



Making humic substances more biodegradable by Judel Ann Buls

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in
Environmental Engineering - Civil
Montana State University
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Abstract:

Humic substances are complex, large molecules, which vary from water source to water source, and are related to a wide variety of water quality problems. They are related to a health risk because they react with chlorine to form chlorinated hydrocarbons, which are considered to be carcinogenic. Additionally, they can be available as a food source for microorganisms, which can lead to the development of biofilm on surfaces, causing a problem called biofouling. Therefore, researchers have attempted to eliminate humic substances using several different methods. One method involves removing the biodegradable fraction through a biofilter, which limits the use of the humic material by microbes downstream. Increasing the biodegradable fraction of humic material through the use of a pre-oxidant can optimize this process, which will allow for higher removal rates, thus decreasing the overall fraction of organic matter that may be available for use at some point in a treatment train.

In this study, four pre-oxidants were studied for their ability to increase the biodegradable fraction of humic substances, which include ozone, chlorine, monochloramine, and UV-light. These oxidants were applied to a humic solution with a 4 ppm TOC concentration, and the treated solution was fractionated based on molecular weight, hydrophobic character, biodegradability, and aromatic character. The results found that ozone was the most effective oxidant, although chlorine and UV-light also showed some ability to make humic substances more biodegradable. Ozone, however, was the only oxidant capable of increasing biodegradability at practical doses commonly used in water treatment facilities. Monochloramine did not show any increases in biodegradability making this oxidant the most stable oxidant used in the study, and useful for purposes where increasing the biodegradability of a humic substance can be detrimental to treatment goals.

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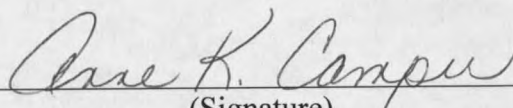
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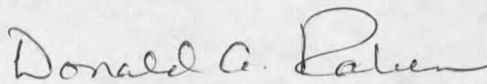
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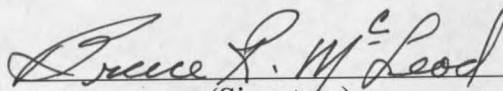
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ABSTRACT

Humic substances are complex, large molecules, which vary from water source to water source, and are related to a wide variety of water quality problems. They are related to a health risk because they react with chlorine to form chlorinated hydrocarbons, which are considered to be carcinogenic. Additionally, they can be available as a food source for microorganisms, which can lead to the development of biofilm on surfaces, causing a problem called biofouling. Therefore, researchers have attempted to eliminate humic substances using several different methods. One method involves removing the biodegradable fraction through a biofilter, which limits the use of the humic material by microbes downstream. Increasing the biodegradable fraction of humic material through the use of a pre-oxidant can optimize this process, which will allow for higher removal rates, thus decreasing the overall fraction of organic matter that may be available for use at some point in a treatment train.

In this study, four pre-oxidants were studied for their ability to increase the biodegradable fraction of humic substances, which include ozone, chlorine, monochloramine, and UV-light. These oxidants were applied to a humic solution with a 4 ppm TOC concentration, and the treated solution was fractionated based on molecular weight, hydrophobic character, biodegradability, and aromatic character. The results found that ozone was the most effective oxidant, although chlorine and UV-light also showed some ability to make humic substances more biodegradable. Ozone, however, was the only oxidant capable of increasing biodegradability at practical doses commonly used in water treatment facilities. Monochloramine did not show any increases in biodegradability making this oxidant the most stable oxidant used in the study, and useful for purposes where increasing the biodegradability of a humic substance can be detrimental to treatment goals.

CHAPTER 1

Introduction

As water treatment processes for supplying drinking water or providing ultrapure water for industrial purposes become better understood, the role that natural organic matter (NOM) plays in these processes has become increasingly important. For example, NOM has the potential to transport pollutants, such as metals, in natural water supplies (MacCarthy and Suffet, 1989). In drinking water treatment, NOM has been linked to several problems including foul taste and odor, displeasing color, increases in chemical disinfectant demand, increased organism counts, and the formation of chlorinated disinfection by-products (DBPs) (Amy, et al., 1987).

DBP formation is a public health risk since DBPs are considered to be carcinogenic (or cancer causing). Therefore, the EPA has recently adopted new regulations concerning their concentrations. The D/DBP Rule imposes stricter disinfection requirements, and also sets limits regulating the allowable concentration of DBPs released into a distribution system. As a result of this new rule, many municipal water treatment facilities are now out of compliance, increasing the need for alternative water treatment processes for NOM removal prior to the disinfection step.

In both drinking water and in industrial settings, another interest in NOM removal has evolved because NOM is the major food source for microorganisms. These organisms are usually found in the form of a biofilm on surfaces such as reverse osmosis (RO) membranes, pipes walls, and in holding tanks. If the purpose of water treatment is to produce ultrapure water, microbial growth on system elements can cause

contamination problems. Additionally, biofouling, or the buildup of biofilms on surfaces such as RO membranes, can occur. This raises pressure requirements making these units more costly to operate, and ultimately makes replacement necessary (Whittaker, 1984).

In response to these problems, researchers have begun to investigate NOM, its chemical makeup, characteristics that make it difficult to remove, and removal methods that are inexpensive, easy to implement, and effective. Identifying specific fractions of NOM has proven to be quite useful in understanding how and why it may or may not be easily removed. A major fraction of NOM is humic material, which is the constituent most commonly associated with the problems generated by NOM (Beckett, 1990). Researchers studying the removal of humic substances have considered a variety of methods such as enhanced coagulation, membrane processes, adding additional treatment steps, and biological removal (Pontius, 1990; Crozes, 1995; LeChevallier, et al., 1992; Wang, et al., 1995; Bouwer and Crowe, 1988). Biological methods have become quite promising since they are often less expensive, less chemically intensive, and more easily implemented than other methods. Biological treatment research has also recently shown that humic substances can be manipulated, through the use of ozone, to become more biodegradable (Owen, et al., 1993; Cipparone, 1997; LeChevallier, 1992; Goel et al., 1995; Langlais, 1991; Gilbert, 1987; Kruithof, et al., 1989). Ozonation increases the amount of NOM removed by biological treatment, making it an even more attractive technology.

Methods of oxidation other than ozonation are currently being used widely in water treatment. These include, but are not limited to, chlorine, monochloramine, and UV-light. Each of these methods can be a cheaper alternative to ozone, and may be

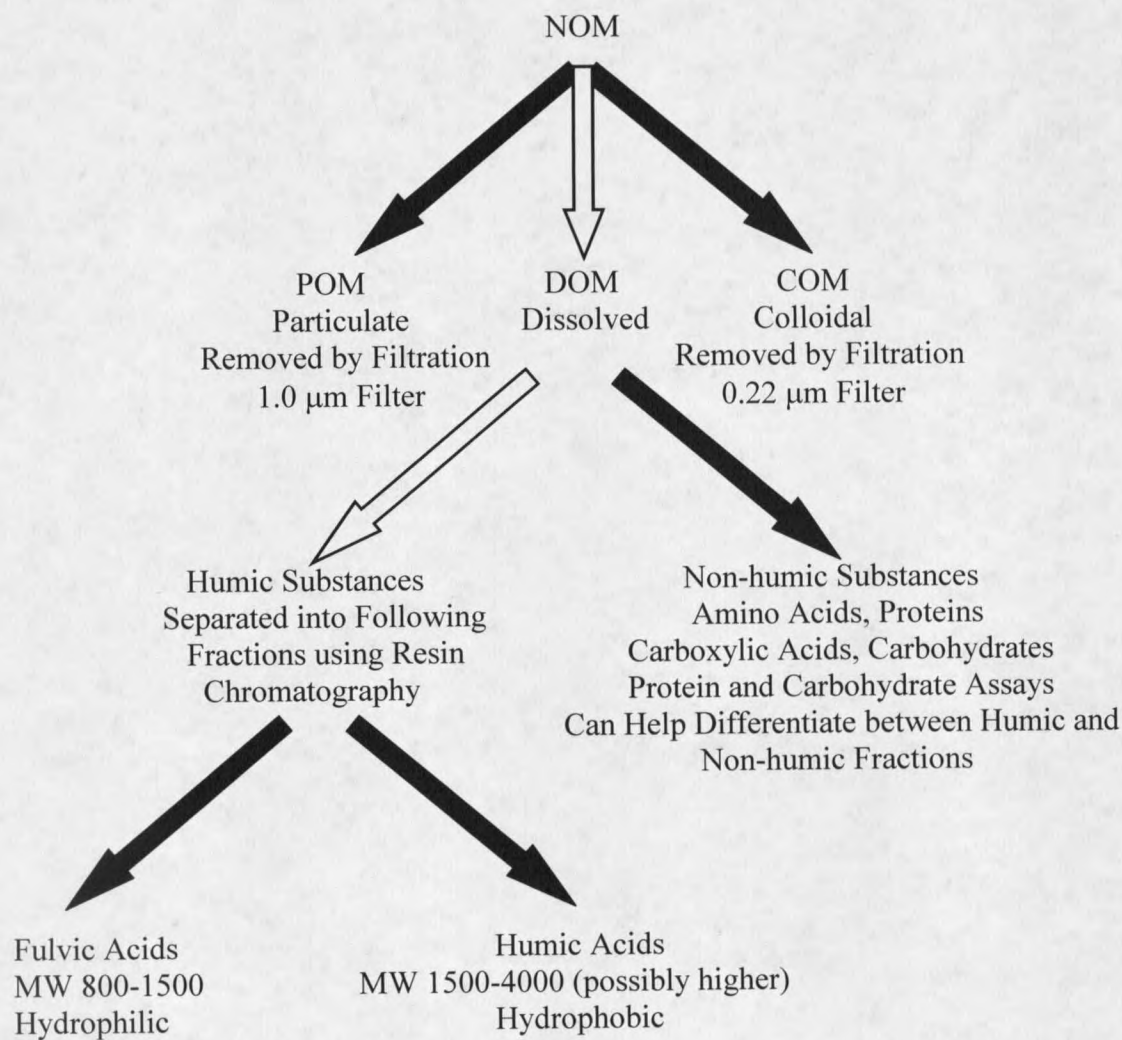
easier to implement. Additionally, most water treatment plant operators are more familiar with one or more of these alternatives, decreasing training requirements. However, limited research has been done regarding their ability to oxidize NOM. In this study, a comparison of oxidizing potential for increasing the biodegradability of NOM was drawn between ozone, chlorine, monochloramine, and UV-light. Ultimately, the most effective oxidant will be used in combination with a biofilter to optimize the process of biofiltration for TOC removal.

Humic Substances

Natural organic matter (NOM) is comprised of all organic material present in a natural water source, and is often the cause of color, taste, and odor properties. It appears to be derived mostly from decaying plant and animal matter; however, other sources such as human pollution may contribute (Bouwer and Crowe, 1988; MacCarthy, 1989). NOM can be dissolved (DOM), colloidal (COM), or particulate (POM) in nature. The particulate and colloidal fractions are seldom a problem in water treatment since removal is often easy to achieve through coagulation/flocculation/sedimentation/filtration (Owen et al., 1993, and 1995). NOM is so diverse in composition that it is defined based mostly on separation techniques used within a laboratory. Figure 1, developed in part by Owen et al. (1995), demonstrates the various major fractions of NOM, as well as the laboratory techniques for isolating these fractions. The white arrows represent fractions that can be further separated into other categories, while the black arrows represent fractions that may or may not be further separated depending on the direction of a study, or the detail required.

Since COM and POM can be removed by filtration, removal studies most often focus on DOM. DOM is commonly measured as dissolved organic carbon (DOC), ranging from low concentrations, of 0.1 mg/L (Pettersson, 1994) in aged groundwater sources, to as high as 50 mg/L in surface water sources affected by spring runoff events (Beckett, 1990). The majority of waters, however, fall between 2 and 10 mg/L DOC (Beckett, 1990; Pettersson, 1994; Owen et al., 1993).

Figure 1. Diagram of NOM Fractions



DOM is further characterized based on its humic fraction. This fraction is highly dependent on the water source, and can range from 40-90% of the total DOC. It is commonly estimated to be around 50% of the total DOC, with higher concentrations seen only in waters that are considerably colored. It can be separated from the total DOM concentration through a DAX-8 resin adsorption procedure (discussed in detail in Chapter 2). The remaining DOC is made up of amino acids, carboxylic acids, proteins, and carbohydrates (Amy, et al., 1987).

Humic substances can again be divided into three more fractions. Humin is considered the fraction that is not soluble at any pH, and is usually particulate in nature. Humic acids comprise the fraction that precipitates out of a water source at a pH below 2, and fulvic acids comprise the fraction that remains in solution at every pH (Beckett, 1990). Although the concentration of each fraction depends largely on the water source, it is often safe to assume that in highly colored waters, the dominant fraction is the humic acids, and in uncolored waters, the dominant fraction is the fulvic acids (Beckett, 1990).

Since humic substances contain several different functional groups such as carboxyls, phenols, hydroxyls, carbonyls, ethers, and esters, it is difficult, and most likely impossible, to establish a common molecular structure. Their molecular weight covers a wide range of sizes, differing both within and between water sources. Molecular weight ranges from 500 to 200,000 Da have been estimated (Thurman, 1985; Beckett, 1990). Other researchers have found that for fulvic acids, a common range is 800-1500 Da, and for humic acids, a reasonable range is from 1500-4000 Da (Beckett, et al., 1987; Beckett, 1989). Still others have found MW ranges through 30,000 Da and higher (Owen, et al., 1993). The methods used for this type of characterization, however, vary widely, and are

often quite expensive to do correctly. Additionally, it is a common belief that these fractions can change quite readily through water treatment processes where pH is often altered, oxidizing chemicals are added, and microorganisms degrade certain fractions. This only compounds the problem of accurately representing molecular weight fractions within a water source, let alone between water sources.

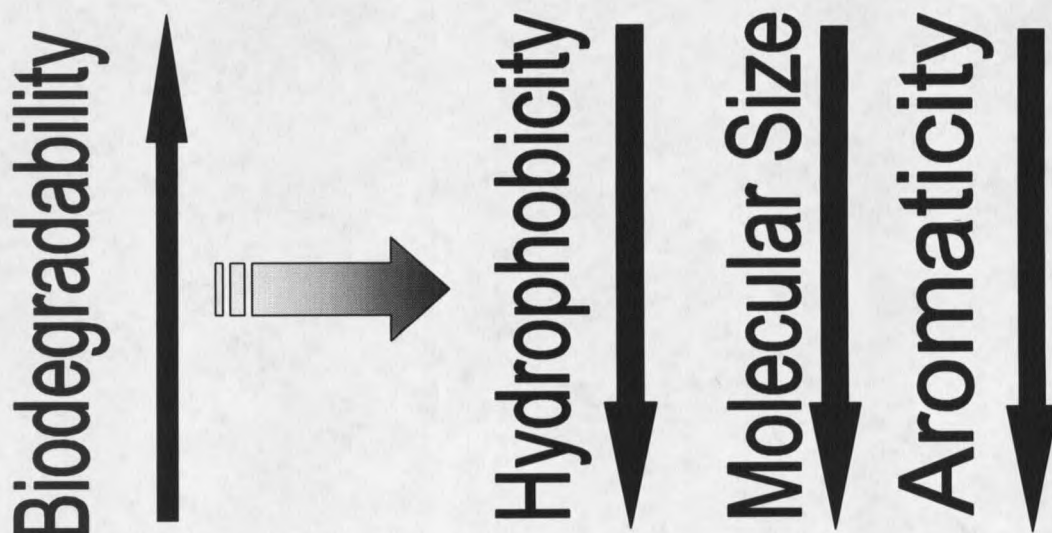
The conformational structure of humic substances has also been a subject of debate. Current beliefs are that humic substances are comprised of coiled, long-chained molecules, which may be cross-linked (Stevenson, 1994). These coils are most dense at high concentrations, low pH, and high ionic strength, and are much more flexible at neutral pH, low ionic strength, and low concentration. It has also been suggested that humic substances are comprised of low molecular weight molecules that are held together by hydrophobic forces, which are stronger at low pH and high ionic strength. This conformation is responsible for "apparently high-molecular weight" molecules (Conte and Piccolo, 1999).

Due to the above complexities of humic substances, researchers have undertaken considerable efforts to develop universal methods for measuring them. Currently, TOC measurements seem to be the general consensus, however, other researchers use the absorbance of UV-light at a wavelength of 254 nm as an alternative (Dobbs, et al., 1972). This method has one major advantage over TOC measurements because it only takes a few seconds to perform a measurement. However, its major drawback is that the only material absorbing UV-light at this wavelength is the aromatic fraction of a sample. This may or may not be related to the TOC of a sample because aromatic character is highly dependent on a water source (Owen, et al, 1993; Dobbs, et al., 1972). In fact, many water

sources do not exhibit a large aromatic character, unless it is highly colored, which is commonly caused by a very high fraction of organic matter derived from soil and plant debris, such as a swamp (Beckett, 1990). UV-254 can also be a valuable measurement when it is done in conjunction with TOC. The comparison of the two measurements provides knowledge of how aromaticity and organic carbon content are related (Dobbs, et al., 1972).

Although the characteristics of humic substances are far from being completely understood, the research that has been done regarding these characteristics has given researchers some insight into the relationship between them and the biodegradability of humic substances. Figure 2 shows general relationships between hydrophobicity, aromaticity, and molecular weight. Additionally, it is believed that tightly coiled, highly complex, large molecular weight humic material is the most difficult to break down (Owen et al., 1993; Conte and Piccolo, 1999).

Figure 2. Relationship between Biodegradability and Humic Properties



Owen, et al. (1993), used laboratory methods based on the characteristics shown in Figure 2, to develop a reliable protocol for analyzing the biodegradability of humic substances. The combination of protocols is necessary because simply measuring biodegradability has proven to be difficult.

Researchers (Servais, et al., 1989; Block, et al., 1992; Volk, et al., 1997, and 1993; Goel, et al., 1995) have attempted to develop standard methods for determining biodegradable dissolved organic carbon (BDOC). However, since these tests are largely dependent on the water source being studied, as well as the organisms doing the degradation, the water quality community has been hesitant to adopt a standard method (Gjessing, et al., 1999). This is largely due to the fact that the BDOC methods may be difficult to duplicate both between and within laboratories at confidence intervals reliable enough to be considered standard protocol.

At this time, most BDOC methods being employed involve sampling a water source, measuring the initial DOC, allowing the sample to incubate for a set period of time, and then measuring the remaining DOC. This process produces somewhere between 11-35% DOC reduction (Bouwer and Crowe, et al., 1988; Martin-Mousset, et al., 1997). This fraction, termed the BDOC, depends on the length of time the test is run, which varies from a few days to a month, or until a steady DOC concentration is reached. It also depends on the type of inoculation utilized. If a biomass support medium is used, it is important to note that different media exhibit different adsorption properties and biomass support capabilities. Therefore, researchers commonly develop BDOC protocol based on the following principles. Sand that has been inoculated with indigenous microorganisms should be used for the media. This media should preferably be low in

organic carbon content to limit contamination of a sample. The organisms should be capable of degrading similar concentrations and types of NOM. Additionally, initial tests should be done to establish the effluent DOC concentration over time. This will establish the amount of time that is necessary to meet a constant DOC concentration. Finally, all samples should be filtered to prevent biological contamination (Servais, et al., 1989; Block, et al., 1992; Volk, et al., 1997, and 1993; Goel, et al., 1995).

Since BDOC measurements are difficult to quantify, the ability to correlate an increase in biodegradability of humic substances to the humic properties discussed above, may provide supporting evidence that biodegradation of the NOM is occurring.

Methods for Removing Humic Substances

There are a variety of alternative water treatment options available to municipalities and industry. The best choice for each facility, however, is likely to be different and depends on several parameters. First, the level of removal that has to be met may require more than one method to be employed. Additionally, the space available at the facility for implementing new treatment steps should be considered. Other water quality parameters must be considered to ensure that they will not interfere with the treatment option chosen, and most importantly, the cost of implementation should be considered. Since there is a wide range of treatment trains used today, it is first necessary to analyze each process, and find an effective alternative that will fit the specific facility. If the facility in question is planned from scratch, with the primary treatment goal being the removal of humic substances, a combination of alternatives would be very advantageous, and conducive to process optimization. The following

discussion explores the advantages and disadvantages of the alternatives currently available.

Enhanced Coagulation

Enhanced coagulation is the addition of excess or alternative coagulants for the increased removal of DBP precursors (mostly NOM). This process can be a fast, cheap alternative for facilities already doing coagulation/flocculation. In order to implement this process effectively, simple jar tests investigating a range of pH values, a variety of coagulants, and a range of coagulant doses must be done. These tests can evaluate the most effective coagulation process for a specific water source, thus optimizing coagulation and flocculation for a given water (Pontius, 1990; Tambo, et al., 1989).

However, this alternative is not possible in all water treatment facilities. For example, facilities that operate by direct in-line filtration often skip flocculation and sedimentation steps, and may even limit coagulation processes. Additionally, facilities that are treating water for industrial purposes may not use coagulation at all, or may not want the chemicals in their water stream. Therefore, these facilities have very limited options regarding enhanced coagulation, and other alternatives will be better (Crozes, 1995).

Rapid Rate Filters

For treatment facilities that require additional removal beyond enhanced coagulation, or treatment facilities that operate with direct in-line filtration, an option might be to change the media used during the filtration step. Media such as granular activated carbon (GAC) may be more effective since it can adsorb a fraction of NOM.

This provides for both filtration of particulate matter, and adsorption of dissolved organic matter, thus reducing taste and odor problems as well as organic compound concentrations. However, some precautions must be taken when using GAC. Very high organic carbon waters can exhaust the GAC much faster than a sand/anthracite combination. This could be costly because the GAC would have to be replaced much more frequently. Therefore, it is important to note that GAC used in this manner requires substantial control measures for limiting biological activity, in order to enhance adsorption properties. Also, an additional pre-disinfection step may be necessary to control microbial growth within the filter, and/or increased disinfection may be required after filtration to combat sloughed organisms. Some facilities may have to be concerned about backwashing, as well, because GAC has a lower density than anthracite (Pontius, 1990).

Ion Exchange

Ion exchange has also been used for TOC removal; however, this process requires a macroporous resin specifically designed to attract polyvalent humate and fulvate anions. The resin can be expensive, is very weak since it consists of large molecules, and can be difficult to regenerate due to a strong attraction of the NOM for the aromatic resin matrix. Additionally, ion exchange may require space within the treatment facility that is not available.

Ion exchange has shown promise by significantly decreasing chlorine demand during the disinfection process. However, long-term operation can be a problem due to fouling and resin losses caused by the problems associated with regeneration.

Additionally, Fu and Symons (1988) measured the effectiveness of ion exchange on different molecular weight fractions of humic substances and found that limited adsorption was achieved in MW fractions below 5000 Da. Another problem is waste disposal of the regenerant, which may add to shipping and disposal requirements (Pontius, 1990).

Membrane Processes

Membrane processes have also been used for NOM removal, and are very effective for preventing downstream biofouling, and removing DBP precursors (Thorsen, 1999). There are many different membrane processes. One option is reverse osmosis, which is a pressure-driven process that removes virtually everything within a water sample. The membrane modules for this process are very expensive and sensitive, making this process less practical for the removal of NOM than other options since high molecular weights can cause fouling due to buildup on the membrane surface. Electrodialysis involves the transfer of ions through membranes via a direct electric current. This method is not commonly used for NOM removal since it is based on charged particles, and humic substances and other organic matter are usually weak in charge (Pontius, 1990).

A much more effective membrane process for NOM removal is ultrafiltration (UF). This is another pressure driven process, but is much more applicable for NOM removal since the membrane's molecular weight cutoff (MWC) ranges from 10,000 to 300,000 Da. This range is much larger than an RO membrane, and can be adjusted according to the purpose of treatment. Particulate and colloidal material,

microorganisms, and organic constituents larger than the MWC of the membrane are removed (Dietrich, 1995). This option may not always be the best one, however, since the membranes have additional pressure requirements within the plant, and must be replaced or cleaned routinely due to fouling. This fouling could be caused by scale, colloidal deposition, silt layer formation, metal oxide and silica buildup, and microbial buildup (biofouling) (Conlon, 1990; Thorsen, 1999). Additionally, the filtration apparatuses usually have considerable space requirements. Waste disposal of the concentrated water stream can be a concern as well.

However, as research in the area of UF continues, the process has become more and more economical, and process control has improved (Conlon, 1990; Thorsen, 1999). This method will likely become a very popular method for NOM removal in the future (Pontius, 1990), especially as research redirects itself to combine it with additional processes, such as upstream biological pretreatment. This combination has the potential to optimize membrane life, decrease additional power requirements, and improve the overall stability of water source (Conlon, 1990; Dietrich, 1995; Pontius, 1990; Thorsen, 1999).

Oxidation/Disinfection Alternatives

Several chemicals used in water treatment, commonly employed for disinfection purposes, can act as oxidizing chemicals, which can influence and possibly even cause the removal of NOM concentrations. If NOM removal to prevent downstream biofouling is the main objective of treatment, specific primary disinfection processes may be implemented, which usually involves the addition of a disinfection chemical prior to the

filtration step. If this method is used, the chemical can actually act much more like an oxidizer, as opposed to a disinfectant. As an oxidizer, the chemical can alter the makeup of NOM and make it more biodegradable for organisms in the filter. Additionally, primary disinfectants can be removed within the filter, along with their reaction byproducts to eliminate adverse effects later in the treatment process (Crozes, 1995; Pontius, 1990).

If preventing disinfection by-product formation is the goal, changing disinfectants can be effective. At this stage of treatment, NOM removal is probably not happening, and oxidation is not advisable. It is more productive to maintain the stability of the NOM by apply a disinfectant chemical that does not react with humic substances. Chloramines, most commonly monochloramine, offer a great advantage over chlorine, since they do not react with humic substances as readily, virtually eliminating the formation of DBPs, and maintaining a residual much longer. However, it is not as effective a disinfectant as chlorine, and contact times, for specific disinfection criteria are impossible to meet (Crozes, 1995; Pontius, 1990).

Ozonation is another alternative, and can be quite effective since ozone is capable of providing fast and efficient kills, while considerably decreasing the DBP formation in most waters. However, in highly brominated waters, hypobromous acid formation may actually compromise ozone's effectiveness. Additionally, cost and space requirements may make ozonation an unfeasible option (Crozes, 1995).

Although there are other disinfection chemicals available, the ones mentioned above are most applicable for the majority of water treatment facilities with regards to potential NOM removal or chemical modification. Each of the disinfection alternatives

will decrease the concentration of chlorine required for post filtration disinfection, thus decreasing the formation of DBPs. However, chlorine continues to be the most widely used disinfection chemical. Although changing disinfection chemicals may be cheap and easy to implement, optimizing preceding steps within the treatment process for the removal of DBP precursors prior to disinfection (such as doing enhanced coagulation) is a much more attractive option. It will allow for much better process control and performance because it not only removes known DBP precursors, but can also remove unknown precursors. This is a big advantage because little is known about DBPs formed during chlorination, and even less is known about potential DBPs formed during other disinfection processes such as ozonation (Crozes, 1995, Pontius, 1990). Therefore, if oxidation/disinfection processes are to be implemented, primary disinfection processes, as discussed above, likely provide the most effective option for oxidant application to enhance NOM removal because the formation of DBPs is limited.

Biofiltration

The final method for discussion, and the method being studied in this project is biofiltration. This process involves the use of a biofilter, which is comprised of a porous medium that has become colonized with a biofilm. Sand is commonly employed as the medium, and more recently, biologically activated carbon (BAC), or GAC that has become biologically spent during the filtration step of water treatment, has been used. The media are continuously colonized with organisms that are indigenous to the treated water supply. These organisms will then biodegrade a fraction of the NOM, thus

lowering the total concentration available to react with disinfectants during the final or secondary disinfection process.

Biofiltration has significant advantages over the treatment methods discussed above. First, it is more desirable than disinfection alternatives because instead of simply avoiding the formation of disinfection by-products, biofiltration provides removal of NOM, therefore making it applicable for both biofouling prevention and DBP precursor removal. The advantage over membrane processes and ion-exchange is that biofiltration is usually cheaper to implement, does not require as much maintenance, and does not produce a waste stream that must be disposed of. In many cases, implementing biofiltration does not even require replacement of media due to the low carbon concentrations being treated in water treatment facilities thus minimizing operating and maintenance costs. In water treatment facilities that do not practice coagulation, or that do minimal coagulation, biofiltration is often much easier, and cheaper to implement than an advanced coagulation process due to space requirements for coag/floc/sed chambers. Overall, biofilters are relatively inexpensive, do not require much space for operation (depths of less than 1 meter are usually sufficient), require minimal chemical use, and are simple to operate (Bouwer and Crowe, 1988).

BAC is currently considered to be one of the best media for biofiltration. This is because BAC has a larger surface area and better surface texture than most other media, therefore having the potential to hold a greater amount of biomass. Many researchers have shown that during similar empty bed contact times (EBCT), BAC filters performed better for NOM removal than sand or sand-anthracite dual-media filters (Wang, 1995).

Additionally, LeChevallier, et al (1988), showed that as EBCT in BAC filters increased from 5 to 20 minutes, TOC removal increased from 29.0% to 51.2%.

Another medium that has gained positive recognition is iron-oxide coated sand. The basis for the development of this medium stems from relationships between cast iron pipe used in distribution systems, and natural organic matter. This relationship provides a favorable environment for biofilm growth, therefore increasing microbial activity within a distribution system. Several findings in research, including the following, provide evidence for this fact.

A survey conducted by LeChevallier et al., in 1996, suggested that distribution systems with over 1000 miles of unlined cast iron pipe had coliform levels three times greater than systems with less than 200 miles of unlined cast iron pipe. Additionally, long-term studies on bacterial growth vs. substratum material showed that exposed iron sustained more growth than cement, glass, and other materials (Delanoue et al., 1997). Another study conducted by Martin, et al., 1982, and further substantiated by LeChevallier, et al., in 1990, provides indirect evidence that using corrosion inhibitors, for reducing corrosion of iron pipes in a distribution system can provide for reduction or control of bacterial regrowth.

This research has culminated in the belief that corrosion events in iron pipes (providing for the formation of iron-oxides) are related to biological regrowth problems. The exact phenomenon responsible for this relationship has been questioned. Initially, researchers attributed it to the chlorine demand of iron and its corrosion products, believing that this lowered the chlorine residual within a distribution system allowing for less disinfectant efficiency (LeChevallier et al., 1990, 1993; Chen et al., 1993). However,

later research has shown that in a system where corrosion control was employed, and chlorine residuals were maintained at a consistent concentration, iron pipe surfaces allowed for faster biofilm development, as well as a more diverse population than PVC (Camper, 1997; LeChevallier, 1997). Therefore, another mechanism may be responsible. This mechanism, in fact, is related to corrosion products of iron, which provide significant surface area for colonization by bacteria, provide protection for cells from mechanical detachment (scrubbing), provide some disinfectant neutralization, and collect nutrients on the surface utilizable by biofilms (Qi, 1999).

Further studies of the relationship between iron-oxide and NOM have shown that iron-oxide coated materials have increased adsorptive capabilities regarding humic substances (Qi, 1999; Chang, 1992; Benjamin, et al., 1997). This adsorptive capability makes iron-oxide coated materials ideal as biofilter media because improved adsorption allows for increased exposure for biofilms to biodegradable NOM. This can then provide for an increase in biomass, therefore improving biofilter performance.

Since biomass accumulation is directly related to filter effectiveness, other pretreatment steps can have a large effect on the efficiency of a biofilter. Research suggests that substrate loading rate effects biomass accumulation significantly (Wang, 1995). Therefore, any pretreatment process that alters the loading rate to a biofilter can affect the biofilter performance. Even more importantly, any pretreatment process that alters the fraction of biodegradable substrate can significantly impact biofilter performance. One optional pretreatment method that has been shown to improve biofilter performance is the application of a preoxidant (LeChevallier, et. al., 1992). Initially, preoxidation of a water source was done to provide increased disinfection, for the control

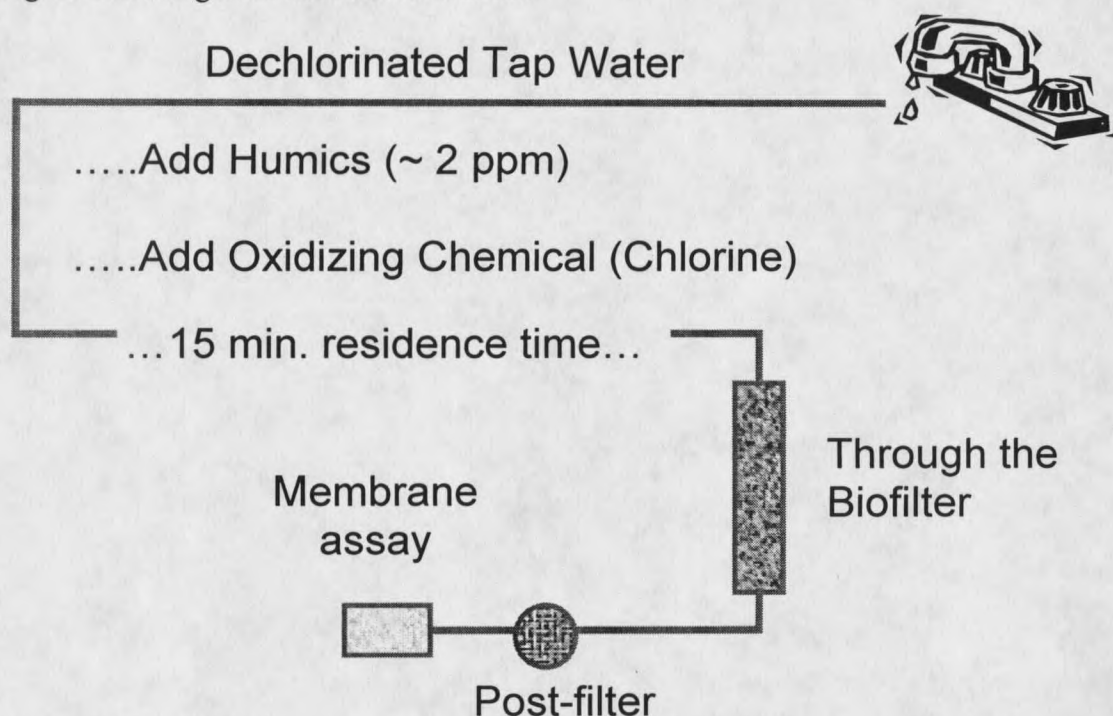
of taste and odor, to provide increased chemical conditioning of the water, or to control other problem chemicals within a water source such as iron and manganese (LeChevallier, 1992). However, an additional benefit to preoxidation exists. Preoxidation can provide for oxidation of NOM, which may increase the low molecular weight fraction of NOM, decrease the hydrophobicity, and decrease the aromaticity, therefore increasing the fraction of biodegradable NOM (Cipparone, et al., 1997; LeChevallier, et al., 1992).

Based on these concepts, a biological pretreatment train to remove NOM that may contribute to downstream membrane fouling was developed at the Center for Biofilm Engineering by Ph.D. candidate Chris Wend. Several treatment trains were developed around a biofilter, for the purpose of treating low carbon level drinking water (less than 4 mg/L TOC). Figure 3 shows a schematic of the entire possible treatment process. Each treatment train studied eliminated one or more of the steps shown below. The system was fed with Bozeman City tap water, which carried a TOC concentration around 1 mg/L during the majority of the year, and up to 6 mg/L during spring runoff (Wend, 1998). The water was dechlorinated through a BAC column with a short residence time. A concentrated humic amended water source was then pumped into the water stream. The targeted TOC content of the complete feed water was between 2 and 3 mg/L TOC.

The stream was then split, and $\frac{1}{2}$ of the stream was treated with a preoxidant (Cl_2). The concentration of chlorine added was based on the concentration of chlorine that would react entirely with the humic substances in 15 minutes. This concentration ranged from 0.3 and 0.5 mg/L free Cl_2 , depending on the humic concentration and makeup during application. This requirement was necessary to prevent chlorine from

entering the biofilter, which has been shown to significantly reduce filter performance (Wang, 1995; LeChevallier, 1992).

Figure 3. Biological Pretreatment Process



The water then passed through a biofilter with either BAC or iron-oxide coated sand as the media. The stream was split again, and $\frac{1}{2}$ of it passed through an ultrafilter to prevent sloughed microorganisms from travelling downstream. This step was vital to the performance of the process, especially if the intent was to reduce biofouling. If organisms were allowed to slough off a biofilter, they might actually compound a biofouling problem, instead of improving it.

The final step in the process involved several assays for measuring biofouling capacity. Bead assays were done so total cell counts could be performed, and some

visual analysis of the biofouling layer could be done. Additionally, a membrane assay was designed to measure pressure changes across the membrane.

Preliminary results from this work showed that the biological filtration has a significant impact on TOC removal, as does the ultrafiltration step. In fact, the ultrafilter has basically become a second biological reactor. It is significant to note that plugging of the biofilters and the ultrafiltration membranes did not occur during the two years of operation for this system. Results were inconclusive regarding the impact of the preoxidation step (chlorination) that was studied, necessitating further research.

Preoxidation Research

Ozone

The inconclusive nature of the research described above gave rise to the need for additional analysis of preoxidation techniques. An extensive literature search revealed several papers showing that ozone is capable of altering NOM characteristics related to biodegradability (Owen, et al., 1993; Cipparone, et al., 1997; LeChevallier, et al., 1992; Goel, et al., 1995; Langlais, 1991; Gilbert, 1987; Kruithof, et al., 1989). In fact, even though methods of measuring biodegradation in each of these sources, including AOC (assimilable organic carbon), BDOC, DBP formation, and adsorption capacity comparisons, were different, the overwhelming conclusion from all studies is that ozone increases the biodegradability of dissolved natural organic matter.

Several researchers (Owen, et al., 1993; Kruithof, et al., 1989) showed that little DOC destruction occurred during ozonation, however, lower molecular weight and non-humic (often fulvic acids, and hydrophilic in nature) fractions increased, which correlated

to a decrease in aromatic character. Goel, et al. (1995), examined ozone doses from 0 to 7.3 mg ozone/mg TOC, and found that increased ozone dose correlated to improved BDOC production, especially for water containing NOM with high levels of high-molecular-weight organics. Additionally, this study noted that NOM with low aromatic character and higher fractions of low-molecular-weight organics biodegraded more readily.

Cipparone, et al. (1997), also found similar results. This study, and others found that removal of the biodegradable fraction of TOC was maximized at an ozone dose of 1 mg ozone/mg TOC (Langlais, 1991). Additionally, chlorine demand was decreased and chlorine residuals lasted longer in water treated with both ozone and biological processes, as opposed to ozonated waters only. DBP formation was also reduced significantly for water that was ozonated then subsequently biologically treated. Based on these findings, it is reasonable to conclude that ozone is capable of increasing the biodegradable fraction of humic substances, thus improving biofiltration processes.

The exact mechanism responsible for this seems to be based on the nature of certain molecules within the NOM. Studies have shown that simple aromatic compounds exposed to high doses of ozone demonstrated significant ring cleavage, which can be associated with biodegradability (Langlais, 1991). This is also true in natural waters, and enhances the finding that at a dose of 1 mg ozone/mg TOC, BDOC formation is likely to be complete, therefore resulting in a maximum TOC removal rate at this dose (Langlais, 1991). This dose is currently the most commonly applied dose within full-scale treatment plants (Langlais, 1991).

Chlorine

The oxidizing capabilities of chlorine have been much less studied than ozone. In fact, only four studies examined the ability of chlorine to oxidize humic substances (LeChevallier, 1992; Kruithof et al., 1989; Reckhow, et al., 1990; Hanna, et al., 1991). Kruithof and Reckhow examined only the DBP formation potential of chlorinated humic substances. Reckhow, et al. (1990) applied a chlorine dose of 20 mg/L TOC and the results suggested that chlorine consumption is due to reactions with activated aromatic structures, indicating that aromatic character could change with chlorination. Kruithof, et al. (1989) applied a chlorine dose of 1.7 mg/L TOC and found that chlorination resulted in the formation of THMs, non-volatile high-molecular-weight organohalides, and mutagenic compounds. Hanna (1991) examined the actual reaction species formed and found that after chlorination, a reduction in phenols, methoxyl carbon, aryl-C carbon, and ketones occurred along with a relative increase in carbohydrates and alkyl carbon. It is most important to note that these studies show production of primarily lower molecular weight molecules, as well as species (carbohydrates) that are easier to metabolize than the untreated humic substances.

LeChevallier (1992), related oxidation with chlorine to the ability of a biofilter to remove NOM. He applied a given dose of chlorine, and then dechlorinated the water after 10 minutes. His findings indicated that chlorination did provide for a decrease in TOC across a biofilter, although not as significantly as ozone. He also suggested that chlorine might be superior to ozone since the chlorinated biological filter effluent produced a lower AOC concentration than the ozonated effluent. This phenomenon indicates that even though ozone can reduce the overall TOC concentration of a water

source better than other oxidants, it may still be more problematic because it can alter the humic material so much that biofilter effluents still contain higher concentrations of assimilable organic carbon, allowing for increased organism growth downstream.

Other studies done on distribution system regrowth indicated that at certain concentrations, and under certain water quality conditions, chlorine may have the ability to oxidize humic substances providing food sources for biofilms downstream of disinfection processes (Abernathy, 1998; LeChevallier, 1990). This phenomenon was seen when the oxidizing power of chlorine exceeded its disinfection capabilities.

Monochloramine

Only one study examined the ability of monochloramine to oxidize humic substances (LeChevallier, 1992). It was the intent of this study however, to allow the residual to penetrate a biological filter. The findings indicated that since monochloramine is much more stable than chlorine, residuals were found in the effluent of the biofilter whereas chlorine residuals were eliminated near the top of the biofilter. Additionally, the presence of monochloramine inhibited bacterial growth within the filter, preventing it from performing as well as the prechlorinated system.

UV-light

Research concerning UV-light and its ability to breakdown humic substances has been limited as well. One study involving the influence of natural UV-irradiation on sea water showed that low-molecular-weight carbonyl compounds were formed due to the absorption of sunlight by humic substances (Kieber, et al., 1990).

Another study examined the effects of UV-light disinfection on biofilm formation in a distribution system. This study concluded that UV-light may have a bactericidal effect on organisms because results showed considerably less production of biofilm within pipelines supplied with UV-treated water, as compared to ozonated water (Lund, et al., 1995). Additional research by this team investigated this aspect of UV-treatment further. Their conclusion that an inhibitory effect on heterotrophic bacteria persists after UV-irradiation of water containing humic material was based on the formation of hydroxyl radicals which have been shown to be toxic to cells and bacterial spores (Lund, et al., 1994). However, at high organic matter concentrations, and in systems where biofilm was well established, the effect was not nearly as pronounced. Lund, et al. (1994) hypothesized that this could be due to UV-irradiation making humic substances more biodegradable.

One study directed at investigating the effects of artificial UV-irradiation of NOM utilized low-pressure mercury-vapor ultraviolet irradiation at 254 nm and a dose of 130 mW*s/cm² to oxidize four water sources and examine their biological regrowth potentials. The results found no statistical evidence that UV-treatment increased BDOC or AOC, however, a few selected samples indicated that it may be possible for the NOM in certain water sources, to become more biodegradable after treatment. Therefore, the research team recommended that all utilities considering UV-treatment should be aware of the possibility that biodegradability may increase, and test their sources accordingly (Shaw, et al., 2000).

Based on the above investigations, it is apparent that UV-light at 254 nm can alter the makeup of humic substances. The overall effect, however, is not well understood, and is likely to be very dependent on water source.

Summary

Based on the findings of other researchers, the scope of this project was specified as a comparison of the ability of ozone, chlorine, monochloramine, and UV-light to increase the fraction of biodegradable humic substances. It was hypothesized that ozone would outperform the other oxidants, however, to what extent this would occur was questionable. Could chlorine, monochloramine, or UV-light provide enough change in humic character and biodegradability to make cost consideration the driving factor for choosing a preoxidation process? Furthermore, could the effects of these oxidants be optimized to perform at levels consistent with what had been previously found for ozone?

To answer these questions, the study examined each oxidant for its ability to not only increase the BDOC of a humic solution, but also to alter humic characteristics related to hydrophobicity, aromaticity, and molecular size. Additionally, different doses were analyzed for each oxidant to assess a realistic range for pretreatment based on the application.

The results will present overall TOC reduction, TOC reduction based on specific fractions, MW distribution chromatograms, and UV-254 adsorption before and after each preoxidation method. These results will then be analyzed to predict the oxidant's ability to improve biofilter performance, for utilization as a deterrent for biofouling on sensitive surfaces. For biofiltration options used in water treatment facilities, this study will

provide some understanding of how preoxidation techniques could lower disinfectant requirements, in turn limiting DBP formation. And finally, this study will provide insight into the relationship between oxidizing disinfectants and distribution system regrowth events.

CHAPTER 2

Materials and Methods

The purpose of this research was to compare the ability of ozone, chlorine, monochloramine, and UV-light to oxidize a humic solution, and increase its biodegradability. This was accomplished by oxidizing a 4 ppm humic solution with each of the four treatments, and measuring a variety of parameters believed to be associated with an increase in the biodegradable fraction of NOM. The parameters measured include aromaticity, MW fractions, humic acid fractions, and BDOC fractions. Aromaticity was measured using UV-254 analysis, which measures the sample absorbency of UV-light at 254 nm. MW fractions were measured using High Performance Size Exclusion Chromatography (HPSEC). Humic acid fractions were measured using DAX-8 resin chromatography, and BDOC fractions were measured using sand columns inoculated with organisms capable of removing the biodegradable fraction of each sample. The following discussion provides detailed procedures for each of the above tests, as well as a variety of other methods used in the lab to complete this project.

Humic Solution Recipe

A humic solution was prepared using Elliot Silt Loam Soil, available from the International Humic Substances Society (St. Paul, MN) in quantities of 500 grams/yr. The soil has been used in the Center for Biofilm Engineering for other experimental purposes, so fractionating it not only provided information for the results of this project, but also provided a database of humic properties useful for future Center research.

A stock solution was prepared by adding 50 g of soil to 500 mL of 0.1 N NaOH, and mixing for 48 hours. This slurry was then centrifuged at 10,000 rpm, and a g value of 4, at 4°C for 20 minutes to separate out the solid particles, leaving a thick elixir, with a concentration of approximately 1000 mg/L TOC. The stock solution was stored in the dark at 4°C.

A 20 L carboy was baked at 500°C for 24 hours, and filled with distilled water. 80 mL of stock solution was stirred in to provide a bulk solution concentration of ~4 mg/L TOC. This bulk solution was stored in the dark at 4°C.

Material Selection

Since this research involved measuring low carbon levels, materials for this project were selected based on carbon contamination potential. All containers were PyrexTM or borosilicate glass containers, capable of being acid washed and fired. Additionally, all tubing selected for the project was silicone or PTFE tubing. Pipette tips were kept in sterile boxes that had never been autoclaved since autoclaving causes carbon to leach from the plastic. All reagents were ordered specially for this project to prevent contamination from outside sources, and to ensure the lowest carbon levels possible. And finally, ultrapure water was used for all rinsing and sample preparation purposes.

Glassware Preparation

Since carbon levels were often measured below 100 ppb TOC, glassware preparation was paramount to the success of the project. All glass was acid washed for at least 8 hours. Volumetric glassware was then rinsed several times with ultrapure water

and covered with tin foil. All other glassware was rinsed with ultrapure water, covered with tin foil, and baked for a minimum of 24 hours at 350 ° C. Gloves were worn when handling clean glassware to prevent contamination from hands.

Ozone Oxidation Technique

Oxidation with ozone was done using a Coralife Electronic Ozonizer Model # 50 (Energy Savers Unlimited, Inc., Harbor City, CA). The generator was set on high output, and connected to a laboratory air outlet. Since the laboratory air is not purified, and can contain a variety of unknown particles, a filter column (approximately 1.5 feet long) was made using a Labclear Gas Filter Column (Oakland, CA) packed with air drying crystals, which changed from blue to purple as absorbency was depleted. A portion of the crystals were removed (from the middle of the filter column), and a GAC trap approximately 6 inches long was inserted to remove organic compounds, providing a clean air stream to travel through the ozone generator.

Ozone output was measured using Standard Method 2350E, Ozone Demand/Requirement—Semi-Batch Method (Standard Methods, 1995). This protocol involved the use of two serial KI traps with 500 mL of 2% KI. Ozone was bubbled into continuously stirred traps for a period of 10-15 minutes. 10 mL of 2N H₂SO₄ was added to each trap, and the contents were titrated with 0.005N sodium thiosulfate until the yellow color almost disappeared. 2 mL of 0.5% starch indicator was then added, and titration was resumed until the resulting blue color disappeared. The ozone dose was calculated according the following equation supplied by Standard Methods (1995).

$$\text{Ozone dose, mg/min} = (A + B) * N * 24 / T$$

Where: A = mL titrant for Trap A

B = mL titrant for Trap B

N = normality of sodium thiosulfate (0.005)

T = ozone time, min

Trap B was always found to have no ozone in it. Therefore, only one trap was used for capture. Once the ozone dose was measured, ozone was applied to a humic solution, making sure to keep the air flowrate constant. The generator was attached to an airtight bottle capable of holding 2 L of sample. The sample was continuously stirred. An acid washed and baked, glass sparging tube was used to bubble the O₃ into the sample. This produced many fine bubbles, optimizing the process of oxidation. A KI trap similar to Trap A and B was used to measure the unreacted ozone in the headspace of the sample. This trap was titrated according to the procedure above, and the result was subtracted from the total ozone dose. The ozone application rate for a given time period was then calculated. The total ozone applied in mg/L was also calculated, and divided by the TOC concentration in mg/L. The final dose was recorded in mg O₃/mg TOC.

The oxidized sample was allowed to set after oxidation for at least one hour and ozone was measured routinely using HACH method 450 on a HACH DR/2000 to ensure that no ozone residual remained.

Ozone oxidation of TOC was analyzed at three doses, 0.5, 1, and 2 mg O₃/mg TOC. 0.5 mg O₃/mg TOC was chosen because this dose has been used in other studies, and is less than the common dose in water treatment facilities. 1 mg O₃/mg TOC was chosen because this is the most typical municipal water treatment dose. 2 mg O₃/mg

TOC was selected because this is usually the maximum dose applied at municipal water treatment facilities due to cost of application.

Chlorine Oxidation Technique

Oxidation with chlorine was done using a sodium hypochlorite bleach solution. 5 grams of bleach solution was added to ultrapure water with a resulting residual of 250 mg/L Cl_2 . 1 mL of this stock solution was added to 250 mL of sample to give a 1 mg/L Cl_2 concentration. The stock solution was made fresh at each application, and concentrations were measured each time to ensure similar results.

Selected concentrations were then applied to the humic solution and allowed to react until the free and total Cl_2 concentrations were below the detection limit for the diethyl-p-phenylene diamine (DPD) method. Chlorine was dosed at 4 mg/L Cl_2 since this is the maximum regulated dose allowed for chlorine under the D/DBP Rule. It was also dosed at 0.5 mg/L Cl_2 since this was the dose applied in a companion study, which was chosen to meet the criteria that no residual be measured after 15 minutes of contact time between the humic solution and chlorine.

A HACH DR/2000 was used to measure residuals using free and total chlorine packets designed for the DPD method (HACH method 80). Chlorine demand curves were generated for each concentration by measuring the chlorine concentration at various time intervals until the residual was undetectable.

The DR/2000 was routinely checked for accuracy using a potassium permanganate standard taken from Standard Methods 4500-Cl G, DPD Colorimetric Method (Standard Methods, 1995). 891 mg KMnO_4 was added to 1 L of ultrapure water

to make stock #1. 10.0 mL stock #1 solution was diluted with 100 mL of ultrapure water to make stock #2, and 1 mL of stock #2 was diluted with 100 mL ultrapure water to give a chlorine equivalent of 1.00 mg/L using the DPD method. A standard curve was developed from 0.2 to 2.0 mg/L, and compared against the DR/2000. Correlation between the curve and the instrument were always satisfactory.

Before oxidized samples were applied to BDOC columns, total and free chlorine concentrations were measured to ensure no residual.

Monochloramine Oxidation Technique

Monochloramine was made in the lab according to the following procedure. A 1 L phosphate buffer was made using 0.5 g dibasic potassium phosphate and 1 L of ultrapure water. The pH of the buffer was checked, and adjusted with 0.1N NaOH to a pH of 8.9 to 9.2. Next, 0.11 g ammonium chloride was mixed with 100 mL of the phosphate buffer. 1 mL of ~4% sodium hypochlorite bleach solution was added to the 100 mL solution. It was imperative to add this very slowly (less than 20 μ L every 6 seconds). The solution was allowed to stir for 30 minutes before measuring total and free chlorine residuals.

Total and free chlorine measurements were done on the HACH DR/2000 using DPD powder packets and the HACH method 80. Monochloramine measurements were done by adding a KI crystal to the free chlorine test and measuring it on the DR/2000 using HACH method 80. The difference between the total and free chlorine measurements was considered the monochloramine concentration. The recipe used above generated a monochloramine concentration of 400-500 mg/L.

46 mL of the 100 mL stock solution was added to a 6 L volume of the humic solution to give a final concentration of ~ 4 mg/L NH_2Cl , and allowed to react for ten days. This dose was chosen because it is the maximum monochloramine dose that can be applied based on regulations set by the D/DBP Rule. Residuals were measured daily to develop a demand curve. At ten days approximately 60% of the NH_2Cl remained in the sample, so that it was necessary to neutralize remaining NH_2Cl to terminate the experiment. 0.1N sodium thiosulfate was titrated into each 2 L sample to eliminate the residual. Special care was taken to ensure that little or no excess thiosulfate was available since other research, conducted by Michele Prevost has indicated that thiosulfate may cause problems with the BDOC test.

UV-light Application

UV-light application was based on inactivation tables for common bacteria, viruses, protozoa, yeasts, and molds found in drinking water systems. Table 1 (Tipton, 1998, http://www.tiptonenv.com/uv_technical.html#tableI; Shaw, et al., 2000; Atlantic Ultraviolet Co.) shows the incident energies necessary to inhibit colony formation in more than 99.9% (3-log removal) of microorganisms at a wavelength of 253.7 nm. The UV-dose is reported in $\mu\text{W}\cdot\text{s}/\text{cm}^2$. The dose required to inactivate the Tobacco Mosaic virus (highlighted in Table 1) was selected as a dose of 1X for this project since that is the maximum known dose required to inactivate all microorganisms listed. However, it is important to understand that this dose is not necessarily the minimum application dose required for drinking water treatment. Standards for UV disinfection are currently set at the federal level at a minimum of 16,000 $\mu\text{W}\cdot\text{s}/\text{cm}^2$, which is based on a dose that

inactivates *Saccharomyces cerevisiae* (highlighted in Table 1). However, it is evident from Table 1 that this is not sufficient to effectively treat several organisms, and therefore some states have adopted higher standards up to 38,000 $\mu\text{W}\cdot\text{s}/\text{cm}^2$ which will inactivate *Bacillus subtilis* spores (Parrotta, et al., 1998). It was previously believed that *Cryptosporidium* and *Giardia* (highlighted in Table 1) were limiting factors when considering UV treatment (Karanis, et al., 1992; Shaw, et al., 2000). However, recent research has shown that these oocysts are much easier to treat than previously thought (Mofidi, et al., 1999; Braur, 2000; Shaw, et al., 2000). Therefore, I did not consider these organisms when selecting a starting dose. I chose 440,000 $\mu\text{W}\cdot\text{s}/\text{cm}^2$ as a dose of 1X because it set a theoretical maximum UV-dose to be used in a municipal water treatment facility for disinfection purposes and was easy to achieve with the UV-light apparatus being used in the lab.

The UV-apparatus utilized was a Minipure, MIN-1, with a Ster-L-Ray low-pressure mercury-vapor lamp (Atlantic Ultraviolet Co., Hauppauge, New York). The lamp was encased in a stainless steel chamber and silicone tubing was used to deliver sample through the system. The minimum dose of 40,000 $\mu\text{W}\cdot\text{s}/\text{cm}^2$ is obtained at a flowrate of 1 gal/min, according to the manufacturer. Changing the flowrate through the apparatus allowed the dose to be increased linearly. A curve based on absorbance of UV-254 was developed for doses from 1X to 230X by circulating a water sample through the UV chamber for approximately 24 hours. This curve was used to identify doses of UV that would provide for significant changes in UV-254 absorbance. Based on these results, doses at 1X, 10X, and 53X were chosen. These doses were applied by

establishing flowrates of 320 mL/min, 32 mL/min, and 6.0 mL/min respectively, and using a continuously flowing system to oxidize the sample.

Table 1: Incident Energies for Various Bacteria, Molds, Viruses, Protozoa, and Yeasts	
Ultraviolet Energy Levels at 2537 Angstrom Units Wavelength required for 99.9% Destruction of Various Microorganisms	
UV Energy in Microwatt-seconds per Square Centimeter	
BACTERIA	
Agrobacterium Lumetaciens	8500
Bacillus anthracis	8700
Bacillus megaterium (vegetative)	2500
Bacillus megaterium (spores)	5200
Bacillus subtilis (vegetative)	11000
Bacillus subtilis (spores)	22000
Clostridium tetani	23100
Corynebacterium diphtheriae	6500
Escherichia coli	6100
Legionella species	2760-5500
Leptospira interrogans (infectious jaundice)	6000
Mycobacterium tuberculosis	10000
Neisseria catarrhalis	8500
Proteus vulgaris	6600
Pseudomonas aeruginosa (laboratory strain)	3900
Pseudomonas aeruginosa (envir. Strain)	10500
Rhodospirillum rubrum.	6200
Salmonella species	7000-15200
Sarcina Lutea	26400
Serratia marcescens	6160
Shigella species	4200-7000
Staphylococcus species	6600-10000
Streptococcus species	5500-8800
Veridans streptococci	3800
Vibrio cholerae	6500
MOLD SPORES	
Aspergillus flavus (yellowish green)	99000
Aspergillus glaucus (bluish green)	88000
Aspergillus niger (black)	330000
Mucor racemosus (A & B) (white gray)	35200
Penicillium species	22000-

Rhizopus nigricans (black – Cheese mold)	88000 220000
ALGAE	
Chlorella vulgaris (algae)	22000
PROTOZOA	
Nematode eggs	40000
Paramecium	200000
Cryptosporidium parvum 4-log inactivation (Shaw, et al., 2000)	<10000
Giardia lamblia 4-log inactivation (Shaw, et al., 2000)	4000
VIRUSES	
Bacteriophage (E. coli)	6600
Hepatitis virus	8000
Influenza virus	3400
Polio virus (Poliomyellitis)	21000
Rotavirus	24000
Tobacco mosaic virus	440000
YEAST	
Baker's yeast	8800
Brewer's yeast	6600
Common yeast cake	13200
Saccharomyces cerevisiae and ellipsoideus	13200
Saccharomyces sp	17600

http://www.tiptonenv.com/uv_technical.html#tableI
(Shaw, et al., 2000; Atlantic Ultraviolet Co.)

TOC

TOC was measured as Non-Purgeable Organic Carbon (NPOC), using a high-temperature catalytic method (TOC-5000A Total Organic Carbon Analyzer, Shimadzu Scientific Instrument Inc., Columbia, MD) with a high sensitivity catalyst to allow for low carbon level analysis. Sample volumes of 40 mL were collected in acid washed and baked vials. They were acidified with 0.2 mL of 2N HCl, and stored at 4°C prior to analysis. During measurement, they were sparged for 5 minutes to remove all inorganic and volatile organic carbon.

A standard curve was developed using potassium hydrogen phthalate $\text{KC}_8\text{H}_5\text{O}_4$, which was baked in an oven for at least one week, or until dehydrated. 1.0626 g was added to 1.0 L of ultrapure water to make stock solution #1 at a concentration of 1000 mg/L as C. 25 mL of stock #1 was added to 1.0 L of ultrapure water to make stock solution #2 at a concentration of 25 mg/L as C. Standards were made from stock #2 in volumes of 200 mL. Table 2 gives the concentrations commonly used for standards, and the corresponding volumes of stock #2 required to meet those concentrations. A four point linear regression was performed using four of the five concentrations listed below. Each time new standards were made, or instrument maintenance took place, a new standard curve was created. One standard per run (16 samples) was used, along with one duplicate and three ultrapure water samples to assess instrument operation.

Table 2. Standard Recipes for TOC Measurement

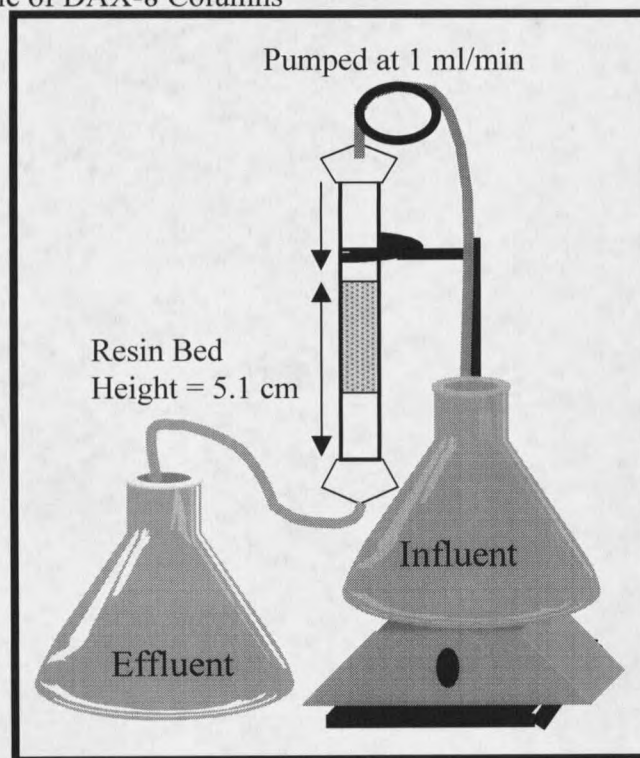
Standard Concentration (ppb)	Volume Stock #2 Required (mL)
250	2
500	4
1000	8
2000	16
4000	32

DAX-8 Resin Chromatography

DAX-8 resin chromatography was used to separate the humic solution based on hydrophobic-hydrophilic fractions. This method is also commonly referred to as humic acid/fulvic acid separation, or non-humic/humic separation. It is based on the property of humic substances that at a $\text{pH} = 2.0$, humic acids (or the hydrophobic fraction or humic

fraction) separates out and the fulvic acids (or hydrophilic fraction or non-humic fraction) remain dissolved (Thurman, et al., 1981; Owen, et al., 1993; Debroux, et al., 1995). Figure 4 shows a schematic of the column and laboratory setup. Previous protocol called for the use of Amberlite XAD-8 resin. However, this resin is no longer manufactured through Amberlite. Supelco (St. Louis, MO) now carries the resin under the name Supelite DAX-8 resin.

Figure 4. Schematic of DAX-8 Columns



Omnifit (10 mm x 150 mm) columns (Omnifit, Cambridge, England) were purchased because the columns were made of borosilicate glass making it possible to acid wash and bake them between each use. Additionally, all endfittings were made of Teflon, with Teflon tubing, o-rings, and miscellaneous fittings. Frits were made in the

lab from 100 mesh stainless steel screen and fired glass wool. This was necessary because all frits manufactured by Omnifit prevented passage of a fraction of humic substances. The size of the column was selected based on the amount of sample available to do the test. A balance between sample volume, sample run time, and TOC strength of the sample must be met. Appendix A has a detailed discussion concerning column selection, as well as additional sources for further information on this topic.

Since the resin was not chemically inert, an extensive cleaning process was required to lower background carbon levels. A background level of 0.05 mg/L TOC was considered to be low enough to prevent noticeable contamination. To achieve this, the following protocol was followed. Resin was rinsed in 6 consecutive 0.1N NaOH baths, each a minimum of 12 hours. The resin was then transferred to a Soxhlet Extractor (Fisher, 09-571B), and extracted for 48 hours each with methanol, acetonitrile, and methanol. Cleaned resin was stored in methanol. At least 24 hours before use, cleaned resin was rinsed of methanol several times with ultrapure water. The resin was then placed in ultrapure water at 60°C to drive off all remaining methanol, changing the ultrapure water at least 3 times over this 24 hour period. Acid washed and baked columns were then packed with the clean resin and washed with a series of 3 column volumes each of 0.1N NaOH, ultrapure water, 0.1N HCl, and ultrapure water again, a total of 3 times. Ultrapure water was run through the column a final time, and TOC samples were collected to measure background column concentrations. If these backgrounds were 0.1mg/L TOC over that of the rinse water, additional washes with NaOH and HCl were done and background concentrations were measured again.

Once the columns were ready for sample application, 1L samples were acidified to a pH = 2.0 with 6N HCl. pH measurements were done with an ATIO Orion PerpHect Meter (Model 370, Boston, MA). The samples were then placed on stir plates to prevent settling of separated fractions, and pumped through the columns at approximately 1 ml/min. Column effluent was collected in an acid washed and baked 1L flask. 40 mL of this hydrophilic fraction was analyzed for TOC, 100 mL was saved for duplicate measurements and UV-254 analysis, and the remaining volume was recorded and disposed of.

After separation, the column contained approximately 1-2 cm of brown colored resin. This retained fraction was eluted from the column at 0.5 mL/min with 0.1N NaOH. Approximately 30-50 mL of sample was collected, and then diluted at a ratio of either 2:40 or 4:40, depending on the level of discoloration, for TOC analysis. The volume was measured, and the sample was saved for UV-254 analysis and duplicate TOC measurements if necessary.

A mass balance was then done on the resulting TOC measurements to ensure that at least 90% of the bulk TOC was accounted for after separation. The following calculation was programmed into an Excel TM spreadsheet, where mass balances and the resulting hydrophobic fractions were calculated.

$$TOC_{in} - TOC_{non-humic} = TOC_{eluant} (Vol_{eluant} / Vol_{sample})$$

The resin was then rinsed into a bottle containing methanol, and the cleaning process was repeated as necessary. Columns were then acid washed and baked to clean. Maintenance was done, as required, on end pieces and tubing. Replacement parts were always kept on hand.

MW Fractionation

Ultrafiltration. Ultrafiltration was initially chosen for MW fractionation. The method was based on a protocol commonly used at the University of Colorado—Boulder by Gary Amy, et al. (Amy, et al., 1987; Collins, et al., 1986; Owen, et al., 1993). Amicon YM and YC series membranes (Millipore, San Jose, CA) were used, and were chosen because they are classified as hydrophilic, so little or no adsorption of hydrophobic acids should occur. The membranes were chosen to accommodate the following MWCs (molecular weight cutoffs), <500 Da (YC05), < 1000 Da (YM1), <3000 Da (YM3), < 10,000 Da (YM10), and < 30,000 Da (YM30). Samples were processed through Millipore 300 mL stainless steel stirred cells (Millipore, San Jose, CA) with Teflon o-rings and a Teflon stir bar, chosen to prevent carbon leaching from plastic materials.

Since a separate aliquot of sample was processed through each membrane, the corresponding fractions were cumulative fractions, comprising all MWs below the respective cutoff. Discrete fractions could be calculated by taking the difference between TOC measurements of neighboring samples. Some researchers, in fact, have discouraged attempts to find discrete fractions because variability between membranes of the same nominal size can cause slightly different separation, giving varied results (Owen, et al., 1993). Therefore the focus of these fractions is on MW trends, as opposed to exact quantitative results.

The stirred cell was rinsed with one cell volume (300 mL) of ultrapure water, then it was rinsed with about 50 mL of sample before sample collection was started. The sample size was 140 mL, allowing 50 mL for the initial rinse, 40 mL for TOC analysis and UV-254 measurements, and 50 mL of retentate (sample remaining in the stirred cell).

Mass balances could be performed routinely on retentate, effluent, and bulk samples to ensure no loss or gain of carbon throughout the filtration process. Flow through the cell was driven by pressure using high-purity nitrogen at 55 psi. It was also important to minimize the vortex caused by the stir bar. This vortex was not allowed to exceed 2 cm. Membranes could be reused several times (until mass balances indicated contamination, or upon discoloration), and were stored in acid washed and baked containers containing ultrapure water at 4°C.

This method was studied for nearly 6 months, after which time it was decided that for low carbon levels, such as the levels being used for this experiment, considerable contamination resulted from the membranes. Since continuous washing and treatment with mild NaOH and HCl did not eliminate the contamination, it was decided to discontinue ultrafiltration for MW fractionation.

HPSEC. High performance size exclusion chromatography (HPSEC) was also used for molecular weight separation. Figure 5 is a diagram of the system employed for this project. HPSEC involves separating a water sample into fractions based on molecular size by utilizing a column capable of retaining MW fractions below a specific limit. The separated sample is then processed through a detector, and the output is displayed on a computer as peaks, showing zones of greatest concentration for a period of time, depending on the size of the molecules being analyzed. Molecules larger than the pore size of the packing material will be pushed through the column first, and smaller molecules, which are retained within the packing material pore matrix are attenuated, and take longer to elute (Her, et al., 1999). This is demonstrated in Figure 6.

Figure 5. Schematic of HPSEC Instrumentation

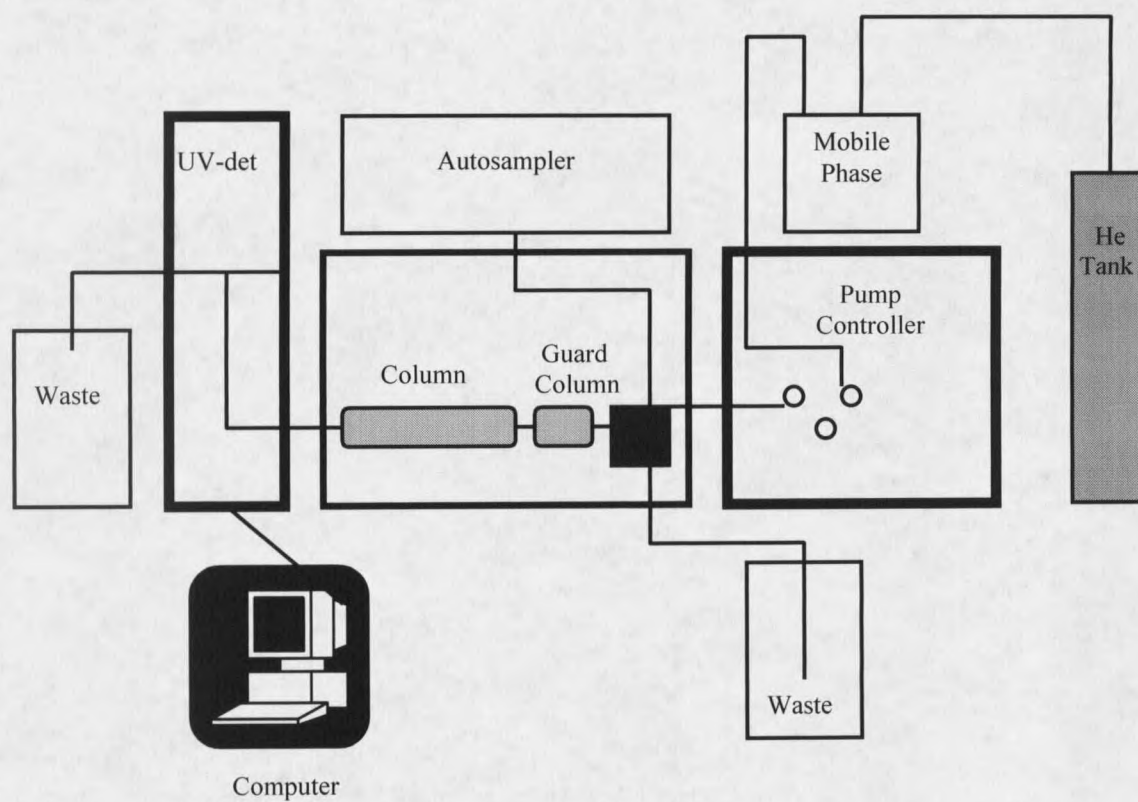
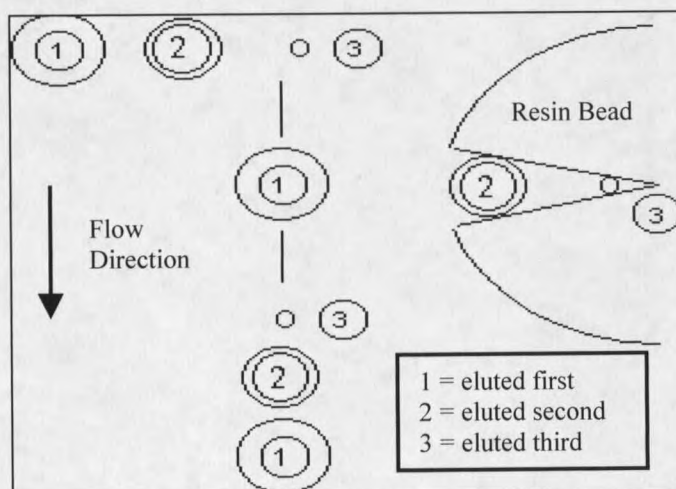


Figure 6. Principle of HPSEC Separation



Adapted from Shodex Instruments (JM Science, Grand Island, NY)

The success of this method for fractionating NOM is highly dependent on mobile phase selection, column selection, proper standard selection, sample injection size, mobile phase flow-rate, and detection method. Therefore, the following sources were carefully examined and a method was adopted based on the previously listed parameters, and instrument limitations in the lab (Her, et al., 1999; Becher, et al., 1985; Marquet, et al., 1999; Chin, et al., 1991; Chin, et al., 1994; Conte and Piccolo, 1999; Rausa, et al., 1991).

The instruments employed for this method include a Dionex DX 300 Series High Performance Liquid Chromatograph in conjunction with a Dionex AD20 variable wavelength absorbance detector (Sunnyvale, CA). The HPLC machine delivers a mobile phase to a column where separation occurs. The mobile phase is sparged with helium gas to eliminate inorganic carbon and oxygen, which may cause interference with detection processes. An automated sampler (Model # ASM-2) is used to deliver sample to a column. The UV-detector is connected to the effluent of the column, and was programmed according to the following parameters. The lamp intensity was set on low, and it was operated at a wavelength of 254 nm. The measurement sensitivity was set to 0.2 absorbance units (AU), to minimize background fluctuations in the resulting chromatogram.

A 500 μ L sample loop delivered 0.5 mL of a 5 mL sample to a Shodex KW-802.5 silica-gel packed guard column (8.0 mm x 50 mm) and column (8.0 mm x 300 mm) (JM Science, Grand Island, NY). The column was selected based on the packing material, retention time, and the MW range for separation in Daltons (Da). The packing material was designed specifically for separation of protein-type molecules with a maximum MW

exclusion limit of 150,000 Da. The retention time was based on the column capacity, as well as the recommended flowrate across the column. The column manufacturer recommended a flowrate of 1 mL/min giving a maximum elution time of 15 minutes. Additionally, column capacity dictated the maximum injection volume for the column, which was limited to 500 μ L. The major drawback of the column was pH concerns, since the packing media dissolves in solutions with a pH higher than 7.5. Therefore, a strongly buffered mobile-phase was used to ensure pH stability.

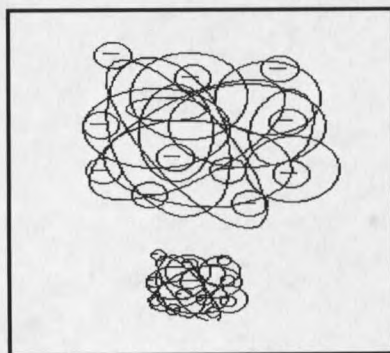
Increasing the sample size would improve resolution. Therefore, an additional column was special ordered to accommodate a sample size of 2 mL. This column was packed with TSK-50S Toyoperl resin (TOSHAAS) (Watrex International, Inc., Pittsford, NY), which was housed in a stainless steel reservoir (20 mm x 250 mm) (Watrex International, Inc., Pittsford, NY), recommended by Nam Guk Her, et al., 1999. However, the column was selected to optimize detection on a TOC detector, which was not available in our labs. Therefore, when used with our UV-detector, peak widths were very broad, and difficult to interpret. Additionally, several parameters, such as flowrate, pressure requirements, injection loop size, and mobile phase selection needed to be modified for proper operation of this column. Because this optimization process is quite time consuming, it was considered to be beyond the scope of this project, and the use of this column was abandoned. Therefore, all samples were separated on the Shodex column.

Mobile phase selection was based on manufacturer recommendations. It consisted of a 50 mM phosphate buffer + 0.5 M NaCl. The phosphate buffer prevented

pH values higher than 7.5 from crossing the column, and the sodium chloride minimized the detection peak width to allow for the highest possible resolution.

Several researchers have spent considerable time examining MW standards capable of accurately representing humic substance MW (Becher, et al., 1985; Chin, et al., 1991; Her, et al., 1999; Conte and Piccolo, 1999). Since humic substances are so diverse and complex, a surrogate compound is needed. Currently, globular proteins, polysaccharides, polyethylene glycol (PEG), and polystyrene sulfonates (PSS) are commonly used as standards. Selecting the proper standard depends not only on how accurately the standard represents the humic substances being studied, but also on the available detection methods. Globular proteins and polystyrene sulfonates are capable of being detected with UV-254, however, both have been criticized by researchers because they tend to over or underpredict MW fractions of humic substances (Chin, et al., 1991; Conte and Piccolo, 1999). This is partly because the molecular size (or hydrodynamic radius) of humic substances and other molecules can be dramatically affected by properties such as pH and ionic strength. Figure 7 shows how a molecule with the same MW can appear drastically different at varying ionic strengths.

Figure 7. Large Apparent Molecular Size Due to pH and Ionic Strength



Courtesy of Shodex Instruments

This not only enhances the necessity of preparing a mobile phase with a representative ionic strength, but it also highlights the importance of choosing standards least affected by these properties. So, while the hydrodynamic radius and interaction with the stationary phase of any compound is bound to be different from those of humic substances, polysaccharides and PEG seem to be less affected by pH and ionic strength than the other standards. This makes them more consistent for use with a variety of water sources and humic substances (Conte and Piccolo, 1999).

Even though PSS has been recommended over globular proteins by several researchers (Conte and Piccolo, 1999; Chin, et al., 1991), another problem is its insolubility in water. Since the original goal of this method was to collect each MW fraction from the effluent of the UV-detector and measure the TOC background on the Shimadzu TOC 5000-A, PSS was not selected as the standard for this project since the undissolved polystyrene beads have been known to damage the high sensitivity catalyst used in the TOC machine. Therefore, since the only detector available in our labs was a UV-detector, and original intention of this method were to use the Shimadzu TOC machine, protein globules were chosen as MW standards in this project. Table 3 shows the standards chosen, and their corresponding MWs (Sigma, St. Louis, MO).

Table 3. MW standards and Respective MWs

Standard	MW (Daltons)
Vitamin B-12	1,350
Aprotinin	6,500
Cytochrome C	12,400
Carbonic Anhydrase	29,000
Albumin	66,000
Blue Dextran	2,000,000

Results were recorded on Dionex software in the form of chromatograms. Since there was a small response from ultrapure water samples on the UV-detector, sample chromatograms were adapted by subtracting out the water chromatogram baselines. The resulting chromatograms are displayed, as well as tables showing respective areas for selected peaks. Number-averaged and weight-averaged MWs are calculated according to the following equations, where h_i is the detector response corresponding to the elution time (i).

Number-averaged MW

$$M_n = \frac{\sum_{i=1}^N h_i}{\sum_{i=1}^N (h_i/M_i)}$$

Weight-averaged MW

$$M_w = \frac{\sum_{i=1}^N (h_i M_i)}{\sum_{i=1}^N h_i}$$

BDOC

Biodegradable Dissolved Organic Carbon (BDOC) measurements can be done using several methods. However, the success of each method highly depends on the organisms that do the biodegradation, the water sample being studied, and the ability of the researcher to control various types of carbon contamination that may be caused by the method itself. The basic protocol involves inoculating a water sample with organisms capable of degrading the entire biodegradable fraction of organic carbon. The inoculation process varies from protocol to protocol.

Three protocols were considered for this project. The first utilized a suspended biomass for inoculation. 2 mL of naturally inhabited water (i.e. untreated surface water),

were used to inoculate a 200 mL sample. The inoculum was filtered through a 2 μ m filter to remove particulate material and protozoa. The sample was filtered through a 0.2 μ m membrane filter to remove any bacterial population already in the water sample, and an initial TOC measurement was done. The samples were allowed to sit for 4 weeks at room temperature in the dark, and then final TOC measurements were done. The difference between the initial TOC and the final TOC was termed to be the BDOC (Servais, et al., 1989). One advantage of this method is that it causes very little carbon contamination to a water sample, as compared to other methods. However, the major disadvantage is that it takes one month to complete the biodegradation of a sample. Therefore, due to time considerations, this method was not chosen as the BDOC method for this project.

The other two methods considered involved an inoculum of fixed biomass on sand. For the first method, the sand was colonized with organisms indigenous to Bozeman city tap water. The sand column was placed online behind a biologically active carbon (BAC) column so the water sample was dechlorinated. Consequently, the organisms were adapted to live on extremely low levels of carbon (less than 500 ppb). When needed, 100 g of sand was removed from the inoculated column and placed into a 500 mL acid washed and baked flask. The sand was gently rinsed three times with ultrapure water to eliminate any extraneous carbon contamination, while keeping the organisms attached to the sand. Then, it was rinsed once with sample to prevent sorption of carbon from the sample, which could skew the carbon measurement by as much as 20-30% (which could be more than the BDOC of the sample). Then, 300 mL of sample was added to the flask, and allowed to sit for three to five days. The actual time required

to biodegrade the sample was measured initially by collecting samples for TOC analysis daily until a baseline carbon concentration was reached. This indicated that carbon degradation was complete.

Before TOC analysis, the samples were to be filtered using Fisher brand 0.2 μm nylon filters to remove potentially suspended biomass, which would be measured in the carbon analysis. Carbon contamination from the filters was a problem as well, so a filter rinsing process was developed that consistently lowered the background carbon from the filters below 100 ppb. 30 mL plastic syringes were filled 3 times with 0.1N HCl and rinsed through the filter. Then, the filter was rinsed three times with ultrapure water. A final rinse of ultrapure was saved for TOC analysis, so background concentrations could be measured.

This protocol was developed based on previous methods used by Servais, et al. (1989), and Volk, et al. (1993). However, significant problems were encountered in the use of this method. For example, using the city tap water as an inoculum for the sand column increased sample sorption problems mentioned previously, and also may have caused nutrient-shocking to the biomass due to the large difference in TOC concentrations between the feed water to the column (< 0.5 ppm) and the sample water (4 ppm). Building a new feed water system minimized this problem. The new system involved inoculating a 10 L carboy of distilled water with 2 L of surface water from the duck pond at Montana State University. This water is untreated, high in biological activity, and high in carbon content. The biomass inoculum was fed to 2 columns filled with silica sand for one week to establish biomass. Then, the columns were fed with a 4 ppm humic stock solution identical to the solution that was used for oxidation

experiments. This stock solution was recirculated through the columns for approximately a week, and then replaced with a fresh solution. This was done in order to provide a somewhat consistent level of carbon to the organisms, that was similar in concentration and make-up of sample water. The higher carbon level allowed sorption problems to be minimized, and prevented nutrient-shocking from taking place.

Another problem that may have been associated with the second method was uneven contact between the sample and the fixed biomass since the sand was at the bottom of the water sample and there was still a significant amount of water above the sand surface. This may or may not have been a problem, however, it was decided that a better method for providing contact between the sample and the fixed biomass would be to recirculate the sample through a smaller column (100 g) of sand for a given period of time. This third method, involving the new inoculating process and the smaller recirculating sand columns (adapted from Cipparone, et al., 1997), provided much more consistent and believable results.

For this third method, initial and final TOC measurements were done, and the difference was termed the BDOC fraction. Additionally, samples needed to be filtered according to the method described above to prevent biomass contamination and carbon contamination from the filter itself. Carbon removal in the BDOC columns was consistently between 20 and 50% depending on the water sample and the oxidation method, and a decrease in carbon over a period of time was always seen. Samples were measured in triplicate to ensure consistency from column to column, and experiment to experiment. UV-254 analysis was done occasionally to establish a relationship between

BDOC and aromatic character. All other carbon characterization data were compared to BDOC results to establish trends between techniques.

UV-254 Analysis

The aromatic character of the treated and non-treated humic solution was measured using UV-254 analysis. The method was based on the characteristic of humic and fulvic acids to absorb ultraviolet light in proportion to their TOC concentration. The protocol for UV-254 was taken from Standard Methods, Method 5910 B, Ultraviolet Absorption Method (1993). It was measured at 254 nm on a Milton Roy spectrophotometer (Rochester, NY) with a 1-cm quartz cell. Results were reported as cm^{-1} . No sample treatment was done prior to measuring absorbance. However, eluant DAX-8 column samples were occasionally diluted to be read on the UV-spec. Additionally, since the pH of effluent DAX-8 column samples were below 2, and at low pH values, the accuracy of UV-254 analysis diminishes, these samples were not measured for UV-absorbance.

Statistical Analysis

To test for a significant dose-response effect and to estimate the slope of the dose-response line, an analysis of covariance was used, where the fraction change of each parameter (hydrophobicity/hydrophilicity, aromaticity, TOC reduction, and biodegradability) was the response, the bulk solution treated (carboy) was the random factor, and the dose was the covariate. The p-value associated with dose pertains to the null hypothesis that the true slope of the dose-response line is zero. Additionally, another p-value was generated that is associated with bulk solution (carboy), which pertains to the

null hypothesis that there is no variability among carboys. The latter p-value consistently showed no variability among carboys, so these values are not discussed in detail in the text of the paper, but are reported in Appendix B. The analysis was performed using the General Linear Model program in MINITAB (version 12.1, Minitab, Inc.). A p-value less than 0.05 indicated a significant effect (or discredited the null hypothesis).

CHAPTER 3

Experimental Results

The experimental results of this study examine both methods development and oxidation. The methods developed in this study concern molecular weight (MW) separation, a potentially powerful tool in describing the fate of high molecular weight compounds upon oxidation.

Oxidation demand curves were generated for UV-light, chlorine, and monochloramine to identify proper dosing techniques and ozone application rate tables were developed to ensure proper and consistent ozone dosing levels. Both the original and oxidized humic solutions were characterized based on the properties listed below.

- Hydrophobic/hydrophilic character
- MW distribution
- Aromaticity
- Overall TOC content

Additional comparisons were made based on removal rates of each of the assays described below. TOC losses after oxidation, most likely due to conversion of organic carbon to CO₂, and hydrophobic/hydrophilic fractions were measured in mg/L TOC. Chromatograms of MW fractions gave insight into potential MW changes that occurred. Aromatic character was measured in cm⁻¹. Tables of results, reported in the text of the paper, display the results as fractions of the initial bulk solution. All concentration tables are available in Appendix B. The results were used to identify the most effective oxidant, as well as the most effective dose applied for each oxidant.

MW Fractionation

Two methods were considered for molecular weight fractionation: ultrafiltration and high performance size exclusion chromatography (HPSEC). Both methods are explained in detail in Chapter 2. Ultrafiltration involves the use of stirred cells and membranes with MW cutoffs ranging from 500 to 30,000 Da, and HPSEC involves separation through an SEC column running in conjunction with an HPLC machine. The benefit of ultrafiltration was that TOC measurements could be done on membrane effluents, so mass balances could be performed between the total carbon in the sample, and the sum of the carbon levels in each respective fraction. A considerable portion of this project was dedicated to eliminating background carbon contamination throughout the ultrafiltration system to ensure that no carbon contamination was influencing the results. Table 4 shows a range of background TOC measured from each of the membranes, which were stored in carbon-free glass containers and ultrapure water at 4°C (n = the number of background measurements per membrane). The membranes were also subjected to several washes with ultrapure water through the stirred cell to try and eliminate any carbon that may have been trapped within the membrane structure.

Table 4. Ultrafiltration Membrane Backgrounds After Several Rinses with Ultrapure Water.

Membrane (MW Cutoff – Da)	Background Carbon Range (µg/L TOC)
YM30 (30,000)	32 – 98 n=7
YM10 (10,000)	62 – 1006 n=4
YM3 (3,000)	86 – 890 n=8
YM1 (1,000)	173 – 970 n=12
YC05 (500)	189 – 1142 n=5

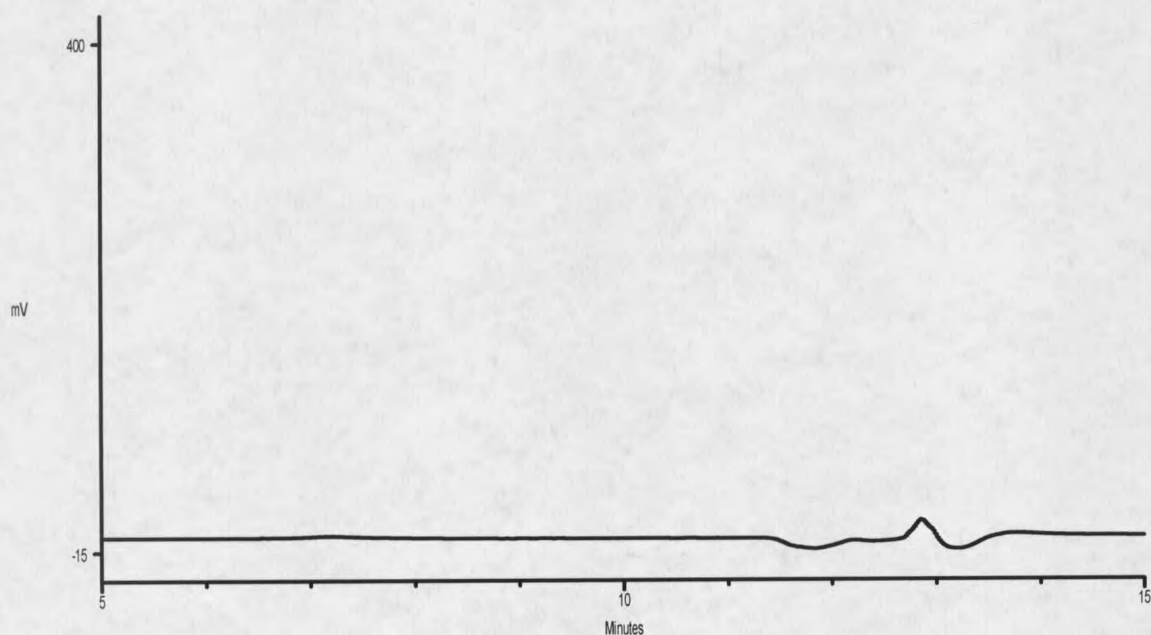
The lowest levels of TOC contamination for each of the highest MW cutoff membranes seemed to be reasonable enough to run the tests. In fact, the YM30 membrane was consistently clean, mass balances between the eluant and the retentate were closed within 10%, and results seemed reliable with 40% of the humic solution being retained by the membrane. However, for the YM 10 and YM3 membranes, high levels of contamination were much more frequent; it was quite common to see a membrane with less than 100 ppb TOC be rinsed again, with a resulting background of 500 ppb. The inconsistency was too difficult to control, and therefore made evaluation of collected fractions difficult to rely on. YM1 and YC05 membranes also demonstrated this inconsistency, and their backgrounds barely went below 200 ppb on any given run. Since the bulk solution concentration was between 2 and 4 mg/L TOC, 200 ppb was not acceptable, as the total carbon in each of those possible fractions could have been less than the background. Consequently, HPSEC was considered.

High performance size exclusion chromatography, although used in research for many years, has only recently been considered useful to humic substance researchers. Detection processes have limited researchers flexibility, until just recently, therefore resulting in methods limited use for humic substance research. As detection processes have become more sensitive and flexible, HPSEC has become more valuable as a tool for measuring MW fractions of humic substances. Current detection processes include but are not limited to UV-254, Refractive Index (RI)-detection, and on-line TOC detection. It is widely accepted that detection should be done using two or more detectors (i.e. UV-det and one other), since UV only measures the UV-absorbing fraction of humic

substances, which can vary widely from water source to water source. However, due to laboratory equipment limitations, this project only investigated UV-detection.

The SEC column was rinsed three times before each run with ultrapure water, and Figure 8 is an example chromatogram of the third rinse for each run. This chromatogram was subtracted from each of the chromatograms displayed in the results of this paper.

Figure 8. Background Ultrapure Water Chromatogram



Figures 9A and 9B show the chromatogram used to develop the standard curve for the HPSEC method, and the standard curve, respectively. The standard curve was developed by measuring the MW distribution of five protein globules with selected MWs, listed on the chromatogram. This chromatogram shows the peaks and retention times developed from one run. However, standards were run throughout the project, and retention times for each of the standards remained identical throughout the entire project. Therefore, no error is reported in any point used in the generated standard curve.

The MWs were plotted vs. retention time to generate Figure 9B. Generating a standard curve proved to be a bit difficult since the last two points did not allow for a good linear regression fit. Therefore, in order to capture behavior between standard points, several regression curves were attempted, including an exponential regression, a power regression, a logarithmic regression, and a point to point regression. Since the exponential, logarithmic, and power regressions did not provide a good fit either, a point to point regression was chosen. This is shown in Figure 9B by two linear regressions (one for the four lower MW standards, and one for the two high MW standards). These curves were used to calculate number averaged and weight averaged MWs for each sample. These weights are displayed in tables under their respective treatment methods, and calculation tables are shown in Appendix B.

Figure 9A. Standard Curve for HPSEC

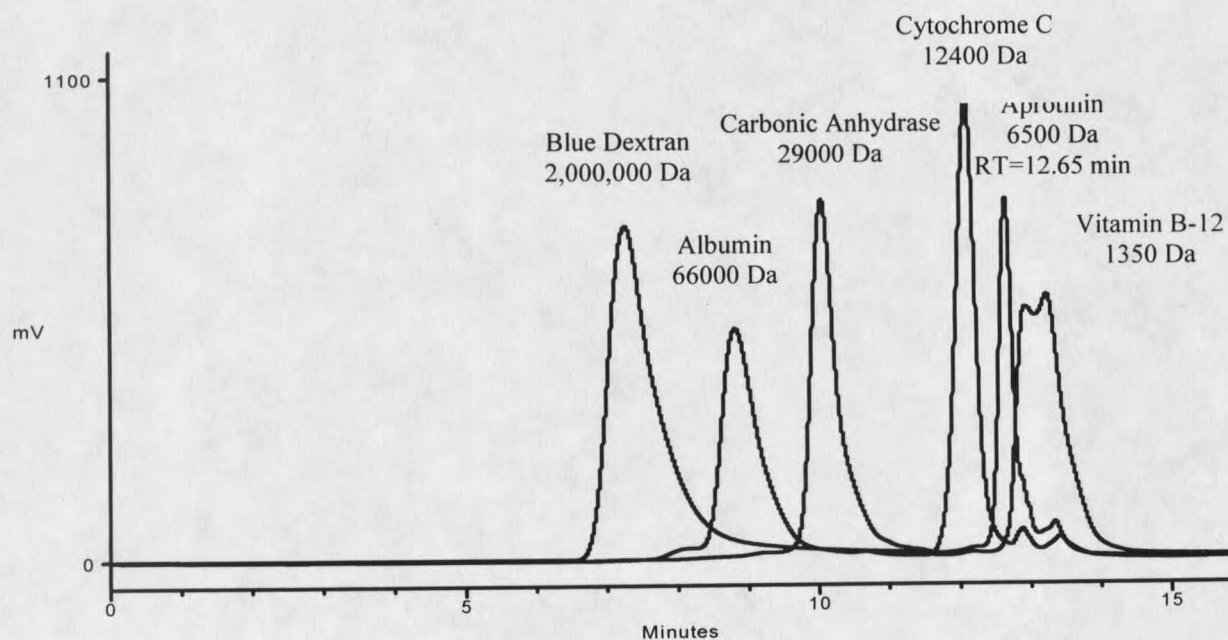
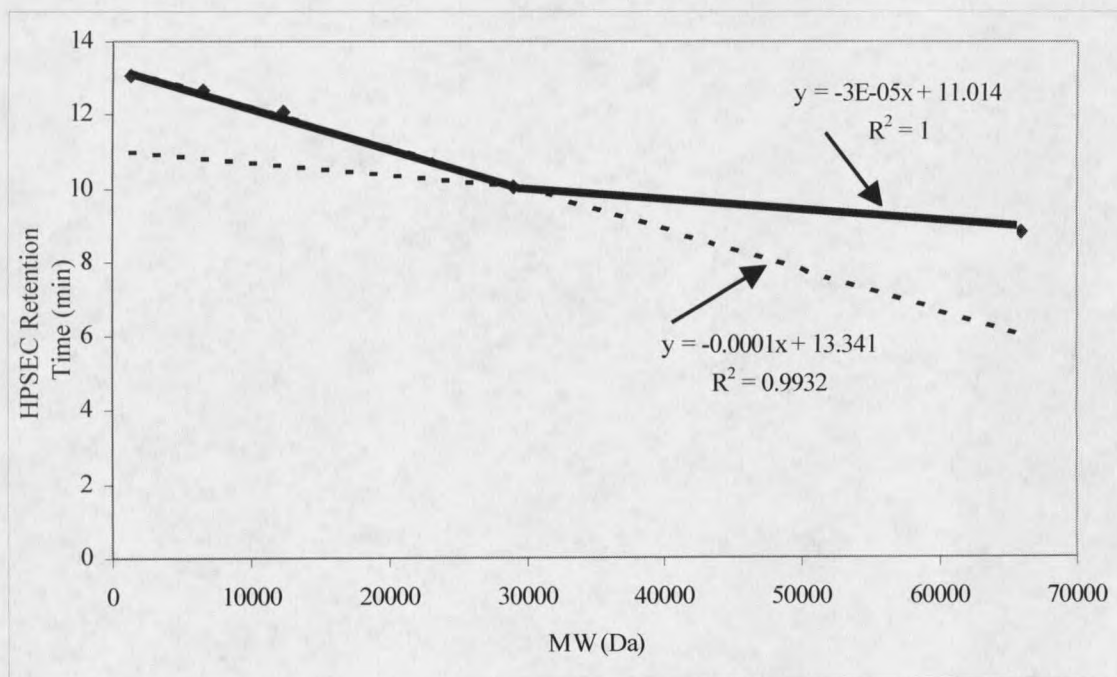


Figure 9B. Standard Curve with Equations used to Calculate MWs



Although results from this method were largely qualitative in nature, they were easily reproducible, and gave insight into the changes that occurred in the aromatic fraction of the humic substances. This insight can be considerably useful in understanding what happens to the molecules in a solution as oxidation occurs. As explained in Chapter 1, humic substances are large, complex molecules built of many aromatic rings, which give rise to aromatic character. The effect of oxidation on these aromatic rings is called cleavage, or breaking of the aromatic rings into less complex and potentially easier degraded fractions. It follows then that if aromatic character decreases, the number of aromatic rings decreases. However, with this decrease in aromatic rings, there is an increase in smaller “pieces,” or smaller molecular weight molecules. Therefore, although only a UV-detection unit is used for analysis in this project, it is

reasonable to infer that if a chromatogram shows a decrease in aromatic character, that the fraction that is missing from the chromatogram has become smaller MW "pieces," which no longer absorb light at 254 nm.

Without any other measurements, complete destruction of organic carbon to CO_2 cannot be eliminated as a cause of decreased aromaticity. However, this phenomenon was not considered plausible in any experiments run in this project because TOC levels were also measured both before and after treatment. Since very little TOC destruction occurred during all oxidation techniques, and aromatic character was destroyed almost completely at varying doses it was possible to conclude that a decrease in aromatic character represents organic carbon becoming less aromatic, not being converted to CO_2 .

Ozonation

Ozonation tables were generated using the methods described in Chapter 2. During each application, varying airflow and ozone generator behavior caused application rates to vary. Therefore, to measure the amount of ozone actually being consumed by each solution, a maximum application rate was measured each time oxidation occurred. This rate was then applied to a humic solution, and unreacted ozone levels were measured. The difference between the maximum and unreacted ozone levels was considered the ozone consumption of the solution, which is reported as $\text{mg O}_3/\text{mg TOC}$. The oxidation tables and corresponding calculations for each time step are displayed in Appendix B. Table 5 shows the ozone application rates for each ozone experiment. The targeted doses include 0.5, 1.0, and 2.0 $\text{mg O}_3/\text{mg TOC}$.

Table 5. Ozone Oxidation Rates for Each Experiment

Date Oxidized	Bulk TOC Conc. (mg/L)	Volume Treated (L)	Mg Ozone/mg TOC
3/14/2000	3.612	2	0.57
4/3/2000	3.427	2	0.48
4/3/2000	3.427	2	0.56
1/31/2000	3.824	2	1.03
2/14/2000	3.770	2	1.05
3/14/2000	3.612	2	1.19
3/14/2000	3.612	2	2.07
4/3/2000	3.427	2	1.84*
4/3/2000	3.427	2	1.88

*Although the oxidation rate is ~ 2 mg O_3 /mg TOC, the complete results indicate that a higher application rate occurred in this sample. This may be the result of a leak during the measurement of the application rate.

Fractionation Results

Table 6 shows the calculated fractions of NOM corresponding to each ozone dose based on hydrophilic/hydrophobic behavior, biodegradability, and aromaticity. The influence of ozone on the bulk solution was calculated by dividing the oxidized bulk TOC concentration by the unoxidized bulk TOC concentration. The hydrophobic and hydrophilic fractions were calculated by dividing the respective measured TOC concentrations by the remaining TOC concentration after oxidation. The BDOC fraction was calculated by dividing the nonbiodegradable TOC concentration by the bulk TOC concentration and subtracting the resulting fraction from 1.0. The aromatic fraction was calculated by dividing the absorbance after oxidation by the absorbance before oxidation. The p-value for each fraction is listed at the bottom of each row in Table 6, representing the probability that dosage had no effect on that fraction. Bulk concentration tables are available in Appendix B.

The shaded data represents data that was excluded from statistical analysis and results interpretation for this project. 1.84 mg O₃/mg TOC dose was excluded because it is believed that there was a tubing leak during application that allowed ozone to escape during the initial application rate measurements, resulting in a higher application rate than what was calculated. The 0.57 mg O₃/mg TOC was excluded because of the BDOC measurement, which was less than the unoxidized control. It is possible that contamination occurred due to filtration methods used on BDOC samples. It is also possible that the media in the column used to process this sample was contaminated, increasing the non-biodegradable fraction.

Table 6. Measured Fractions after Ozone Oxidation Based on Dose

Dose (mg O ₃ /mg TOC)	Hydrophobic Fraction	Hydrophilic Fraction	BDOC Fraction	Aromatic Fraction	Fraction Removed Via Oxidation
0.0	0.59	0.49	0.27	1.00	0.00
0.0	0.62	0.42	0.36	1.00	0.00
0.0	0.54	0.30	0.28	1.00	0.00
0.0	0.53	0.43	0.28	1.00	0.00
0.57	0.49	0.58	0.19	0.73	0.01
0.56	0.48	0.52	0.33	0.68	-0.03
0.48	0.47	0.57	0.31	0.63	0.00
1.03	0.32	0.76	0.39	0.56	0.00
1.05	0.22	0.77	0.42	0.56	0.07
1.19	0.26	0.70	0.36	0.53	0.11
2.07	0.20	0.79	0.31	0.41	0.13
1.84	0.11	0.89	0.40	0.23	0.15
1.88	0.21	0.79	0.34	0.41	0.02
p-value	0.001	0.001	0.067	<0.001	0.018

The shaded lines show data that was excluded from the statistical analysis and data analysis.

The fraction values in Table 6 can also be graphed to identify trends associated with dose. Several different carboys of bulk solution were used and each bulk solution

had a slightly different starting concentration, and a somewhat different chemical make-up. Therefore, the curves have been grouped according to the bulk solution treated for that experiment, and are represented in the figure legend by the corresponding date of treatment and a different symbol. No error bars are included on these plots as each individual point is displayed. Linear regressions were done on each data set to demonstrate general trends.

Figures 10-14 show the general trends of each fraction after oxidation with ozone. As oxidizing dose increases, hydrophobicity decreases and the hydrophilic fraction increases (p -value = 0.001) (Figures 10 and 11). The aromatic fraction decreases (Figure 12) as dose increases (p -value < 0.001). BDOC trends (Figure 13) indicate that as dose increases, biodegradability increases. However, since the p -value for this plot is 0.067, additional BDOC tests should be done to strengthen these findings. And finally, as ozone dose increases (Figure 14), TOC concentration decreases (p -value = 0.013).

Figure 10. Ozonated Hydrophobic Fractions Grouped by Bulk Solution

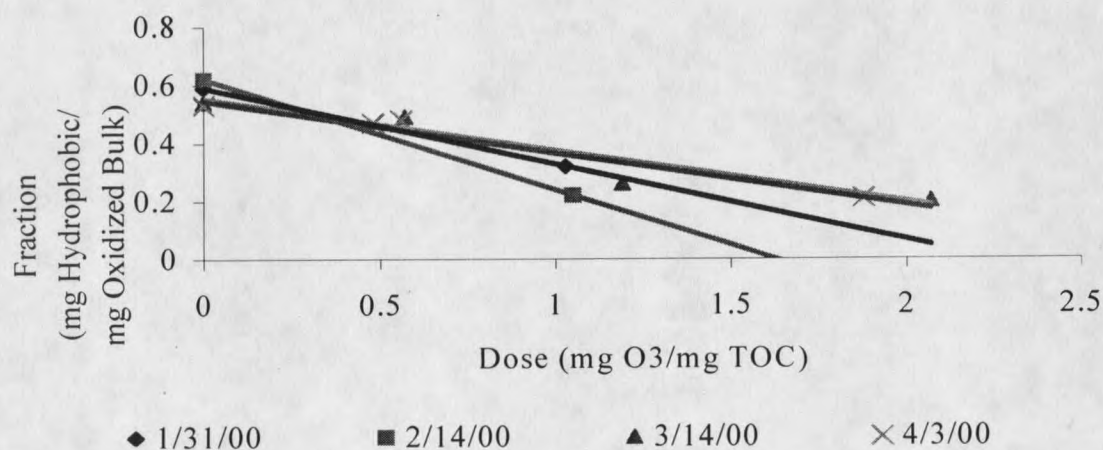


Figure 11. Ozonated Hydrophilic Fraction Grouped by Bulk Solution

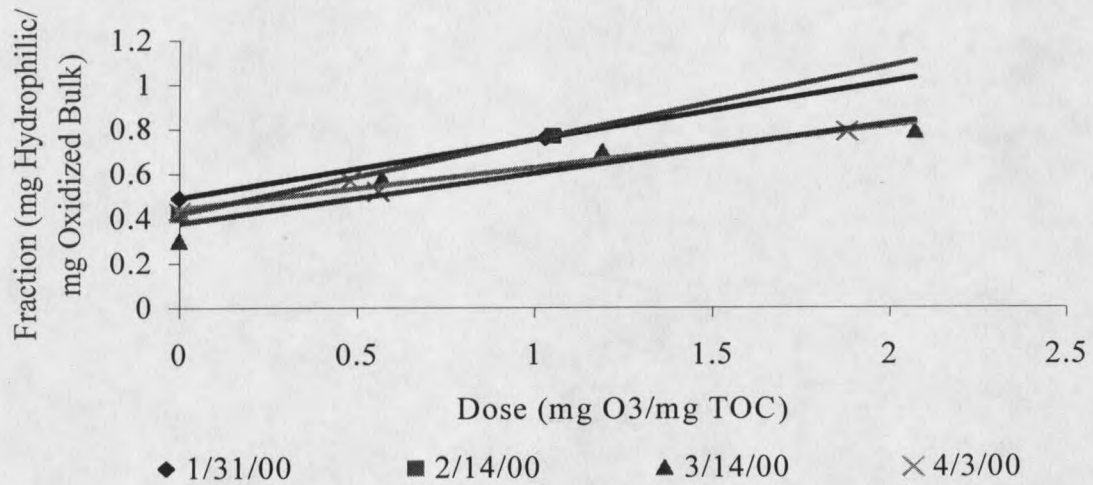


Figure 12. Ozonated Aromatic Fractions Grouped by Bulk Solution

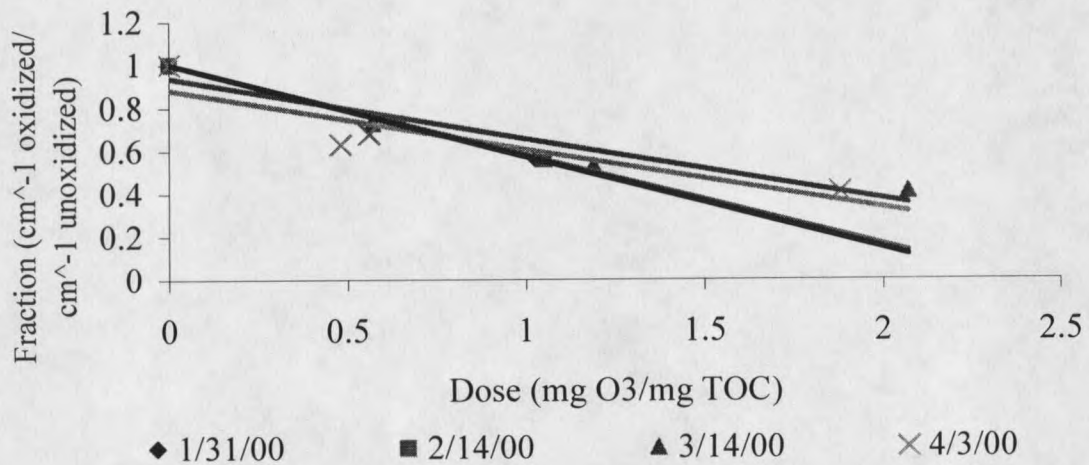


Figure 13. Ozonated BDOC Fraction Grouped by Bulk Solution

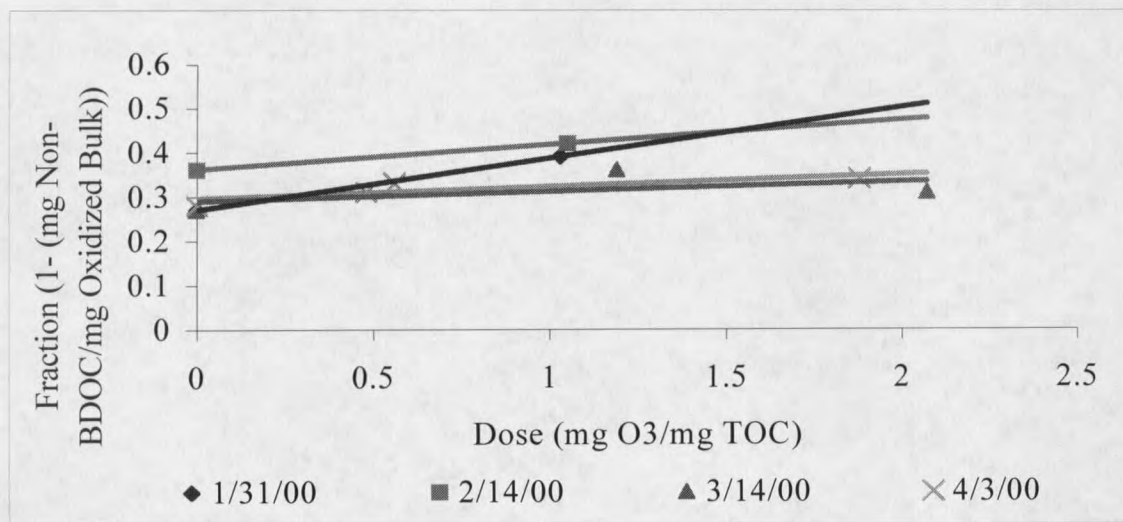
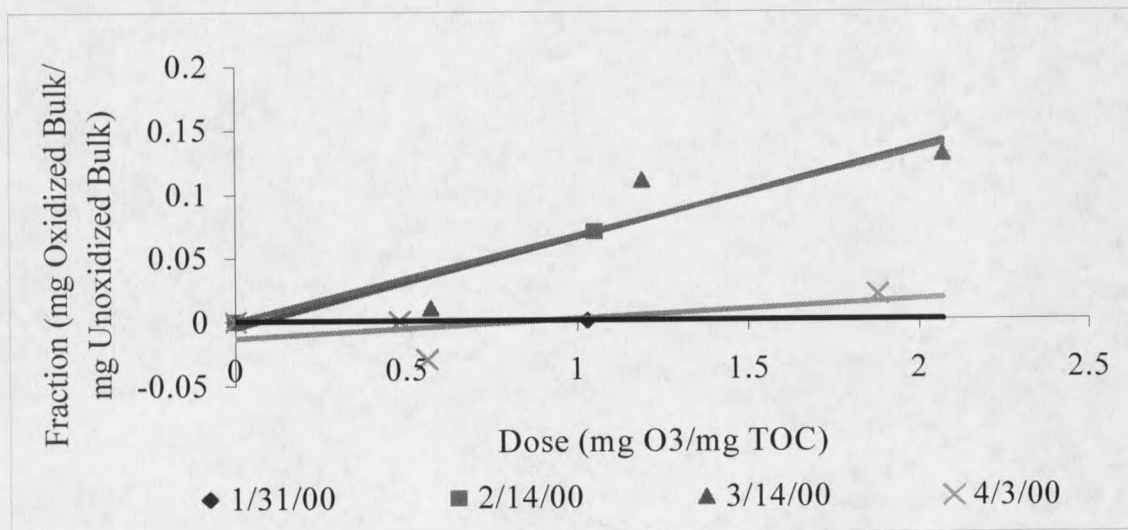


Figure 14. Overall TOC Reduction Caused by Ozonation



The final fractionation method used in this project involves HPSEC, or MW fractionation. Figures 13-15 show chromatograms for each dose applied, compared to the bulk solution. Each line represents a separate experiment. Table 7 gives the number averaged and weight averaged MWs for each dose.

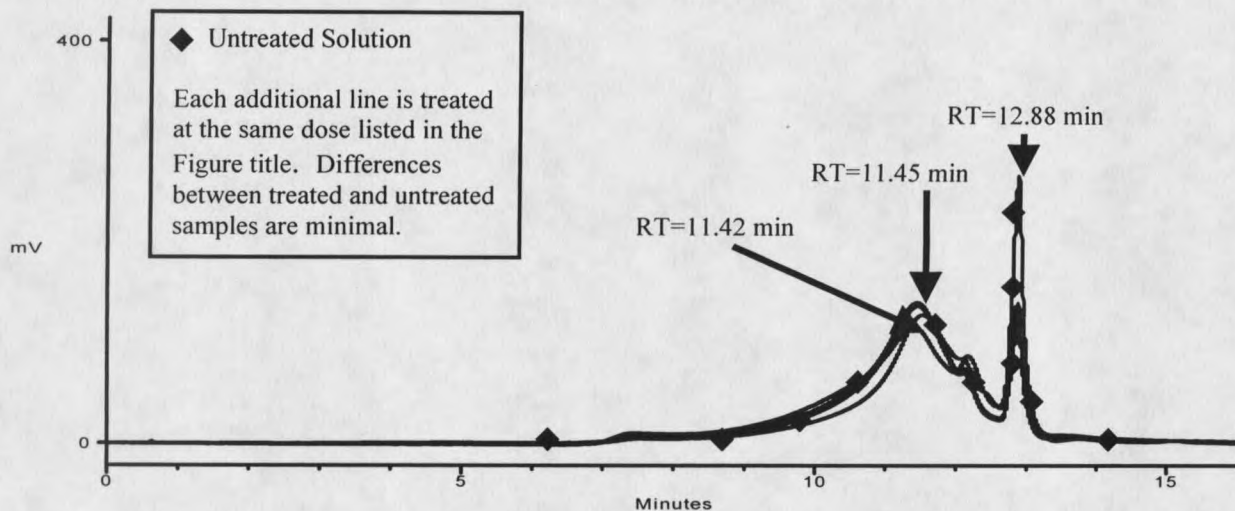
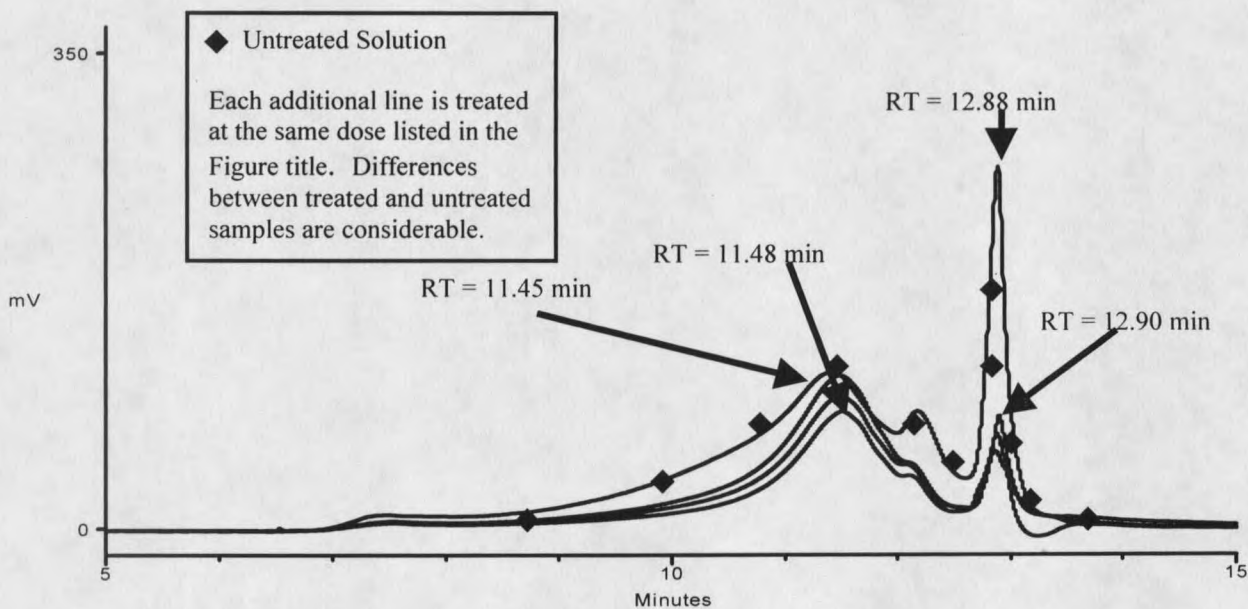
Figure 15. HPSEC Chromatogram for Ozone Dose of 0.5 mg O₃/mg TOC.Figure 16. HPSEC Chromatogram for Ozone Dose of 1.0 mg O₃/mg TOC.

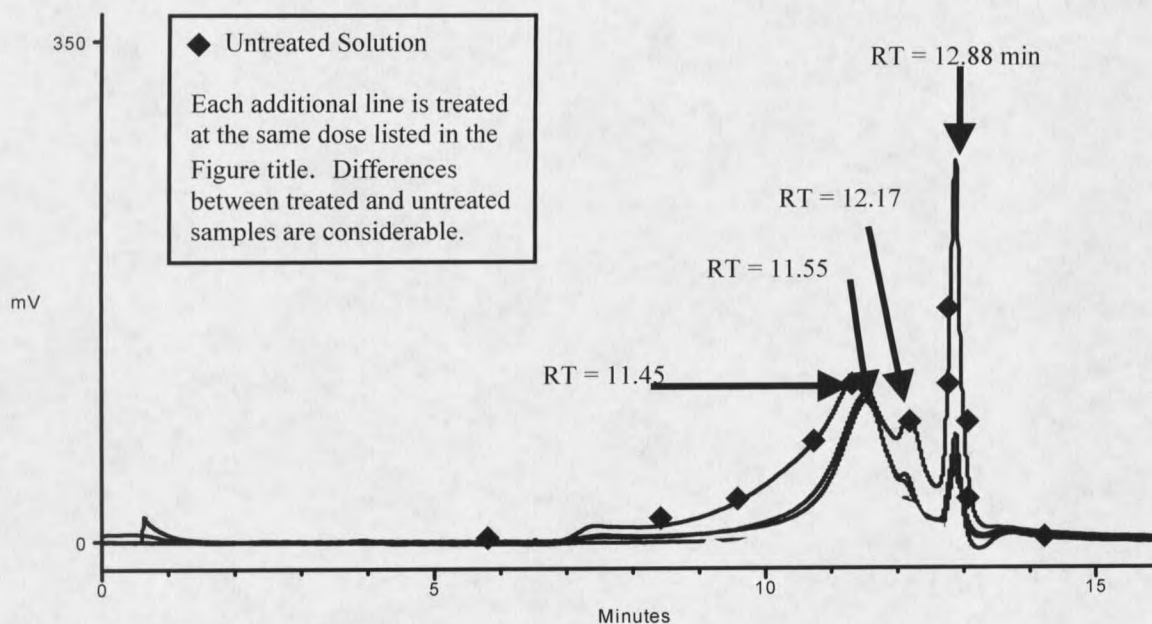
Figure 17. HPSEC Chromatogram for Ozone Dose of 2.0 mg O₃/mg TOC.

Table 7. Number and Weight Averaged MWs for Ozone Experiments.

Dose (mg O ₃ /mg TOC)	Number Averaged MWs	Weight Averaged MWs
0	7192	23934
0.57	8496	21252
0.56	9558	22751
0.48	9536	23648
1.03	9850	22419
1.05	5076	21995
1.19	15747	24532
2.07	14744	22399
1.88	8889	20952

The equations used to calculate the MWs in Table 7 are shown in Chapter 2 under the section HPSEC, and the tables and calculations used to generate the values are located in Appendix B. The variables required for the calculations are M_i and h_i . The standard curve in Figure 9B was used to generate the MWs based on elution time (M_i), and h_i , which is the height of the chromatogram at each elution time, was taken from the chromatograms at various time intervals.

It is evident from the chromatograms that oxidation with ozone decreases the aromatic fraction of the sample (shown by the difference between the untreated chromatogram, and each of the treated chromatograms), therefore lowering the height of the detected peaks in each chromatogram. This can be interpreted to mean that smaller MW fractions are being formed as oxidation with ozone occurs. Additionally, the movement of peaks from a shorter retention time to a longer retention time, which is evident in some of these chromatograms, may also reinforce these findings.

It is much more difficult to understand how the number averaged and weight averaged MWs relate to changes in MWs after oxidation, especially with only one detection method available. For samples where decreases in aromatic character are considerable, such as the 1.0 and 2.0 mg O₃/mg TOC doses, these calculations can be quite misleading because they cannot take into account the entire mass of molecules being measured since some of the mass becomes undetectable using UV-254. Therefore, there are too many inferences that must be made to use them to confirm or reject any trends that exist.

Based on the above results, an ozone dose of 1 mg O₃/mg TOC was chosen as the most effective ozone dose for use in conjunction with a biofilter for the purposes of removing NOM. At this dose, 53% of the original hydrophobic fraction was removed (from 0.57 to 0.27), 45% of the original aromatic fraction was removed (from 1.00 to 0.55), and the molecular weight chromatogram shows a slight shift in peaks, as well as a significant decrease in chromatogram height. These trends relate to an increase in biodegradability of approximately 11% over the untreated BDOC fraction (from 0.28 to 0.39). These results are much higher than those found for the 0.5 mg O₃/mg TOC

treatment. Oxidation at this dose produced a 16% decrease in the hydrophobic fraction (from 0.57 to 0.48), a 32% decrease in aromatic character, and little or no shift in MW, as well as minimal decrease in chromatogram height. This corresponded to an increase in the untreated biodegradable fraction of only 4% (from 0.28 to 0.32). Conversely, at the 2.0 mg O₃/mg TOC treatments, although the hydrophobic fraction was reduced by 65% (from 0.57 to 0.21), the aromatic fraction was decreased by 59% (from 1.0 to 0.41), and the chromatogram favors the formation of smaller MW molecules, biodegradability only increased by 4.5% over the untreated BDOC fraction (from 0.28 to 0.325). An example of the calculations used to derive each of the above numbers is shown in Appendix B.

Therefore, 1.0 mg O₃/mg TOC was chosen as the most effective application dose since it was considerably more effective than the 0.5 mg O₃/mg TOC treatment, and appeared to perform equally as well, or even better than the 2.0 mg O₃/mg TOC treatment.

Chlorination

Chlorine dosing concentrations were selected as 0.5 and 4.0 mg/L Cl₂ and applied using methods described in Chapter 2 under Chlorine Oxidation Techniques. Figure 18 shows four different chlorine demand curves for concentrations ranging from 0.0 to 1.0 mg/L Cl₂. These curves were used to find a concentration that corresponded to no residual after 15 minutes. Subsequent tests confirmed that 0.35-0.5 mg/L Cl₂ was the target dose, depending on the exact concentration of the humic solution being tested. Therefore, 0.5 mg/L Cl₂ was chosen as the minimum dose used in this project. Figure 19 shows the chlorine demand curve for a dose of 4.0 mg/L only. A control was done on an

ultrapure water sample to ensure that all chlorine disappearance was due only to reaction with the humic material.

Figure 18. Chlorine Demand Curves for 3.0 – 4.0 ppm Humic Solution at Various Chlorine Doses.

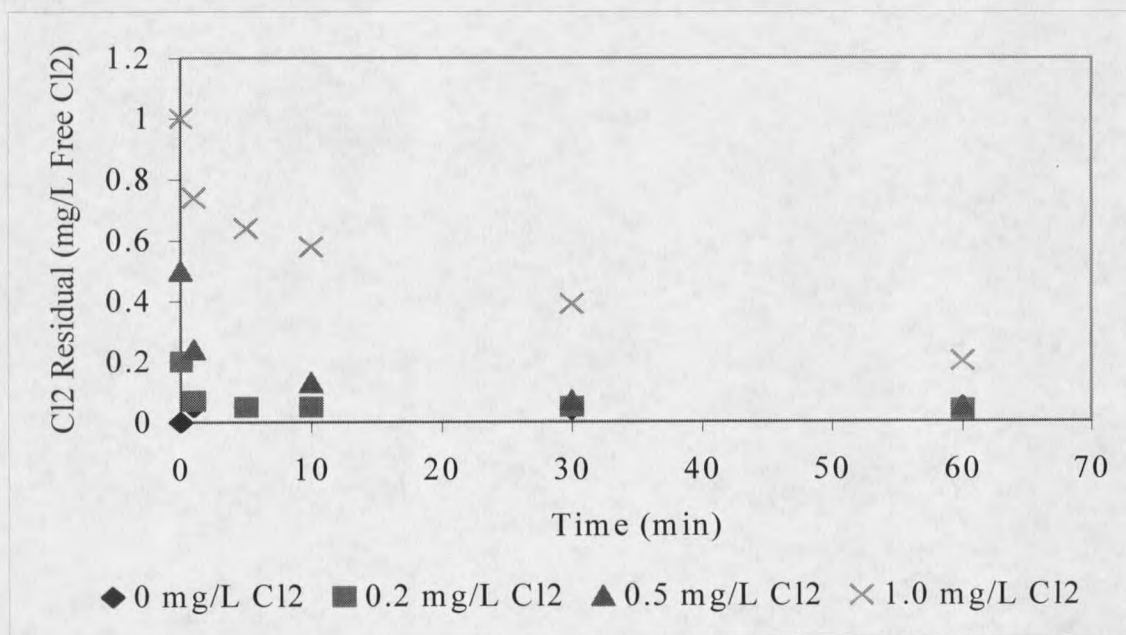
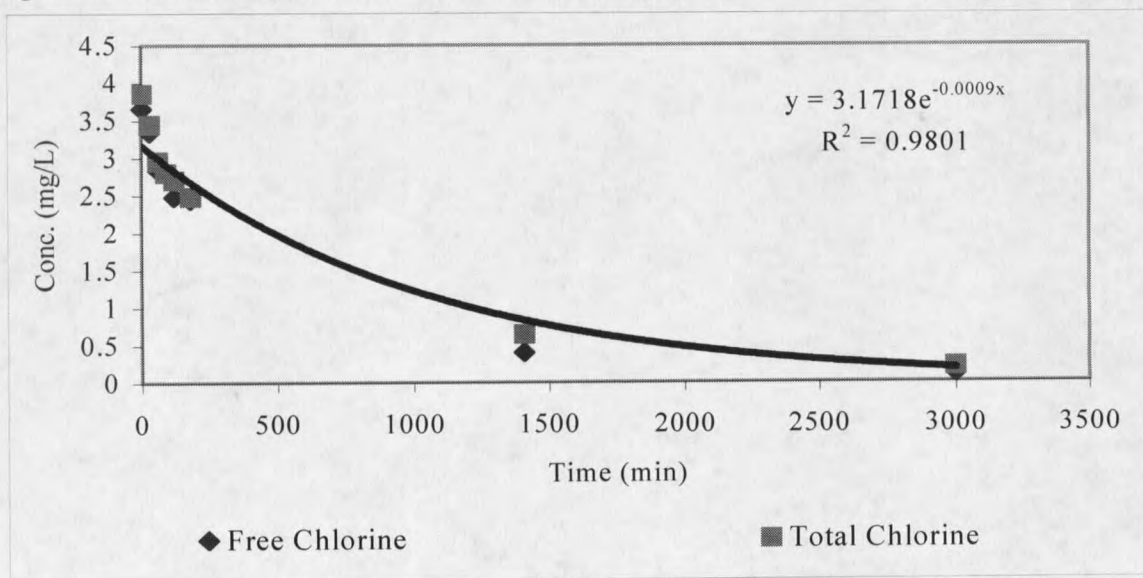


Figure 19. Chlorine Demand Curve at 4.0 mg/L Cl₂



Fractionation Results

Table 8 shows the calculated fractions corresponding to each dose based on hydrophilic/hydrophobic behavior, biodegradability, and aromaticity. The calculations were done identically to those for ozone. The p-value for each fraction is listed at the bottom of each row. Bulk concentration tables are available in Appendix B.

The values in Table 8 can be graphed to identify trends associated with dose for each of the above fractions. For hydrophobic/hydrophilic fractionation (Figures 20-21), the trend indicates that as dose increases, hydrophobicity decreases and hydrophilicity increases. It is interesting to note that even though two trend lines show a negative slope on the hydrophilic plot, the increase is still statistically significant (p-value = 0.005). These two lines, however, are represented by points collected from 0.5 mg/L Cl₂ tests.

Table 8. Measured Fractions After Chlorine Oxidation Based on Dose

Dose (mg/L Cl ₂)	Hydrophobic Fraction	Hydrophilic Fraction	BDOC Fraction	Aromatic Fraction	Fraction Removed Via Oxidation
0	0.59	0.49	0.27	1.00	0.00
0	0.54	0.44	0.34	1.00	0.00
0	0.62	0.42	0.36	1.00	0.00
0	0.53	0.47	0.28	1.00	0.00
0	0.54	0.30	0.28	1.00	0.00
0.5	0.55	0.46	0.24	0.99	0.01
0.5	0.56	0.40	0.29	1.03	0.02
0.5	0.49	0.40	0.30	0.95	0.02
4	0.42	0.58	0.42	0.74	0.04
4	0.30	0.62	0.39	0.73	0.06
4	0.40	0.61	0.27	0.74	0.07
p-value	0.007	0.005	0.399	<0.001	0.001

This is significant to note because it is possible that the effects of chlorine at 0.5 mg/L Cl₂ are not great enough to differentiate between the nontreated sample and the treated

sample. Therefore, an apparent increase (as seen in Figure 21), may only represent differences caused by instrument error or differences in collection processes, which is considered to be 10%. The 10% is based on variations seen within the hydrophobic/hydrophilic fractionation method, as well as variations seen from sample to sample on the TOC instrument.

The aromatic trend (Figure 22) indicates that as dose increases, the aromatic fraction decreases ($p\text{-value} < 0.001$). BDOC trends (Figure 23) appear similar to the hydrophilic trends because all 0.5 mg/L Cl_2 points appear to be negative in slope, even though tests that were extended out to 4 mg/L Cl_2 appear to show an increase in biodegradability with an increase in dose. This inconsistency is confirmed by a $p\text{-value}$ for this plot of 0.339. And finally, trends associated with overall TOC reduction (Figure 24) due to oxidation with chlorine show that as dose increases, overall TOC reduction increases. This is confirmed statistically, as well ($p\text{-value} = 0.001$).

The format for each graph is identical to those displayed for ozone. Therefore, the data has been grouped based on each bulk solution, and corresponding date. Linear regressions have been done through each experiment, to show general trends associated with an increase in dose. Additionally, since all points have been included on each plot, there are no error bars associated with any points displayed.

Figure 20. Chlorinated Hydrophobic Fractions Grouped by Bulk Solution.

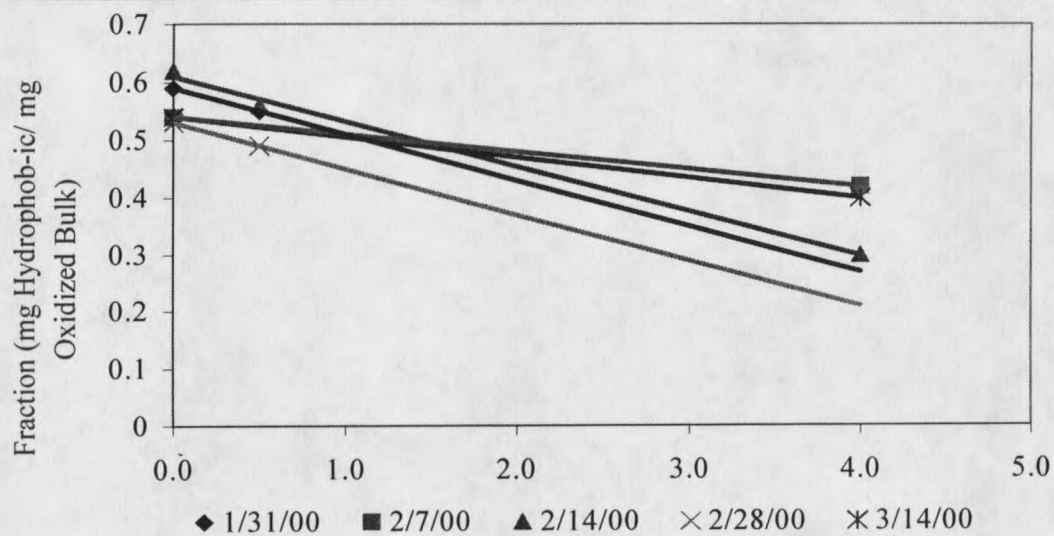


Figure 21. Chlorinated Hydrophilic Fraction Grouped by Bulk Solution

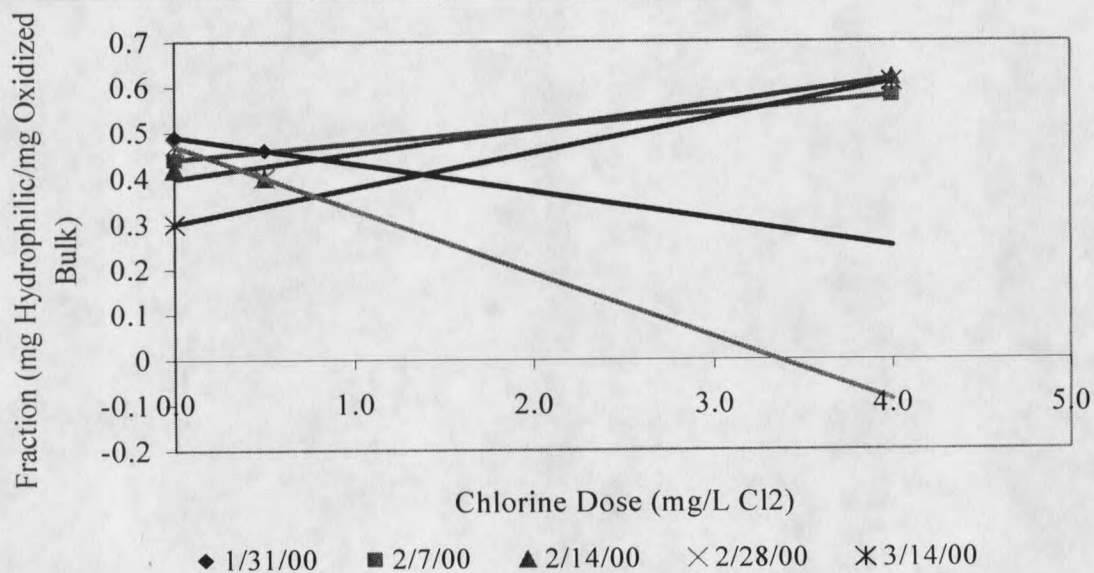


Figure 22. Chlorinated Aromatic Fractions Sorted by Bulk Solution.

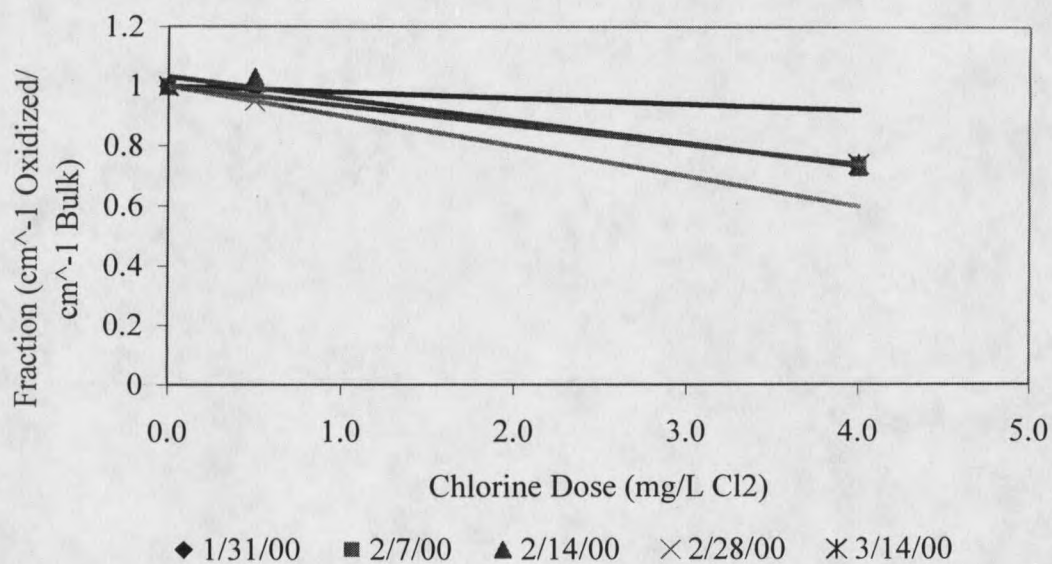


Figure 23. Chlorinated BDOC Fraction Grouped by Bulk Solution.

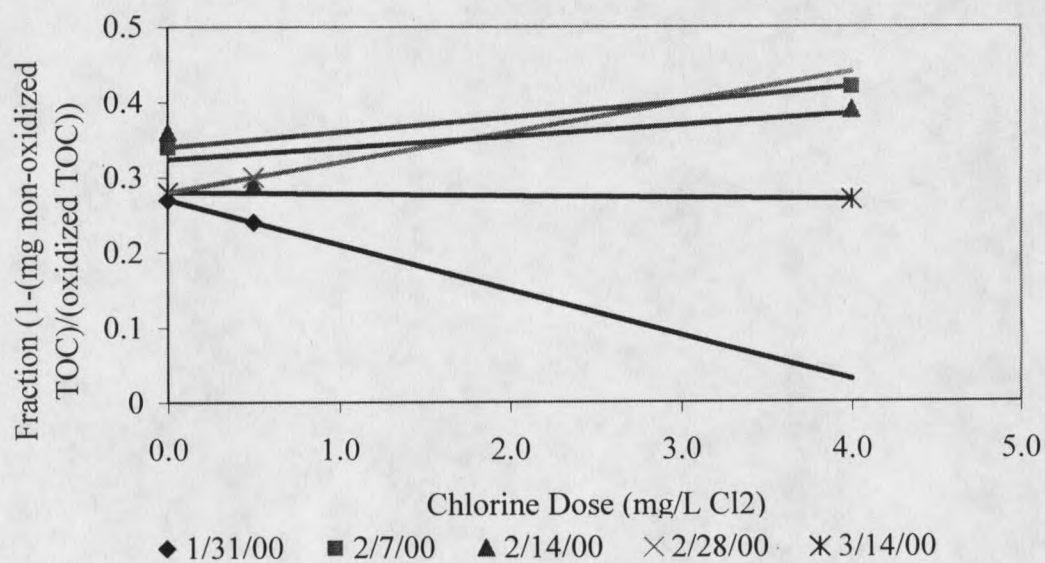
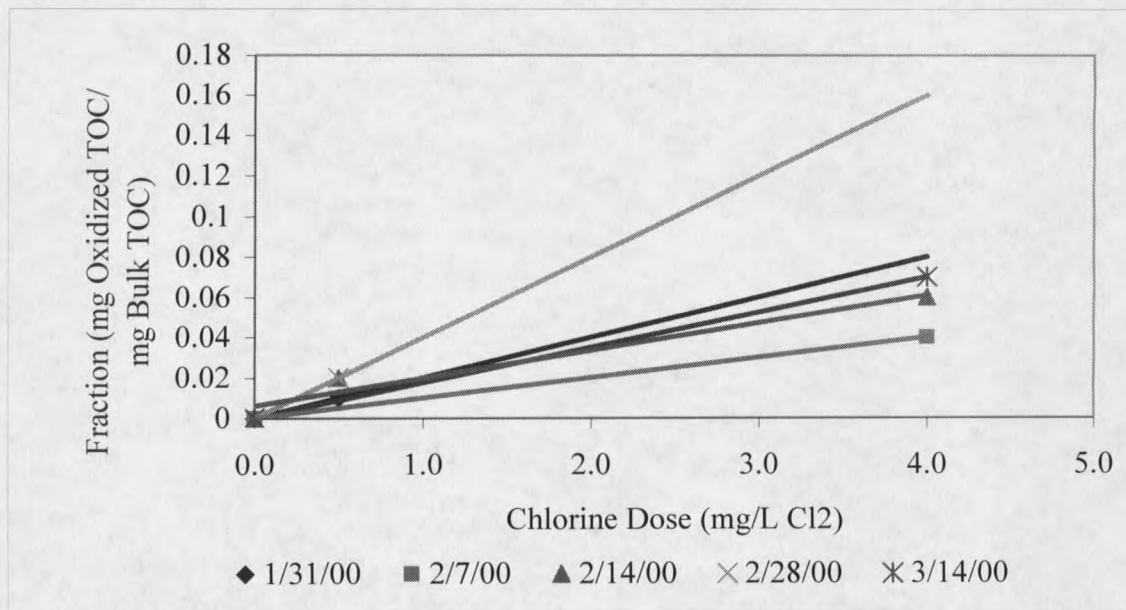


Figure 24. Overall TOC Reduction Caused by Chlorination.



Figures 25 and 26 show MW fractionation chromatograms for each dose, compared to the bulk solution. The bulk solution chromatogram is displayed in each of the plots for comparison purposes, and is noted by the diamond symbols.

The chromatograms indicate that no real MW changes are evident for treatment with 0.5 mg/L Cl₂, and that a small change in MWs may have occurred during treatment with 4.0 mg/L Cl₂. This is also reinforced by UV-254 analysis, which shows that at the maximum dose, there was more of an effect on aromatic character than at the lower dose. It is also evident that shifting of the 11.42 minute peak occurred in the 4 ppm chlorine treatment, indicating that smaller MW aromatic fractions were possibly formed. This may indicate that chlorine can break down less complex bonds within a humic molecule, but has difficulty cleaving the aromatic bonds present.

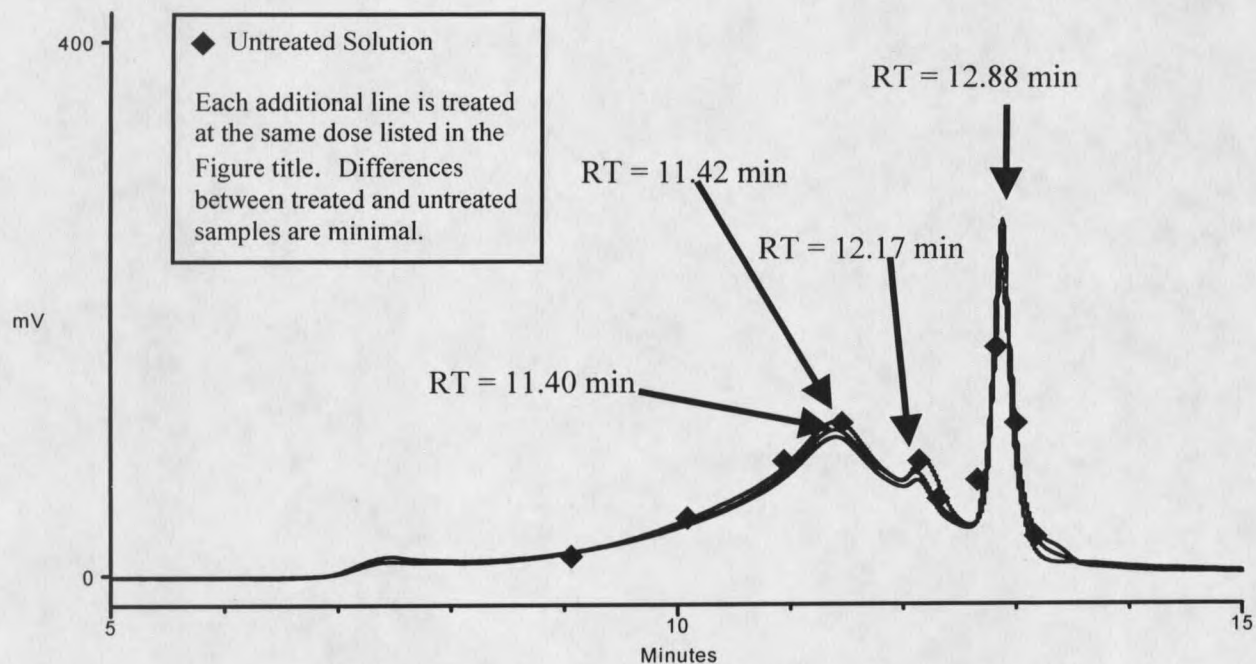
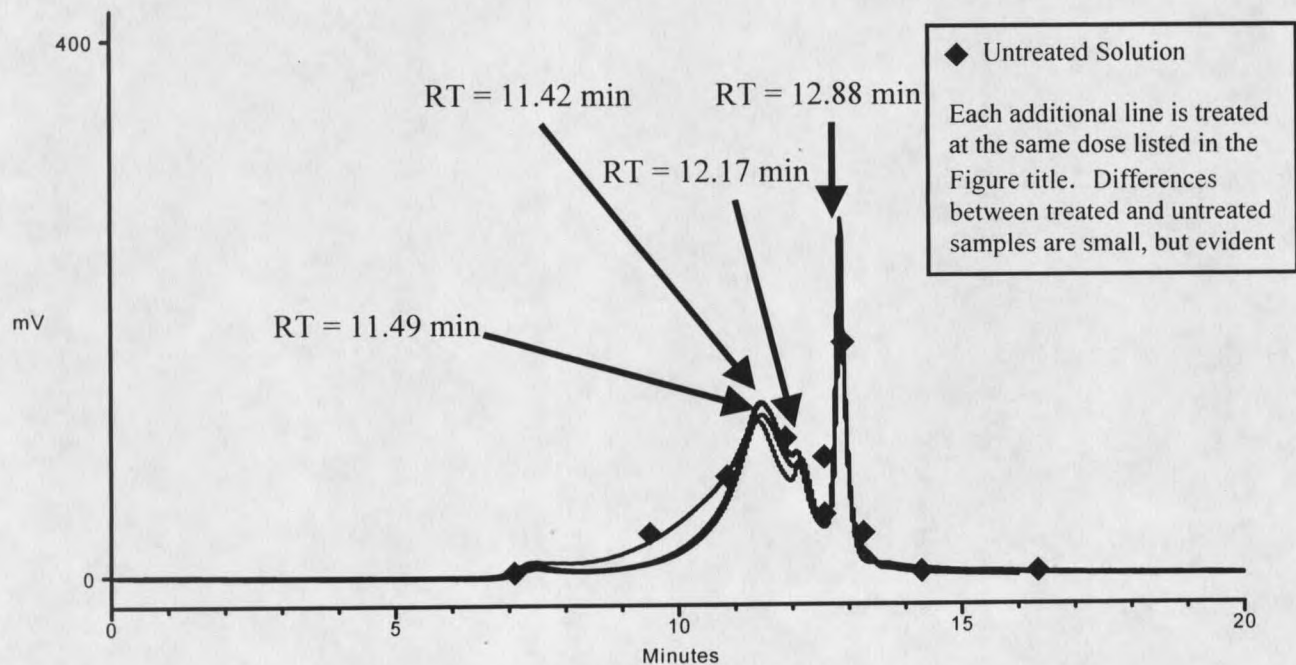
Figure 25. HPSEC Chromatogram for Chlorine Dose of 0.5 mg/L Cl_2 .Figure 26. HPSEC Chromatogram for Chlorine Dose of 4.0 mg/L Cl_2 .

Table 9 gives the number averaged and weight averaged MWs for each of the above chromatograms. The table was generated identically to Table 7, which displays the number and weight averaged MWs for the ozone experiments. Calculation tables for the following values are available in Appendix B.

Table 9. Number and Weight Averaged MWs for Chlorine Experiments.

Dose (mg/L Cl ₂)	Number Averaged MWs	Weight Averaged MWs
0	7192	23934
0.5	4349	22973
0.5	5828	25147
0.5	5828	25156
4.0	5552	17868
4.0	4974	17691
4.0	4702	18347

The number averaged and weight averaged MWs may be more applicable for chlorine analysis since minimal aromatic character was lost due to oxidation. Based on these numbers, at the 4 ppm treatment, both the number averaged and weight averaged MWs decreased, which likely correlates to the dip in the 4 ppm chromatogram seen between 7 and 10 minutes, as well as the peak shift from 11.42 to 11.49 minutes.

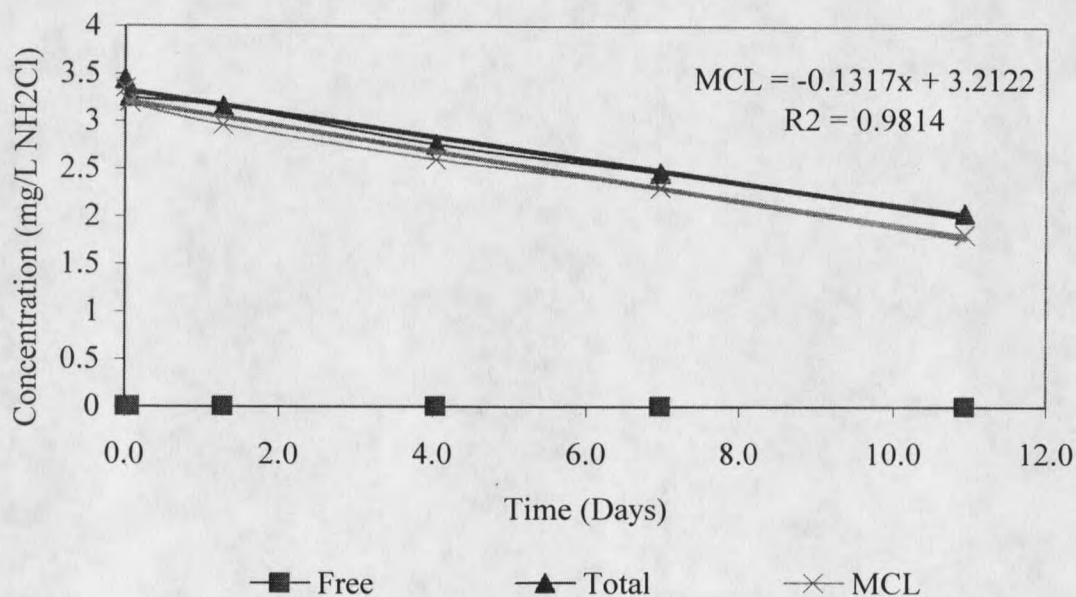
Based on the above results, it appears that chlorine can have a significant effect on humic substances and biodegradation properties at high enough concentrations. This is apparent because the initial hydrophobic character was decreased by 33% (from 0.56 to 0.37), the initial aromatic character was decreased by 26% (from 1.0 to 0.74), and a change in the treated chromatogram indicates a shift to lower MW molecules. These trends correlate to a 5% increase over the untreated biodegradable fraction (from 0.31 to 0.36). What is more important, however, is that this effect is probably still happening at

the lower concentration, however, laboratory analysis used in this project is not sensitive enough to differentiate between an actual effect and laboratory error. For example, at 0.5 mg/L Cl_2 , the initial hydrophobic character was only decreased by 5.5% (from 0.56 to 0.53), aromatic character was only reduced by 1% (from 1.0 to 0.99), and the biodegradable fraction was increased over the untreated BDOC concentration by only 3% (from 0.31 to 0.28). Additionally, no difference was evident between the nontreated chromatogram and the treated chromatograms indicating no change in MW.

Monochloramine Oxidation

The maximum monochloramine dose selected was 4.0 mg/L NH_2Cl because affects of chlorine oxidation were difficult to see at doses less than 4.0 mg/L Cl_2 and monochloramine was not expected to react as readily as chlorine. The initial dose of NH_2Cl was 3.44 mg/L, and after ten days, the monochloramine residual (1.80 mg/L NH_2Cl) was removed using 0.9 mL sodium thiosulfate. The resulting final oxidant demand was 1.64 mg/L NH_2Cl . The monochloramine demand curve is shown in Figure 27. Additionally, a control was run for three days, which involved oxidizing ultrapure water with NH_2Cl . This control indicated that 0.05 mg/L NH_2Cl disappeared in three days, which correlates to a 0.18 mg/L NH_2Cl disappearance over the 10 day treatment period making the theoretical minimum reacted concentration equal to 1.46 mg/L NH_2Cl .

Figure 27. Monochloramine Demand Curve at 1.64 mg/L NH_2Cl .



Fractionation Results

Table 10 shows the calculated fractions corresponding to no treatment and treatment with 1.64 mg/L NH_2Cl based on hydrophilic/hydrophobic behavior, biodegradability, and aromaticity. The calculations were done identically to those calculations done for ozone. Since only one dose was studied on three different 2 L volumes, and each oxidation was done on the same day using the same bulk solution, statistical analysis was simplified since it was not necessary to correct each value for the bulk solution. Only a linear regression was necessary between zero and the three points that were collected at the specified dose. Therefore, both a p-value and R^2 values for each test are listed at the bottom of each row. Bulk concentration tables are available in Appendix B.

Table 10. Measured Fractions after Monochloramine Oxidation Based on Dose.

Dose (mg/L NH_2Cl)	Hydrophobic Fraction	Hydrophilic Fraction	BDOC Fraction	Aromatic Fraction	Fraction Removed Via Oxidation
0	0.54	0.46	0.28	1.00	0.00
4	0.39	0.54	0.25	1.16	-0.06
4	0.42	0.54	0.31	1.03	-0.04
4	0.41	0.63	0.31	1.07	-0.06
p-value	0.017	0.017	0.826	0.337	0.057
R^2 value	0.966	0.966	0.03	0.389	0.889

Figure 28 shows the general trends of each fraction calculated in Table 10 after monochloramine oxidation. For hydrophobic/hydrophilic fractionation, the trend indicates that with oxidation, hydrophobicity decreases and the hydrophilic fraction increases (p-value=0.017). The aromatic trend indicates that there might actually be an increase in the aromatic fraction with oxidation. However, this trend is not confirmed

statistically (p -value = 0.337). Additionally, there was an *increase* in overall TOC concentration after oxidation (p -value = 0.057), which indicates that some kind of organic carbon contamination may have influenced the data slightly. This is most likely due to the fact that the aromaticity and TOC concentration did not change significantly enough to be distinguishable from the nontreated sample, which is reinforced by the p -values (p -values >0.05). Additionally, each of these samples are less than 10% different from the bulk solution, which was explained previously to be the operator/instrument error for this project. BDOC trends indicate no relation after oxidation with monochloramine (p -value = 0.826).

Figure 28. Monochloramine Oxidized Fractions.

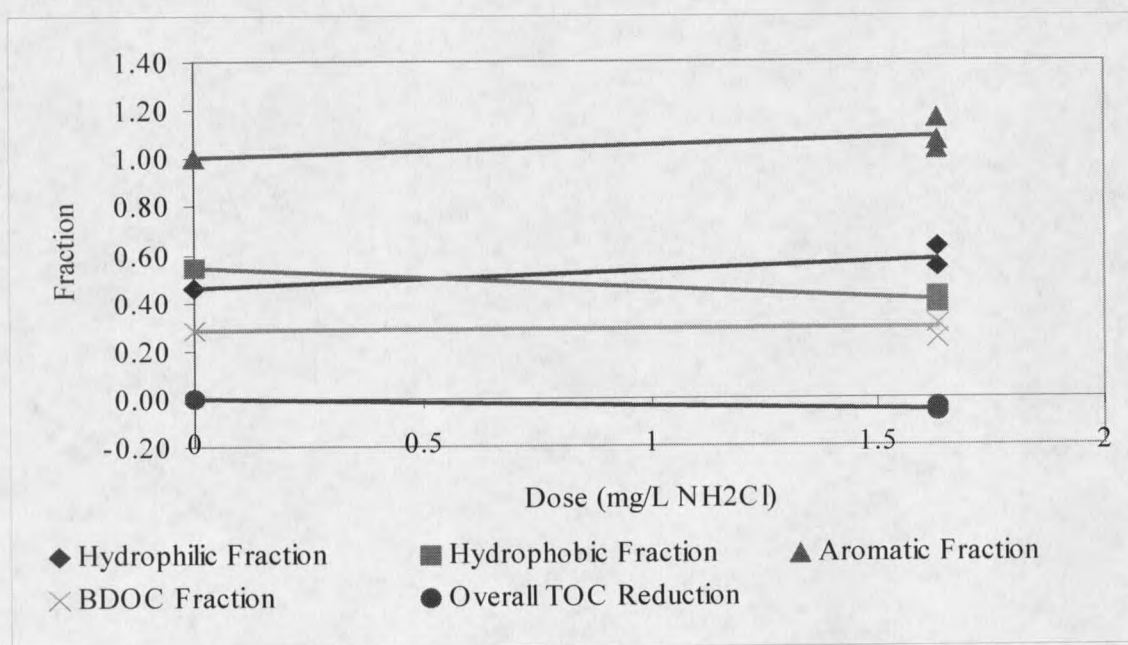


Figure 29 shows MW fractionation chromatograms for the oxidation experiment with monochloramine, compared to the bulk solution. Table 11 gives the number averaged and weight averaged MWs for each dose. Only two curves showing oxidized

samples are shown in Figure 29 for the monochloramine experiments. The third curve had an extra peak in it, which is attributed to a contamination event. Also, it is interesting to note that at 12.88 min, the two monochloraminated curves are higher than the bulk solution curve. This is thought to be caused by the addition of sodium thiosulfate, since a chromatogram of the oxidized bulk (shown in Appendix B) shows similar peaks to the bulk solution at 12.88 mins, and the only difference between the two samples was the addition of sodium thiosulfate.

Figure 29. HPSEC Chromatogram for Monochloramine Oxidation.

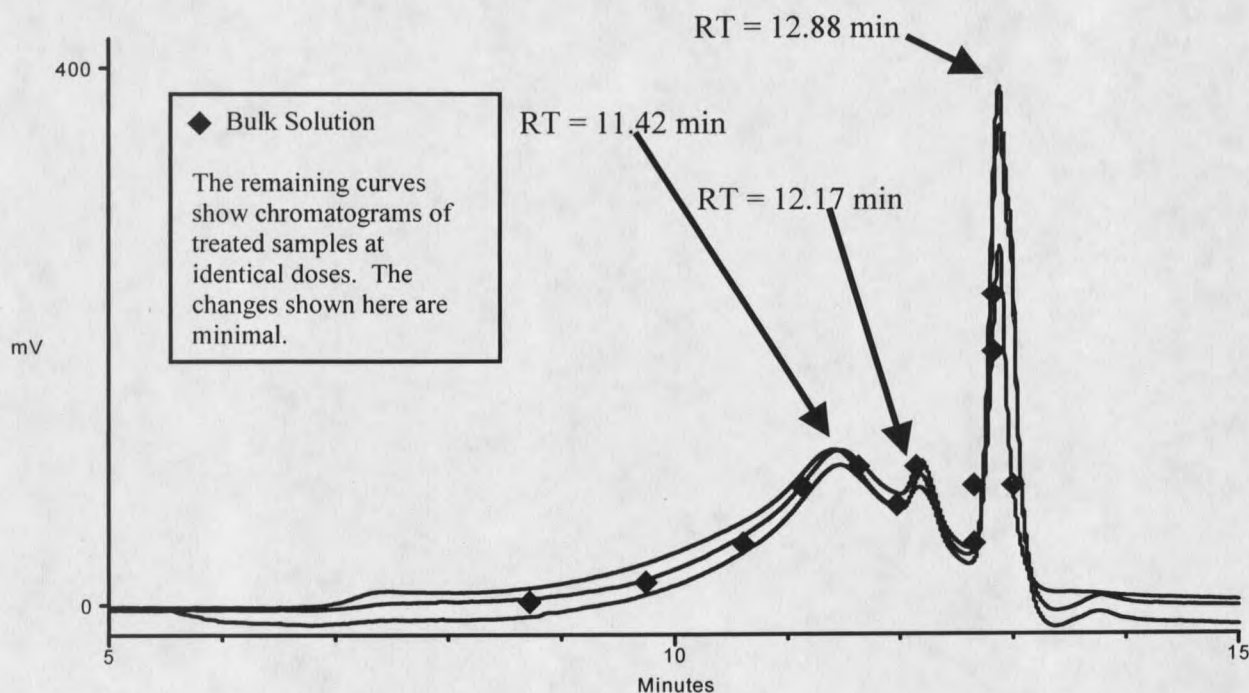


Table 11. Number and Weight Averaged MWs for Monochloramine Experiments.

Dose (mg/L NH_2Cl)	Number Averaged MWs	Weight Averaged MWs
0	7192	23934
1.64	8738	16853
1.64	8809	15096

The values in Table 11 were calculated identically to those in Table 7 for ozone. The calculation tables used to generate Table 10 are located in Appendix B. The results based on these chromatograms indicate that very little changes occurred in MW fractions after oxidation. Although weight averaged MWs seem to indicate a decrease based on treatment with monochloramine, this is misleading. The decrease is caused by the larger peaks at 12.88 minutes for the samples, as opposed to the bulk solution. The larger peaks influence the calculation significantly by placing more weight on the lower MW fractions as opposed to the higher MW fractions, lowering the weight averaged MWs. Additionally, this decrease is not consistent with the increase seen for the number averaged MWs, and therefore the disagreement between the two calculations discounts the predictions of trends that may be present.

Overall, monochloramine oxidation was not an effective method for increasing the biodegradability of humic substances. Even though there was a 24% decrease in the initial fraction of hydrophobicity (from 0.54 to 0.41), there was an increase in aromaticity (from 1 to 1.09), no apparent changes in MW fractions, and only a 1% increase over the untreated biodegradable TOC concentration (from 0.28 to 0.29). This indicates that monochloramine is not an effective oxidant for humic substances.

UV-light Oxidation

UV-light doses were selected by developing a curve based on aromatic character destruction using the methods described in Chapter 2 under UV-light Oxidation Techniques. Figures 30 and 31 show the results of this test. The difference between the two curves is that Figure 30 appears to become asymptotic in nature towards the end of

the experiment, however, since tests were performed at doses less than 53X, the linear relationship seen in the lower doses was much more accurate at estimating outcomes. Based on this table, target rates were selected of 1X (480 mW*sec/cm²), 10X (4800 mW*sec/cm²), and 53X (25,600 mW*sec/cm²).

Figure 30. UV-light Curve Based on Aromatic Destruction at 254 nm.

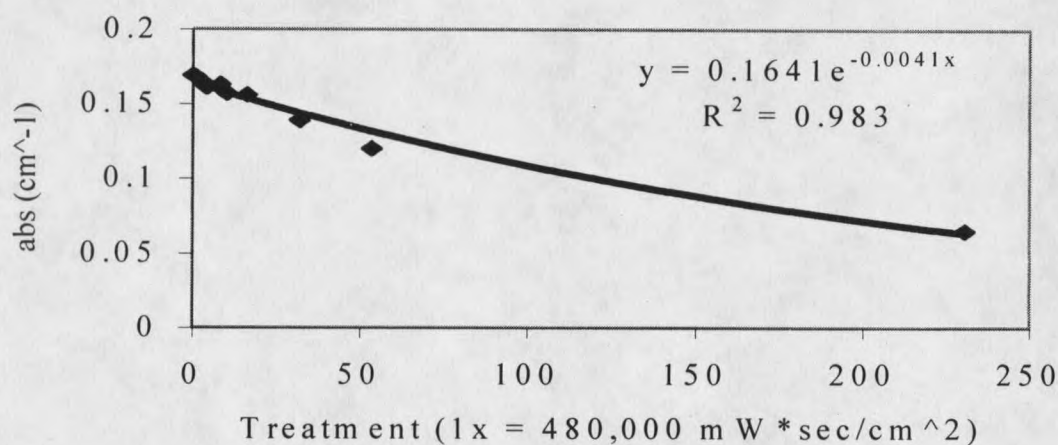
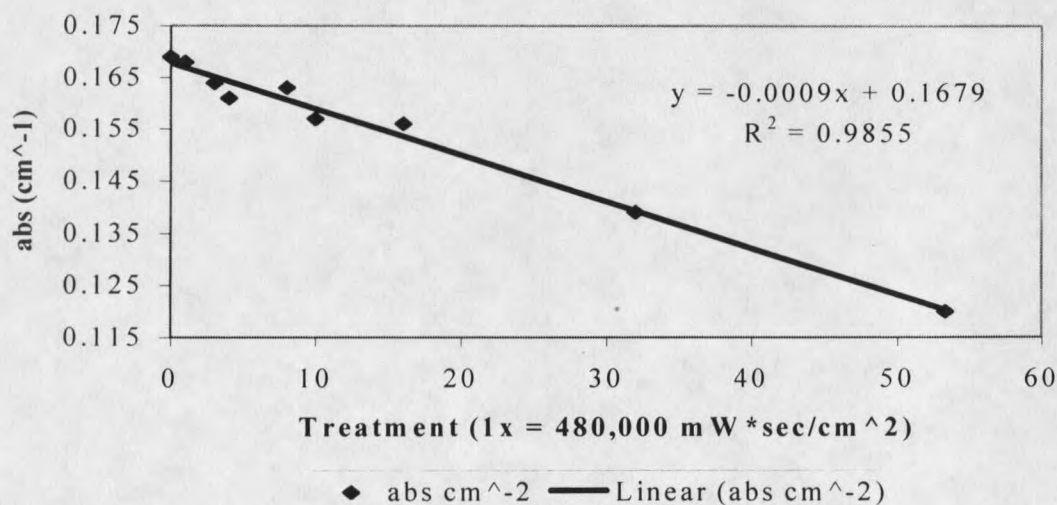


Figure 31. UV-light Curve Based on Aromatic Destruction at 254 nm for Doses less than 64X.



Fractionation Results

Table 12 shows the calculated fractions corresponding to each dose based on hydrophilic/hydrophobic behavior, biodegradability, and aromaticity. The calculations of each fraction were done identically to the calculations done in the ozone experiments. The p-value for each fraction is listed at the bottom of each row. Bulk concentration tables are available in Appendix B.

Table 12. Measured Fractions After UV-light Oxidation Based on Dose.

Dose (mW*sec/ cm ²)	Hydrophobic Fraction	Hydrophilic Fraction	BDOC Fraction	Aromatic Fraction	Fraction Removed Via Oxidation
0.0	0.53	0.47	0.28	1.00	0.00
0.0	0.53	0.43	0.28	1.00	0.00
0.0	0.51	0.56	0.29	1.00	0.00
480	0.47	0.46	0.31	1.01	0.03
480	0.38	0.39	0.34	1.05	0.01
480	0.49	0.45	0.24	0.85	-0.10
4800	0.50	0.54	0.33	0.93	-0.05
4800	0.49	0.57	0.28	0.93	-0.05
4800	0.48	0.57	0.28	0.90	-0.04
25,600	0.32	0.72	0.45	0.71	0.01
25,600	0.36	0.74	0.54	0.71	0.06
25,600	0.27	0.71	0.52	0.72	0.03
p-value	<0.001	<0.001	<0.001	<0.001	0.046

UV-light oxidation did not change the bulk solution significantly until the final dose of 25,600 mW*sec/cm² (53X). The hydrophobic fraction was decreased by only 7% for 480 mW*sec/cm² (1X) (from 0.51 to 0.47) and 6% for 4800 mW*sec/cm² (10X) (from 0.51 to 0.49). The aromatic fraction actually increased at the 1X treatment, and decreased by 7% for the 10X treatment (from 1.0 to 0.93). Further, the biodegradable fraction of the overall TOC content changed by less than 2% during the 1X

and 10X treatments. Conversely, after treatment at 53X, the hydrophobic fraction decreased by 62% (from 0.51 to 0.32), the aromatic character decreased by 29%, and the biodegradable fraction increased by 24% over the BDOC of the untreated sample (from 28% to 54%).

The values in Table 12 can also be graphed to identify trends associated with dose for each of the above fractions. The format for the following graphs is identical to the format used for ozone, so the data has been grouped by bulk solution, and the corresponding oxidation date. However, only one trend line has been added to each of these plots since the effects of UV-light on the humic sample were so minimal at 1X and 10X that results were within 15% of the bulk sample. It was not until the dose of 53X was applied that collected data produced significant enough changes to the solution to see true trends.

For hydrophobic/hydrophilic fractionation (Figures 32 and 33), the trend indicates that as oxidizing dose increases, hydrophobicity decreases and the hydrophilic fraction increases (p-value < 0.001). The aromatic trend (Figure 34) indicates that as dose increases, the aromatic fraction decreases (p-value < 0.001). BDOC trends (Figure 35) indicate that as dose increases, biodegradability increases (p-value < 0.001). And finally, trends associated with overall TOC reduction (Figure 36) due to oxidation with UV-light show a positive trend as dose increases (p-value = 0.046). It is significant to note that although this positive trend exists, several increases in TOC concentration after treatment were recorded (indicated by the negative fractions). To explain this, one must consider the possible effects of carbon contamination at doses where the effects of UV are minimal. This is quite possible because although the UV system was rinsed with

ultrapure before use, other experiments utilized the same apparatus as this study, and therefore, the humic solution could have picked up some residue that was unable to be removed from the apparatus during rinsing.

Figure 32. UV-light Oxidized Hydrophobic Fractions Grouped by Bulk Solution

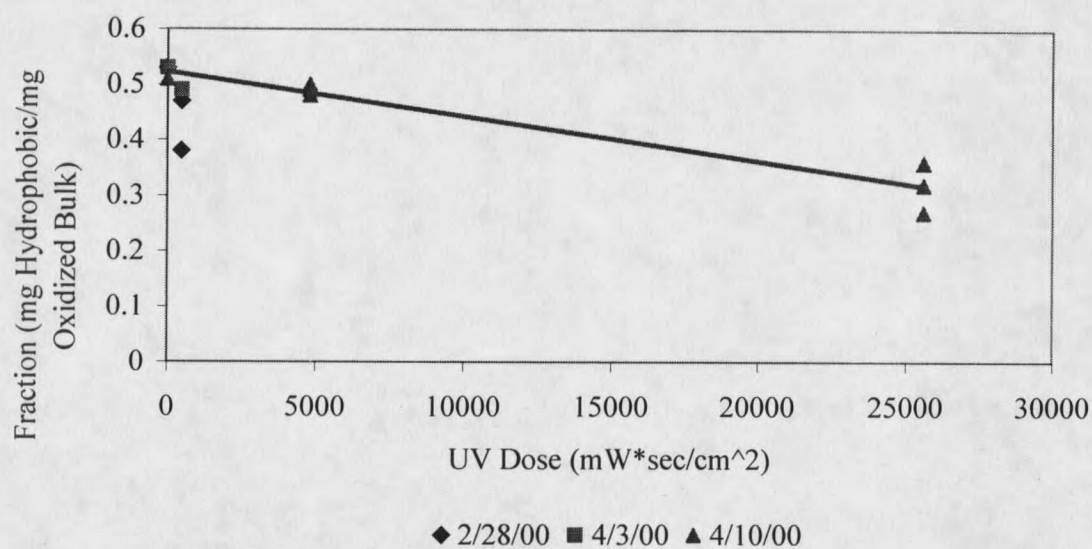


Figure 33. UV-light Oxidized Hydrophilic Fraction Grouped by Bulk Solution.

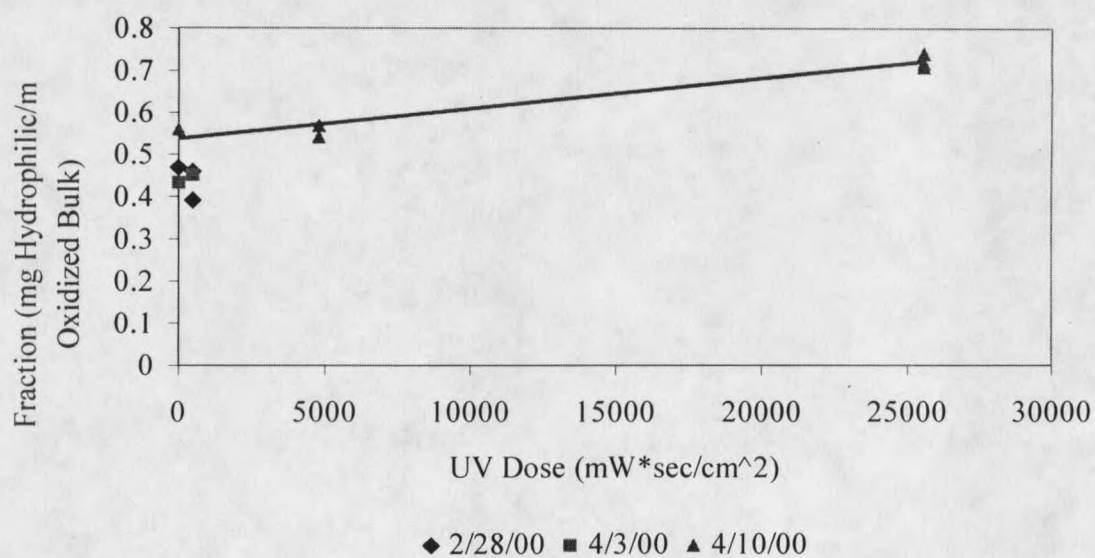


Figure 34. UV-light Oxidized Aromatic Fractions Grouped by Bulk Solution.

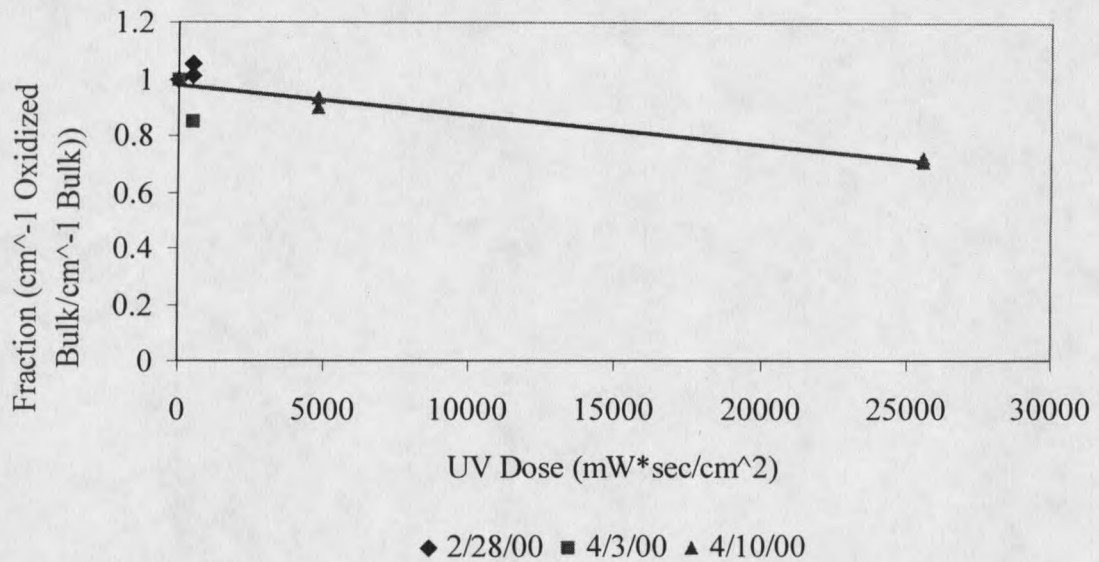


Figure 35. UV-light Oxidized BDOC Fractions Grouped by Bulk Solution.

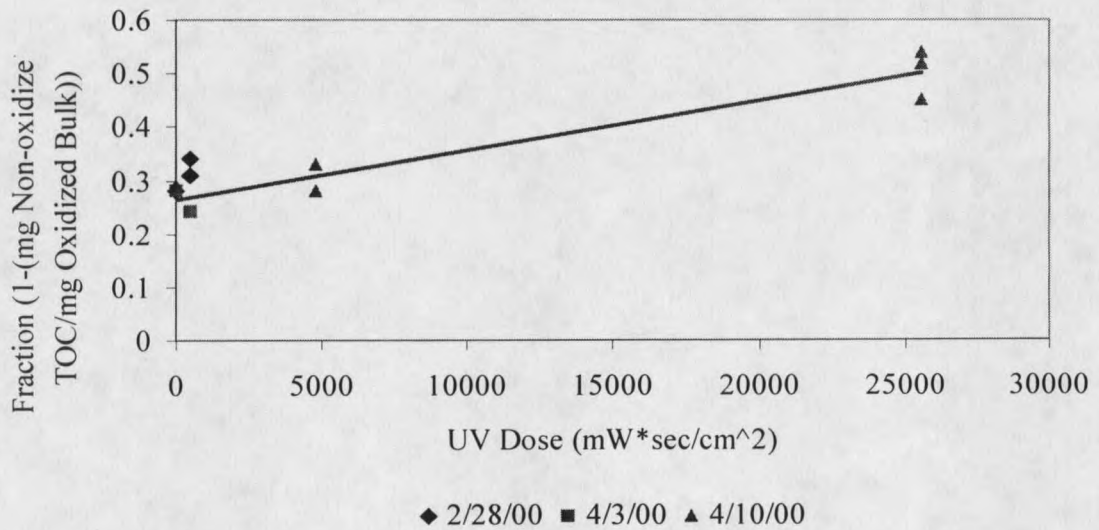
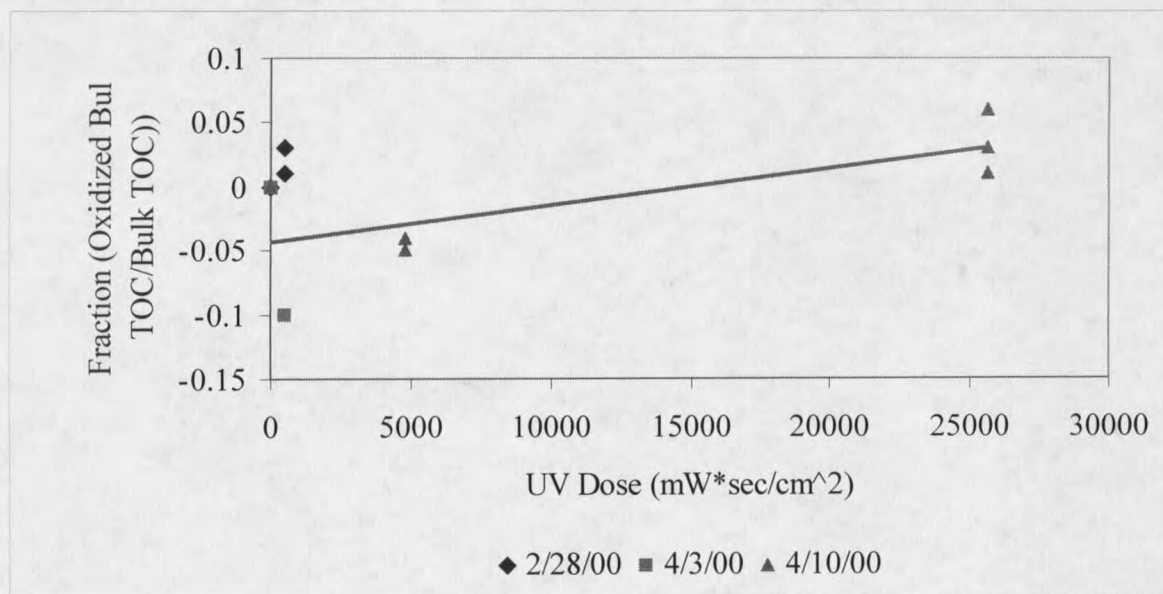


Figure 36. Overall TOC Reduction Caused by UV-light Oxidation.



Figures 37-39 show chromatograms of each dose, compared to the bulk solution.

Table 13 gives the number averaged and weight averaged MWs for each dose.

Figure 37. HPSEC Chromatogram for UV-light Dosed at 1X.

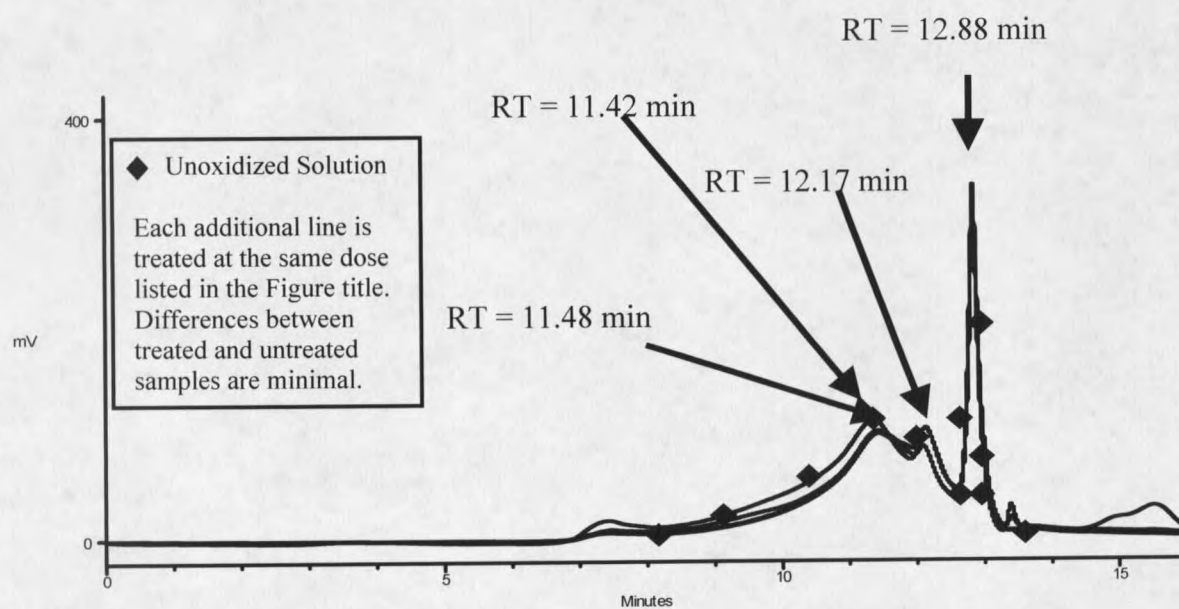


Figure 38. HPSEC Chromatogram for UV-light Dosed at 10X.

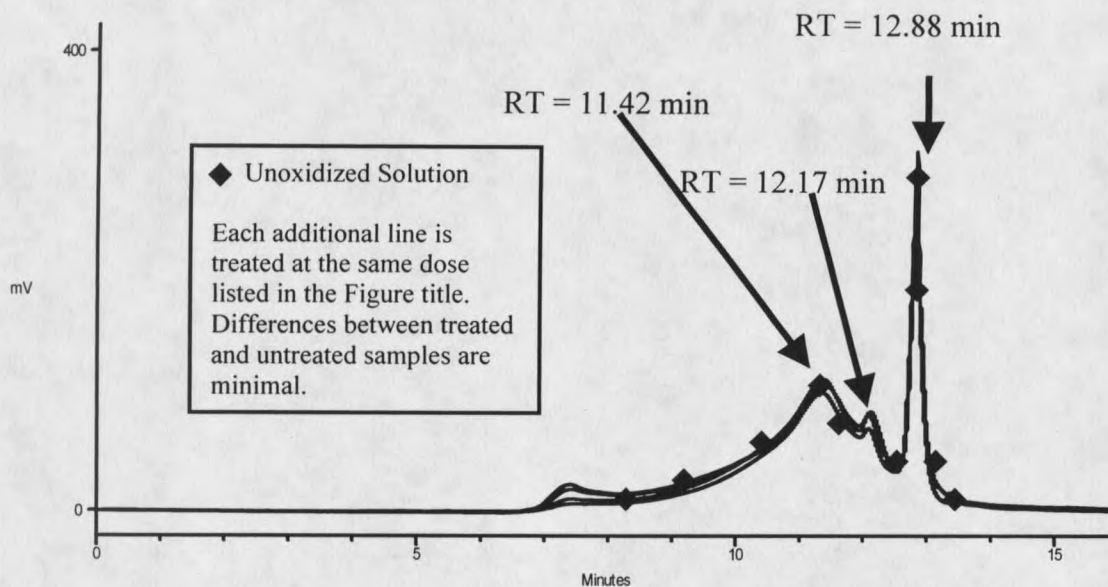


Figure 39. HPSEC Chromatogram for UV-light Dosed at 53X.

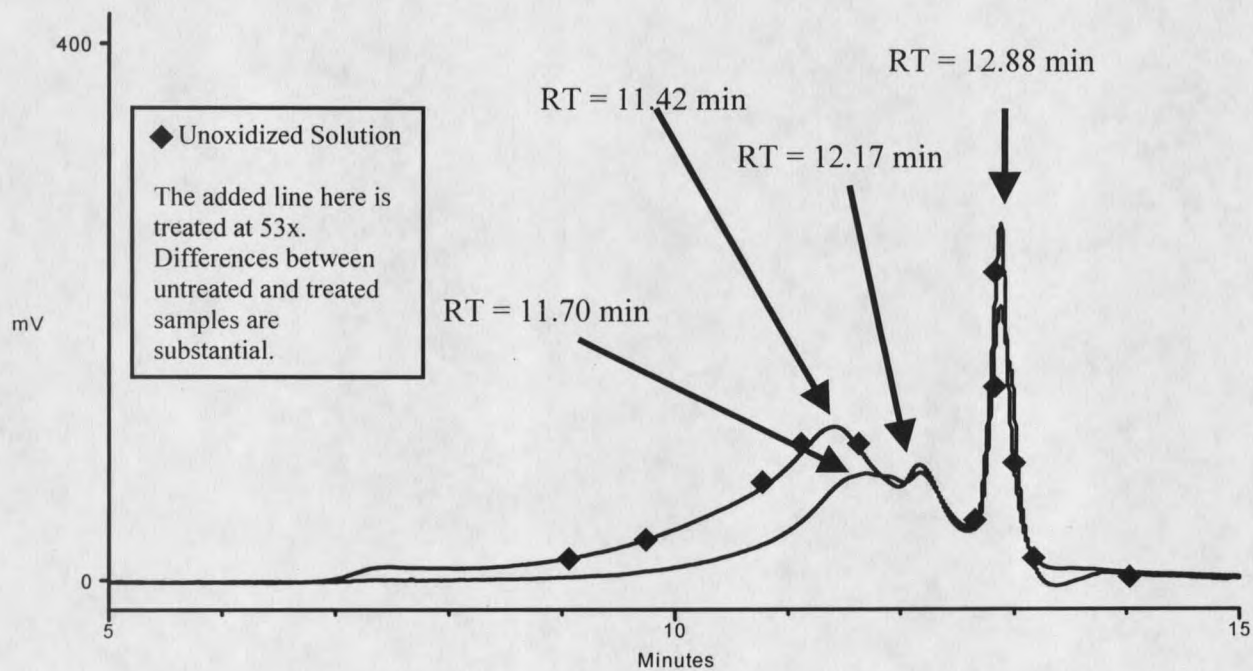


Table 13. Number and Weight Averaged MWs for UV Experiments.

Dose (mW*sec/cm ²)	Number Averaged MWs	Weight Averaged MWs
0	7192	23934
480	6460	19799
480	5977	17218
480	5882	19417
4800	5479	27501
4800	5312	25641
4800	6818	20257
25,600	8515	14500

The values in Table 13 were calculated identically to those in Table 7 for ozone. The calculation tables used to generate Table 13 are located in Appendix B. At a dose of 25,600 mW*sec/cm², there was only one chromatogram run. This is because a 6 L bulk solution was treated at one time, and then split into three 2 L samples. The chromatogram was run on the 6 L bulk solution.

The results of each chromatogram are consistent with aromatic fractions measured using the UV-254 analysis, which suggests that as dose goes up, MW fractions are becoming smaller. However, this trend cannot be reinforced by the calculated number and weight averaged MWs for reasons similar to those explained in the ozonation section. Since aromatic character is changing significantly after treatment, the aromatic fraction that is missing cannot be accounted for in the calculation. This therefore causes overestimations of the averaged MWs for the sample, preventing the results from accurately representing the MW breakdown after oxidation.

Summary

The results for this project show that oxidation with ozone and UV-light can reduce the aromatic character and the hydrophobic fraction, change the high MWs to lower MWs, and increase the biodegradability of the solution. However, whether these results can be achieved realistically and economically in the field, will predict the applicability of each treatment process for NOM removal or prevention of regrowth events within the distribution system.

Additionally, the results also indicate that chlorine, at high doses, may increase the biodegradability of NOM in water although levels applicable for NOM removal would be excessive. The results achieved from chlorine experiments strengthen the theory that chlorine disinfection processes may be partially responsible for regrowth events within distribution systems, and further necessitate that water treatment facility operators be aware of these effects to improve process control within their systems.

Results of the monochloramine experiment indicate that monochloramine has minimal, if any, effect on properties associated with biodegradability, therefore its use in NOM removal applications would not be advisable. Additionally, the decreased effect as compared to chlorine is further evidence that for disinfection processes where regrowth is limiting factor for effective disinfection, monochloramine can be a better option for both decreasing the formation of disinfection by-products and limiting regrowth in treated water supplies.

Finally, it is important to note that the results of this project would not have been as strong without the application of various methods for measuring humic fractions, and the understanding of how each fraction relates to biodegradability.

CHAPTER 4

Discussion

Due to the difficulty associated with measuring the biodegradability of a water sample, considerable effort was put into developing a set of protocols that was reliable, easy to run, and relatively fast. Additionally, low carbon analysis required special attention when selecting the best methods. Due to these considerations, some method adaptation was done to ensure that all the needs of this project were met. These adaptations are discussed in the Methods Analysis section of this chapter, along with possible directions for further improvement. A discussion will also be presented on the overall effectiveness of the protocols chosen in this project, and whether this set of protocols was an effective process for analyzing biodegradability. Additionally, Appendix A contains the step-by-step protocol for each of the methods discussed below, not only as a reference for this project, but also for further use within the CBE for humic fractionation related experiments.

Each oxidation procedure studied in this project, whether results showed that biodegradation was increased or not, can be directly applied to some area of water treatment. These applications will be discussed in Oxidation Procedure Analysis section of this chapter. For each oxidant, the discussion will include ease of application, whether the oxidant can be effective at changing NOM character at realistic doses, and how results of this project compare to results of similar projects (reviewed in Chapter 1 under each respective oxidant heading).

And finally, a comparison of each oxidation procedure for use in various water treatment purposes will be discussed in the Comparison of Techniques section of this chapter. These results will be analyzed for comparison to one study completed by LeChevallier, et al. (1992), which looked at the effects of preoxidation using ozone, chlorine, and monochloramine on a biofilter system. Additionally, this section will include a discussion of dosing relationships, and how dose may or may not be applicable as a tool for predicting biodegradation trends. This discussion can be useful for treatment plant operators looking for a quick evaluation of possible oxidant applications to meet the specific treatment needs of their facility.

Methods Analysis

Considerable time and research was required to successfully test appropriate methods for characterizing the humics before and after oxidation. Initially, an extensive literature search was done to select the methods, but extensive laboratory time to understand and adapt each method to best meet the needs of this project was also required. The hydrophobic/hydrophilic fractionation method, using DAX-8 resin, required a few simple adaptations for low carbon level analysis. The BDOC test required some modification to produce a more reliable test, and considerable time was spent to find a MW fractionation method that would meet the needs of this project most effectively. Each of these methods, and the changes that were made, will be discussed below.

Humic Character Separation Chromatography

DAX-8 resin chromatography was adapted from Malcolm and Thurman (1981), Owen, et al. (1993), and Debroux et al. (1995). The method is explained in detail in Chapter 2, and a step by step procedure is available in Appendix A. Very little adaptation was necessary, however, a few considerations based on safety and low carbon analysis were required. First, during the initial cleaning process of the resin itself, safety requirements required us to utilize different solvents for soxhlet extraction. Although the above citations call for a variety of solvents, including diethyl ether and hexane, we extracted our resin with a methanol, acetonitrile, methanol sequence.

The need to measure carbon at low levels also warranted special selection of materials to ensure that no extraneous contamination occurred. Glass columns were acid washed and rinsed before each application, and special frits were built within the lab using fired glass wool and stainless steel screen. This was necessary because frits obtained from the manufacturer were hydrophobic in nature, and prevented the passage of the humic fraction, retaining in on the frit instead of on the resin. This complicated mass balances because not all of the humic fraction could be collected.

No other adaptations to the methods referred to above were necessary for the success of this method. In fact, mass balances were commonly closed within 90%, and tests were repeatable between experiments.

BDOC Method

BDOC analysis, as discussed in Chapter 1, can be very difficult since NOM characteristics are highly dependent on source water, and the test is highly dependent on

the organisms doing the degradation. Two methods were tested during this project including those of Volk et al. (1993), and Cipparone et al. (1997). Detailed protocols are available in Chapter 2, and a step by step procedure for the Cipparone method is available in Appendix A.

As described in Chapter 2, there were several complications with the Volk method, including sorption problems and possible nutrient-shocking of the biomass due to large differences in TOC concentrations between the inoculating water source (Bozeman City tap water), and the sample water (see Chapter 2, BDOC section, pg. 50-51). Therefore, adaptations were made regarding the method for inoculating the sand (an untreated surface water replaced the tap water), and for the method of sample application (a recirculating column was used, as opposed to a stationary, 500 mL flask). Based on the problems that were encountered during BDOC analysis, the following considerations for setting up a laboratory scale experiment are advisable.

1. Sand columns should be inoculated with water that has similar organic carbon levels and make-up to sample water to ensure that the biomass is capable of degrading the sample.
2. Sample application should be optimized for sample/media contact to ensure the most efficient biodegradation possible.

MW Fractionation

Developing a MW fractionation technique that suited all aspects of this project including low carbon levels, mass balance capabilities, and repeatability was the most

difficult methods development step. Although the results were acceptable, even the final method did not meet all of the above requirements.

Ultrafiltration, as mentioned in Chapter 2, was the first choice of methods because it provided mass balance calculation capabilities. However, the method was quite difficult to do with low carbon levels, and the results were continuously unreliable. Numerous attempts were made to clean the membranes to levels acceptable for low carbon analysis, however, since the project needed a method sensitive enough to differentiate between carbon levels as small as 100 ppb, and carbon leaching from membranes seldom dropped below 200 ppb, this method was not acceptable. It is my opinion that this method should be reserved for analyses of water samples with TOC levels greater than 10 ppm. If the carbon content in the water sources being studied is less than this, and this method is still desired, then the samples should be concentrated. The ultrafiltration method was ultimately replaced with high performance size exclusion chromatography.

High performance size exclusion chromatography, however, still did not provide all of the desired outcomes for this project. This method was extremely repeatable, and was capable of measuring small fractions. However, due to equipment limitations, mass balance calculations were not possible. Therefore, the results of this method are quite qualitative in nature. Additionally, the results may be somewhat misleading without a good understanding of the detection process, the possible effects of an oxidant on humic substances, and the interactions between the column, the mobile phase, and the water sample.

First, the detection process has significant effects on the results of this method. For example, since UV-detection was used for this project, and UV character changed after oxidation, it was often not possible to evaluate the resulting chromatograms on a mass basis. This is because the peak areas could not always account for the entire mass before and after treatment. Furthermore, it is quite likely that not all organic carbon was accounted for in nontreated samples as well, since not all initial organic carbon is aromatic in nature. Therefore, it is more desirable to have two or more detection processes available, which can accurately account for the entire humic content.

The results based on HPSEC would have been much more useful had an on-line TOC analysis incorporated with High Performance Liquid Chromatography (HPLC) been available. One unit does exist on the campus of the University of Colorado—Boulder, however, this unit is a prototype from Ionics—Seivers (Boulder, CO). The unit has also required that considerable methods development be done to optimize the chromatography for low carbon analysis. According to Her, et al. (1999), this methods development is moving in the positive direction. Due to time constraints, optimization of this method was not explored intricately. Further research may provide more insight into an alternative detection method to improve quantitative studies. The following list incorporates the areas of research that may be of interest to others working with fractionating humic substances.

1. Fraction collection from the HPSEC column for measurement on the Shimadzu TOC-5000A.
2. Modification of an on-line TOC machine for TOC analysis.
3. Addition of a refractive index detector.

4. Exploration of several different size exclusion columns.
5. Concentration of a low carbon sample to improve detection and eliminate baseline contamination.

Additionally, once HPSEC has been chosen for MW fractionation of humic substances, there are several factors that one should consider. Several of these factors are listed in Chapter 2, however, they are reiterated here, along with some additional information important to the proper development of this method.

1. Standards should be chosen to closely represent humic substances, as well as meet detection process requirements.
2. Columns should be chosen to provide separation of humic substances type molecules. Protein separation columns work well.
3. Column size should be maximized to accept large sample volumes. Depending on the column packing-material, the sample concentration, and the detection process, this can have a great effect on the detection response.
4. Mobile phase should be chosen so that the least possible interference is caused with the detection process, yet peak resolution is unaffected.
5. Detection parameters (such as absorbance unit setting for UV-254), should be optimized for maximum peak resolution, and minimum background influence.
6. Sample loop size and sample size should be chosen to maximize the detector response (especially for low carbon analysis).
7. Flowrate should be considered. This will affect the amount of time it takes for each test to run, as well as chromatography resolution.

Manufacturers have optimized many of these operational parameters for specific columns, however, their optimization has been most commonly for protein analysis, which is quite different than the needs of humic substance fractionation. It is important to read as much as possible about how other researchers have accomplished the task of optimizing SEC columns for humic substance fractionation, as well as allow time for experimentation within your own lab to provide for the most efficient method possible.

Protocol Analysis

In humic substance research, settling on a set of protocols for measuring fractions and successfully predicting biodegradation trends is a hot topic. Most researchers agree that additional sample analysis is required beyond BDOC tests, however, the exact combination of protocols has not been determined. As previously mentioned, desirable qualities for the protocol include simplicity, time requirements, reliability, and consistent relationships to biodegradability. In this project, it was determined that an increase in biodegradability could be predicted with a decrease in hydrophobicity and aromatic character, as long as these two parameters happened simultaneously, and the decrease in hydrophobic character was greater than 10%. Additionally, in general, an increase in the hydrophilic fractions correlated to an increase in biodegradability, although for exact analysis of the hydrophilic character, an additional separation step must be added involving separation through an XAD-4 resin, which was not performed during this project. This may help to account for some of the discrepancies that resulted in hydrophilic trends when changes in the hydrophobic/hydrophilic fractions were close to or less than 10%.

MW analysis was far more difficult to use as a predictor, although it appears that when considerable changes are seen in the chromatograms developed from UV-detection, these changes correlate with increased biodegradation. Another detection process would provide a more solid set of criteria for comparison.

The most important question to answer, however, is whether three alternative parameters are enough to provide insight into biodegradation trends of carbon in a water sample. If the protocols selected to measure the three parameters are capable of measuring at sensitive enough levels required for the successful analysis, I believe that three additional parameters, plus the BDOC test, can provide a more solid analysis of biodegradation trends. However, unless changes in these parameters are extreme, and all correlate to increased biodegradability, I would not recommend relying upon these methods without a BDOC test to confirm the relationship, especially for chlorine and UV-light applications. This is due mostly to the fact that water sources can vary considerably, and humic substances may react differently under different circumstances. In a laboratory setting, however, these additional parameters provide a level of certainty and confidence that cannot be reached with only the BDOC analysis.

Therefore, the protocol used in this project to predict biodegradability should be used in combination with BDOC analysis, unless considerable changes support increased biodegradation in all other parameters. These parameters are much more useful as fast, reliable ways to track biodegradation behavior once the initial relationship has been established for a specific water source.

Analysis of Oxidation Procedures

Ozonation

The results of the ozonation experiments show that as ozone dose increases, hydrophobic character decreases, hydrophilic character increases, aromatic character decreases, and lower MW fractions increase. The results also indicate that as all of these trends are occurring, biodegradability is increasing, although the results of the BDOC tests were not considered to be statistically different ($p\text{-value} = 0.067$). It is likely that performing the BDOC tests a few more times would improve the $p\text{-value}$, and support the trends seen above. This assumption is based on complications encountered in ozone dosing causing the omission of two points from the statistical analyses. The average % changes of the three experiments run at each dose are displayed in Table 14. A (-) indicates a decrease, and no sign indicates an increase in the fraction.

Table 14. Average % Changes Due to Oxidation with Ozone Arranged by Dose.

Ave. Dose (mg O ₃ /mg TOC)	Ave. % Change in Hydrophobicity	Ave. % Change in Hydrophilicity	Ave. % Change in Aromaticity	Ave. % Reduction in TOC	Ave. % Change in BDOC Fraction
0.5	-16	38	-32	-1	4
1.0	-53	80	-45	6	11
2.0	-65	93	-59	8	4.5

Results also showed that as ozone dose increased, overall TOC reduction increased. This indicates that ozone has some destructive power, however, its destruction capabilities are limited in comparison to other changes recorded. TOC destruction via ozonation with realistic doses should not be considered a reliable method for TOC reduction.

Results found in this study also imitate findings from Cipparone, et al (1997), which show that ozone can significantly increase biodegradability. However, at doses greater than 1 mg O₃/mg TOC, there is a leveling effect. In fact, results of this project show that although the decrease in aromaticity and hydrophobic character continues to grow (see Table 14), biodegradability actually decreases. This decrease is considered to be partially influenced by the fact that one sample at the 2.0 mg O₃/mg TOC dose was neglected, however, it is still significant to note that there is no substantial increase associated with the doubling of the dose.

As explained by Langlais (1991), this leveling phenomenon has been seen by many and is thought to be a result of ozone being able to reach a "maximum" breakdown of humic substances around 1 mg O₃/mg TOC. This finding may also be, in part, responsible for the contradictory p-values since the statistical analysis tests a linear slope against a slope of zero. Therefore, if a leveling effect is possible, a slope might be skewed by the results of the highest dose.

What is even more encouraging, is that ozone experiments were run at doses easily attainable in a municipal water treatment facility. This implies that similar results could be attained, quickly, and without much added expense at facilities already utilizing ozone for disinfection processes, or possibly NOM removal. This finding, in combination with the positive trends (in relation to increasing biodegradability) that were measured, makes ozonation promising as a preoxidation step for biofiltration optimized for the purposes of removing NOM. This confirms experimentation that has shown that ozone, in conjunction with a biofilter, optimizes biofiltration for NOM removal (Goel, et

al., 1995; Cipparone, et al., 1997; Langlais, 1991; Owen, et al., 1993; Kruithof, et al., 1989).

The potential water quality implications of ozone preoxidation to biofiltration include prevention of biofouling on sensitive membrane surfaces (such as RO), allowing for increased membrane life, and cheaper operation costs. Additionally, in a water treatment facility, two implications exist. The first is in regards to the application of ozone without biofiltration. In this case, it is important for water treatment facility operators to understand that additional disinfection must be done to prevent biofilm growth, since ozonation can increase biodegradability of NOM, making it available to potential microorganism growth downstream from the treatment plant. The second implication is for applications that utilize a biofilter after a conventional filtration step, but before disinfection. In this case, the effects of ozone can significantly improve water quality, lower required disinfection residuals, and decrease the formation of disinfection by-products, making effluents safer and healthier for human consumption.

Overall, ozone is capable of improving water treatment processes currently used for a variety of applications. This has been utilized extensively in European countries and implementation in American water facilities is just now becoming common. The increased use of ozone will allow for improved finished water, and decreased operating costs for advanced filtration treatment processes. It will also be a valuable step for reclaiming wastewater, a treatment process that will likely occur in the next century to extend water supplies.

Chlorination

The results of the chlorination experiments show that at high enough doses, chlorine decreases hydrophobic character, increases hydrophilic character, decreases aromatic character slightly, and slightly increases the low MW fraction of NOM. Statistically, however, it is not possible to relate these changes to biodegradability. Therefore, more assumptions must be drawn from the results of the surrogate parameters that were collected, which overall, tend to support a probable increase in biodegradability. The average changes in NOM character from the three experiments run at the two chlorine doses are displayed in Table 15. As before, a (-) indicates a decrease in the fraction in question.

Table 15. Average % Changes Due to Oxidation with Chlorine Arranged by Dose.

Ave. Dose (mg/L Cl ₂)	Ave. % Change in Hydrophobicity	Ave. % Change in Hydrophilicity	Ave. % Change in Aromaticity	Ave. % Reduction in TOC	Ave. % Change in BDOC Fraction
0.5	-5.5	0	-1	2	3
4.0	-33	42	-26	6	5

The results of the BDOC and hydrophilic tests show, along with various treatments, that the 0.5 mg/L treatment appears to actually have a slight negative effect on biodegradability (see negative slopes in Figures 20-24, Chapter 3). Additionally, data collected from the 4.0 mg/L treatment always appears to have a positive effect on biodegradability. This trend contributes to the inconsistency seen in the statistical analysis, which is based on a linear analysis between dose and BDOC.

It may be that at the lower dose, there is not enough chlorine available to react effectively with the humic substances, and the resulting by-products may actually be higher molecular weight, more complex, compounds. This theory is supported by Kruithof, et al. (1989) who found that the production of these molecules is possible during chlorination. This theory is not intended to imply that no changes in humic substances occurred which could make them more biodegradable at 0.5 mg/L, or even to imply that biodegradability may decrease after oxidation with chlorine at low doses. It is meant to suggest that the effects of chlorine at a low dose may not be evident enough to be statistically different from no treatment, and even more, that the possible generation of large MW molecules could counteract the positive effects of chlorine on biodegradability.

At a higher dose of 4.0 mg/L, it is probable that there was a breakdown of simpler bonds within the humic substance (indicated by the MW changes), and some (although only 25%) of the aromatic bonds, which influenced biodegradability in a much more positive manner. Therefore, it may be better to analyze the results from the 0.5 mg/L and 4.0 mg/L doses separately. This would allow for statistical verification that chlorine, at higher doses, does provide oxidation of humic substances, making them more biodegradable.

The potential water quality implications of these findings are that chlorine, even though capable of some breakdown of humic substances, is probably not a good choice as an oxidant for optimizing NOM removal via a biofilter. Furthermore, in the drinking water treatment industry, these results show that chlorine treatment (most commonly in disinfection processes) can cause the formation of more biodegradable fractions, increasing the potential for microbial growth within the drinking water distribution

system. This finding is important because water treatment facility operators need to know and understand how to control potential regrowth problems before they start.

Other researchers (Abernathy, 1998; LeChevallier, et al., 1990) have found increased biofilm formation in water treated with low concentrations of chlorine. Although this project does not directly support the findings of these researchers, their results cannot be discounted. It is possible that low doses of chlorine still have a very small oxidizing effect on humic substances. This small effect, added up over time, could produce the increased biofilm growth seen in these previous experiments. Additionally, the organic matter in the water sources studied in those projects could possibly have been more conducive to breakdown by chlorine. This is a good reminder that the results of this research, although useful for making general recommendations, are really only applicable to the water source being studied, or other water sources similar in composition.

Monochloramine Oxidation

Monochloramine oxidation was the least effective treatment for increasing the biodegradability of humic substances of all of the treatments. The average removals of the three experiments run at the trial dose are displayed in Table 16. As stated above, a (-) indicates a decrease in the fraction in question.

Table 16. Average % Changes Due to Oxidation with NH_2Cl Arranged by Dose.

Ave. Dose (mg/L NH_2Cl)	Ave. % Change in Hydrophobicity	Ave. % Change in Hydrophilicity	Ave. % Change in Aromaticity	Ave. % Reduction in TOC	Ave. % Change in BDOC Fraction
1.64	-24	2	9	-5	1

Results show that at the applied dose, hydrophobicity decreases and hydrophilicity increases. Aromaticity actually seemed to increase with oxidation, as did the overall TOC concentration. These latter two changes in these fractions were less than 10%, and it is quite possible that these discrepancies are due to instrument and sampling errors. Additionally, no significant changes in MW were noted, which correlates to the biodegradation tests that showed no differences after treatment. These results indicate that although hydrophobic and hydrophilic changes tend to support possible increased biodegradation, no other parameter supports this change, and therefore, this method is the least effective for increasing the fraction of biodegradable humic substances. It is important, however, to remember that a very limited effect may still be possible, which is supported by the hydrophilic/hydrophobic fractionation tests.

This finding has some positive implications for water treatment processes. Monochloramine, being the least reactive with humic substances, is likely to be the most effective oxidant for secondary disinfection purposes. Accordingly, there is far less possibility of altering humic substances during disinfection with monochloramine than with chlorine. Additionally, the dose applied in this study was the maximum dose that can be applied in a municipal water treatment facility, and the length of the test was 10-days. In a distribution system, the residence time in the distribution system is usually less than this, and monochloramine doses applied for municipal water treatment are usually lower. Therefore, the effects of monochloramine on humic substances and the potential effects on microbial growth within a distribution system are likely to be even less pronounced than the effects that were recorded in this project.

UV-light Oxidation

UV-light oxidation presented some very interesting results. There was a strong correlation between UV-light and all trends associated with biodegradability. As dose increased, hydrophobic character decreased and hydrophilic character increased. Aromatic character decreased, MW fractions became smaller, and biodegradability increased. In fact, at 25600 mW*sec/cm² (53X), biodegradability of the humic substances changed by as much as 24%. The average changes in humic character of the three experiments run at each dose are displayed in Table 17. A (-) indicates a decrease in the fraction in question.

Table 17. Average % Changes Due to Oxidation with UV-light Arranged by Dose.

Ave. Dose (mW*sec/ cm ²)	Ave. % Change in Hydrophobicity	Ave. % Change in Hydrophilicity	Ave. % Change in Aromaticity	Ave. % Reduction in TOC	Ave. % Change in BDOC Fraction
480	-13	-10	-3	-2	2
4800	-6	15	-8	-5	2
25600	-38	49	-29	3	24

It is significant to note, that very little overall TOC destruction occurred during treatment with UV-light at 254 nm, meaning that oxidation with UV-light is not strong enough to cause the complete destruction of organic carbon to CO₂. Additionally, it is interesting that even with a 24% increase in biodegradability, there was only a 25% change in aromaticity. During ozonation, there was a much greater change in aromatic character, and a much smaller effect on biodegradation. This indicates that although aromatic rings may still be present, there may be some sort of critical chemical alteration (but not breakdown) of the aromatic rings within the sample, which makes these rings

more susceptible to microbial degradation. These changes seen during UV-light oxidation were the most pronounced of any treatment, which may make it the most effective treatment for increasing biodegradability of humic substances.

However, the major drawback of this sort of oxidation was the extremely high dose requirements necessary to cause significant changes in biodegradation. In Chapter 2, under oxidation techniques for UV-light, there was a large discussion on dosing levels and how dosing was chosen for this project. It is important to note that the starting dose for oxidation (1X) was chosen as a theoretical maximum dose in a municipal water treatment system for deactivation of all known microbes. Results of this project correlate with results found in the research conducted by Shaw, et al. (2000), which indicated that, at a dose of $130 \text{ mW}\cdot\text{s}/\text{cm}^2$, an increase in biodegradability of humic substances was not apparent. Since no significant changes were seen in any parameters at doses applied in this project as high as 10X ($4800 \text{ mW}\cdot\text{s}/\text{cm}^2$), the results are confirmed. It would be quite rare for a facility to dose at levels higher than this, let alone 53 times this dose, making UV-light for municipal water treatment applications an unattractive option for use as a pre-oxidant to a biofiltration step. UV-light is not likely to be used for oxidation of humic substances in most applications because dosing requirements to provide significant changes in humic substance make-up are most likely too expensive, and difficult to meet in large scale applications.

In a laboratory system, however, UV may be applicable to specific purposes. One specific application that may have been useful would be to include UV in the preparatory steps for BDOC sand media. By oxidizing the feed water continuously with UV, the nonbiodegradable fraction would be substantially decreased, which could extend

the life of feed water used to prepare sand media by increasing the biodegradable fraction.

Comparison of Oxidation Techniques

Since the ultimate goal of this project was to contrast and compare each of the above treatments and their ability to increase the biodegradable fraction of humic substances, the following criteria were developed in order to choose the most effective overall treatment. First, the treatment objective needed to be identified. Is increased or decreased biodegradability the desired goal? Next, some measure of biodegradability, such as detected via the BDOC test was needed. Finally, the dosing requirements to provide significant results needed to be reasonable for applications in a municipal water treatment facility. These requirements gave the following two rankings: one for increased biodegradability (applicable for NOM removal applications, in conjunction with a biofilter) and the other for decreased biodegradability (applicable for distribution system regrowth control, in systems using these oxidants for disinfection purposes).

Table 18. Ranking of Oxidation Treatments

Increased Biodegradability	Decreased Biodegradability
Ozone	Monochloramine
UV-light	Chlorine
Chlorine	UV-light
Monochloramine	Ozone

Only the study conducted by LeChevallier, et al in 1992 compared any of these treatments on a specific water source with relation to increasing the biodegradable fraction of humic substances. The main difference between his study and the study

conducted for this paper is that his study included a biofilter after the preoxidation step. His conclusions stated that preozonation at an average dose of 0.5 mg O₃/mg TOC provided for similar TOC reduction to prechlorination/dechlorination processes where chlorine doses were applied at 5 mg/L, and dechlorination was done after 10 minutes with an average residual around 3 mg/L. Additionally, monochloramine provided less TOC removal than the other two alternatives. It was suggested that monochloramine did not perform as well because it was allowed to penetrate the filter entirely. It is significant to note that chloramine concentrations in the effluent of the biofilter were around 0.1 mg/L, indicating that biofilters can still operate, although not at optimized conditions, under the influence of a disinfectant. That study also found that filter effluent AOC levels for preozonated water were consistently higher than filter effluent AOC levels for chlorinated and chloraminated water sources, even though TOC removal was often comparable or higher. This is potentially problematic in systems where biofouling prevention is the main treatment objective because increased biofilm growth could occur downstream if higher carbon concentrations are "available" after biofilter treatment (LeChevallier, 1992).

The results of this project found similar results for similar dosing levels. Ozone treatment at 0.5 mg O₃/mg TOC gave similar results to chlorine oxidation at 4.0 mg/L, and monochloramine was much less effective, in changing NOM properties than the other oxidants. In the present study, more doses were explored, which made it easier to distinguish between the above treatments, clearly identifying the applicability of ozone above that of chlorine.

The study of several doses also made it possible to analyze the relationship between dosing and biodegradation trends. The most important trend, apparent with all oxidants (except monochloramine, which was only studied at one dose), is that as dose increases, an increase in biodegradability is probable. Therefore, water treatment plant operators must be aware that increasing doses of disinfectants may actually be counterproductive for decreasing regrowth problems within a distribution system, depending on the chemical makeup of the water source.

Additionally, as dose increased, it was quite common to see changes in the other parameters, which support the occurrence of biodegradation. It was also apparent that as oxidizing dose increased, overall TOC removal increased as well. This decrease was very small and it should not be used to justify the use of oxidants for TOC removal without a biofilter.

Whether the increased biodegradable fractions that were observed in this project would be directly removed through a biofilter is not possible to predict accurately at this time. There are too many other variables that affect the operation of a biofilter. One of the most important variables for increasing removal rates is the empty bed contact time (EBCT), which can have a considerable effect on the efficiency of the biofiltration system. Since different biodegradable fractions take different amounts of time to degrade, an EBCT that is too short may not allow enough time for degradation of the increased biodegradable fraction. It is therefore important to optimize each system before application, to ensure that flowrates, treatment goals, and cost, are balanced for the best possible operation. The only way to know whether preoxidation techniques have an

effect on biofilter performance is to use them in conjunction with a biofilter and measure removal rates.

CHAPTER 5

Conclusion

Based on the results of this project, ozone was chosen as the best oxidation technique for increasing the biodegradable fraction of NOM. The next step is to apply this oxidant as a pretreatment step to a biofiltration system. This task is currently being undertaken by Melanie Nowlin, an undergraduate in Bio-Resources Engineering at the Center for Biofilm Engineering—Montana State University. This companion project utilizes the biofilter system built by Chris Wend, and an ozone contact chamber built to allow for a maximum ozone dose of 1.5 mg O_3 /mg TOC to be applied to the influent water source. The column has been designed so that minimum ozone doses should rarely drop below 1.0 mg O_3 /mg TOC. The doses were chosen based on results of this project and other similar projects (Cipparone, et al., 1995).

The ozone results can then be compared to those previously attained where chlorine was used as the preoxidant. It is anticipated from results of this study that ozone will provide for an overall increase in TOC removal, over untreated and prechlorinated treatment processes.

Additionally, further analysis of these oxidants will ultimately require a cost analysis of each process. Based on current practices in water treatment facilities, which are usually optimized for cost, ozone most closely meets current treatment practices. What will be interesting to find is whether preoxidation with ozone increases the TOC removal capabilities of a biofilter beyond that of a biofilter with no preoxidation step. This is especially important if the goal is to increase removals to justify the cost of

implementing an ozone generator capable of treating high volumes of water commonly treated in a municipal water treatment system.

Finally, when choosing preoxidation as an alternative treatment process, or examining the potential for disinfection chemicals to increase microbial growth within a distribution system, it is important to remember that each water source is different, and will probably require some sort of analysis before choosing the best option. Given this requirement, possibly the most important part of this project, as well as the most important part of continued research in this area of water treatment, involves developing protocols for measuring biodegradability, that are easy, quick, and inexpensive to perform. Once these tests are perfected, water treatment system operators can extend their level of control over water quality, and public health concerns.

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APPENDICES

APPENDIX A
Step-by-Step Protocol

CHROMATOGRAPHY COLUMN SET-UP FOR SEPARATION OF HUMIC/NON-HUMIC FRACTIONS

RESIN STORAGE

- 1) Acid bath two 500 ml bottles for 24 hours. Rinse with UP water, and then bake for 24 hours.
- 2) Fill both bottles with about 300-ml of methanol.
- 3) Label one "clean resin" and the other "used resin".
- 4) Change methanol occasionally in the "used resin" bottle (as significant discoloration occurs).
- 5) Never place anything in "clean resin" bottle to prevent contamination.

RESIN CLEANING PROCEDURE

- 1) Remove resin from storage (in excess methanol) and rinse several times with hot deionized water (~60 degrees C) to drive off methanol.
- 2) Place resin in 1000 ml flask and add 0.1 N NaOH in excess. Swirl and let sit.
- 3) After a minimum of 12 hours, swirl and decant NaOH allowing fines to be decanted (goal being to not only clean resin but to obtain uniformly sized resin beads). Add fresh NaOH. Repeat for a minimum of six decants.
- 4) Decant NaOH for the final time and rinse with UP water at room temperature several times.
- 5) Transfer resin into a Soxhlet extraction thimble. The thimbles for the water lab's Soxhlet extractor hold approximately 30 g of resin. Allow about ½" of headspace to minimize loss of resin to splashing. Place methanol in Soxhlet round bottom flask. Fill to about ¾ full. Add about 10 small glass beads to act as boiling chips.
- 6) Place Soxhlet extractor in hood. Connect Soxhlet components, turn on water for adequate flow, turn heating mantle to about 4-4.5 units. Allow the extractor to operate for about 10 minutes and check drip flow rate out of condenser (should be approx. 1 drop/sec). Do not leave unattended until operation is consistent. Soxhlet extractor may explode or boil over if temperature is too high.
- 7) Extract with methanol for a minimum of 48 hours. Discard methanol and fill flask to mark with acetonitrile.
- 8) Extract with acetonitrile for a minimum of 48 hours. Discard in same waste container as methanol.
- 9) Transfer "cleaned" resin to clean methanol storage bottle.

COLUMN DESIGN AND SET-UP

- 1) $ID_{\text{column}} \geq 20 \times OD_{\text{bead}}$ (to minimize wall effects)
- 2) No specific column diameter to length ratio is specified.
- 3) Choose a sample volume. This volume depends on the TOC strength of your sample, the volume that can be collected, the method for TOC measurement, and the amount of time available to run the sample. The following parameters were for my system. Since I worked with a low bulk TOC concentration, I chose to run a large volume of sample (see thesis for discussion of chromatography column size vs. sample size). I also wanted to set my column up to run overnight, thus the 16-hour sample run time (5:00 P.M. to 9:00 A.M.).

Sample Size: 1.0 LTOC strength of bulk: ~ 2.0 mg/L

Volume that can be collected: variable

Sample run time: 16 hours

TOC measurement method: Shimadzu TOC-5000A

When choosing this volume, it is important you should also consider the bed volume you may be working with. Your sample volume should be at least 10 x the resin bed to minimize the effects of dilution due to water used to wet the bed, sample, and eluant. These volumes could be considered to improve a mass balance, and must be considered if the sample volume is less than 10 x the resin bed.

- 4) Next, calculate a bed volume (BV) based on the flowrate. The flowrate should not exceed 15 BV/hour. For a 16-hour run and 1000 ml of sample, 240 BV will be needed which gives a BV of 4.17 ml.

$$15 \text{ BV/hour} \times 16 \text{ hours} = 240 \text{ BV}$$

$$1000 \text{ ml} / 240 \text{ BV} = 4.17 \text{ ml} / \text{BV} \sim 4.0 \text{ ml}$$

- 5) Find a brand of glass columns (I chose OmniFit glass columns), that can provide a BV a little bigger than the volume given above. (Allow for some headspace above column for an additional water column. This water column will help distribute the sample evenly across surface of the resin bed). The following column parameters are the parameters that I chose for my column.

Column diameter = 1 cm

Column length = 15 cm

Column volume = 9.5 cm

- 6) It is important that you consider all column materials. I found that OmniFit columns worked well, however, I could not use the frits that were supplied with the column for sample distribution due to contamination problems, and separation problems caused

by the frit attracting the humics. Therefore, I used the OmniFit column (1X15 cm), built my own frit for the bottom plug, and did not use a frit in the top plug.

- 7) The bottom frit was built using fired glass wool (conventional oven for 24+ hours), and a fine meshed stainless steel screen to prevent the loss of resin or uneven distribution of resin on the bottom. The screen was cut to fit the inside diameter of the column. The plug was stuffed with the glass wool, and then capped with the screen.
- 8) The glass column should be soaked in an acid bath for 8+ hours, rinsed with UP water, and baked for 8+ hours to eliminate contamination. Tubing should be changed upon discoloration.
- 9) To insert the tubing into the small stainless steel cap, slice the tubing at a sharp angle, then grab the sliced end through the cap with pliers and pull through until tubing can be cut flush with the face of the cap.

ISOLATION PROCEDURE

- 1) Obtain cleaned DAX-8 from methanol storage container. Pour resin into acid-washed flask or beaker with a stir bar. Pour excess methanol back into container. Clean methanol from resin by rinsing repeatedly (until methanol is removed) with heated (~60 degrees C) UP water (I did this for one full day with periodic water changes—at least 4).
- 2) Pack column with appropriate volume of resin.
- 3) Rinse column with 250 ml of U.P. at 15 BV/hr and measure effluent DOC. If it is not close to U.P. (I went to about 0.1 mg/L TOC), methanol has not been sufficiently removed. Continue rinsing column.
- 4) Rinse column with 3 column volumes of 0.1 N NaOH (50 ml) at 15 BV/hr. (approx. 30 min).
- 5) Rinse column with 3 column volumes of U.P.
- 6) Rinse column with 3 column volumes of 0.1 N HCl.
- 7) Rinse column with 3 column volumes of U.P.
- 8) Repeat steps 4-7 two more times for a total of 3 rinses. Collect ~40 ml of U.P. after rinse is completed and measure TOC. If TOC level is greater than 0.05 mg/L, do an additional rinse cycle and measure again.
- 9) Obtain a 1.0 L sample volume (or sample size you have settled on). Filter sample through a 0.45 μ m filter. Be sure to test filter for the possibility of carbon contamination. Collect a portion of this sample for UV and DOC analysis. Acidify the sample to a pH = 2.0 with 6.0 N HCl (use a low carbon grade HCl). Collect another sample for DOC and UV analysis.

- 10) Run measured volume through column. Collect effluent into an acid washed glass container. Keep track of the volume collected. This is easiest by pre-weighing the glass container and then measuring the final volume. You will see a significant color change during this step. As the adsorption sites in the resin begin to fill, they will show a dark brown color. In my column set-up, this color usually developed from about 1/3 to 1/2 the distance down the column.
- 11) Reverse flow to upward direction. Flush column with 5-10 column volumes (dependent on the elution efficiency of specific sample source) at 5-7.5 BV/hr with 0.1 N NaOH. Collect eluted sample in pre-weighed, acid washed glass container or graduated cylinder. When eluted sample appears clear in the water column above resin, this is a good indication that elution has been completed. Be sure to consider the volume of water in the water column before elution. It may be best to eliminate this column before this step is completed. This way, no dilution (or minimal dilution will occur due to this).
- 12) The dark colored elution must be diluted to meet the constraints set by the method of measuring TOC. The Shimadzu TOC-5000A can only measure levels below 3-4 mg/L. I always dilute my samples to be around 1-2 mg/L.
To calculate this dilution, use the following method:

$$DOCo = [DOC_{eluant} * V_{eluant} + DOC_{effluent} * V_{effluent}] / Vo$$
 Solve for DOC_{eluant}
 Divide DOC_{eluant} by desired TOC value, and this will give you your dilution.
- 13) After the dilution, collect a sample for UV analysis. Then, make sure to acidify the sample to a pH of 2.0 before running through the TOC machine (measure this sample with a pH probe to ensure proper acidification since the eluant pH is around 11 or 12).
- 14) Use the above equation, except solve for the DOCo using the measured effluent and eluant values. Compare this to the measured DOCo and calculate the % error in the reading. Try and close the mass balance within 90%, even better would be 95%.
- 15) Place used resin in container for cleaning. Acid-wash columns, and clean plugs.

BDOC ANALYSIS

MATERIALS

Silica Sand

1-2 columns, at least 500 ml

Specified number of 100 ml columns for types of water being tested (these are the BDOC columns)

10-20 Liter Carboy

Tubing

Pumps

The purpose of the inoculating columns is to prepare a media (in our case, silica sand) that has a consortium of organisms growing on it that can effectively biodegrade a sample. Feeding the organisms water that is similar to your sample is the most effective way to achieve this. Properties that should be considered when choosing your media include TOC level, temperature, particulate fraction and, depending on your research, various other properties.

The inoculating column is necessary because the organisms must be exposed to a continuous food source, and most bench scale systems are not equipped to run a continuous test. The major advantage of this method, as opposed to other BDOC methods, is that sorption problems are eliminated, nutrient-shocking problems are limited, and using organisms familiar with the type of sample being tested optimizes organic carbon removal. Additionally, the test can be completed in a week or less and sample size is not of concern because the ratio of sand to sample is not significant for this method.

A continuously recirculating feed can be effectively substituted (provided frequent water changes) to represent a continuous feed situation without interfering with supply limitations, as are often encountered in lab-scale systems. The sand can be transferred to smaller columns when need be and a more controlled BDOC test can be performed.

SET UP FOR INOCULATING COLUMNS

- 1) Use 1 or 2 500 ml columns.

GROWING THE ORGANISMS

- 2) Pack the columns with sand (you might need to build screens to prevent the loss of sand from the column).
- 3) Mount the columns where they are easily accessible and in a bath or sink where spills can be contained.
- 4) Fill the carboy with approximately 2-L of unchlorinated, "dirty" water (we used water from the MSU duck pond). Fill the rest of the carboy with deionized water.
- 5) Recirculate this water through the inoculating columns continuously for approximately one week.

FEEDING THE ORGANISMS

- 6) Empty the carboy and refill with feed water that closely resembles sample concentration of TOC.
- 7) Recirculate through the columns for one week.
- 8) Replace the feed water with fresh feed water.

DETERMINING THE AMOUNT OF TIME BETWEEN FEED WATER CHANGES

- 9) Measure initial TOC.
- 10) Track the TOC concentration until it no longer changes. This final reading will be the number of days between feed water changes. It will also give you some indications of how long to run the BDOC test.
- 11) If the organism count seems to be a little low (which can be determined by doing intermittent Total Cell Counts), you may need to add some fresh organisms (from your dirty water source) to the feed water (approx. 1-L of dirty water).

SET UP FOR BDOC COLUMNS

- 1) Rinse the BDOC column with ultrapure (UP) water several times.
- 2) Take one inoculating column apart and fill the BDOC column with 100g of inoculated sand.
- 3) Refill the inoculating column with new silica sand. Remember to alternate columns to ensure a biologically activated media.

SAMPLE APPLICATION

- 4) Collect 1-L of your sample. Set aside approx. 100 ml to do initial TOC measurements.
- 5) Recirculate the remaining sample through the columns.
- 6) Measure the TOC concentration daily on the first sample to establish a run time.
- 7) When TOC concentration stabilizes, the run is finished. After the first time running your sample, you only have to collect a sample on the last day.
- 8) Upon completion, empty the sand into the garbage and rinse the columns with UP water several times.

FILTERING SAMPLES

- 1) The initial TOC sample does not have to be filtered because it will not have been exposed to the sand. However, subsequent samples must be filtered.

- 2) We used 0.2 micrometer nylon filters (available from Fisher) and plastic 30 ml syringes.
- 3) Rinse the syringe and filter with one bed volume (BV) UP water.
- 4) Rinse with 3 BV 0.1N HCl.
- 5) Rinse with 3 BV UP water.
- 6) Rinse with approx. 5 ml of the sample.
- 7) Filter the sample into an acid washed and baked container.
- 8) Discard filter and repeat (all samples should be done in duplicate).

HPSEC

COLUMN SELECTION

A column should be selected that can separate a MW range from 1000 Da or less to 100,000 Da or less. If you prefer to have a more precise separation, it may be best to use two or more columns, within this range.

Additionally, the column medium should be conducive to separating molecules such as proteins. This is commonly a silica-gel, however, other media are available for these processes, and can be explored.

Finally, selecting a column may also depend somewhat on how low in carbon your sample is, and how sensitive your detection process is. This may take a bit of experimentation on your part, as well as reading to get a better idea how these parameters go together.

This is a very important part of this process since the columns are usually over \$1000.00 a piece. So, it is best to select a column and design other parameters around it.

The column I chose was a Shodex KW802.5 column. This was packed with a silica-gel media, and the column dimensions were (8.0 mm x 50 mm) for the guard column and (8.0 mm x 300 mm) for the separation column (JM. Science, Grand Island, NY).

A guard column is not necessary, however, they protect the main column from contamination, so they can be useful.

INSTRUMENT SETUP

The following parameters were used for the HPSEC method done in this project, however, these parameters can be modified with to improve your selected process.

UV-detector (Dionex AD20) was set to:

0.2 AU

254 nm

low intensity UV-lamp

HPLC unit (Dionex DX300 Series HPLC) was set to:

Flowrate = 1.0 mL/min

Pressure = 540 psi

SAMPLE AND INJECTION LOOP SIZE

To optimize the process, the largest sample size that can be applied to the column (0.5 ml) was used. Therefore, the following size sample loop was chosen

PEEK 0.5 mL sample loop

To accommodate this size loop, special sample vials were also purchased that were large enough to apply the size sample necessary to the column. Therefore, 5 mL sample vials and caps that work with the ASM-2 automated sampler were used.

STANDARD SELECTION

Standards should be chosen based on detection. Therefore, Polystyrene Sulfone or protein globules are best with only UV-detection. Others are better for different detection methods.

Protein Globules were selected from 1350-2,000,000 Da

Vitamin B12

Aprotinin

Cytochrome C

Carbonic Anhydrase

Albumin

Blue Dextran

MOBILE PHASE

The mobile phase should be selected to prevent interference with the detection process. I chose the following recipe to run with only UV-detection:

50 mM phosphate buffer + 0.5 M NaCl

2.00 g NaH_2PO_4

8.94 g $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$

17.53 g NaCl

1.0 L Ultrapure

SAMPLE PREPARATION

Samples were run without any preparation. However, the following sampling schedule was followed:

3 UP
1 Standard (containing 2 peaks)
Samples
2 UP

APPENDIX B
Additional Results Information

Calculations for Ozone Oxidation Tables

Time Interval = Measured with a Timer

mL titrant = Measured through Titration

$\text{mg O}_3 \text{ in Trap} = 24 * 0.005 * \text{mL titrant (equation from Standard Methods)}$

$\text{mg O}_3/\text{min} = \text{mg O}_3 \text{ in trap}/\text{Time (min)}$

$\text{mg O}_3 \text{ reacted} = (\text{App. Rate (mg O}_3/\text{min)} - \text{mg O}_3/\text{min (each time step)}) * \text{time}$
(this column is a cumulative calculation so at each new time step, add the previous calculation to the new one)

$\text{mg O}_3/\text{mg TOC} = \text{mg O}_3 \text{ reacted} / (\text{Bulk Conc} * \text{Treated Volume}) = \text{mg O}_3/\text{mg TOC}$

Table 19: 0.5 mg O₃/mg TOC Oxidation Table						
	Time (min)	ml titrant	mg O₃ in trap	mg O₃/min	mg O₃ Reacted	mg O₃ mg TOC
App. Rate #1	11	20.3	2.44	0.22		Bulk conc.
App. Rate #2						3.612 mg/L
	10	6	0.72	0.07	1.49	0.207
	34	49	5.88	0.17	1.65	0.228
	15	19.5	2.34	0.16	0.98	0.136
	<u>59</u>				<u>4.13</u>	<u>0.57</u>
	0.98					
	Time (min)	ml titrant	mg O₃ in trap	mg O₃/min	mg O₃ Reacted	mg O₃ mg TOC
App. Rate #1	30	56	6.72	0.22		Bulk conc.
App. Rate #2						3.427 mg/L
	45	56.5	6.78	0.15	3.30	0.481
	<u>45</u>				<u>3.30</u>	<u>0.48</u>
	0.75					
	Time (min)	ml titrant	mg O₃ in trap	mg O₃/min	mg O₃ Reacted	mg O₃ mg TOC
App. Rate #1	30	56	6.72	0.224		Bulk conc.
App. Rate #2						3.427 mg/L
	52.5	66	7.92	0.151	3.84	0.560
	<u>52.5</u>				<u>3.84</u>	<u>0.56</u>

Table 20: 1 mg O₃/mg TOC Oxidation Table

	Time (min)	ml titrant	mg O ₃ in Trap	mg O ₃ /min	mg O ₃ reacted	mg O ₃ mg TOC
App. Rate #1	15	25.5	3.06	0.20		Bulk conc.
App. Rate #2	15	42	5.04	0.34		3.824 mg/L
	16.5	19	2.28	0.14	1.09	0.142
	18.75	25.7	3.08	0.16	0.74	0.097
	19.5	29.1	3.49	0.18	0.49	0.064
	16.5	25	3.00	0.18	0.37	0.048
	8.75	14.1	1.69	0.19	0.09	0.012
	15	27.87	3.34	0.22	1.70	0.222
	15.5	34.6	4.15	0.27	1.06	0.138
	11	26.5	3.18	0.29	0.52	0.067
	15	26.6	3.19	0.21	1.85	0.242
	136.5				7.89	1.03
	2.28					
	Time (min)	ml titrant	mg O ₃ in Trap	mg O ₃ /min	mg O ₃ reacted	mg O ₃ mg TOC
App. Rate #1	14	29.4	3.53	0.25		Bulk conc.
App. Rate #2						3.770 mg/L
	28	52	6.24	0.22	0.82	0.108
	28	53	6.36	0.23	0.70	0.092
	28	44.1	5.29	0.19	1.76	0.234
	33	51.7	6.20	0.19	2.11	0.280
	28.5	44.8	5.38	0.19	1.81	0.240
	12.5	20.4	2.45	0.20	0.70	0.093
	158				7.90	1.05
	2.63					
	Time (min)	ml titrant	mg O ₃ in Trap	mg O ₃ /min	mg O ₃ reacted	mg O ₃ mg TOC
App. Rate #1	27	64	7.68	0.284		Bulk conc.
App. Rate #2						3.612 mg/L
	45	94.5	11.34	0.252	1.46	0.202
	79	128	15.36	0.194	7.11	0.984
	124				8.57	1.19

Table 21: 2.0 mg O3/mg TOC Oxidation Table

	Time (min)	ml titrant	mg O3 in trap	mg O3/min	mg O3 reacted	mg O3 mg TOC
App. Rate #1	19	41.5	4.98	0.26		Bulk conc.
App. Rate #2						3.612 mg/L
	53	72.3	8.68	0.16	5.22	0.722
	45.5	69.2	8.30	0.18	3.62	0.501
	46.5	73.1	8.77	0.19	3.42	0.473
	20	35.6	4.27	0.21	0.97	0.134
	24.5	39	4.68	0.19	1.74	0.241
	189.5				14.96	2.07
	3.16					
	Time (min)	ml titrant	mg O3 in trap	mg O3/min	mg O3 reacted	mg O3 mg TOC
App. Rate #1	22	36.5	4.38	0.20		Bulk conc.
App. Rate #2	30	56	6.72	0.22		3.427 mg/L
	71	98.6	11.83	0.17	2.30	0.336
	50.5	56.8	6.82	0.13	3.24	0.472
	52	58.5	7.02	0.14	3.33	0.486
	41	72	8.64	0.21	0.54	0.079
	36	40.5	4.86	0.14	3.20	0.467
	250.5				12.62	1.84
	4.18					
	Time (min)	ml titrant	mg O3 in trap	mg O3/min	mg O3 reacted	mg O3 mg TOC
App. Rate #1	30	56	6.72	0.224		Bulk conc.
App. Rate #2						3.427 mg/L
	81	91.3	10.96	0.135	7.19	1.049
	57.5	59.9	7.19	0.125	5.69	0.830
	138.5				12.88	1.88

Table 22: Bulk Solution Raw Data Sorted by Date

Date	Sample	TOC (mg/L)	UV-254 (cm⁻¹)
1/31/00	Bulk	3824	0.157
	Hydrophilic	1856	
	Hydrophobic	2263	0.116
	Final BDOC	2788	
2/7/00	Bulk	4374	0.161
	Hydrophilic	1946	
	Hydrophobic	2363	0.105
	Final BDOC	2895	0.153
2/14/00	Bulk	3770	0.140
	Hydrophilic	1580	
	Hydrophobic	2346	0.108
	Final BDOC	2429	
2/28/00	Bulk	3413	0.148
	Hydrophilic	1616	
	Hydrophobic	---	---
	Final BDOC	---	2350
3/14/00	Bulk	3612	0.169
	Hydrophilic	1079	
	Hydrophobic	1954	0.119
	Final BDOC	2592.1	
4/3/00	Bulk	3427	0.180
	Hydrophilic	1459	
	Hydrophobic	1831.4	0.114
	Final BDOC	2459	0.138
4/10/00	Bulk	4220	0.168
	Hydrophilic	2349	
	Hydrophobic	2139	0.118
	Final BDOC	3006	

Table 23: Ozonated Raw Data Sorted by Date

Date	Sample	TOC (mg/L)	UV-254 (cm ⁻¹)
1/31/00	Bulk	3824	0.157
1 mg/mg	Oxidized Bulk	3829	0.088
	Hydrophilic	2895	
	Hydrophobic	1233	0.051
	Final BDOC	2340	
2/14/00	Bulk	3770	0.140
1 mg/mg	Oxidized Bulk	3522	0.078
	Hydrophilic	2707	
	Hydrophobic	781.5	0.034
	Final BDOC	2037	
3/14/00	Bulk	3612	0.169
1 mg/mg	Oxidized Bulk	3231	0.09
	Hydrophilic	2251	
	Hydrophobic	841	0.045
	Final BDOC	2071.1	
3/14/00	Bulk	3612	0.169
0.5 mg/mg	Oxidized Bulk	3569	0.124
	Hydrophilic	2065	
	Hydrophobic	1756	0.079
	Final BDOC	2905	
3/14/00	Bulk	3612	0.169
2 mg/mg	Oxidized Bulk	3157	0.069
	Hydrophilic	2488	
	Hydrophobic	633.5	0.033
	Final BDOC	2193	
4/3/00	Bulk	3427	0.180
2 mg/mg	Oxidized Bulk	2911	0.042
	Hydrophilic	2584	
	Hydrophobic	324.4	0.017
	Final BDOC	1754	0.061
4/3/00	Bulk	3427	0.180
2 mg/mg	Oxidized Bulk	3360	0.073
	Hydrophilic	2656	
	Hydrophobic	704	0.036
	Final BDOC	2228	0.074
4/3/00	Bulk	3427	0.180
0.5 mg/mg	Oxidized Bulk	3541	0.122
	Hydrophilic	1837	
	Hydrophobic	1704	0.048
	Final BDOC	2370	0.116
4/3/00	Bulk	3427	0.180
0.5 mg/mg	Oxidized Bulk	3431	0.114
	Hydrophilic	1947	
	Hydrophobic	1628	0.076
	Final BDOC	2370	0.094

Table 24- HPSEC Number Averaged and Weight Averaged MW Calculation Tables at 0.5 mg O3/mg TOC														
0.57					0.56					0.48				
Time	Height (hi)	Mwi	Mwi*hi	Mwi/hi	Time	Height (hi)	Mwi	Mwi*hi	Mwi/hi	Time	Height (hi)	Mwi	Mwi*hi	Mwi/hi
7.1	1363.0	1.32E+05	1.79E+08	0.0	7.1	795.0	1.32E+05	1.05E+08	0.0	7.1	2016	1.32E+05	2.65E+08	0.0
7.2	3631.0	1.27E+05	4.62E+08	0.0	7.2	1906.0	1.27E+05	2.42E+08	0.0	7.2	3207	1.27E+05	4.08E+08	0.0
7.3	5649.0	1.23E+05	6.93E+08	0.0	7.3	3168.0	1.23E+05	3.89E+08	0.0	7.3	4518	1.23E+05	5.54E+08	0.0
7.5	6599.0	1.18E+05	7.80E+08	0.1	7.5	4028.0	1.18E+05	4.76E+08	0.0	7.5	5379	1.18E+05	6.36E+08	0.0
7.6	6379.0	1.14E+05	7.26E+08	0.1	7.6	4078.0	1.14E+05	4.64E+08	0.0	7.6	5469	1.14E+05	6.22E+08	0.0
7.7	5500.0	1.09E+05	6.01E+08	0.1	7.7	3858.0	1.09E+05	4.22E+08	0.0	7.7	5309	1.09E+05	5.81E+08	0.0
7.9	5050.0	1.05E+05	5.30E+08	0.0	7.9	3838.0	1.05E+05	4.03E+08	0.0	7.9	5279	1.05E+05	5.54E+08	0.1
8.0	4650.0	1.00E+05	4.67E+08	0.0	8.0	3998.0	1.00E+05	4.02E+08	0.0	8.0	5499	1.00E+05	5.52E+08	0.1
8.1	4431.0	9.60E+04	4.25E+08	0.0	8.1	4109.0	9.60E+04	3.95E+08	0.0	8.1	5809	9.60E+04	5.58E+08	0.1
8.3	4480.0	9.16E+04	4.10E+08	0.0	8.3	4519.0	9.16E+04	4.14E+08	0.0	8.3	6149	9.16E+04	5.63E+08	0.1
8.4	4780.0	8.71E+04	4.16E+08	0.1	8.4	5120.0	8.71E+04	4.46E+08	0.1	8.4	6750	8.71E+04	5.88E+08	0.1
8.5	5250.0	8.27E+04	4.34E+08	0.1	8.5	5890.0	8.27E+04	4.87E+08	0.1	8.5	7641	8.27E+04	6.32E+08	0.1
8.7	5829.0	7.82E+04	4.56E+08	0.1	8.7	6801.0	7.82E+04	5.32E+08	0.1	8.7	8702	7.82E+04	6.81E+08	0.1
8.8	6609.0	7.38E+04	4.88E+08	0.1	8.8	7842.0	7.38E+04	5.79E+08	0.1	8.8	9882	7.38E+04	7.29E+08	0.1
8.9	7498.0	6.94E+04	5.20E+08	0.1	8.9	9133.0	6.94E+04	6.33E+08	0.1	8.9	11354	6.94E+04	7.87E+08	0.2
9.1	8577.0	6.49E+04	5.57E+08	0.1	9.1	10695.0	6.49E+04	6.94E+08	0.2	9.1	13105	6.49E+04	8.51E+08	0.2
9.2	9886.0	6.05E+04	5.98E+08	0.2	9.2	12417.0	6.05E+04	7.51E+08	0.2	9.2	14736	6.05E+04	8.91E+08	0.2
9.3	11254.0	5.60E+04	6.30E+08	0.2	9.3	14509.0	5.60E+04	8.13E+08	0.3	9.3	16878	5.60E+04	9.46E+08	0.3
9.5	12843.0	5.16E+04	6.62E+08	0.2	9.5	16711.0	5.16E+04	8.62E+08	0.3	9.5	19310	5.16E+04	9.96E+08	0.4
9.6	14581.0	4.71E+04	6.87E+08	0.3	9.6	19233.0	4.71E+04	9.07E+08	0.4	9.6	22042	4.71E+04	1.04E+09	0.5
9.7	16600.0	4.27E+04	7.09E+08	0.4	9.7	21966.0	4.27E+04	9.38E+08	0.5	9.7	25145	4.27E+04	1.07E+09	0.6
9.9	18868.0	3.82E+04	7.22E+08	0.5	9.9	25319.0	3.82E+04	9.68E+08	0.7	9.9	28537	3.82E+04	1.09E+09	0.7
10.0	21475.0	3.38E+04	7.26E+08	0.6	10.0	28863.0	3.38E+04	9.76E+08	0.9	10.0	32341	3.38E+04	1.09E+09	1.0
10.1	24363.0	3.21E+04	7.81E+08	0.8	10.1	32917.0	3.21E+04	1.06E+09	1.0	10.1	36654	3.21E+04	1.18E+09	1.1
10.3	27610.0	3.07E+04	8.49E+08	0.9	10.3	37641.0	3.07E+04	1.16E+09	1.2	10.3	41558	3.07E+04	1.28E+09	1.4
10.4	31366.0	2.94E+04	9.22E+08	1.1	10.4	43067.0	2.94E+04	1.27E+09	1.5	10.4	47062	2.94E+04	1.38E+09	1.6
10.5	35772.0	2.81E+04	1.00E+09	1.3	10.5	49363.0	2.81E+04	1.39E+09	1.8	10.5	53458	2.81E+04	1.50E+09	1.9
10.7	41197.0	2.67E+04	1.10E+09	1.5	10.7	56980.0	2.67E+04	1.52E+09	2.1	10.7	61244	2.67E+04	1.64E+09	2.3
10.8	48241.0	2.54E+04	1.23E+09	1.9	10.8	66480.0	2.54E+04	1.69E+09	2.6	10.8	70801	2.54E+04	1.80E+09	2.8
10.9	58012.0	2.41E+04	1.40E+09	2.4	10.9	78682.0	2.41E+04	1.89E+09	3.3	10.9	82951	2.41E+04	2.00E+09	3.4
11.1	71620.0	2.27E+04	1.63E+09	3.1	11.1	94037.0	2.27E+04	2.14E+09	4.1	11.1	98464	2.27E+04	2.24E+09	4.3
11.2	89714.0	2.14E+04	1.92E+09	4.2	11.2	112004.0	2.14E+04	2.40E+09	5.2	11.2	116408	2.14E+04	2.49E+09	5.4
11.3	109576.0	2.01E+04	2.20E+09	5.5	11.3	128380.0	2.01E+04	2.58E+09	6.4	11.3	132401	2.01E+04	2.66E+09	6.6
11.5	124243.0	1.87E+04	2.33E+09	6.6	11.5	135627.0	1.87E+04	2.54E+09	7.2	11.5	139006	1.87E+04	2.61E+09	7.4
11.6	124932.0	1.74E+04	2.18E+09	7.2	11.6	127519.0	1.74E+04	2.22E+09	7.3	11.6	129989	1.74E+04	2.26E+09	7.5
11.7	114541.0	1.61E+04	1.84E+09	7.1	11.7	109392.0	1.61E+04	1.76E+09	6.8	11.7	111514	1.61E+04	1.79E+09	6.9
11.9	98136.0	1.47E+04	1.45E+09	6.7	11.9	88751.0	1.47E+04	1.31E+09	6.0	11.9	91989	1.47E+04	1.36E+09	6.2
12.0	85817.0	1.34E+04	1.15E+09	6.4	12.0	71004.0	1.34E+04	9.52E+08	5.3	12.0	74555	1.34E+04	1.00E+09	5.6
12.1	82720.0	1.21E+04	9.99E+08	6.8	12.1	69122.0	1.21E+04	8.35E+08	5.7	12.1	73794	1.21E+04	8.91E+08	6.1
12.3	63527.0	1.07E+04	6.82E+08	5.9	12.3	50764.0	1.07E+04	5.45E+08	4.7	12.3	54598	1.07E+04	5.87E+08	5.1
12.4	51428.0	9.41E+03	4.84E+08	5.5	12.4	31465.0	9.41E+03	2.96E+08	3.3	12.4	34302	9.41E+03	3.23E+08	3.6
12.5	42057.0	8.08E+03	3.40E+08	5.2	12.5	24639.0	8.08E+03	1.99E+08	3.1	12.5	27126	8.08E+03	2.19E+08	3.4
12.7	34753.0	6.74E+03	2.34E+08	5.2	12.7	23798.0	6.74E+03	1.60E+08	3.5	12.7	26456	6.74E+03	1.78E+08	3.9
12.8	91912.0	5.41E+03	4.97E+08	17.0	12.8	73837.0	5.41E+03	3.99E+08	13.6	12.8	82211	5.41E+03	4.45E+08	15.2
12.9	123174.0	4.08E+03	5.02E+08	30.2	12.9	89412.0	4.08E+03	3.65E+08	21.9	12.9	98444	4.08E+03	4.01E+08	24.1
13.1	38780.0	2.74E+03	1.06E+08	14.1	13.1	22326.0	2.74E+03	6.12E+07	8.1	13.1	24454	2.74E+03	6.71E+07	8.9
13.2	12154.0	1.41E+03	1.71E+07	8.6	13.2	7162.0	1.41E+03	1.01E+07	5.1	13.2	7841	1.41E+03	1.11E+07	5.6
13.3	4301.0	7.70E+01	3.31E+05	55.9	13.3	4469.0	7.70E+01	3.44E+05	58.0	13.3	4858	7.70E+01	3.74E+05	63.1
	1821728.0		3.87E+10	214.4		1847634.0		4.20E+10	193.3		1987195		4.70E+10	208.4404
Number Ave.	8495.5				9558.2					9533.6				
Weight Ave.	21252				22751					23648				

Table 25: HPSEC Number Averaged and Weight Averaged MW Calculation Tables at 1.0 mg O3/mg TOC

1.03					1.05					1.19				
Time	Height (hi)	Mwi	Mwi*hi	Mwi/hi	Time	Height (hi)	Mwi	Mwi*hi	Mwi/hi	Time	Height (hi)	Mwi	Mwi*hi	Mwi/hi
7.2	1713.0	1.27E+05	2.18E+08	0.0	7.1	384.0	1.32E+05	5.05E+07	0.0	7.1	794.0	1.30E+05	1.04E+08	0.0
7.3	3271.0	1.24E+05	4.05E+08	0.0	7.2	1782.0	1.27E+05	2.27E+08	0.0	7.2	2532.0	1.27E+05	3.22E+08	0.0
7.5	4150.0	1.17E+05	4.86E+08	0.0	7.3	3030.0	1.23E+05	3.72E+08	0.0	7.3	4311.0	1.24E+05	5.34E+08	0.0
7.7	3780.0	1.10E+05	4.18E+08	0.0	7.5	3770.0	1.18E+05	4.46E+08	0.0	7.5	5310.0	1.17E+05	6.22E+08	0.0
7.8	3401.0	1.07E+05	3.64E+08	0.0	7.6	3909.0	1.14E+05	4.45E+08	0.0	7.6	5499.0	1.14E+05	6.26E+08	0.0
8.0	3201.0	1.00E+05	3.22E+08	0.0	7.7	3330.0	1.09E+05	3.64E+08	0.0	7.7	4940.0	1.10E+05	5.46E+08	0.0
8.2	3231.0	9.38E+04	3.03E+08	0.0	7.9	3090.0	1.05E+05	3.24E+08	0.0	7.9	4560.0	1.04E+05	4.73E+08	0.0
8.3	3451.0	9.05E+04	3.12E+08	0.0	8.0	2941.0	1.00E+05	2.95E+08	0.0	8.0	4400.0	1.00E+05	4.42E+08	0.0
8.5	3920.0	8.38E+04	3.28E+08	0.0	8.1	2831.0	9.60E+04	2.72E+08	0.0	8.1	4350.0	9.71E+04	4.23E+08	0.0
8.7	4410.0	7.71E+04	3.40E+08	0.1	8.3	2911.0	9.16E+04	2.67E+08	0.0	8.3	4490.0	9.05E+04	4.06E+08	0.0
8.8	5079.0	7.38E+04	3.75E+08	0.1	8.4	3120.0	8.71E+04	2.72E+08	0.0	8.4	4840.0	8.71E+04	4.22E+08	0.1
9.0	5948.0	6.71E+04	3.99E+08	0.1	8.5	3360.0	8.27E+04	2.78E+08	0.0	8.5	5280.0	8.38E+04	4.42E+08	0.1
9.2	6997.0	6.05E+04	4.23E+08	0.1	8.7	3660.0	7.82E+04	2.86E+08	0.0	8.7	5819.0	7.71E+04	4.49E+08	0.1
9.3	8265.0	5.71E+04	4.72E+08	0.1	8.8	4029.0	7.38E+04	2.97E+08	0.1	8.8	6509.0	7.38E+04	4.80E+08	0.1
9.5	9614.0	5.05E+04	4.85E+08	0.2	8.9	4419.0	6.94E+04	3.06E+08	0.1	8.9	7298.0	7.05E+04	5.14E+08	0.1
9.7	11252.0	4.38E+04	4.93E+08	0.3	9.1	4938.0	6.49E+04	3.21E+08	0.1	9.1	8217.0	6.38E+04	5.24E+08	0.1
9.8	13160.0	4.05E+04	5.33E+08	0.3	9.2	5507.0	6.05E+04	3.33E+08	0.1	9.2	9336.0	6.05E+04	5.65E+08	0.2
10.0	15368.0	3.34E+04	5.13E+08	0.5	9.3	6156.0	5.60E+04	3.45E+08	0.1	9.3	10515.0	5.71E+04	6.01E+08	0.2
10.2	17945.0	3.14E+04	5.64E+08	0.6	9.5	6895.0	5.16E+04	3.56E+08	0.1	9.5	11874.0	5.05E+04	5.99E+08	0.2
10.3	21251.0	3.04E+04	6.46E+08	0.7	9.6	7714.0	4.71E+04	3.64E+08	0.2	9.6	13342.0	4.71E+04	6.29E+08	0.3
10.5	25497.0	2.84E+04	7.24E+08	0.9	9.7	8663.0	4.27E+04	3.70E+08	0.2	9.7	15071.0	4.38E+04	6.60E+08	0.3
10.7	31480.0	2.64E+04	8.31E+08	1.2	9.9	9722.0	3.82E+04	3.72E+08	0.3	9.9	16979.0	3.71E+04	6.30E+08	0.5
10.8	39991.0	2.54E+04	1.02E+09	1.6	10.0	11010.0	3.38E+04	3.72E+08	0.3	10.0	19157.0	3.38E+04	6.48E+08	0.6
11.0	52317.0	2.34E+04	1.22E+09	2.2	10.1	12588.0	3.21E+04	4.04E+08	0.4	10.1	21695.0	3.24E+04	7.03E+08	0.7
11.2	69399.0	2.14E+04	1.49E+09	3.2	10.3	14425.0	3.07E+04	4.43E+08	0.5	10.3	24722.0	3.04E+04	7.52E+08	0.8
11.3	87938.0	2.04E+04	1.79E+09	4.3	10.4	16812.0	2.94E+04	4.94E+08	0.6	10.4	28328.0	2.94E+04	8.33E+08	1.0
11.5	97518.0	1.84E+04	1.80E+09	5.3	10.5	19918.0	2.81E+04	5.59E+08	0.7	10.5	32874.0	2.84E+04	9.34E+08	1.2
11.7	87119.0	1.64E+04	1.43E+09	5.3	10.7	24053.0	2.67E+04	6.43E+08	0.9	10.7	38599.0	2.64E+04	1.02E+09	1.5
11.8	65353.0	1.54E+04	1.01E+09	4.2	10.8	29675.0	2.54E+04	7.54E+08	1.2	10.8	46242.0	2.54E+04	1.18E+09	1.8
12.0	47922.0	1.34E+04	6.43E+08	3.6	10.9	37305.0	2.41E+04	8.98E+08	1.5	10.9	56403.0	2.44E+04	1.38E+09	2.3
12.2	42967.0	1.14E+04	4.90E+08	3.8	11.1	47652.0	2.27E+04	1.08E+09	2.1	11.1	69840.0	2.24E+04	1.57E+09	3.1
12.3	23669.0	1.04E+04	2.46E+08	2.3	11.2	60864.0	2.14E+04	1.30E+09	2.8	11.2	86125.0	2.14E+04	1.84E+09	4.0
12.5	15527.0	8.41E+03	1.31E+08	1.8	11.3	74906.0	2.01E+04	1.50E+09	3.7	11.3	102290.0	2.04E+04	2.09E+09	5.0
12.7	16287.0	6.41E+03	1.04E+08	2.5	11.5	84872.0	1.87E+04	1.59E+09	4.5	11.5	111832.0	1.84E+04	2.06E+09	6.1
12.8	55594.0	5.41E+03	3.01E+08	10.3	11.6	82316.0	1.74E+04	1.43E+09	4.7	11.6	106716.0	1.74E+04	1.86E+09	6.1
13.0	42108.0	3.41E+03	1.44E+08	12.3	11.7	68584.0	1.61E+04	1.10E+09	4.3	11.7	90092.0	1.64E+04	1.48E+09	5.5
13.2	13190.0	1.41E+03	1.86E+07	9.4	11.9	52156.0	1.47E+04	7.69E+08	3.5	11.9	70180.0	1.44E+04	1.01E+09	4.9
13.3	8665.0	4.10E+02	3.55E+06	21.1	12.0	39782.0	1.34E+04	5.33E+08	3.0	12.0	52936.0	1.34E+04	7.10E+08	3.9
0.0	0.0	1.00E+00	0.00E+00	0.0	12.1	38534.0	1.21E+04	4.65E+08	3.2	12.1	47701.0	1.24E+04	5.92E+08	3.8
0.0	0.0	1.00E+00	0.00E+00	0.0	12.3	26340.0	1.07E+04	2.83E+08	2.5	12.3	33514.0	1.04E+04	3.49E+08	3.2
0.0	0.0	1.00E+00	0.00E+00	0.0	12.4	14715.0	9.41E+03	1.38E+08	1.6	12.4	19566.0	9.41E+03	1.84E+08	2.1
0.0	0.0	1.00E+00	0.00E+00	0.0	12.5	11879.0	8.08E+03	9.59E+07	1.5	12.5	15580.0	8.41E+03	1.31E+08	1.9
0.0	0.0	1.00E+00	0.00E+00	0.0	12.7	14855.0	6.74E+03	1.00E+08	2.2	12.7	15270.0	6.41E+03	9.79E+07	2.4
0.0	0.0	1.00E+00	0.00E+00	0.0	12.8	44945.0	5.41E+03	2.43E+08	8.3	12.8	40947.0	5.41E+03	2.22E+08	7.6
0.0	0.0	1.00E+00	0.00E+00	0.0	12.9	55491.0	4.08E+03	2.26E+08	13.6	12.9	46162.0	4.41E+03	2.04E+08	10.5
0.0	0.0	1.00E+00	0.00E+00	0.0	13.1	22465.0	2.74E+03	6.16E+07	8.2	13.1	6439.0	2.41E+03	1.55E+07	2.7
0.0	0.0	1.00E+00	0.00E+00	0.0	13.2	10750.0	1.41E+03	1.52E+07	7.6	0.0	0.0	1.33E+05	0.00E+00	0.0
0.0	0.0	1.00E+00	0.00E+00	0.0	13.3	8963.0	7.70E+01	6.90E+05	116.4	0.0	0.0	1.33E+05	0.00E+00	0.0
0.0	971948.0		2.18E+10	98.7		1022016.0		2.25E+10	201.3		1339476.0		3.29E+10	85.1
Number Ave. 9849.63					5076.395					15746.8				
Weight Ave. 22418.6					21994.55					24532.7				

Table 26: HPSEC Number Averaged and Weight Averaged MW Calculation Tables at 2.0 mg O3/mg TOC									
2.07					1.88				
Time	Height (hi)	Mwi	Mwi*hi	wi/hi	Time	Height (hi)	Mwi	Mwi*hi	Mwi/hi
7.1	764.0	1.32E+05	1.01E+08	0.0	7.1	615.0	1.32E+05	8.09E+07	0.0
7.2	1803.0	1.27E+05	2.29E+08	0.0	7.2	935.0	1.27E+05	1.19E+08	0.0
7.3	2842.0	1.23E+05	3.49E+08	0.0	7.3	1556.0	1.23E+05	1.91E+08	0.0
7.5	3501.0	1.18E+05	4.14E+08	0.0	7.5	2166.0	1.18E+05	2.56E+08	0.0
7.6	3851.0	1.14E+05	4.38E+08	0.0	7.6	2376.0	1.14E+05	2.70E+08	0.0
7.7	3431.0	1.09E+05	3.75E+08	0.0	7.7	2146.0	1.09E+05	2.35E+08	0.0
7.9	3202.0	1.05E+05	3.36E+08	0.0	7.9	1806.0	1.05E+05	1.89E+08	0.0
8.0	3042.0	1.00E+05	3.06E+08	0.0	8.0	1886.0	1.00E+05	1.89E+08	0.0
8.1	2952.0	9.60E+04	2.83E+08	0.0	8.1	1886.0	9.60E+04	1.81E+08	0.0
8.3	2982.0	9.16E+04	2.73E+08	0.0	8.3	2156.0	9.16E+04	1.97E+08	0.0
8.4	3052.0	8.71E+04	2.66E+08	0.0	8.4	2016.0	8.71E+04	1.76E+08	0.0
8.5	3272.0	8.27E+04	2.71E+08	0.0	8.5	2476.0	8.27E+04	2.05E+08	0.0
8.7	3481.0	7.82E+04	2.72E+08	0.0	8.7	2666.0	7.82E+04	2.09E+08	0.0
8.8	3721.0	7.38E+04	2.75E+08	0.1	8.8	3287.0	7.38E+04	2.43E+08	0.0
8.9	4061.0	6.94E+04	2.82E+08	0.1	8.9	3747.0	6.94E+04	2.60E+08	0.1
9.1	4510.0	6.49E+04	2.93E+08	0.1	9.1	4157.0	6.49E+04	2.70E+08	0.1
9.2	4970.0	6.05E+04	3.01E+08	0.1	9.2	4788.0	6.05E+04	2.90E+08	0.1
9.3	5519.0	4.01E+04	2.21E+08	0.1	9.3	5848.0	5.60E+04	3.28E+08	0.1
9.5	6129.0	3.87E+04	2.37E+08	0.2	9.5	6549.0	5.16E+04	3.38E+08	0.1
9.6	6838.0	3.74E+04	2.56E+08	0.2	9.6	7599.0	4.71E+04	3.58E+08	0.2
9.7	7618.0	3.61E+04	2.75E+08	0.2	9.7	9020.0	4.27E+04	3.85E+08	0.2
9.9	8567.0	3.47E+04	2.98E+08	0.2	9.9	10531.0	3.82E+04	4.03E+08	0.3
10.0	9686.0	3.34E+04	3.24E+08	0.3	10.0	12192.0	3.38E+04	4.12E+08	0.4
10.1	11084.0	3.21E+04	3.56E+08	0.3	10.1	14234.0	3.21E+04	4.57E+08	0.4
10.3	12843.0	3.07E+04	3.95E+08	0.4	10.3	16395.0	3.07E+04	5.04E+08	0.5
10.4	15241.0	2.94E+04	4.48E+08	0.5	10.4	19347.0	2.94E+04	5.69E+08	0.7
10.5	18358.0	2.81E+04	5.15E+08	0.7	10.5	23410.0	2.81E+04	6.57E+08	0.8
10.7	22604.0	2.67E+04	6.04E+08	0.8	10.7	28493.0	2.67E+04	7.62E+08	1.1
10.8	28458.0	2.54E+04	7.23E+08	1.1	10.8	35257.0	2.54E+04	8.96E+08	1.4
10.9	36711.0	2.41E+04	8.84E+08	1.5	10.9	44393.0	2.41E+04	1.07E+09	1.8
11.1	48230.0	2.27E+04	1.10E+09	2.1	11.1	56481.0	2.27E+04	1.28E+09	2.5
11.2	63436.0	2.14E+04	1.36E+09	3.0	11.2	71661.0	2.14E+04	1.53E+09	3.3
11.3	80301.0	2.01E+04	1.61E+09	4.0	11.3	87712.0	2.01E+04	1.76E+09	4.4
11.5	93699.0	1.87E+04	1.76E+09	5.0	11.5	99209.0	1.87E+04	1.86E+09	5.3
11.6	94208.0	1.74E+04	1.64E+09	5.4	11.6	96847.0	1.74E+04	1.69E+09	5.6
11.7	80191.0	1.61E+04	1.29E+09	5.0	11.7	80487.0	1.61E+04	1.29E+09	5.0
11.9	58881.0	1.47E+04	8.68E+08	4.0	11.9	59473.0	1.47E+04	8.77E+08	4.0
12.0	43325.0	1.34E+04	5.81E+08	3.2	12.0	44754.0	1.34E+04	6.00E+08	3.3
12.1	39768.0	1.21E+04	4.80E+08	3.3	12.1	45144.0	1.21E+04	5.45E+08	3.7
12.3	26950.0	1.07E+04	2.90E+08	2.5	12.3	30724.0	1.07E+04	3.30E+08	2.9
12.4	17588.0	9.41E+03	1.66E+08	1.9	12.4	17446.0	9.41E+03	1.64E+08	1.9
12.5	14172.0	8.08E+03	1.14E+08	1.8	12.5	13974.0	8.08E+03	1.13E+08	1.7
12.7	12783.0	6.74E+03	8.62E+07	1.9	12.7	14064.0	6.74E+03	9.48E+07	2.1
12.8	34343.0	5.41E+03	1.86E+08	6.3	12.8	45644.0	5.41E+03	2.47E+08	8.4
12.9	37940.0	4.08E+03	1.55E+08	9.3	12.9	56101.0	4.08E+03	2.29E+08	13.8
13.1	4151.0	2.74E+03	1.14E+07	1.5	13.1	13703.0	2.74E+03	3.76E+07	5.0
0.0	0.0	1.33E+05	0.00E+00	0.0	13.2	4467.0	1.41E+03	6.30E+06	3.2
0.0	0.0	1.33E+05	0.00E+00	0.0	13.3	3147.0	7.70E+01	2.42E+05	40.9
	995059.0		2.23E+10	67.5		1114971.0		2.34E+10	125.4
Number Ave.	14744				Number Ave.	8889.3			
Weight Ave.	22399				Weight Ave.	20952			

Table 27: Chlorinated Raw Data Sorted by Date

Date	Sample	TOC (mg/L)	UV-254 (cm⁻¹)
1/31/00	Bulk	3824	0.157
0.5 ppm	Oxidized Bulk	3788	0.155
	Hydrophilic	1750	
	Hydrophobic	2074	0.132
	Final BDOC	2868	
2/7/00	Bulk	4374	0.161
4 ppm	Oxidized Bulk	4197	0.119
	Hydrophilic	2420	
	Hydrophobic	1769	0.073
	Final BDOC	2444	0.136
2/14/00	Bulk	4374	0.161
4 ppm	Oxidized Bulk	4120	0.117
	Hydrophilic	2540	
	Hydrophobic	1216	0.050
	Final BDOC	2531.5	
2/14/00	Bulk	3770	0.140
0.5ppm	Oxidized Bulk	3691	0.144
	Hydrophilic	1487	
	Hydrophobic	2056	0.109
	Final BDOC	2608	
3/14/00	Bulk	3612	0.169
4ppm	Oxidized Bulk	3369	0.125
	Hydrophilic	2062	
	Hydrophobic	1353	0.066
	Final BDOC	2457.6	
2/28/00	Bulk	3413	0.148
0.5ppm	Oxidized Bulk	3346	0.140
	Hydrophilic	1333	
	Hydrophobic	1655	0.107
	Final BDOC	2357	

Table 29: HPSEC Number Averaged and Weight Averaged MW Calculation Tables at 4.0 mg/L C12															
Time	Height (hi)	Mwi	Mwi*hi	MWi/hi	Time	Height (hi)	Mwi	Mwi*hi	MWi/hi	Time	Height (hi)	Mwi	Mwi*hi	MWi/hi	
7.1	1223.0	1.29E+05	1.58E+08	0.0	6.9	414.0	1.36E+05	5.63E+07	0.0	7.1	3761.0	1.32E+05	4.95E+08	0.0	
7.3	2621.0	1.25E+05	3.29E+08	0.0	7.1	1913.0	1.32E+05	2.52E+08	0.0	7.2	6617.0	1.27E+05	8.41E+08	0.1	
7.4	3879.0	1.22E+05	4.72E+08	0.0	7.2	3721.0	1.27E+05	4.73E+08	0.0	7.3	8196.0	1.23E+05	1.01E+09	0.1	
7.5	4698.0	1.18E+05	5.56E+08	0.0	7.3	5070.0	1.23E+05	6.22E+08	0.0	7.5	8356.0	1.18E+05	9.88E+08	0.1	
7.6	4818.0	1.15E+05	5.53E+08	0.0	7.5	5729.0	1.18E+05	6.77E+08	0.0	7.6	7117.0	1.14E+05	8.10E+08	0.1	
7.7	3969.0	1.11E+05	4.41E+08	0.0	7.6	5110.0	1.14E+05	5.82E+08	0.0	7.7	5718.0	1.09E+05	6.25E+08	0.1	
7.8	3480.0	1.08E+05	3.74E+08	0.0	7.7	4150.0	1.09E+05	4.54E+08	0.0	7.9	4979.0	1.05E+05	5.22E+08	0.0	
7.9	3160.0	1.04E+05	3.29E+08	0.0	7.9	3661.0	1.05E+05	3.84E+08	0.0	8.0	4270.0	1.00E+05	4.29E+08	0.0	
8.0	2801.0	1.00E+05	2.81E+08	0.0	8.0	3231.0	1.00E+05	3.25E+08	0.0	8.1	3840.0	9.60E+04	3.69E+08	0.0	
8.1	2571.0	9.69E+04	2.49E+08	0.0	8.1	2942.0	9.60E+04	2.82E+08	0.0	8.3	3621.0	9.16E+04	3.32E+08	0.0	
8.2	2431.0	9.34E+04	2.27E+08	0.0	8.3	2902.0	9.16E+04	2.66E+08	0.0	8.4	3611.0	8.71E+04	3.15E+08	0.0	
8.3	2441.0	8.98E+04	2.19E+08	0.0	8.4	3012.0	8.71E+04	2.62E+08	0.0	8.5	3780.0	8.27E+04	3.13E+08	0.0	
8.4	2521.0	8.62E+04	2.17E+08	0.0	8.5	3261.0	8.27E+04	2.70E+08	0.0	8.7	4030.0	7.82E+04	3.15E+08	0.1	
8.5	2691.0	8.27E+04	2.23E+08	0.0	8.7	3641.0	7.82E+04	2.85E+08	0.0	8.8	4430.0	7.38E+04	3.27E+08	0.1	
8.6	2911.0	7.91E+04	2.30E+08	0.0	8.8	4130.0	7.38E+04	3.05E+08	0.1	8.9	4899.0	6.94E+04	3.40E+08	0.1	
8.7	3200.0	7.56E+04	2.42E+08	0.0	8.9	4770.0	6.94E+04	3.31E+08	0.1	9.1	5718.0	6.49E+04	3.71E+08	0.1	
8.9	3560.0	7.20E+04	2.56E+08	0.0	9.1	5569.0	6.49E+04	3.61E+08	0.1	9.2	6508.0	6.05E+04	3.94E+08	0.1	
9.0	4019.0	4.38E+04	1.76E+08	0.1	9.2	6488.0	6.05E+04	3.92E+08	0.1	9.3	7447.0	5.60E+04	4.17E+08	0.1	
9.1	4568.0	4.27E+04	1.95E+08	0.1	9.3	7627.0	5.60E+04	4.27E+08	0.1	9.5	8555.0	5.16E+04	4.41E+08	0.2	
9.2	5228.0	4.17E+04	2.18E+08	0.1	9.5	8926.0	5.16E+04	4.60E+08	0.2	9.6	9934.0	4.71E+04	4.68E+08	0.2	
9.3	6007.0	4.06E+04	2.44E+08	0.1	9.6	10594.0	4.71E+04	4.99E+08	0.2	9.7	11632.0	4.27E+04	4.97E+08	0.3	
9.4	6845.0	3.95E+04	2.71E+08	0.2	9.7	12602.0	4.27E+04	5.38E+08	0.3	9.9	13800.0	3.82E+04	5.28E+08	0.4	
9.5	7834.0	3.85E+04	3.01E+08	0.2	9.9	14940.0	3.82E+04	5.71E+08	0.4	10.0	16507.0	3.38E+04	5.58E+08	0.5	
9.6	8953.0	3.74E+04	3.35E+08	0.2	10.0	17787.0	3.38E+04	6.01E+08	0.5	10.1	19774.0	2.94E+04	5.80E+08	0.7	
9.7	10231.0	3.63E+04	3.72E+08	0.3	10.1	21274.0	2.94E+04	6.25E+08	0.7	10.3	23789.0	2.49E+04	5.93E+08	1.0	
9.8	11819.0	3.53E+04	4.17E+08	0.3	10.3	25450.0	2.49E+04	6.34E+08	1.0	10.4	28564.0	2.05E+04	5.85E+08	1.4	
9.9	13626.0	3.42E+04	4.66E+08	0.4	10.4	30495.0	2.05E+04	6.24E+08	1.5	10.5	34408.0	1.60E+04	5.51E+08	2.1	
10.0	15784.0	3.31E+04	5.23E+08	0.5	10.5	36549.0	1.60E+04	5.86E+08	2.3	10.7	41670.0	1.16E+04	4.82E+08	3.6	
10.1	18340.0	3.21E+04	5.88E+08	0.6	10.7	43972.0	1.16E+04	5.09E+08	3.8	10.8	50911.0	7.13E+03	3.63E+08	7.1	
10.2	21316.0	3.10E+04	6.61E+08	0.7	10.8	53383.0	7.13E+03	3.81E+08	7.5	10.9	62978.0	2.41E+04	1.52E+09	2.6	
10.3	24802.0	2.99E+04	7.43E+08	0.8	10.9	65611.0	2.41E+04	1.58E+09	2.7	11.1	78801.0	2.27E+04	1.79E+09	3.5	
10.5	28826.0	2.89E+04	8.32E+08	1.0	11.1	81465.0	2.27E+04	1.85E+09	3.6	11.2	98180.0	2.14E+04	2.10E+09	4.6	
10.6	33610.0	2.78E+04	9.35E+08	1.2	11.2	100777.0	2.14E+04	2.16E+09	4.7	11.3	116861.0	2.01E+04	2.35E+09	5.8	
10.7	39292.0	2.67E+04	1.05E+09	1.5	11.3	119169.0	2.01E+04	2.39E+09	5.9	11.5	127359.0	1.87E+04	2.39E+09	6.8	
10.8	46253.0	2.57E+04	1.19E+09	1.8	11.5	128720.0	1.87E+04	2.41E+09	6.9	11.6	123653.0	1.74E+04	2.15E+09	7.1	
10.9	54692.0	2.46E+04	1.35E+09	2.2	11.6	123924.0	1.74E+04	2.16E+09	7.1	11.7	112655.0	1.61E+04	1.81E+09	7.0	
11.0	65168.0	2.35E+04	1.53E+09	2.8	11.7	112126.0	1.61E+04	1.80E+09	7.0	11.9	99209.0	1.47E+04	1.46E+09	6.7	
11.1	77951.0	2.25E+04	1.75E+09	3.5	11.9	98249.0	1.47E+04	1.45E+09	6.7	12.0	89350.0	1.34E+04	1.20E+09	6.7	
11.2	92492.0	2.14E+04	1.98E+09	4.3	12.0	88529.0	1.34E+04	1.19E+09	6.6	12.1	90978.0	1.21E+04	1.10E+09	7.5	
11.3	106623.0	2.03E+04	2.17E+09	5.2	12.1	90187.0	1.21E+04	1.09E+09	7.5	12.3	69771.0	1.07E+04	7.50E+08	6.5	
11.4	116730.0	1.93E+04	2.25E+09	6.1	12.3	68368.0	1.07E+04	7.34E+08	6.4	12.4	49692.0	9.41E+03	4.68E+08	5.3	
11.5	119047.0	1.82E+04	2.17E+09	6.5	12.4	48268.0	9.41E+03	4.54E+08	5.1	12.5	40542.0	8.08E+03	3.27E+08	5.0	
11.6	113434.0	1.71E+04	1.94E+09	6.6	12.5	39087.0	8.08E+03	3.16E+08	4.8	12.7	41351.0	6.74E+03	2.79E+08	6.1	
11.7	104466.0	1.61E+04	1.68E+09	6.5	12.7	40066.0	6.74E+03	2.70E+08	5.9	12.8	122135.0	5.41E+03	6.61E+08	22.6	
11.8	93041.0	1.50E+04	1.40E+09	6.2	12.8	115443.0	5.41E+03	6.25E+08	21.3	12.9	194229.0	4.08E+03	7.92E+08	47.6	
11.9	84722.0	1.39E+04	1.18E+09	6.1	12.9	183536.0	4.08E+03	7.48E+08	45.0	13.1	57673.0	2.74E+03	1.58E+08	21.0	
12.1	80967.0	1.29E+04	1.04E+09	6.3	13.1	53373.0	2.74E+03	1.46E+08	19.5	13.2	24459.0	1.41E+03	3.45E+07	17.3	
12.2	81317.0	1.18E+04	9.60E+08	6.9	13.2	22033.0	1.41E+03	3.11E+07	15.6	13.3	16257.0	7.70E+01	1.25E+06	211.1	
12.3	64209.0	1.07E+04	6.90E+08	6.0	13.3	14610.0	7.70E+01	1.12E+06	189.7						
12.4	47372.0	9.68E+03	4.58E+08	4.9											
12.5	38304.0	8.61E+03	3.30E+08	4.4											
12.6	34738.0	7.54E+03	2.62E+08	4.6											
12.7	43087.0	6.48E+03	2.79E+08	6.7											
12.8	118747.0	5.41E+03	6.42E+08	21.9											
12.9	188615.0	4.34E+03	8.19E+08	43.4											
13.0	93281.0	3.28E+03	3.06E+08	28.5											
13.1	36107.0	2.21E+03	7.98E+07	16.3											
13.2	19299.0	1.14E+03	2.21E+07	16.9											
13.3	13197.0	7.70E+01	1.02E+06	171.4											
		2247934.0		4.02E+10	404.9			1.95E+06		3.44E+10	3.91E+02	4.70E+03		3.62E+10	419.5
Number Ave.	5551.89						4.97E+03					1.83E+04			
Weight Ave.	17867.8						1.77E+04								

Table 30: NH₂Cl Raw Data Sorted by Date

Date	Sample	TOC (mg/L)	UV-254 (cm⁻¹)
4/13/00	Bulk	3775	0.152
	Oxidized Bulk	3989	0.176
	Hydrophilic	2156	
	Hydrophobic	1548	
	Final BDOC	3005	
4/13/00	Bulk	3775	0.152
	Oxidized Bulk	3929	0.157
	Hydrophilic	2132	
	Hydrophobic	1637	
	Final BDOC	2726	
4/13/00	Bulk	3775	0.152
	Oxidized Bulk	3992	0.162
	Hydrophilic	2504	
	Hydrophobic	1656	0.050
	Final BDOC	2770	

Table 31: HPSEC Number Averaged and Weight Averaged MW Calculation Tables for Monochloramine Oxidation										
Time	Height (hi)	Mwi	Mwi*hi	Mwi/hi	Time	Height (hi)	Mwi	Mwi*hi	Mwi/hi	
7.3	3.40E+01	1.23E+05	4.17E+06	0.0	9.5	1.29E+03	5.16E+04	6.67E+07	0.0	
7.5	1.11E+03	1.18E+05	1.32E+08	0.0	9.6	3.46E+03	4.71E+04	1.63E+08	0.1	
7.6	1.50E+03	1.14E+05	1.71E+08	0.0	9.7	6.59E+03	4.27E+04	2.81E+08	0.2	
7.7	1.63E+03	1.09E+05	1.79E+08	0.0	9.9	9.98E+03	3.82E+04	3.81E+08	0.3	
7.9	1.84E+03	1.05E+05	1.93E+08	0.0	10.0	1.37E+04	3.38E+04	4.61E+08	0.4	
8.0	2.49E+03	1.00E+05	2.50E+08	0.0	10.1	1.80E+04	3.21E+04	5.76E+08	0.6	
8.1	2.14E+03	9.60E+04	2.06E+08	0.0	10.3	2.29E+04	3.07E+04	7.04E+08	0.7	
8.3	2.55E+03	9.16E+04	2.34E+08	0.0	10.4	2.82E+04	2.94E+04	8.31E+08	1.0	
8.4	2.87E+03	8.71E+04	2.50E+08	0.0	10.5	3.46E+04	2.81E+04	9.72E+08	1.2	
8.5	3.40E+03	8.27E+04	2.81E+08	0.0	10.7	4.15E+04	2.67E+04	1.11E+09	1.6	
8.7	4.00E+03	7.82E+04	3.13E+08	0.1	10.8	4.87E+04	2.54E+04	1.24E+09	1.9	
8.8	4.78E+03	7.38E+04	3.53E+08	0.1	10.9	5.82E+04	2.41E+04	1.40E+09	2.4	
8.9	6.10E+03	6.94E+04	4.23E+08	0.1	11.1	6.99E+04	2.27E+04	1.59E+09	3.1	
9.1	7.19E+03	6.49E+04	4.67E+08	0.1	11.2	8.37E+04	2.14E+04	1.79E+09	3.9	
9.2	9.07E+03	6.05E+04	5.48E+08	0.1	11.3	9.68E+04	2.01E+04	1.94E+09	4.8	
9.3	1.06E+04	5.60E+04	5.92E+08	0.2	11.5	1.03E+05	1.87E+04	1.93E+09	5.5	
9.5	1.26E+04	5.16E+04	6.48E+08	0.2	11.6	9.72E+04	1.74E+04	1.69E+09	5.6	
9.6	1.49E+04	4.71E+04	7.00E+08	0.3	11.7	8.64E+04	1.61E+04	1.39E+09	5.4	
9.7	1.80E+04	4.27E+04	7.67E+08	0.4	11.9	7.55E+04	1.47E+04	1.11E+09	5.1	
9.9	2.15E+04	3.82E+04	8.23E+08	0.6	12.0	7.01E+04	1.34E+04	9.40E+08	5.2	
10.0	2.57E+04	3.38E+04	8.67E+08	0.8	12.1	8.75E+04	1.21E+04	1.06E+09	7.2	
10.1	3.01E+04	3.21E+04	9.67E+08	0.9	12.3	8.50E+04	1.07E+04	9.13E+08	7.9	
10.3	3.49E+04	3.07E+04	1.07E+09	1.1	12.4	4.89E+04	9.41E+03	4.60E+08	5.2	
10.4	4.08E+04	2.94E+04	1.20E+09	1.4	12.5	3.34E+04	8.08E+03	2.69E+08	4.1	
10.5	4.69E+04	2.81E+04	1.32E+09	1.7	12.7	3.23E+04	6.74E+03	2.18E+08	4.8	
10.7	5.36E+04	2.67E+04	1.43E+09	2.0	12.8	2.14E+05	5.41E+03	1.16E+09	39.6	
10.8	6.10E+04	2.54E+04	1.55E+09	2.4	12.9	3.34E+05	4.08E+03	1.36E+09	82.0	
10.9	6.99E+04	2.41E+04	1.68E+09	2.9	13.1	1.08E+05	2.74E+03	2.95E+08	39.3	
11.1	8.12E+04	2.27E+04	1.85E+09	3.6	13.2	7.24E+02	1.41E+03	1.02E+06	0.5	
11.2	9.50E+04	2.14E+04	2.03E+09	4.4	10.8	6.68E+04	2.54E+04	1.70E+09	2.6	
11.3	1.08E+05	2.01E+04	2.17E+09	5.4	10.9	7.40E+04	2.41E+04	1.78E+09	3.1	
11.5	1.14E+05	1.87E+04	2.14E+09	6.1	11.1	8.31E+04	2.27E+04	1.89E+09	3.7	
11.6	1.09E+05	1.74E+04	1.90E+09	6.3	11.2	9.36E+04	2.14E+04	2.00E+09	4.4	
11.7	9.78E+04	1.61E+04	1.57E+09	6.1						
11.9	8.68E+04	1.47E+04	1.28E+09	5.9						
12.0	8.14E+04	1.34E+04	1.09E+09	6.1						
12.1	9.81E+04	1.21E+04	1.19E+09	8.1						
12.3	9.56E+04	1.07E+04	1.03E+09	8.9						
12.4	6.03E+04	9.41E+03	5.67E+08	6.4						
12.5	4.51E+04	8.08E+03	3.64E+08	5.6						
12.7	4.34E+04	6.74E+03	2.92E+08	6.4						
12.8	2.03E+05	5.41E+03	1.10E+09	37.5						
12.9	3.12E+05	4.08E+03	1.27E+09	76.5						
13.1	1.08E+05	2.74E+03	2.96E+08	39.4						
13.2	1.17E+04	1.41E+03	1.65E+07	8.3						
	2.24E+06		3.78E+10	256.5			2.23E+06	3.37E+10	253.3	
Number Ave. 8738.16					8808.7659					
Weight Ave. 16852.7					15095.727					

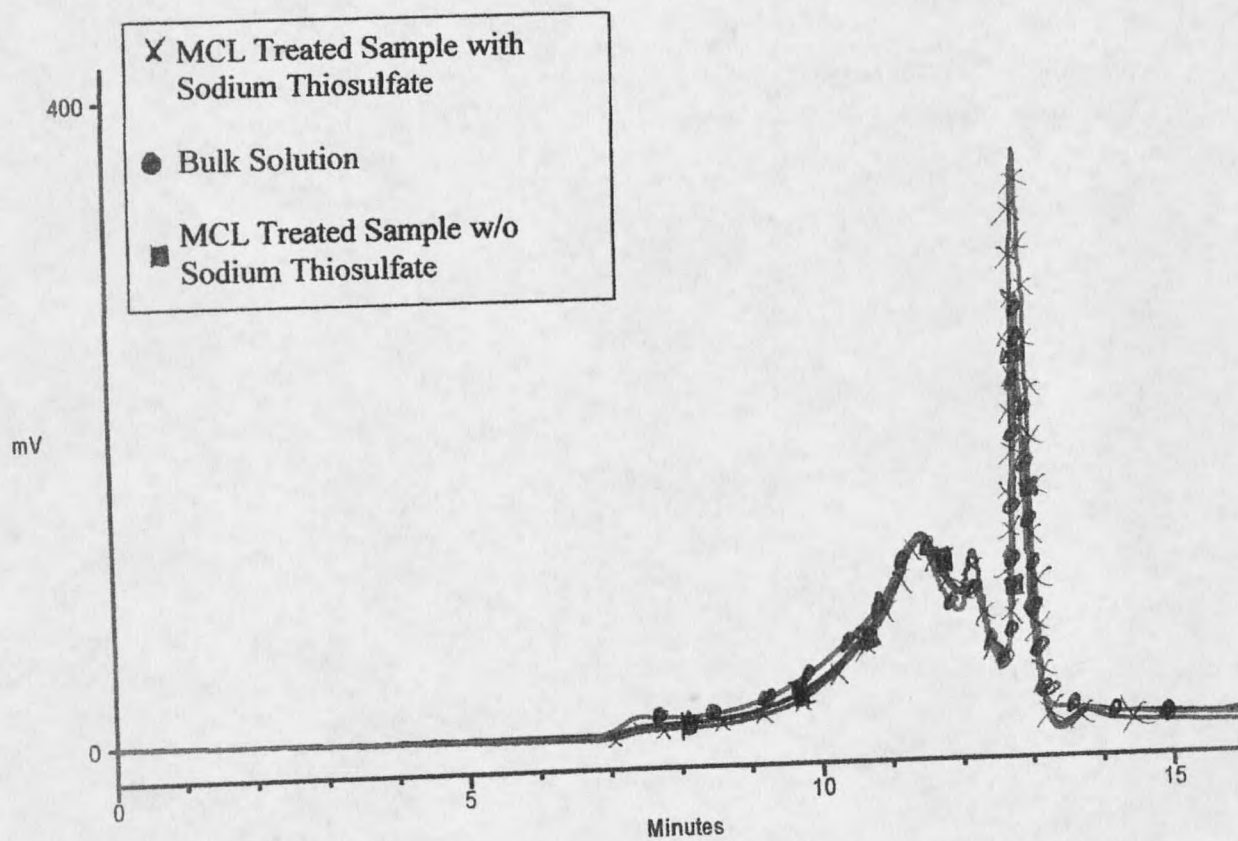


Table 32: UV-light Raw Data Sorted by Date

Date	Sample	TOC (mg/L)	UV-254 (cm ⁻¹)
2/7/00	Bulk	4374	0.161
0.5x	Oxidized Bulk	4544	0.156
	Hydrophilic	1879	
	Hydrophobic	2427	0.111
	Final BDOC	2729	
2/28/00	Bulk	3413	0.148
1x	Oxidized Bulk	3314	0.15
	Hydrophilic	1527	
	Hydrophobic	1549	0.091
	Final BDOC	2298	
2/28/00	Bulk	3413	0.148
1x	Oxidized Bulk	3389	0.156
	Hydrophilic	1308	
	Hydrophobic	1286	0.07
	Final BDOC	2241	
4/3/00	Bulk	3427	0.18
1x	Oxidized Bulk	3757	0.153
	Hydrophilic	1703	
	Hydrophobic	1851	0.11
	Final BDOC	2840	
4/10/00	Bulk	4220	0.168
10x	Oxidized Bulk	4416	0.157
	Hydrophilic	2397	
	Hydrophobic	2222	0.101
	Final BDOC	2963	
4/10/00	Bulk	4220	0.168
10x	Oxidized Bulk	4448	0.157
	Hydrophilic	2544	
	Hydrophobic	2200	0.063
	Final BDOC	3224	
4/10/00	Bulk	4220	0.168
10x	Oxidized Bulk	4387	0.152
	Hydrophilic	2489	
	Hydrophobic	2086	0.098
	Final BDOC	3148	
4/13/00	Bulk	4220	0.168
50x	Oxidized Bulk	4163	0.12
	Hydrophilic	3005	
	Hydrophobic	1333	
	Final BDOC	2309	
4/13/00	Bulk	4220	0.168
50x	Oxidized Bulk	3956	0.12
	Hydrophilic	2945	
	Hydrophobic	1443	
	Final BDOC	1816	
4/13/00	Bulk	4220	0.168
50x	Oxidized Bulk	4095	0.121
	Hydrophilic	2888	
	Hydrophobic	1119	
	Final BDOC	1970	

Table 33: HPSEC Number Averaged and Weight Averaged MW Calculation Tables 1X UV-light Treatment														
Time	Height (hi)	Mwi	Mwi*hi	Mwi/hi	Time	Height (hi)	Mwi	Mwi*hi	Mwi/hi	Time	Height (hi)	Mwi	Mwi*hi	Mwi/hi
7.1	2.17E+03	1.32E+05	2.86E+08	0.0	7.1	8.24E+02	1.32E+05	1.08E+08	0.0	7.1	1.10E+03	1.32E+05	1.45E+08	0.0
7.2	4.39E+03	1.27E+05	5.58E+08	0.0	7.2	2.89E+03	1.27E+05	3.68E+08	0.0	7.2	4.00E+03	1.27E+05	5.09E+08	0.0
7.3	5.86E+03	1.23E+05	7.19E+08	0.0	7.3	4.43E+03	1.23E+05	5.44E+08	0.0	7.3	6.71E+03	1.23E+05	8.23E+08	0.1
7.5	6.31E+03	1.18E+05	7.46E+08	0.1	7.5	4.84E+03	1.18E+05	5.72E+08	0.0	7.5	8.27E+03	1.18E+05	9.78E+08	0.1
7.6	6.02E+03	1.14E+05	6.85E+08	0.1	7.6	4.68E+03	1.14E+05	5.33E+08	0.0	7.6	8.02E+03	1.14E+05	9.13E+08	0.1
7.7	5.58E+03	1.09E+05	6.10E+08	0.1	7.7	4.40E+03	1.09E+05	4.81E+08	0.0	7.7	7.34E+03	1.09E+05	8.03E+08	0.1
7.9	5.26E+03	1.05E+05	5.52E+08	0.1	7.9	4.14E+03	1.05E+05	4.35E+08	0.0	7.9	7.05E+03	1.05E+05	7.40E+08	0.1
8.0	5.07E+03	1.00E+05	5.09E+08	0.1	8.0	3.94E+03	1.00E+05	3.96E+08	0.0	8.0	6.72E+03	1.00E+05	6.75E+08	0.1
8.1	4.99E+03	9.60E+04	4.79E+08	0.1	8.1	3.84E+03	9.60E+04	3.69E+08	0.0	8.1	6.60E+03	9.60E+04	6.34E+08	0.1
8.3	5.15E+03	9.16E+04	4.72E+08	0.1	8.3	4.01E+03	9.16E+04	3.67E+08	0.0	8.3	6.91E+03	9.16E+04	6.33E+08	0.1
8.4	5.52E+03	8.71E+04	4.81E+08	0.1	8.4	4.38E+03	8.71E+04	3.82E+08	0.1	8.4	7.56E+03	8.71E+04	6.59E+08	0.1
8.5	6.06E+03	8.27E+04	5.01E+08	0.1	8.5	4.91E+03	8.27E+04	4.06E+08	0.1	8.5	8.40E+03	8.27E+04	6.95E+08	0.1
8.7	6.79E+03	7.82E+04	5.31E+08	0.1	8.7	5.63E+03	7.82E+04	4.41E+08	0.1	8.7	9.54E+03	7.82E+04	7.46E+08	0.1
8.8	7.62E+03	7.38E+04	5.62E+08	0.1	8.8	6.48E+03	7.38E+04	4.78E+08	0.1	8.8	1.09E+04	7.38E+04	8.01E+08	0.1
8.9	8.65E+03	6.94E+04	6.00E+08	0.1	8.9	7.52E+03	6.94E+04	5.21E+08	0.1	8.9	1.24E+04	6.94E+04	8.61E+08	0.2
9.1	9.87E+03	6.49E+04	6.41E+08	0.2	9.1	8.80E+03	6.49E+04	5.71E+08	0.1	9.1	1.42E+04	6.49E+04	9.19E+08	0.2
9.2	1.13E+04	6.05E+04	6.83E+08	0.2	9.2	1.03E+04	6.05E+04	6.20E+08	0.2	9.2	1.61E+04	6.05E+04	9.76E+08	0.3
9.3	1.29E+04	4.01E+04	5.17E+08	0.3	9.3	1.19E+04	5.60E+04	6.65E+08	0.2	9.3	1.83E+04	5.60E+04	1.02E+09	0.3
9.5	1.47E+04	3.87E+04	5.70E+08	0.4	9.5	1.37E+04	5.16E+04	7.04E+08	0.3	9.5	2.04E+04	5.16E+04	1.05E+09	0.4
9.6	1.67E+04	3.74E+04	6.25E+08	0.4	9.6	1.56E+04	4.71E+04	7.36E+08	0.3	9.6	2.24E+04	4.71E+04	1.06E+09	0.5
9.7	1.89E+04	3.61E+04	6.83E+08	0.5	9.7	1.79E+04	4.27E+04	7.63E+08	0.4	9.7	2.44E+04	4.27E+04	1.04E+09	0.6
9.9	2.14E+04	3.47E+04	7.42E+08	0.6	9.9	2.04E+04	3.82E+04	7.78E+08	0.5	9.9	2.63E+04	3.82E+04	1.01E+09	0.7
10.0	2.41E+04	3.34E+04	8.04E+08	0.7	10.0	2.31E+04	3.38E+04	7.80E+08	0.7	10.0	2.84E+04	3.38E+04	9.61E+08	0.8
10.1	2.72E+04	3.21E+04	8.72E+08	0.8	10.1	2.63E+04	2.94E+04	7.71E+08	0.9	10.1	3.11E+04	2.94E+04	9.12E+08	1.1
10.3	3.07E+04	3.07E+04	9.45E+08	1.0	10.3	2.98E+04	2.49E+04	7.43E+08	1.2	10.3	3.43E+04	2.49E+04	8.53E+08	1.4
10.4	3.48E+04	2.94E+04	1.02E+09	1.2	10.4	3.40E+04	2.05E+04	6.95E+08	1.7	10.4	3.82E+04	2.05E+04	7.81E+08	1.9
10.5	3.94E+04	2.81E+04	1.11E+09	1.4	10.5	3.87E+04	1.60E+04	6.20E+08	2.4	10.5	4.26E+04	1.60E+04	6.83E+08	2.7
10.7	4.47E+04	2.67E+04	1.19E+09	1.7	10.7	4.40E+04	1.16E+04	5.09E+08	3.8	10.7	4.78E+04	1.16E+04	5.54E+08	4.1
10.8	5.10E+04	2.54E+04	1.29E+09	2.0	10.8	5.03E+04	7.13E+03	3.58E+08	7.0	10.8	5.39E+04	7.13E+03	3.84E+08	7.6
10.9	5.88E+04	2.41E+04	1.42E+09	2.4	10.9	5.81E+04	2.69E+03	1.56E+08	21.6	10.9	6.14E+04	2.41E+04	1.48E+09	2.5
11.1	6.89E+04	2.27E+04	1.57E+09	3.0	11.1	6.80E+04	2.27E+04	1.55E+09	3.0	11.1	7.13E+04	2.27E+04	1.62E+09	3.1
11.2	8.11E+04	2.14E+04	1.74E+09	3.8	11.2	8.01E+04	2.14E+04	1.71E+09	3.7	11.2	8.34E+04	2.14E+04	1.79E+09	3.9
11.3	9.26E+04	2.01E+04	1.86E+09	4.6	11.3	9.16E+04	2.01E+04	1.84E+09	4.6	11.3	9.48E+04	2.01E+04	1.90E+09	4.7
11.5	9.91E+04	1.87E+04	1.86E+09	5.3	11.5	9.81E+04	1.87E+04	1.84E+09	5.2	11.5	1.01E+05	1.87E+04	1.89E+09	5.4
11.6	9.64E+04	1.74E+04	1.68E+09	5.5	11.6	9.56E+04	1.74E+04	1.66E+09	5.5	11.6	9.83E+04	1.74E+04	1.71E+09	5.6
11.7	8.96E+04	1.61E+04	1.44E+09	5.6	11.7	8.91E+04	1.61E+04	1.43E+09	5.5	11.7	9.23E+04	1.61E+04	1.48E+09	5.7
11.9	8.32E+04	1.47E+04	1.23E+09	5.6	11.9	8.27E+04	1.47E+04	1.22E+09	5.6	11.9	8.78E+04	1.47E+04	1.30E+09	6.0
12.0	7.81E+04	1.34E+04	1.05E+09	5.8	12.0	7.75E+04	1.34E+04	1.04E+09	5.8	12.0	8.38E+04	1.34E+04	1.12E+09	6.2
12.1	8.63E+04	1.21E+04	1.04E+09	7.1	12.1	8.58E+04	1.21E+04	1.04E+09	7.1	12.1	9.98E+04	1.21E+04	1.21E+09	8.3
12.3	7.08E+04	1.07E+04	7.60E+08	6.6	12.3	7.09E+04	1.07E+04	7.62E+08	6.6	12.3	9.48E+04	1.07E+04	1.02E+09	8.8
12.4	5.07E+04	9.41E+03	4.77E+08	5.4	12.4	5.06E+04	9.41E+03	4.76E+08	5.4	12.4	6.74E+04	9.41E+03	6.35E+08	7.2
12.5	4.11E+04	8.08E+03	3.32E+08	5.1	12.5	4.08E+04	8.08E+03	3.30E+08	5.1	12.5	5.10E+04	8.08E+03	4.12E+08	6.3
12.7	4.02E+04	6.74E+03	2.71E+08	6.0	12.7	3.99E+04	6.74E+03	2.69E+08	5.9	12.7	4.77E+04	6.74E+03	3.21E+08	7.1
12.8	1.32E+05	5.41E+03	7.12E+08	24.3	12.8	1.29E+05	5.41E+03	6.99E+08	23.9	12.8	1.44E+05	5.41E+03	7.77E+08	26.6
12.9	2.52E+05	4.08E+03	1.03E+09	61.7	12.9	2.57E+05	4.08E+03	1.05E+09	63.2	12.9	2.86E+05	4.08E+03	1.17E+09	70.1
13.1	6.25E+04	2.74E+03	1.71E+08	22.8	13.1	6.41E+04	2.74E+03	1.76E+08	23.4	13.1	8.07E+04	2.74E+03	2.21E+08	29.4
13.2	1.86E+04	1.41E+03	2.62E+07	13.2	13.2	1.85E+04	1.41E+03	2.61E+07	13.1	13.2	2.49E+04	1.41E+03	3.51E+07	17.6
13.3	7.38E+03	7.70E+01	5.68E+05	95.8	13.3	6.62E+03	7.70E+01	5.10E+05	86.0	13.3	9.81E+03	7.70E+01	7.55E+05	127.4
	1.95E+06		3.86E+10	302.1		1.92E+06		3.30E+10	320.6		2.21E+06		4.29E+10	375.7
Number Ave. 6460					5977					5882				
Weight Ave. 19799					17218					19417				

Table 34- HPSEC Number Averaged and Weight Averaged MW Calculation Tables 10X UV-light Treatment															
Time	Height (hi)	Mwi	Mwi*hi	Mwi/hi	Time	Height (hi)	Mwi	Mwi*hi	Mwi/hi	Time	Height (hi)	Mwi	Mwi*hi	Mwi/hi	
6.8	6.64E+02	1.40E+05	9.33E+07	0.0	6.9	9.83E+02	1.36E+05	1.34E+08	0.0	6.9	1.50E+01	#####	2.04E+06	0.0	
6.9	2.76E+03	1.36E+05	3.76E+08	0.0	7.1	5.38E+03	1.32E+05	7.08E+08	0.0	7.1	1.50E+03	#####	1.97E+08	0.0	
7.1	8.23E+03	1.32E+05	1.08E+09	0.1	7.2	1.14E+04	1.27E+05	1.45E+09	0.1	7.2	3.27E+03	#####	4.15E+08	0.0	
7.2	1.55E+04	1.27E+05	1.97E+09	0.1	7.3	1.67E+04	1.23E+05	2.04E+09	0.1	7.3	5.09E+03	#####	6.24E+08	0.0	
7.3	2.11E+04	1.23E+05	2.59E+09	0.2	7.5	1.83E+04	1.18E+05	2.17E+09	0.2	7.5	5.70E+03	#####	6.74E+08	0.0	
7.5	2.25E+04	1.18E+05	2.66E+09	0.2	7.6	1.68E+04	1.14E+05	1.91E+09	0.1	7.6	5.70E+03	#####	6.49E+08	0.1	
7.6	1.98E+04	1.14E+05	2.25E+09	0.2	7.7	1.51E+04	1.09E+05	1.66E+09	0.1	7.7	5.71E+03	#####	6.25E+08	0.1	
7.7	1.74E+04	1.09E+05	1.90E+09	0.2	7.9	1.45E+04	1.05E+05	1.52E+09	0.1	7.9	5.81E+03	#####	6.10E+08	0.1	
7.9	1.63E+04	1.05E+05	1.71E+09	0.2	8.0	1.38E+04	1.00E+05	1.39E+09	0.1	8.0	5.79E+03	#####	5.82E+08	0.1	
8.0	1.54E+04	1.00E+05	1.55E+09	0.2	8.1	1.34E+04	9.60E+04	1.28E+09	0.1	8.1	5.89E+03	#####	5.66E+08	0.1	
8.1	1.47E+04	9.60E+04	1.41E+09	0.2	8.3	1.35E+04	9.16E+04	1.24E+09	0.1	8.3	6.29E+03	#####	5.76E+08	0.1	
8.3	1.48E+04	9.16E+04	1.35E+09	0.2	8.4	1.40E+04	8.71E+04	1.22E+09	0.2	8.4	6.72E+03	#####	5.86E+08	0.1	
8.4	1.52E+04	8.71E+04	1.32E+09	0.2	8.5	1.47E+04	8.27E+04	1.22E+09	0.2	8.5	7.71E+03	#####	6.38E+08	0.1	
8.5	1.59E+04	8.27E+04	1.31E+09	0.2	8.7	1.57E+04	7.82E+04	1.23E+09	0.2	8.7	8.62E+03	#####	6.75E+08	0.1	
8.7	1.69E+04	7.82E+04	1.33E+09	0.2	8.8	1.69E+04	7.38E+04	1.25E+09	0.2	8.8	9.83E+03	#####	7.25E+08	0.1	
8.8	1.82E+04	7.38E+04	1.34E+09	0.2	8.9	1.84E+04	6.94E+04	1.28E+09	0.3	8.9	1.12E+04	#####	7.75E+08	0.2	
8.9	1.97E+04	6.94E+04	1.36E+09	0.3	9.1	2.01E+04	6.49E+04	1.31E+09	0.3	9.1	1.27E+04	#####	8.26E+08	0.2	
9.1	2.15E+04	4.27E+04	9.18E+08	0.5	9.2	2.21E+04	6.05E+04	1.34E+09	0.4	9.2	1.45E+04	#####	8.79E+08	0.2	
9.2	2.36E+04	4.14E+04	9.77E+08	0.6	9.3	2.43E+04	5.60E+04	1.36E+09	0.4	9.3	1.66E+04	#####	9.27E+08	0.3	
9.3	2.58E+04	4.01E+04	1.03E+09	0.6	9.5	2.67E+04	5.16E+04	1.38E+09	0.5	9.5	1.89E+04	#####	9.73E+08	0.4	
9.5	2.82E+04	3.87E+04	1.09E+09	0.7	9.6	2.93E+04	4.71E+04	1.38E+09	0.6	9.6	2.16E+04	#####	1.02E+09	0.5	
9.6	3.07E+04	3.74E+04	1.15E+09	0.8	9.7	3.21E+04	4.27E+04	1.37E+09	0.8	9.7	2.47E+04	#####	1.05E+09	0.6	
9.7	3.35E+04	3.61E+04	1.21E+09	0.9	9.9	3.53E+04	3.82E+04	1.35E+09	0.9	9.9	2.81E+04	#####	1.07E+09	0.7	
9.9	3.64E+04	3.47E+04	1.27E+09	1.0	10.0	3.86E+04	3.38E+04	1.31E+09	1.1	10.0	3.20E+04	#####	1.08E+09	0.9	
10.0	3.96E+04	3.34E+04	1.32E+09	1.2	10.1	4.24E+04	2.94E+04	1.25E+09	1.4	10.1	3.62E+04	#####	1.06E+09	1.2	
10.1	4.32E+04	3.21E+04	1.38E+09	1.3	10.3	4.65E+04	2.49E+04	1.16E+09	1.9	10.3	4.08E+04	#####	1.02E+09	1.6	
10.3	4.70E+04	3.07E+04	1.44E+09	1.5	10.4	5.09E+04	2.05E+04	1.04E+09	2.5	10.4	4.57E+04	#####	9.36E+08	2.2	
10.4	5.13E+04	2.94E+04	1.51E+09	1.7	10.5	5.57E+04	1.60E+04	8.92E+08	3.5	10.5	5.09E+04	#####	8.16E+08	3.2	
10.5	5.59E+04	2.81E+04	1.57E+09	2.0	10.7	6.08E+04	1.16E+04	7.04E+08	5.3	10.7	5.66E+04	#####	6.56E+08	4.9	
10.7	6.10E+04	2.67E+04	1.63E+09	2.3	10.8	6.68E+04	7.13E+03	4.76E+08	9.4	10.8	6.33E+04	#####	1.61E+09	2.5	
10.8	6.68E+04	2.54E+04	1.70E+09	2.6	10.9	7.40E+04	2.41E+04	1.78E+09	3.1	10.9	7.17E+04	#####	1.73E+09	3.0	
10.9	7.40E+04	2.41E+04	1.78E+09	3.1	11.1	8.31E+04	2.27E+04	1.89E+09	3.7	11.1	8.24E+04	#####	1.87E+09	3.6	
11.1	8.32E+04	2.27E+04	1.89E+09	3.7	11.2	9.36E+04	2.14E+04	2.00E+09	4.4	11.2	9.48E+04	#####	2.03E+09	4.4	
11.2	9.36E+04	2.14E+04	2.00E+09	4.4	11.3	1.02E+05	2.01E+04	2.04E+09	5.1	11.3	1.06E+05	#####	2.13E+09	5.3	
11.3	1.02E+05	2.01E+04	2.04E+09	5.1	11.5	1.02E+05	1.87E+04	1.91E+09	5.4	11.5	1.10E+05	#####	2.06E+09	5.9	
11.5	1.02E+05	1.87E+04	1.91E+09	5.4	11.6	9.09E+04	1.74E+04	1.58E+09	5.2	11.6	1.03E+05	#####	1.79E+09	5.9	
11.6	9.09E+04	1.74E+04	1.58E+09	5.2	11.7	7.85E+04	1.61E+04	1.26E+09	4.9	11.7	9.07E+04	#####	1.46E+09	5.6	
11.7	7.85E+04	1.61E+04	1.26E+09	4.9	11.9	6.95E+04	1.47E+04	1.02E+09	4.7	11.9	7.99E+04	#####	1.18E+09	5.4	
11.9	6.96E+04	1.47E+04	1.03E+09	4.7	12.0	6.43E+04	1.34E+04	8.62E+08	4.8	12.0	7.40E+04	#####	9.93E+08	5.5	
12.0	6.44E+04	1.34E+04	8.63E+08	4.8	12.1	7.16E+04	1.21E+04	8.65E+08	5.9	12.1	8.36E+04	#####	1.01E+09	6.9	
12.1	7.16E+04	1.21E+04	8.64E+08	5.9	12.3	5.72E+04	1.07E+04	6.15E+08	5.3	12.3	6.83E+04	#####	7.34E+08	6.4	
12.3	5.71E+04	1.07E+04	6.13E+08	5.3	12.4	4.03E+04	9.41E+03	3.79E+08	4.3	12.4	4.94E+04	#####	4.65E+08	5.2	
12.4	4.01E+04	9.41E+03	3.77E+08	4.3	12.5	3.41E+04	8.08E+03	2.75E+08	4.2	12.5	4.09E+04	#####	3.31E+08	5.1	
12.5	3.41E+04	8.08E+03	2.76E+08	4.2	12.7	3.97E+04	6.74E+03	2.67E+08	5.9	12.7	4.41E+04	#####	2.97E+08	6.5	
12.7	3.97E+04	6.74E+03	2.68E+08	5.9	12.8	1.37E+05	5.41E+03	7.39E+08	25.2	12.8	1.68E+05	#####	9.09E+08	31.1	
12.8	1.40E+05	5.41E+03	7.57E+08	25.9	12.9	1.90E+05	4.08E+03	7.74E+08	46.6	12.9	2.16E+05	#####	8.82E+08	53.1	
12.9	2.00E+05	4.08E+03	8.15E+08	49.0	13.1	6.37E+04	2.74E+03	1.75E+08	23.2	13.1	5.25E+04	#####	1.44E+08	19.1	
13.1	6.63E+04	2.74E+03	1.82E+08	24.2	13.2	2.53E+04	1.41E+03	3.56E+07	17.9	13.2	1.59E+04	#####	2.25E+07	11.3	
13.2	2.62E+04	1.41E+03	3.70E+07	18.6	13.3	1.57E+04	7.70E+01	1.21E+06	203.6	13.3	7.74E+03	#####	5.96E+05	100.6	
13.3	1.61E+04	7.70E+01	1.24E+06	209.1											
	2.27E+06		6.24E+10	414.2			2.20E+06		5.65E+10	414.6		2.12E+06		4.29E+10	310.5
Number Ave. 5475.7					5311.9					6818					
Weight Ave. 27501					25641					20257					

Table 35: HPSEC Number and Weight Averaged MW Calc. Tables 53X UV-light

Time	Height (hi)	Mwi	Mwi*hi	Mwi/hi
7.3	2.44E+02	1.23E+05	2.99E+07	0.0
7.5	2.24E+02	1.18E+05	2.65E+07	0.0
7.6	5.40E+01	1.14E+05	6.15E+06	0.0
7.7	-1.15E+02	1.09E+05	-1.26E+07	0.0
7.9	1.14E+02	1.05E+05	1.20E+07	0.0
8.0	8.40E+01	1.00E+05	8.44E+06	0.0
8.1	4.64E+02	9.60E+04	4.46E+07	0.0
8.3	5.24E+02	9.16E+04	4.80E+07	0.0
8.4	6.74E+02	8.71E+04	5.87E+07	0.0
8.5	9.04E+02	8.27E+04	7.48E+07	0.0
8.7	1.06E+03	7.82E+04	8.33E+07	0.0
8.8	1.44E+03	7.38E+04	1.06E+08	0.0
8.9	1.80E+03	6.94E+04	1.25E+08	0.0
9.1	2.18E+03	6.49E+04	1.42E+08	0.0
9.2	2.82E+03	6.05E+04	1.71E+08	0.0
9.3	3.37E+03	5.60E+04	1.89E+08	0.1
9.5	3.87E+03	5.16E+04	2.00E+08	0.1
9.6	4.89E+03	4.71E+04	2.30E+08	0.1
9.7	6.45E+03	4.27E+04	2.75E+08	0.2
9.9	7.64E+03	3.82E+04	2.92E+08	0.2
10.0	9.30E+03	3.38E+04	3.14E+08	0.3
10.1	1.13E+04	3.21E+04	3.64E+08	0.4
10.3	1.35E+04	3.07E+04	4.16E+08	0.4
10.4	1.63E+04	2.94E+04	4.80E+08	0.6
10.5	1.95E+04	2.81E+04	5.47E+08	0.7
10.7	2.32E+04	2.67E+04	6.21E+08	0.9
10.8	2.74E+04	2.54E+04	6.97E+08	1.1
10.9	3.27E+04	2.41E+04	7.88E+08	1.4
11.1	4.00E+04	2.27E+04	9.11E+08	1.8
11.2	4.95E+04	2.14E+04	1.06E+09	2.3
11.3	6.15E+04	2.01E+04	1.24E+09	3.1
11.5	7.25E+04	1.87E+04	1.36E+09	3.9
11.6	7.77E+04	1.74E+04	1.35E+09	4.5
11.7	7.96E+04	1.61E+04	1.28E+09	5.0
11.9	7.79E+04	1.47E+04	1.15E+09	5.3
12.0	7.45E+04	1.34E+04	9.98E+08	5.6
12.1	7.80E+04	1.21E+04	9.41E+08	6.5
12.3	7.55E+04	1.07E+04	8.11E+08	7.0
12.4	5.66E+04	9.41E+03	5.33E+08	6.0
12.5	4.27E+04	8.08E+03	3.45E+08	5.3
12.7	3.97E+04	6.74E+03	2.68E+08	5.9
12.8	1.23E+05	5.41E+03	6.65E+08	22.7
12.9	1.80E+05	4.08E+03	7.35E+08	44.2
13.1	6.18E+04	2.74E+03	1.69E+08	22.5
13.2	7.76E+03	1.41E+03	1.09E+07	5.5
	1.39E+06		2.02E+10	163.3
Number Ave.	8515.2213			
Weight Ave.	14496.836			

Chlorine Statistics

General Linear Model-BDOC Fraction

Factor	Type	Levels	Values			
BulkSolu	random	5	1/31/00	2/7/00	2/14/00	2/28/00
			3/14/00			

Analysis of Variance for BDOCfrac, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F
P					
Dose(mg/	1	0.008183	0.000934	0.000934	0.85
0.399					
BulkSolu	4	0.018552	0.018552	0.004638	4.23
0.073					
Error	5	0.005483	0.005483	0.001097	
Total	10	0.032218			

Term	Coef	StDev	T	P
Constant	0.29808	0.01304	22.87	0.000
Dose(mg/	0.007002	0.007589	0.92	0.399

Expected Mean Squares, using Adjusted SS

Source	Expected Mean Square for Each Term
1 Dose(mg/	(3) + Q[1]
2 BulkSolu	(3) + 1.9294(2)
3 Error	(3)

Error Terms for Tests, using Adjusted SS

Source	Error DF	Error MS	Synthesis of Error MS
1 Dose(mg/	5.00	0.001097	(3)
2 BulkSolu	5.00	0.001097	(3)

Variance Components, using Adjusted SS

Source	Estimated Value
BulkSolu	0.00184
Error	0.00110

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General Linear Model-Hydrophobic

Factor	Type	Levels	Values
BulkSolu	random	5	1/31/00 2/7/00 2/14/00 2/28/00 3/14/00

Analysis of Variance for Hydropho, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F
P					
Dose(mg/0.007	1	0.071706	0.033192	0.033192	18.84
BulkSolu0.410	4	0.008540	0.008540	0.002135	1.21
Error	5	0.008808	0.008808	0.001762	
Total	10	0.089055			

Term	Coef	StDev	T	P
Constant	0.55723	0.01652	33.73	0.000
Dose(mg/	-0.041751	0.009619	-4.34	0.007

Unusual Observations for Hydropho

Obs	Hydropho	Fit	StDev Fit	Residual	St Resid
10	0.300000	0.364333	0.027417	-0.064333	-2.02R

R denotes an observation with a large standardized residual.

Expected Mean Squares, using Adjusted SS

Source	Expected Mean Square for Each Term
1 Dose(mg/	(3) + Q[1]
2 BulkSolu	(3) + 1.9294(2)
3 Error	(3)

Error Terms for Tests, using Adjusted SS

Source	Error DF	Error MS	Synthesis of Error MS
1 Dose(mg/	5.00	0.001762	(3)
2 BulkSolu	5.00	0.001762	(3)

Variance Components, using Adjusted SS

Source	Estimated Value
BulkSolu	0.00019
Error	0.00176

General Linear Model -- Aromaticity

Factor	Type	Levels	Values
BulkSolu	random	5	1/31/00 2/7/00 2/14/00 2/28/00 3/14/00

Analysis of Variance for UVabs.Fr, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F
P					
Dose(mg/ 0.000	1	0.146138	0.079991	0.079991	164.90
BulkSolu 0.492	4	0.001909	0.001909	0.000477	0.98
Error	5	0.002425	0.002425	0.000485	
Total	10	0.150473			

Term	Coef	StDev	T	P
Constant	1.00588	0.00867	116.02	0.000
Dose(mg/	-0.064814	0.005047	-12.84	0.000

Unusual Observations for UVabs.Fr

Obs	UVabs.Fr	Fit	StDev Fit	Residual	St Resid
3	1.00000	1.03120	0.01562	-0.03120	-2.01R
7	1.03000	0.99880	0.01562	0.03120	2.01R

R denotes an observation with a large standardized residual.

Expected Mean Squares, using Adjusted SS

Source	Expected Mean Square for Each Term
1 Dose(mg/	(3) + Q[1]
2 BulkSolu	(3) + 1.9294(2)
3 Error	(3)

Error Terms for Tests, using Adjusted SS

Source	Error DF	Error MS	Synthesis of Error MS
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1 Dose(mg/	5.00	0.000485	(3)
2 BulkSolu	5.00	0.000485	(3)

Variance Components, using Adjusted SS

Source	Estimated Value
BulkSolu	-0.00000
Error	0.00049

Worksheet size: 100000 cells

General Linear Model - Overall TOC Reduction

Factor	Type	Levels	Values
BulkSolu	random	5	1/31/00 2/7/00 2/14/00 2/28/00 3/14/00

Analysis of Variance for TOCreduc, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F
P					
Dose	1	0.0058799	0.0049633	0.0049633	61.53
0.001					
BulkSolu	4	0.0003168	0.0003168	0.0000792	0.98
0.493					
Error	5	0.0004033	0.0004033	0.0000807	
Total	10	0.0066000			

Term	Coef	StDev	T	P
Constant	0.002673	0.003470	0.77	0.476
Dose	0.013883	0.001770	7.84	0.001

Expected Mean Squares, using Adjusted SS

Source	Expected Mean Square for Each Term
1 Dose	(3) + Q[1]
2 BulkSolu	(3) + 2.0802(2)
3 Error	(3)

Error Terms for Tests, using Adjusted SS

Source	Error DF	Error MS	Synthesis of Error MS
1 Dose	5.00	0.0000807	(3)

2 BulkSolu 5.00 0.0000807 (3)

Variance Components, using Adjusted SS

Source	Estimated Value
BulkSolu	-0.00000
Error	0.00008

General Linear Model -- hydrophilic

Factor	Type	Levels	Values
BulkSolu	random	5	1/31/00 2/7/00 2/14/00 2/28/00 3/14/00

Analysis of Variance for Hydrophi, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F
P					
Dose	1	0.069958	0.067410	0.067410	22.76
0.005					
BulkSolu	4	0.011999	0.011999	0.003000	1.01
0.481					
Error	5	0.014807	0.014807	0.002961	
Total	10	0.096764			

Term	Coef	StDev	T	P
Constant	0.40894	0.02102	19.45	0.000
Dose	0.05117	0.01072	4.77	0.005

Expected Mean Squares, using Adjusted SS

Source	Expected Mean Square for Each Term
1 Dose	(3) + Q[1]
2 BulkSolu	(3) + 2.0802(2)
3 Error	(3)

Error Terms for Tests, using Adjusted SS

Source	Error DF	Error MS	Synthesis of Error MS
1 Dose	5.00	0.002961	(3)
2 BulkSolu	5.00	0.002961	(3)

Variance Components, using Adjusted SS

Source	Estimated Value
BulkSolu	0.00002
Error	0.00296

Ozone Statistics

General Linear Model -- BDOC

Factor	Type	Levels	Values
BulkSolu	random	4	1/31/00 2/14/00 3/14/00 4/3/00

Analysis of Variance for BDOC, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F
P					
Dose	1	0.003652	0.006532	0.006532	5.00
0.067					
BulkSolu	3	0.011477	0.011477	0.003826	2.93
0.122					
Error	6	0.007835	0.007835	0.001306	
Total	10	0.022964			

Term	Coef	StDev	T	P
Constant	0.31257	0.01605	19.47	0.000
Dose	0.03549	0.01587	2.24	0.067

Expected Mean Squares, using Adjusted SS

Source	Expected Mean Square for Each Term
1 Dose	(3) + Q[1]
2 BulkSolu	(3) + 2.5826(2)
3 Error	(3)

Error Terms for Tests, using Adjusted SS

Source	Error DF	Error MS	Synthesis of Error MS
1 Dose	6.00	0.001306	(3)
2 BulkSolu	6.00	0.001306	(3)

Variance Components, using Adjusted SS

Source	Estimated Value
BulkSolu	0.00098
Error	0.00131

General Linear Model - Hydrophobic

Factor	Type	Levels	Values
BulkSolu	random	4	1/31/00 2/14/00 3/14/00 4/3/00

Analysis of Variance for Hydropho, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F
P					
Dose	1	0.234751	0.215321	0.215321	44.14
0.001					
BulkSolu	3	0.002632	0.002632	0.000877	0.18
0.906					
Error	6	0.029271	0.029271	0.004878	
Total	10	0.266655			

Term	Coef	StDev	T	P
Constant	0.55322	0.03102	17.83	0.000
Dose	-0.20374	0.03067	-6.64	0.001

Expected Mean Squares, using Adjusted SS

Source	Expected Mean Square for Each Term
1 Dose	(3) + Q[1]
2 BulkSolu	(3) + 2.5826(2)
3 Error	(3)

Error Terms for Tests, using Adjusted SS

Source	Error DF	Error MS	Synthesis of Error MS
1 Dose	6.00	0.004878	(3)
2 BulkSolu	6.00	0.004878	(3)

Variance Components, using Adjusted SS

Source	Estimated Value
BulkSolu	-0.00155
Error	0.00488

General Linear Model - Hydrophilic

Factor	Type	Levels	Values
BulkSolu	random	4	1/31/00 2/14/00 3/14/00 4/3/00

Analysis of Variance for Hydrophi, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F
P					
Dose	1	0.234751	0.215321	0.215321	44.14
0.001					
BulkSolu	3	0.002632	0.002632	0.000877	0.18
0.906					
Error	6	0.029271	0.029271	0.004878	
Total	10	0.266655			

Term	Coef	StDev	T	P
Constant	0.55322	0.03102	17.83	0.000
Dose	-0.20374	0.03067	-6.64	0.001

Expected Mean Squares, using Adjusted SS

Source	Expected Mean Square for Each Term
1 Dose	(3) + Q[1]
2 BulkSolu	(3) + 2.5826(2)
3 Error	(3)

Error Terms for Tests, using Adjusted SS

Source	Error DF	Error MS	Synthesis of Error MS
1 Dose	6.00	0.004878	(3)
2 BulkSolu	6.00	0.004878	(3)

Variance Components, using Adjusted SS

Source	Estimated Value
BulkSolu	-0.00155
Error	0.00488

General Linear Model--Aromaticity

Factor	Type	Levels	Values				
BulkSolu	random	4	1/31/00	2/14/00	3/14/00	4/3/00	

Analysis of Variance for UVabs, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F
P					
Dose	1	0.52978	0.50467	0.50467	49.48
0.000					
BulkSolu	3	0.01004	0.01004	0.00335	0.33
0.806					
Error	6	0.06120	0.06120	0.01020	
Total	10	0.60102			

Term	Coef	StDev	T	P
Constant	0.94443	0.04486	21.05	0.000
Dose	-0.31192	0.04434	-7.03	0.000

Expected Mean Squares, using Adjusted SS

Source	Expected Mean Square for Each Term
1 Dose	(3) + Q[1]
2 BulkSolu	(3) + 2.5826(2)
3 Error	(3)

Error Terms for Tests, using Adjusted SS

Source	Error DF	Error MS	Synthesis of Error MS
1 Dose	6.00	0.01020	(3)
2 BulkSolu	6.00	0.01020	(3)

Variance Components, using Adjusted SS

Source	Estimated Value
BulkSolu	-0.00265
Error	0.01020

General Linear Model - Overall TOC reduction

Factor	Type	Levels	Values
BulkSolu	random	4	1/31/00 2/14/00 3/14/00 4/3/00

Analysis of Variance for TOC, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F
P					
Dose	1	0.012811	0.009920	0.009920	9.54
0.018					

BulkSolu	3	0.007201	0.007201	0.002400	2.31
0.163					
Error	7	0.007280	0.007280	0.001040	
Total	11	0.027292			

Term	Coef	StDev	T	P
Constant	-0.00551	0.01368	-0.40	0.699
Dose	0.04291	0.01389	3.09	0.018

Expected Mean Squares, using Adjusted SS

Source	Expected Mean Square for Each Term
1 Dose	(3) + Q[1]
2 BulkSolu	(3) + 2.8219(2)
3 Error	(3)

Error Terms for Tests, using Adjusted SS

Source	Error DF	Error MS	Synthesis of Error MS
1 Dose	7.00	0.001040	(3)
2 BulkSolu	7.00	0.001040	(3)

Variance Components, using Adjusted SS

Source	Estimated Value
BulkSolu	0.00048
Error	0.00104

Monochloramine Statistics

Regression

The regression equation is
 $y = 0.280 + 0.0061 x$

Predictor	Coef	StDev	T	P
Constant	0.28000	0.03464	8.08	0.015
x	0.00613	0.02454	0.25	0.826

S = 0.03464 R-Sq = 3.0% R-Sq(adj) = 0.0%

Analysis of Variance

Source	DF	SS	MS	F
P				
Regression	1	0.000075	0.000075	0.06
0.826				
Residual Error	2	0.002400	0.001200	
Total	3	0.002475		

Saving file as: M:\MINITAB\MonoclBDOC.MTW

Saving file as: M:\MINITAB\MonoclHydrophobic.MTW

Macro is running ... please wait

Current worksheet: MonoclHydrophobic.MTW

Regression

The regression equation is
 $y = 0.540 - 0.0818 x$

Predictor	Coef	StDev	T	P
Constant	0.54000	0.01528	35.35	0.001
x	-0.08180	0.01082	-7.56	0.017

S = 0.01528 R-Sq = 96.6% R-Sq(adj) = 94.9%

Analysis of Variance

Source	DF	SS	MS	F
P				
Regression	1	0.013333	0.013333	57.14
0.017				
Residual Error	2	0.000467	0.000233	
Total	3	0.013800		

Saving file as: M:\MINITAB\MonoclUVabs.MTW

Macro is running ... please wait

Current worksheet: MonoclUVabs.MTW

Regression

The regression equation is
 $y = 1.00 + 0.0532 x$

Predictor	Coef.	StDev	T	P
Constant	1.00000	0.06658	15.02	0.004
x	0.05317	0.04717	1.13	0.377

S = 0.06658 R-Sq = 38.9% R-Sq(adj) = 8.3%

Analysis of Variance

Source	DF	SS	MS	F
P				
Regression	1	0.005633	0.005633	1.27
0.377				
Residual Error	2	0.008867	0.004433	
Total	3	0.014500		

Regression

The regression equation is
 $y = 0.0000 - 0.0327 x$

Predictor	Coef	StDev	T	P
Constant	0.00000	0.01155	0.00	1.000

x -0.032720 0.008180 -4.00 0.057

S = 0.01155 R-Sq = 88.9% R-Sq(adj) = 83.3%

Analysis of Variance

Source	DF	SS	MS	F
P				
Regression	1	0.0021333	0.0021333	16.00
0.057				
Residual Error	2	0.0002667	0.0001333	
Total	3	0.0024000		

Saving file as: M:\MINITAB\MonoclTOC.MTW

* NOTE * Existing file replaced.

Worksheet size: 100000 cells

Retrieving project from file: M:\MINITAB\Monochl.MPJ

UV-light Statistics

General Linear Model - Aromaticity

Factor	Type	Levels	Values		
BulkSolu	random	3	2/28/00	4/3/00	4/10/00

Analysis of Variance for UVabs(cm, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F
P					
Dose(mW*	1	0.164703	0.091974	0.091974	216.84
0.000					
BulkSolu	2	0.003001	0.003001	0.001501	3.54
0.087					
Error	7	0.002969	0.002969	0.000424	
Total	10	0.170673			

Term	Coef	StDev	T	P
Constant	1.00065	0.00892	112.24	0.000
Dose(mW*	-0.000010	0.000001	*	*

Unusual Observations for UVabs(cm

Obs	UVabs(cm	Fit	StDev Fit	Residual	St Resid
2	1.00000	1.00000	0.02059	0.00000	* X

X denotes an observation whose X value gives it large influence.

Expected Mean Squares, using Adjusted SS

Source	Expected Mean Square for Each Term
1 Dose(mW*	(3) + Q[1]
2 BulkSolu	(3) + 2.1398(2)
3 Error	(3)

Error Terms for Tests, using Adjusted SS

Source	Error DF	Error MS	Synthesis of Error MS
1 Dose(mW*	7.00	0.000424	(3)
2 BulkSolu	7.00	0.000424	(3)

Variance Components, using Adjusted SS

Source	Estimated Value
BulkSolu	0.00050
Error	0.00042

General Linear Model--Hydrophobicity

Factor	Type	Levels	Values
BulkSolu	random	3	2/28/00 4/3/00 4/10/00

Analysis of Variance for Hydropho, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F
P					
Dose(mW*	1	0.073475	0.054927	0.054927	56.89
0.000					
BulkSolu	2	0.000567	0.000567	0.000284	0.29
0.754					
Error	7	0.006758	0.006758	0.000965	
Total	10	0.080800			

Term	Coef	StDev	T	P
Constant	0.51245	0.01207	42.45	0.000
Dose(mW*	-0.000008	0.000001	-7.54	0.000

Expected Mean Squares, using Adjusted SS

Source	Expected Mean Square for Each Term
1 Dose(mW*	(3) + Q[1]
2 BulkSolu	(3) + 2.2801(2)
3 Error	(3)

Error Terms for Tests, using Adjusted SS

Source	Error DF	Error MS	Synthesis of Error MS
1 Dose(mW*	7.00	0.000965	(3)
2 BulkSolu	7.00	0.000965	(3)

Variance Components, using Adjusted SS

Source	Estimated Value
--------	-----------------

BulkSolu	-0.00030
Error	0.00097

General Linear Model-Hydrophilicity

Factor	Type	Levels	Values
BulkSolu	random	3	2/28/00 4/3/00 4/10/00

Analysis of Variance for Hydrophi, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F
P					
Dose(mW* 0.000	1	0.073475	0.054927	0.054927	56.89
BulkSolu 0.754	2	0.000567	0.000567	0.000284	0.29
Error	7	0.006758	0.006758	0.000965	
Total	10	0.080800			

Term	Coef	StDev	T	P
Constant	0.51245	0.01207	42.45	0.000
Dose(mW*	-0.000008	0.000001	-7.54	0.000

Expected Mean Squares, using Adjusted SS

Source	Expected Mean Square for Each Term
1 Dose(mW*	(3) + Q[1]
2 BulkSolu	(3) + 2.2801(2)
3 Error	(3)

Error Terms for Tests, using Adjusted SS

Source	Error DF	Error MS	Synthesis of Error MS
1 Dose(mW*	7.00	0.000965	(3)
2 BulkSolu	7.00	0.000965	(3)

Variance Components, using Adjusted SS

Source	Estimated Value
BulkSolu	-0.00030
Error	0.00097

General Linear Model - BDOC Test

Factor	Type	Levels	Values
BulkSolu	random	3	2/28/00 4/3/00 4/10/00

Analysis of Variance for BDOCFrac, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F
P					
Dose(mW*	1	0.088341	0.073588	0.073588	58.64
0.000					
BulkSolu	2	0.002948	0.002948	0.001474	1.17
0.363					
Error	7	0.008784	0.008784	0.001255	
Total	10	0.100073			

Term	Coef	StDev	T	P
Constant	0.28329	0.01533	18.47	0.000
Dose(mW*	0.000009	0.000001	7.66	0.000

Unusual Observations for BDOCFrac

Obs	BDOCFrac	Fit	StDev Fit	Residual	St Resid
2	0.280000	0.280000	0.035424	0.000000	* X

X denotes an observation whose X value gives it large influence.

Expected Mean Squares, using Adjusted SS

Source	Expected Mean Square for Each Term
1 Dose(mW*	(3) + Q[1]
2 BulkSolu	(3) + 2.1398(2)
3 Error	(3)

Error Terms for Tests, using Adjusted SS

Source	Error DF	Error MS	Synthesis of Error MS
1 Dose(mW*	7.00	0.001255	(3)
2 BulkSolu	7.00	0.001255	(3)

Variance Components, using Adjusted SS

Source	Estimated Value
--------	-----------------

BulkSolu	0.00010
Error	0.00125

General Linear Model - Overall TOC Reduction

Factor	Type	Levels	Values
BulkSolu	random	3	2/28/00 4/3/00 4/10/00

Analysis of Variance for TOC, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F
P					
Dose (mW*	1	0.005242	0.006751	0.006751	5.57
0.046					
BulkSolu	2	0.006437	0.006437	0.003219	2.66
0.130					
Error	8	0.009688	0.009688	0.001211	
Total	11	0.021367			

Term	Coef	StDev	T	P
Constant	-0.02691	0.01268	-2.12	0.066
Dose (mW*	0.000003	0.000001	2.36	0.046

Unusual Observations for TOC

Obs	TOC	Fit	StDev Fit	Residual	St Resid
2	0.000000	-0.050677	0.024608	0.050677	2.06R
6	-0.100000	-0.049323	0.024608	-0.050677	-2.06R

R denotes an observation with a large standardized residual.

Expected Mean Squares, using Adjusted SS

Source	Expected Mean Square for Each Term
1 Dose (mW*	(3) + Q[1]
2 BulkSolu	(3) + 2.6226(2)
3 Error	(3)

Error Terms for Tests, using Adjusted SS

Source	Error DF	Error MS	Synthesis of Error MS
1 Dose (mW*	8.00	0.001211	(3)
2 BulkSolu	8.00	0.001211	(3)

Variance Components, using Adjusted SS

Source	Estimated Value
BulkSolu	0.00077
Error	0.00121

Calculations of Average Percent Removals Displayed in Text of Chapter 3 and Tables in Chapter 4

Table 6. Measured Fractions after Ozone Oxidation Based on Dose

Dose (mg O ₃ /mg TOC)	Hydrophobic Fraction	Hydrophilic Fraction	BDOC Fraction	Aromatic Fraction	Fraction Removed Via Oxidation
0.0	0.59	0.49	0.27	1.00	0.00
0.0	0.62	0.42	0.36	1.00	0.00
0.0	0.54	0.30	0.28	1.00	0.00
0.0	0.53	0.43	0.28	1.00	0.00
Averages	0.57	0.41	0.28	1.00	0.00
0.57	0.49	0.58	0.19	0.73	0.01
0.56	0.48	0.52	0.33	0.68	-0.03
0.48	0.47	0.57	0.31	0.63	0.00
Averages	0.48	0.55	0.32	0.66	-0.01
1.03	0.32	0.76	0.39	0.56	0.00
1.05	0.22	0.77	0.42	0.56	0.07
1.19	0.26	0.70	0.36	0.53	0.11
Averages	0.27	0.74	0.39	0.55	0.09
2.07	0.20	0.79	0.31	0.41	0.13
1.84	0.11	0.89	0.40	0.23	0.15
1.88	0.21	0.79	0.34	0.41	0.02
Averages	0.205	0.79	0.325	0.41	0.075
p-value	0.001	0.001	0.067	<0.001	0.018

Example calculations done for 1 mg O₃/mg TOC

$$\text{Hydrophobic Change} = 1 - 0.27/0.57 = 0.53$$

$$\text{Hydrophilic Change} = (0.74 - 0.41)/0.41 = 0.80$$

$$\text{BDOC Change} = 0.39 - 0.28 = 0.11$$

$$\text{Aromatic Change} = 1.00 - 0.55 = 0.45$$

$$\text{TOC Reduction} = 0.09 - 0.00 = 0.8$$

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