	2.5×10^{-4} M
$MgSO_4.7H_20$	1.5×10^{-4} M	1.5×10^{-4} M	1.5×10^{-4} M
NaCl	-	-	1.00×10^{-2} M
Major nutrients:			
$Na_2SiO_3.9H_2O$	-	2.0×10^{-4} M	-
H_3BO_3	1.6×10^{-5} M	4.8×10^{-5} M	4.8×10^{-5} M
$NaNO_3$	1.3×10^{-3} M	8.3×10^{-4} M	8.3×10^{-4} M
K_2HPO_4	3.2×10^{-5} M	5.2×10^{-5} M	5.2×10^{-5} M
Trace metals:			
$CuSO_4.5H_20$	4.0×10^{-8} M	3.2×10^{-7} M	3.2×10^{-7} M
$ZnSO_4.7H_20$	8.00×10^{-8} M	8.00×10^{-7} M	8.00×10^{-7} M
$CoCl_2.6H_2O$	5.0×10^{-8} M	1.7×10^{-7} M	1.7×10^{-7} M
$MnSO_4.H_2O$	9.20×10^{-7} M	9.20×10^{-6} M	9.20×10^{-6} M
$Na_2MoO_4.2H_2O$	3.0×10^{-8} M	1.3×10^{-6} M	1.3×10^{-6} M
$FeCl_3.6H_2O$	7.5×10^{-6} M	1.2×10^{-5} M	1.2×10^{-5} M
Metal chelator:			
$Na_2EDTA.2H_2O$	1.48×10^{-5} M	1.48×10^{-5} M	1.48×10^{-5} M
Vitamins:			
B12	1.0×10^{-6} g/L	1.0×10^{-6} g/L	1.0×10^{-6} g/L
Biotin	1.0×10^{-6} g/L	1.0×10^{-6} g/L	1.0×10^{-6} g/L
Thiamine	2×10^{-4} g/L	2×10^{-4} g/L	2×10^{-4} g/L
Characteristics:			
pH	7	6.5	7.5
N/P (mol/mol)	40	16	16
DOC (mgC / L)	1.9	1.9	1.9

APPENDIX B: LIGHT SPECIFICATIONS

Length (inches)	Power (Watts)	Supplier	Type	Part No	Color temperature (Kelvins)	Color Rendition Index	Light output (Lumens)
24	20	GE	Wide spectrum	F20T12 PL/AQ	3050	90	750
24	20	Philips	Cool white	F20T12 CW	4100	62	1200
48	40	GE	Wide spectrum	F40T12 PL/AQ	3050	90	1900

Data were provided by the manufacturers

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Curriculum Vitae

Rodrigue F. Spinette was born in Montreal, Canada, on October 24th, 1978. However, he lived in Saint Symphorien, Belgium, for many years and completed most of his secondary education there. In 1995, he moved to Miami, Florida, where he did his senior year of high school at Coral Gables. After graduating from high school in 1996, he attended Miami Dade Community College (1996-1997) and then transferred to Florida International University, in Miami. In May of 2000, he earned a Bachelor of Science in civil engineering. At the end of his undergraduate education, he decided that he wanted to learn more about environmental sciences and engineering and, in September 2000, he entered the graduate program at the Department of Geography and Environmental Engineering at the Johns Hopkins University in Baltimore. He earned a Master's degree in environmental engineering in 2002. Under the direction of his advisor, Charles R. O'Melia, he pursued a doctoral degree studying the fouling of microfiltration membranes used in potable water treatment by algae and algal organic matter. In January 2008, he completed the requirements for his doctoral degree.