Distribution of Colloidal Material in Activated Sludge as Influenced by Cations

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Abstract

Activated sludge influent and effluent from five municipal wastewater treatment plants were analyzed to further elucidate the roles of aluminum, iron, and the monovalent to divalent cation ratio (M/D) on the influent and effluent characteristics of the systems. The size distribution of organic nitrogen, organic carbon, protein, humic acid, and polysaccharide was examined with respect to the concentration of cations in the activated sludge influent. It was found that the majority of organic nitrogen, organic carbon, protein and polysaccharides were found in material larger than $0.45\mu m$ in activated sludge influent. Humic acids were mostly found in material smaller than $0.45\mu m$. Protein was the largest contributor to organic nitrogen and humic acids were the largest contributor to organic carbon. Using $0.45\mu m$ as a division between particulate and soluble material, the ratio of soluble to particulate material in activated sludge influent was found to be negatively correlated with the ratio of iron to aluminum.

In activated sludge effluent, the majority of the organic nitrogen and protein was found in material larger than $0.45\mu m$, while the majority of the organic carbon, humic acid, and polysaccharide were found in material smaller than 30kDa. Influent aluminum concentration had no observable effect on the concentration or distribution of organic nitrogen or organic carbon. Influent iron appeared to play a role in the flocculation of organic nitrogen and protein containing material between $0.45\mu m$ and 1kDa in size. A high monovalent to divalent cation ratio appeared to play a role in deflocculating organic nitrogen containing material larger than $1.5\mu m$ and increased the concentration of TOC smaller than 1kDa and the total polysaccharide concentration. Tertiary depth filtration removed all organic nitrogen and protein in material larger than $0.45\mu m$, but a poor job of removing organic carbon from and an inconsistent job of removing polysaccharide from effluent

Eight lab-scale activated sludge reactors were also run, but the data was not consistent enough for analysis and comparison with the municipal wastewater treatment plants. This thesis contains a series of four papers that each deal with a different aspect of the role of cations on activated sludge influent and effluent. The first paper focuses on activated sludge influent characteristics, the second on effluent organic nitrogen and organic carbon, the third on effluent EPS, and the

last on the lab-scale reactors. The papers were divided in this way because of the unique analytical obstacles that were encountered with each set of data.

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<u>Introduction</u>

During the activated sludge process, organic matter is removed by the growth of microorganisms. These microorganisms flocculate and can be removed by gravity settling in a clarifier. Multivalent cations in the activated sludge promote good flocculation by providing a bridging network with the negatively charged extracellular polymeric substances (EPS) (Higgins and Novak, 1997a).

When biomass does not settle well, organic matter and associated contaminants are released in the effluent and can cause deterioration of the receiving water body. Protein is a major constituent of EPS and may be a significant source of effluent organic nitrogen. It has been shown that endocrine disrupting chemicals (EDCs) bind to organic matter (Holbrook et al, in press) and may be discharged in this phase with the wastewater effluent. Tertiary treatment can remove some of the effluent and the associated organic nitrogen and EDCs, but disinfection may produce undesireable byproducts, such as N-nitrosodimethylamine (NDMA).

Little research has been done to characterize the size of the activated sludge influent and effluent organic material, especially with respect to organic nitrogen and EDCs. The size of the influent material is important in the design, modeling, and operation of an activated sludge system and the effluent material is important when designing a tertiary treatment system, commonly a filter, to remove this material (Aim et al., 1997, Kobler and Boller, 1997).

<u>Activated Sludge and Clarification</u>

Overview

During the activated sludge wastewater treatment process, organic matter is removed through the growth of microorganisms. These microorganisms bind together into suspended masses called floc and can be removed by gravity settling in a clarifier. Poor flocculation can result in inefficient settling. This may result from a variety of causes, including toxins (Bott and Love, 2002) or a high monovalent to divalent cation ratio (Higgins and Novak, 1997a).

When biomass does not settle well, a significant amount of organic matter remains in the effluent and is discharged from the treatment plant if no tertiary treatment options are used. The organic matter released from a wastewater treatment plant has biological oxygen demand (BOD) and nutrients associated with it. If the BOD and nutrients are high enough relative to the size of the receiving water body, deterioration of that water body can result.

Even when a floc does settle well, some organic matter remains in the effluent as a result of the properties of the materials that remain and the operation of the clarifier. The particles that remain have low settling velocities due to the particle size, density, porosity, concentration (Hilligardt and Hoffmann, 1997) or microbial population (Wilén et al, 2003). The efficient operation of a clarifier is also a function of operational and design parameters, such as the sludge blanket depth and thickness, solids loading, overflow rate, and clarifier shape (Tchobanoglous et al., 2003).

Influent characterization

The ability to design and operate an efficient activated sludge system is dependent upon an accurate characterization of the activated sludge influent (post primary treatment). The ASM 1 model characterizes the biodegradable material in the influent as slowly and readily biodegradable substrate, soluble and particulate organic nitrogen, soluble ammonia nitrogen, and soluble nitrate nitrogen. Representative values for these have been proposed (Bidstrup and Grady, 1988), as well as guidelines for converting the typically monitored parameters of total and volatile solids, biological oxygen demand, chemical oxygen demand, ammonia and total Kjeldahl nitrogen (TKN) into the ASM model parameters (Grady et al, 2003). As stated in Grady et al. (2003), it can be a laborious and inexact process to characterize a wastewater in a form suitable for modeling.

One parameter that needs to be characterized accurately for modeling purposes is the amount of biodegradable soluble and particulate material. When the biodegradable feed is composed completely of particulate material, a longer SRT is required for adequate substrate degradation because the material needs to be hydrolyzed. This time needed for hydrolysis helps smooth out the effects of the typical influent diurnal flow pattern on substrate concentration and oxygen demand (Grady et al., 2003).

Filter types and particle size distribution

Filtration is a common choice for tertiary wastewater treatment. Filters can be roughly categorized as depth, surface, or membrane types. Numerous filter designs and materials have been developed for these broad categories (Tchobanoglous et al., 2003). Tertiary filter performance for various particle sizes has been studied recently, most commonly for depth filters, although most focus on the removal of relatively large particles. The smallest particle size measured by Kaminski et al (1997) was $5\mu m$ and Graff et al (2001) measured down to $2\mu m$. Kaminski found a removal rate of up to 50 percent for particles in the size range $5\text{-}10\mu m$ for a depth filter with a 20cm bed depth, 1.1mm grain size, and a hydraulic loading of 20m/h, after 7 hours of operation. Graff found an increase in particles smaller than $10\mu m$ after 1 hour of operation of a deep bed filter composed of anthracite, quartz sand, and garnet. Kobler and Boller

(1997) examined smaller particles, down to 0.3μm, for four types of filters, and found removal for the smallest particles as high as 70 percent, although most values were closer to 20 percent.

Extracellular Polymeric Substances

Overview

The microorganisms in activated sludge produce EPS that are comprised of proteins, polysaccharides, and humic acids. Urbain et al. (1993), Frølund et al. (1996), Nielsen et al. (1996), Dignac et al. (1998), Wilén et al. (2003), and Leppard et al. (2003) all observed that protein was the dominant constituent of EPS. In addition to being produced during biomass growth, EPS are present in the influent to the activated sludge process and released from lysed cells (Urbain et al, 1993, Dignac et al, 1998). It is hypothesized that small pieces of EPS may make up a large amount of the organic matter that remains in wastewater effluent.

Interaction with Cations

Several researchers have found that EPS have a negative surface charge, as determined by the composition of the EPS (Higgins and Novak, 1997a) and colloid titration (Liao et al, 2002, Wilén et al, 2003). The surface charge may become less negative as the solids retention time (SRT) increases and more positive as the pH decreases (Liao et al, 2002).

Protein contains anionic carboxyl and amino functional groups that cause the negative surface charge. This negative surface charge allows EPS to bind with cations in solution (Bruus et al, 1992). When the activated sludge mixed liquor has a high monovalent to divalent cation ratio (M/D), a weak floc structure and poor settling can result (Higgins and Novak, 1997b). A high M/D has been correlated with high activated sludge effluent COD and biopolymer concentration (Murthy and Novak, 2001). Divalent cations form a cation bridge between negatively charged EPS, helping to flocculate the biomass. Murthy et al (1998) observed an improvement in activated sludge settling after the addition of magnesium in full-scale field trials. The role of calcium in flocculation was supported by Biggs et al (2001), who observed an increase in floc size when calcium was added to an activated sludge solution.

Higgins and Novak (1997a) verified that EPS protein plays a much larger role than polysaccharides in flocculation by looking at the floc response to divalent cation concentrations and protein and polysaccharide degrading enzymes. Dignac et al. (1998) found that electrostatic bonds were evenly distributed in the floc, and confirmed the importance of negatively charged protein in floc structure.

Aluminum and iron are both trivalent cations that are present in wastewater and play a significant role in bioflocculation (Park et al, in press). Novak et al (2003) proposed a model in which cations bind to EPS depending on the source of the material. Based on cation release during aerobic and anaerobic digestion, they hypothesized that the EPS produced by microbial growth is linked to divalent cations, and EPS from the influent and cell lysis products is linked to trivalent cations.

Hydrophobic Interactions

Hydrophobic interactions between AS particles are also important in flocculation. Dignac (1998) highlighted the hydrophobic amino acids of protein, especially alanine, leucine, and glycine, as being involved in hydrostatic bonds. Liao et al. (2001) found that the surface hydrophobicity and the EPS protein to carbohydrate ratio both increased with SRT. Wilén et al (2003) observed an increase in flocculation ability as the hydrophobicity increased.

The hydrophobic activated sludge floc can help entrain hydrophobic contaminants that are present in wastewater. One of the most commonly studied classes of hydrophobic contaminants is endocrine disrupting chemicals (EDCs). While hydrophobicity is important in EDC sorption, recent research has shown that it may not be the dominant mechanism (Holbrook et al, in press). Holbrook et al (in press) did not find a correlation between EDC sorption and the EPS protein and polysaccharide concentration.

<u>Organic Nitrogen in Effluent</u>

Impacts

Since protein is a major constituent of EPS in activated sludge, EPS that is not removed in the clarification process may contain a significant amount of organic nitrogen. Nitrogen discharge is being increasingly regulated because of the harmful environmental and human health impacts high levels of nitrogen discharge can cause. These impacts include eutrophication in water bodies (EPA, 1999), methemoglobinemia in humans (Hay et al, 1998), and the formation of carcinogenic nitrogen-containing byproducts of chloramine disinfection (CDHS, 2004).

One nitrogen-containing disinfection byproduct in wastewater treatment systems is the carcinogen N-nitrosodimethylamine (NDMA). NDMA can be formed when chloramine is used to disinfect water containing organic matter. Specifically, monochloramine can interact with NDMA precursors, such as dimethylamine (DMA) or cationic polymers that contain nitrogen (Najm and Trussel, 2001). The formation of NDMA from DMA in the presence of chloramine may be the most frequently studied NDMA formation reaction, but this transformation fails to account for the majority of NDMA formation potential in activated sludge systems (Mitch et al, 2003). Other

nitrogen-containing compounds must also be precursors to NDMA, but they have not been identified.

Chloramine disinfection is common in both wastewater and drinking water treatment. Production of NDMA from chloramine disinfection can be as high as 100ng/L for secondary wastewater effluent (Najm and Russel, 2001), which is 10 times higher than the State of California's action level for drinking water of 10ng/L (CDHS, 2004). Although the formation of NDMA from surface water has been shown to be lower than the aforementioned action level, the increasing trend of wastewater reuse means a greater potential for higher organic loadings to water treatment plants and potential for NDMA formation.

Size Fractionation

The smallest particles that remain after clarification contain a significant percentage of the oxygen demand and nutrients that are released from the treatment plant. Neis and Tiehm (1997) found that an average of 74 percent of the COD and 36 percent of the phosphorus in the secondary effluent that they sampled was associated with particles smaller than $0.1\mu m$. Holbrook et al (in press), found an average of 8.3mg/L organic carbon smaller than $1.5\mu m$, 5.7mg/L organic carbon smaller than 30kDa, and 2.5mg/L organic carbon smaller than 1kDa in secondary clarifier effluent. In addition, Mitch et al (2003) found that the 47 percent of the NDMA formation potential in activated sludge was in material smaller than $0.7\mu m$ in size. Other than extrapolating the aforementioned studies to make an estimate of the organic nitrogen size distribution, little is known about the size of organic nitrogen containing particles in secondary clarifier effluent.

Endocrine Disrupting Chemicals

Overview and effects

Endocrine disrupting chemicals cause negative health effects by interfering with the operation of the hormonal system in humans and wildlife by exaggerating or minimizing hormone response, or by directly interfering with the hormone system (Roefer et al, 2000). The sources of these compounds are as widely varied as their structures. The list of known EDCs includes herbicides, plasticizers, organochlorine compounds, alkylphenol ethoxylate detergent metabolites, and synthetic hormones such as ethinylestradiol (EE2).

Fish are among the most common species used to study the in vivo effects of EDCs. The level of the egg yolk protein found in female fish, vitellogenin, is often used as the indicator of estrogenic effect (Jones et al, 2000). Significant effects have been observed in fish that were exposed to EDCs in the receiving water body of treatment plants, (Larsson et al, 1999 and Sheahan et al, 2002) or at similar concentrations to those found in the treatment plant effluent (Routledge et al,

1998, Metcalfe et al, 2001). A review of the known effects of a wide range of EDCs on a variety of organisms can be found in Oberdörster and Cheek (2000).

The effects of EDCs in the environment on humans have not been fully determined. In large doses, EDCs have been shown to effect the human endocrine system, as evidenced by the use of ethinylestradiol as a female contraceptive. However, little epidemiologic data exists on the long-term effects of exposure to EDCs, especially at the low levels observed in the natural environment (Birnbaum and Fenton, 2003). The effects of EDCs on humans may be particularly difficult to discern given their widespread occurrence and delayed effects. Kolpin et al (2002) found EDCs in over 60 percent of the streams they surveyed across the United States in 1999 and 2000. Birnbaum and Fenton (2003) state that organisms exposed to EDCs during early development have an increased risk of cancer later in life.

Endocrine disrupters in wastewater treatment
Estrogenic EDCs (EEDCs) are compounds that mimic the functions of estrogen and are likely the most studied EDCs with respect to wastewater treatment processes. The most common EEDCs are estrone (E1), estradiol (E2), estriol (E3), and ethinylestradiol (EE2) (Johnson and Sumpter, 2001 and D'Ascenzo et al, 2003). E1, E2, and E3 typically enter the sewer system in a conjugated form, either as sulfates or glucuronates, but are deconjugated by the time they reach the wastewater treatment plant, presumably by *Escherichia coli* (D'Ascenzo et al, 2003).

Endocrine disrupting chemicals have been found in significant amounts in wastewater treatment plants by a large number of researchers (Desbrow et al, 1998, Matsui, 2001, Holbrook et al, 2002, and Svenson et al, 2003). All of these researchers found estrogenic activity in influents in the range of 2-150ng/L and in effluents in the range of 1-15ng/L, expressed as the equivalent amount of E2.

The activated sludge treatment process has been shown to remove some EDCs by up to 85 percent (Johnson and Sumpter, 2001) through degradation (Ternes et al, 1999, Lee and Liu, 2002, and Onda et al, 2003) and sorption (Johnson and Sumpter, 2001, Schäfer et al, 2002, and Holbrook et al, in press). Sorption may be due to the hydrophobicity of the EDCs (Johnson and Sumpter, 2001), but other research has shown that hydrophobic interactions may not be the dominant sorption mechanism (Holbrook et al, in press). Sorbed EDCs, although removed from the liquid effluent, have environmental implications when biosolids are land-applied (La Guardia et al, 2001). Tertiary treatments such as advanced oxidation (Onda et al., 2002), nanofiltration (Wintgens et al, 2002 and Schäfer et al, 2003), and reverse osmosis (Schäfer et al, 2003) have been shown to further remove EDCs from effluents.

Research Objectives

As nitrogen, NDMA, and EDC contamination in wastewater receiving water bodies becomes a larger environmental and public health issue, it is becoming more important to advance the state of the art in their removal. This requires an increased understanding of what materials are in the activated sludge influent and clarified effluent and the size distribution of these materials. An increased understanding of the roles of cations on flocculation is also needed to improve treatment efficiency. Trivalent cations especially have not been adequately studied in this regard. This research is intended to address these concerns.

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Distribution of Colloidal Material in Activated Sludge Influent as Influenced by Cations

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Abstract

Five wastewater treatment plants were examined to determine the size distribution of TKN, TOC, and the EPS constituents of protein, humic acid, and polysaccharides in the activated sludge influent. The material larger than $0.45\mu m$ contained the largest percentage of organic nitrogen, TOC, proteins, and polysaccharides. The material smaller than $0.45\mu m$ contained the largest percentage of humic acids. Protein was the largest contributor to organic nitrogen and humic acids were the largest contributor to organic carbon. Using $0.45\mu m$ as a division between particulate and soluble material, the ratio of soluble to particulate material (S/P) was found to be negatively correlated with the ratio of iron to aluminum.

Introduction

The ability to design and operate an efficient activated sludge is dependent upon an accurate characterization of the activated sludge influent (after primary treatment). The ASM 1 model characterizes the biodegradable material in the influent as slowly and readily biodegradable substrate, soluble and particulate organic nitrogen, soluble ammonia nitrogen, and soluble nitrate nitrogen. Representative values for these parameters have been proposed (Bidstrup and Grady, 1988), as well as guidelines for converting the typically monitored parameters of total and volatile solids, biological oxygen demand, chemical oxygen demand, ammonia and total Kjeldahl nitrogen (TKN) into the ASM model parameters (Grady et al., 2003). Since the parameters used in the model cannot be directly measured, it can be a laborious and inexact process to characterize a wastewater in a form suitable for modeling (Grady et al., 2003).

One parameter that requires characterization for accurate modeling is the amount of biodegradable soluble and particulate matter. When the biodegradable feed is composed completely of particulate material, a longer solids retention time (SRT) is required for adequate degradation than for soluble feed. This occurs because the particulate matter needs to be hydrolyzed before uptake by biomass. Particulate materials in activated sludge influent also help smooth out the effects of the typical influent diurnal flow pattern on substrate concentration and oxygen demand (Grady et al., 2003). This occurs because the time needed for particulate hydrolysis spreads the influent loading over a longer time period.

Specific constituents such as proteins, humic acids, and polysaccharides, comprise the bulk parameters used in the ASM model, but they are not modeled individually. However, information on these constituents is important for predicting the floc structure of the biomass. Flocculation is important for the efficient performance of the secondary clarifier. Cations have been shown to be important in flocculation due to their interaction with negatively-charged extracellular polymeric substances (EPS) (Higgins and Novak, 1997a). Novak et al. (2003) has proposed a model in which cations bind to EPS depending on whether the EPS was present in the activated sludge influent or was produced during the activated sludge process. They proposed that the EPS produced by microbial growth is linked to divalent cations and EPS from the influent and cell lysis products is linked to trivalent cations. A high monovalent to divalent cation ratio (M/D) appears to disrupt flocculation and cause poor mixed liquor settling (Higgins and Novak, 1997b).

This paper characterizes the TKN, total organic carbon (TOC), and dominant EPS constituents in five activated sludge wastewater treatment plant influents. The quantities and size distribution of these materials are examined with respect to the concentration of cations in the influent.

Methods

Activated sludge influent grab samples were obtained from five wastewater treatment plants and transported to the laboratory either directly (Blacksburg and Peppers Ferry samples) or shipped overnight (Blue Plains, Piscataway, and Parkway samples). The samples that were shipped were transported in coolers containing ice packs to maintain a temperature of approximately 4°C. The samples that were transported directly to the laboratory were stored overnight at 4°C. All samples were transported in plastic containers.

Sample treatment and analysis

After an unfiltered sample was taken (whole fraction), the remainder of the sample was centrifuged for 15 minutes at 7,000 RPM (8,700xg) before filtration by a 1.5μm glass fiber filter, a 0.45μm nitrocellulose membrane filter, a 30,000 Dalton (30kDa) ultrafilter, or a 1,000 Dalton (1kDa) ultrafilter. Samples to be analyzed for TKN, ammonia, TOC, and metals were stored in acid-washed glass containers at 4°C and at a pH less than two. The pH was adjusted to this value with concentrated sulfuric acid. Samples for protein, humic acid, and polysaccharide measurements were stored at -15°C in sterile polypropylene vials.

Total Kjeldahl nitrogen measurements were conducted according to method 4500-N_{org} C. (APHA, 1998) and ammonia measurements were conducted according to method 4500-NH_3 C. (APHA, 1998). Total organic carbon was analyzed with a Sievers 800 TOC Analyzer for fractions smaller

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than 0.45μm and with a Dohrmann Carbon Analyzer for samples larger than 0.45μm. The whole influent samples were digested according to method 3030 E. (APHA, 1998) and analyzed by ICP (Ultima) for sodium, potassium, magnesium, calcium, aluminum and iron. Protein and humic acid measurements were performed according to Frølund et al. (1996) and polysaccharides were performed according to Dubois et al. (1956). Spectroscopic measurements were made using a Beckmann DU640 spectrometer.

Data Analysis

In order to examine the effects of cations on the influent characteristics, the results for the samples of adjoining sizes were subtracted from each other in order to obtain the quantity of material between the two sizes. For example, the TKN for the sample filtered through a 0.45μm filter was subtracted from the TKN for the sample filtered through a 1.5μm filter to obtain the quantity of TKN that is smaller than 1.5μm but larger than 0.45μm. The fractions were also analyzed to determine what percentage of the whole sample they represented. On occasion, these analyses produced a negative and obviously incorrect value. The inconsistent data points were adjusted in order to include as much data as possible into the research efforts.

Data inconsistencies occurred when a sample produced a higher value than a sample from the same source filtered through a more porous membrane; or a value lower than a sample from the same source filtered through a less porous membrane. When inconsistencies occurred, the values that were too high were adjusted to equal the next larger size fraction and the values that appeared to be too low adjusted to equal the next smaller size fraction. For example, if a sample filtered through a 30kDa filter produced a value larger than the sample from the same source filtered through a 0.45μm filter, the data from the 30kDa filter would be adjusted to equal that from the 0.45μm filter. Likewise, if a sample filtered through a 30kDa filter produced a value smaller than the sample from the same source filtered through a 1kDa filter, the data from the 30kDa filter would be adjusted to equal that from the 1kDa filter. This is illustrated in Figure 1. The value for the 0.45μm filter sample did not fit the general trend. Since this value was too low and the values around it confirm that this data point is incongruous, the fraction between 1.5μm and 0.45μm would be too large and the fraction between 0.45μm and 30kDa would be negative. Figure 1b shows how that data point was treated to bring it into line with the other samples from the same source. It was adjusted upwards to equal the 30kDa value.

Figure 1a. Before data adjustment.   Figure 1b. After data adjustment.
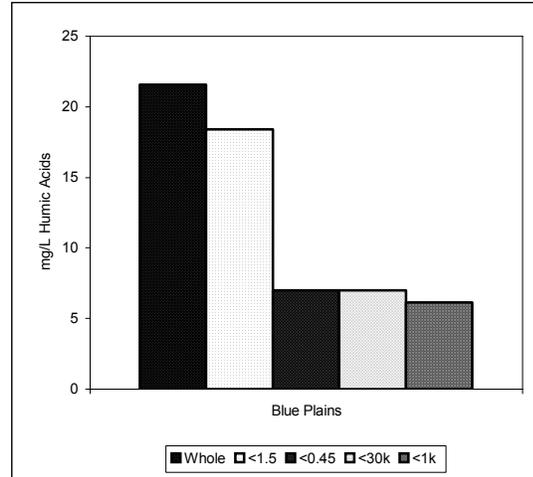
These changes only occurred if it was obvious that one data point did not fit with the other filtered samples. If a general trend could not be determined from the set of filtered data, generally there were multiple inconsistencies and all of the data from that analysis were disregarded.

The TKN was divided into ammonia, soluble organic nitrogen, and particulate organic nitrogen fractions, which can be used in the ASM model. The TOC, protein, humic acid, and polysaccharide data were divided into soluble and particulate fractions as well, although this information cannot be directly used by the ASM model. Since the ratio of soluble to particulate material was used in the analyses for this paper and any inert material was assumed to be uniformly distributed, inert material was not considered in the analyses presented here. Soluble material was defined as material smaller than 0.45μm and particulate material was defined as material larger than 0.45μm. This definition of soluble has a history of being used as the dividing line between particulate and soluble, such as in Randtke and McCarty (1979).

Results

Constituent Distribution

All of the activated sludge influents had approximately the same total TKN (Fig. 2). The influent TKN had an average value of 18.9mg-N/L. Ammonia accounted for an average of 68 percent of this TKN. The material larger than 1.5μm accounted for an average of 13 percent of the total TKN, but the values varied between 4 percent and 25 percent of the total TKN. Three plants, Blue Plains, Pepper's Ferry, and Piscataway, contained a significant amount of material between 1.5μm and 0.45μm, averaging 19 percent of the total TKN, or 3.5mg-N/L. Parkway and Blacksburg contained less than one percent of the total TKN in this size fraction.

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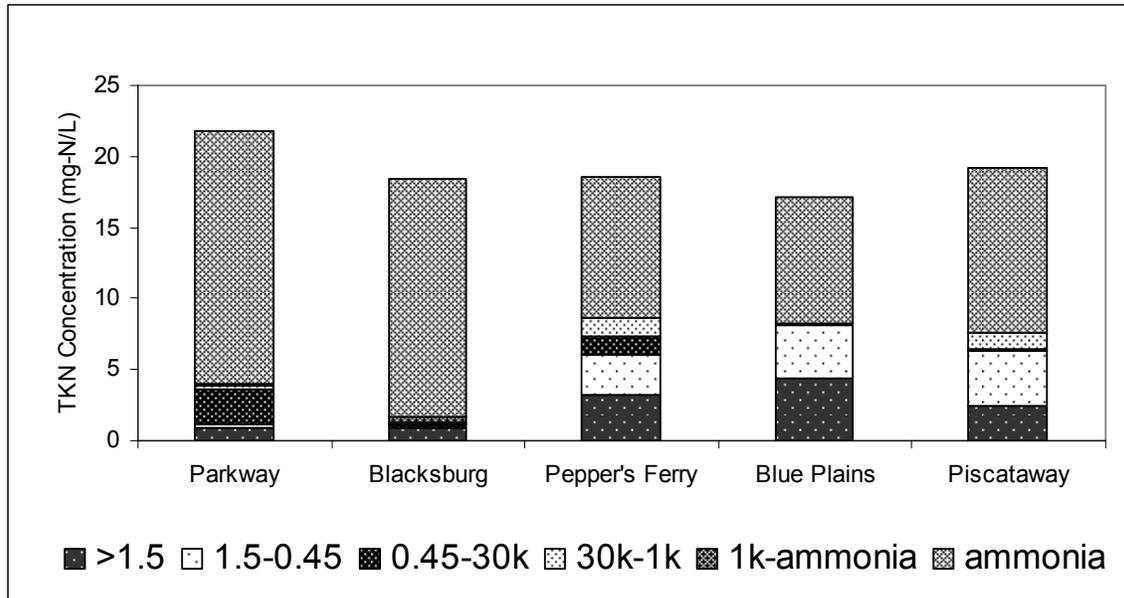
Figure 2. Distribution of TKN in the field sample influents.

The TOC measurements for the unfiltered sample and the sample filtered through a 1.5μm filter from Pepper's Ferry were not conducted and therefore are not included in the TOC analyses. For the four remaining influents, the total TOC varied between 28.1mg-C/L and 58.7mg-C/L with an average of 47.3mg-C/L (Fig 3). The largest percentage of this total was found in the material larger than 1.5μm. This material accounted for an average of 51 percent of the total TOC and only varied between 21.2mg-C/L and 24.7mg-C/L. The material smaller than 1kDa accounted for 11.3mg-C/L, or 21 percent, on average. The material between 1.5μm and 0.45μm and between 30kDa and 1kDa accounted for ten percent and 13 percent of the total TOC, respectively. Material between 0.45μm and 30kDa contained an average of six percent of the total TOC. The magnitudes of the size fractions that were measured for Pepper's Ferry were in line with the values from the other four treatment plants.
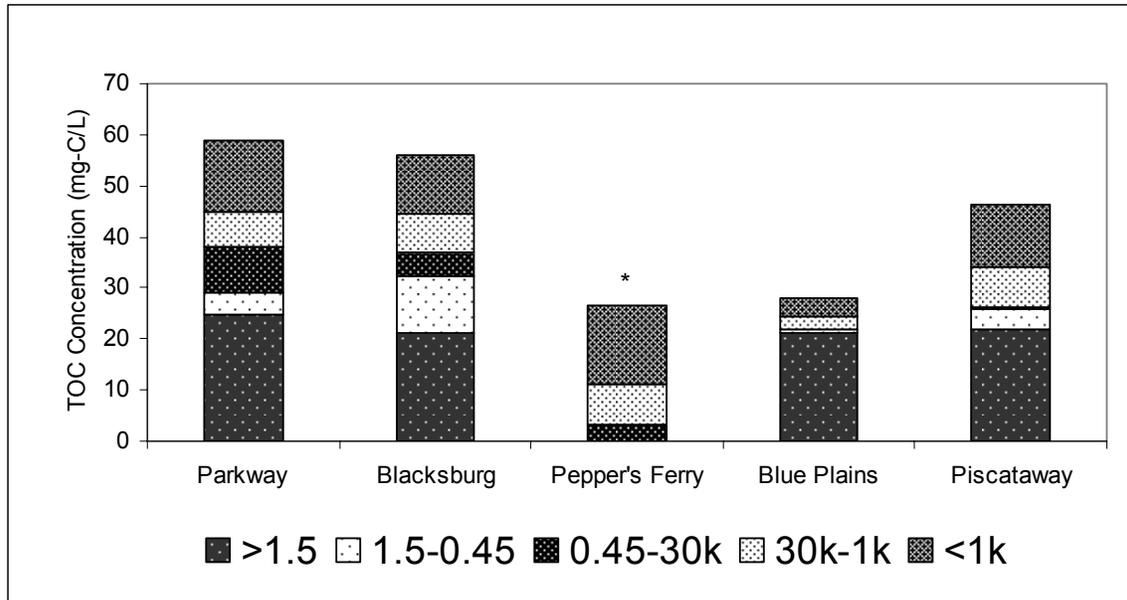
Figure 3. Distribution of TOC in the field sample influents. *The TOC for the size fractions larger than 0.45μm was not measured.

The influent total protein values ranged from 13.8mg/L at Parkway to 29.4mg/L at Piscataway (Fig 4). The average total protein value was 21.1mg/L. The plants all contained roughly the same distribution of protein, with the exception of Piscataway, which contained almost no protein in material larger than 1.5μm. The other four treatment plants contained an average of 13.6mg/L protein, or an average of 71 percent of the total, in material larger than 1.5μm. Piscataway contained 12.1mg/L protein in material between 1.5μm and 0.45μm and 15.7mg/L protein in material between 30kDa and 1kDa. No protein containing material was found between 0.45μm and 30kDa or smaller than 1kDa at Piscataway. Parkway, Blacksburg, and Pepper's Ferry all contained between 1.9mg/L and 2.6mg/L protein in material between 30kDa and 1kDa, while Blue Plains did not contain any in this size fraction at the method detection limit. Pepper's Ferry and Parkway were the only two plants that had a significant amount of protein in the 0.45μm to 30kDa size fraction with 2.6mg/L and 2.1mg/L, accounting for 11 percent and 15 percent of the total protein, respectively.
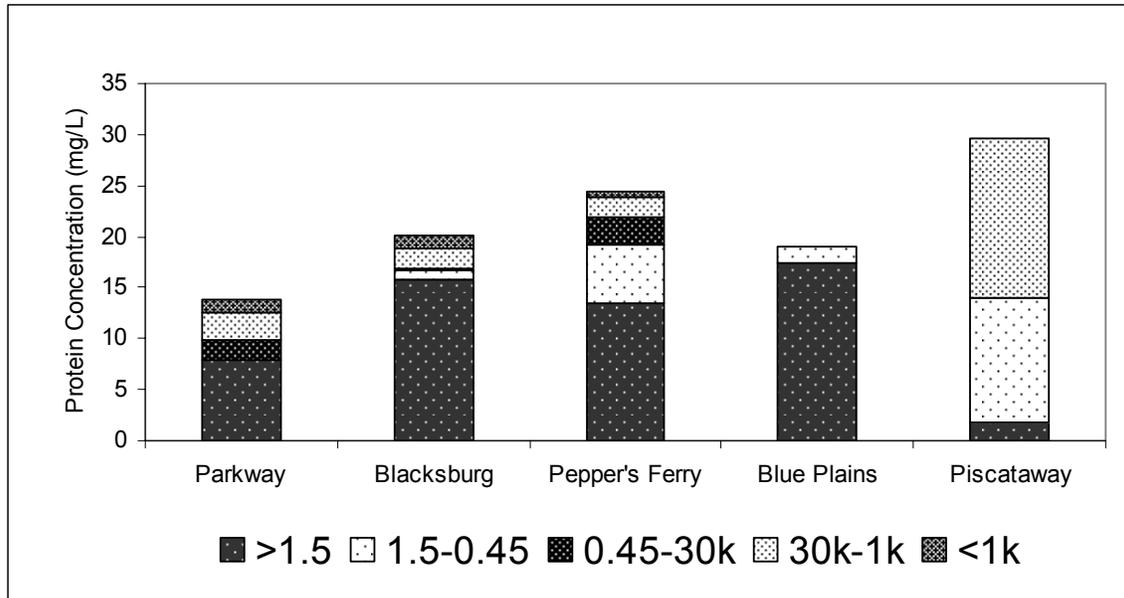
Figure 4. Distribution of protein in the field sample influents.

Total influent humic acid concentration varied between 21.6mg/L at Blue Plains and 85.9 mg/L at Parkway (Fig. 5). The material larger than 1.5μm at Parkway, Pepper's Ferry, and Blacksburg contained 21.1mg/L, 31.9mg/L and 16.6mg/L humic acid, accounting for 25 percent, 23 percent and 45 percent of the total humic acid, respectively. Blue Plains only contained 15 percent, or 3.2 mg/L humic acid, in the fraction larger than 1.5μm, and Piscataway contained no detectable humic acid in this size fraction. The fraction between 1.5μm and 0.45μm was significant at Blue Plains, where it contained 53 percent, or 14.4mg/L of the humic acid. The fraction between 30kDa and 1kDa varied widely in humic acid concentration, as it contained between zero percent and 56 of the total. The fraction smaller than 1kDa contained between 0 percent and 36 percent of the humic acid at the treatment plants studied. The humic acid data from Piscataway was too inconsistent and was not used for further humic acid analysis.
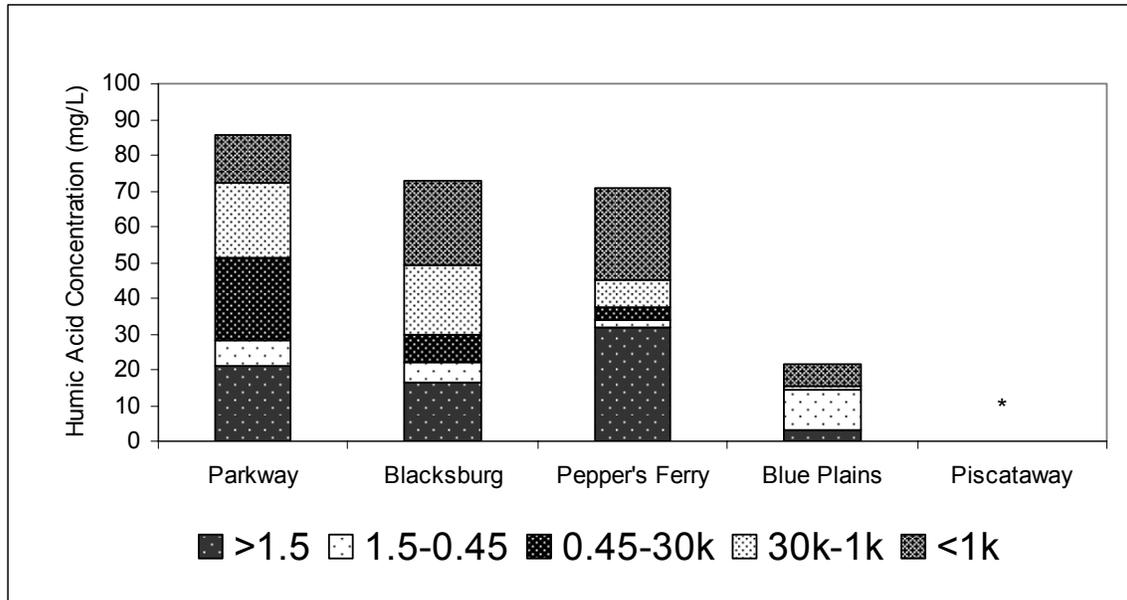
Figure 5. Distribution of humic acids in the field sample influents. *The Piscataway data is not presented.

Although the magnitude of the polysaccharide concentration varied greatly among the treatment plants, from a minimum of 12.9mg/L at Piscataway to a maximum of 58.8mg/L at Pepper's Ferry (Fig. 6), the relative importance of the fraction larger than 1.5μm was consistently high, accounting for between 51 percent and 87 percent of the total polysaccharides. The remainder of the polysaccharides were fairly evenly distributed across the other size fractions for all of the treatment plants.
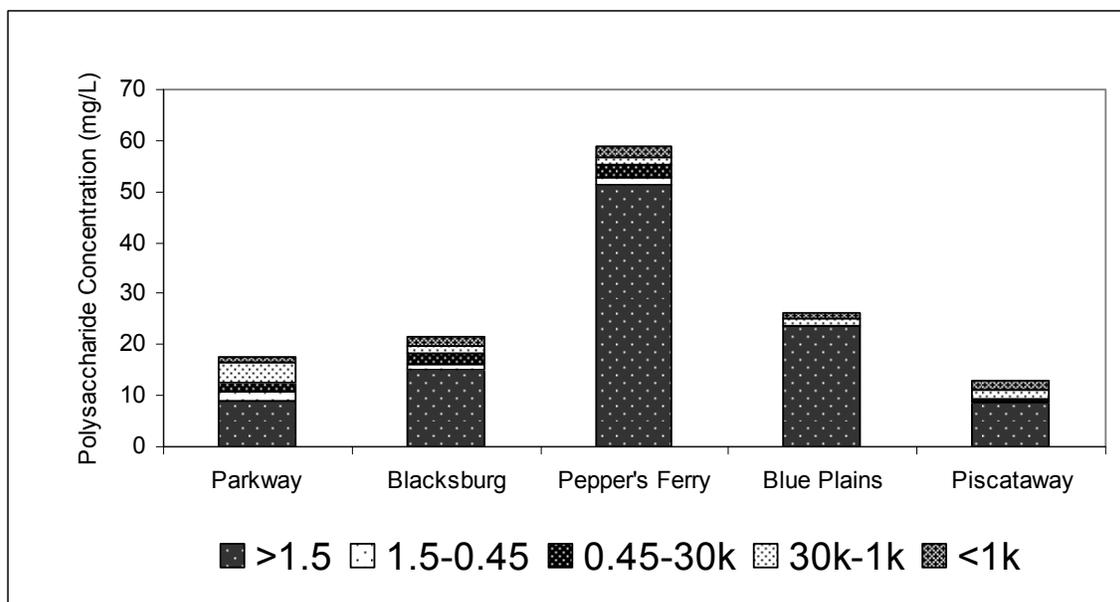
Figure 6. Distribution of polysaccharides in the field sample influents.

A mass balance was performed in an effort to account for the organic nitrogen and organic carbon measured in the samples in terms of the protein, humic acid, and polysaccharide concentrations. Representative formulae for proteins, humic acids, and polysaccharides were used and are found in Table 1.

Table 1. Representative fractions, by weight, of protein (Grady et al, 2003), humic acid (Tan, 2003), and polysaccharide (Grady et al, 2003).

	Protein	Humic Acid	Polysaccharide
C	0.55	0.56	0.40
H	0.07	0.47	0.07
O	0.23	0.36	0.53
N	0.16	0.32	0.00

Using the formulae in Table 1, Figure 7 provides a comparison of the organic nitrogen measured in the whole sample with the cumulative protein, humic acid, and polysaccharide concentration on a nitrogen equivalent basis. The Pepper's Ferry and Blue Plains samples had a higher organic nitrogen concentration than could be accounted for by proteins, humic acids, and polysaccharides. The Parkway and Blacksburg organic nitrogen concentrations were lower than the cumulative nitrogen concentration in the protein, humic acid, and polysaccharide constituents. These results indicate that the organic nitrogen or EPS material was either not accurately measured, or not accurately characterized by the representative formulae used. The humic acid

concentration for Piscataway was omitted due to data inconsistencies. Protein was the largest component of organic nitrogen, accounting for between 44 percent and 80 percent of the total with an average of 61 percent. The humic acids accounted for between 20 percent and 56 percent of the total with an average of 39 percent.
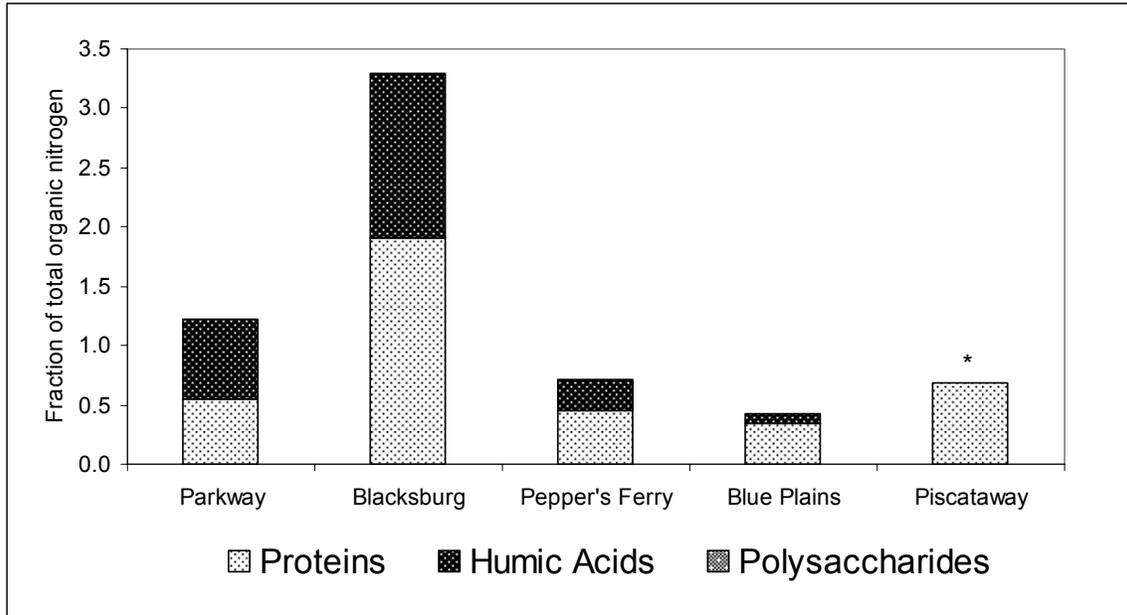
Figure 7. Ratio of the concentration of EPS constituents on a nitrogen equivalent basis to the organic nitrogen concentration in the whole samples. *The humic acid concentration for Piscataway is not presented.

Figure 8 presents the protein, humic acid, and polysaccharide data on a carbon basis in comparison to the TOC measurements for each treatment plant using the representative formulae in Table 2. There was generally good agreement between the total TOC for each plant and the cumulative carbon concentration calculated from the protein, humic acid, and polysaccaride fractions. The cumulative carbon concentrations for Parkway, Blacksburg, and Blue plains are slightly higher than the total TOC for those plants, but not by a large amount. This indicates that most of the carbon in the samples was characterized and the formulae used were fairly accurate for carbon. The total TOC was not measured for Pepper's Ferry and the humic acid concentration for Piscataway was omitted due to data inconsistencies. Humic acids were the largest contributor to TOC, ranging between 38 percent and 77 percent of the total with an average of 61 percent. Proteins and polysaccharides both accounted for about 20 percent of the TOC on average.
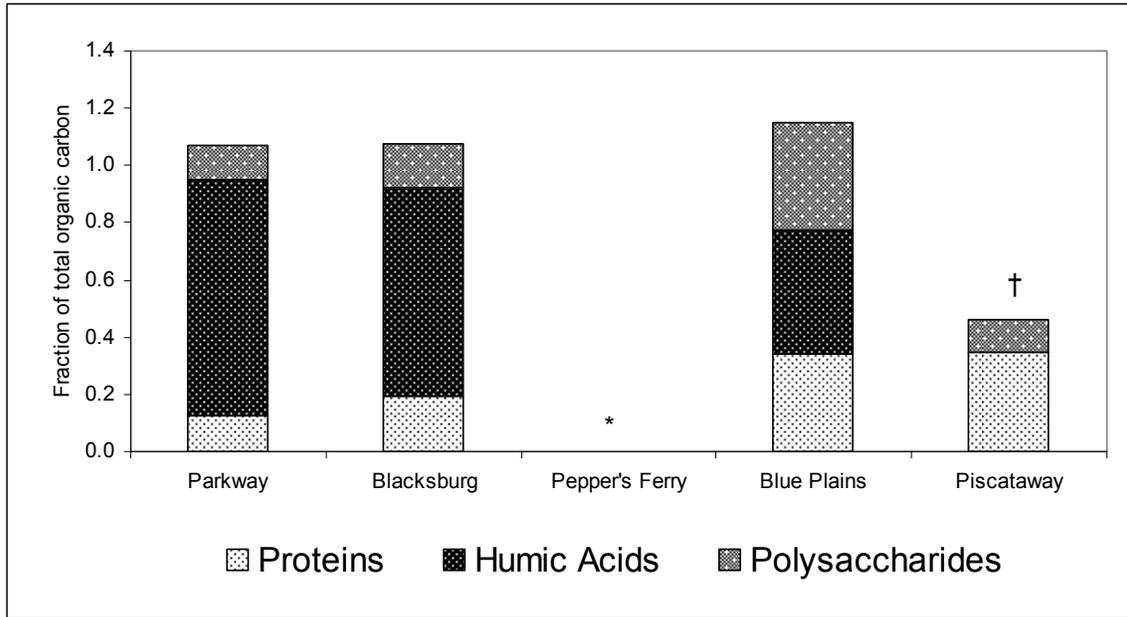
Figure 8. Ratio of the concentration of EPS constituents on a carbon equivalent basis to the organic carbon concentration in the whole samples. *The total TOC was not measured for Pepper's Ferry. †The humic acid concentration for Piscataway is not presented.

Cation Effects

The distribution of the constituents in each of the size fractions was analyzed with respect to the influent aluminum and iron concentrations, the M/D ratio, and the sodium to iron ratio. No consistent trends were found in this analysis, but when the ratio of soluble to particulate material for each constituent was considered with respect to the cations mentioned above, there were trends noted in the aluminum and iron data for organic nitrogen, TOC, humic acids, and polysaccharides. The cation trends can be summarized by the use of the ratio of iron to aluminum (Fe/Al) in the influent. The cation data is shown in Table 2.

Table 2. Treatment plant influent cation concentrations in mg/L and ratios on an equivalent basis.

	Al	Fe	Na	K	Ca	Mg	Fe/Al	M/D
Parkway	1.83	1.14	51.75	11.83	52.23	7.33	0.30	0.79
Blacksburg	0.28	0.34	59.12	12.02	42.02	13.63	0.59	0.89
Pepper's Ferry	1.04	2.70	306.57	11.31	72.42	16.95	1.25	2.72
Blue Plains	0.48	7.21	57.89	12.41	50.74	10.08	7.18	0.89
Piscataway	0.72	2.65	48.61	8.73	78.61	6.13	1.77	0.53

Figure 9 shows the effects of the Fe/Al ratio, on a molar equivalent basis, on the S/P ratios of organic nitrogen and humic acids. Figure 10 shows the effects of the iron to aluminum ratio on TOC and polysaccharides. All of these constituents show a decreasing trend in the S/P ratio with an increasing Fe/Al ratio. No trends were found for the S/P ratio of protein with respect to the Fe/Al ratio.
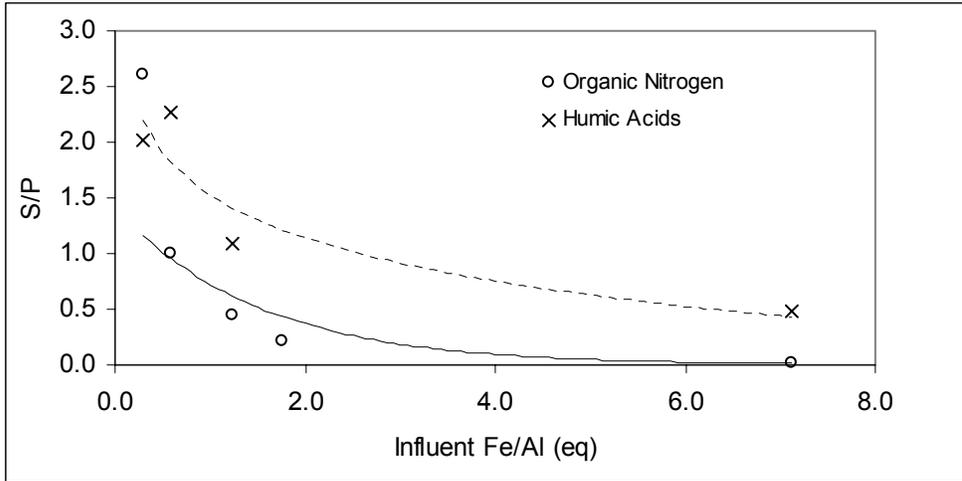
Figure 9. Effects of the influent iron to aluminum ratio (molar equivalent basis) on the S/P ratios of organic nitrogen and humic acids.
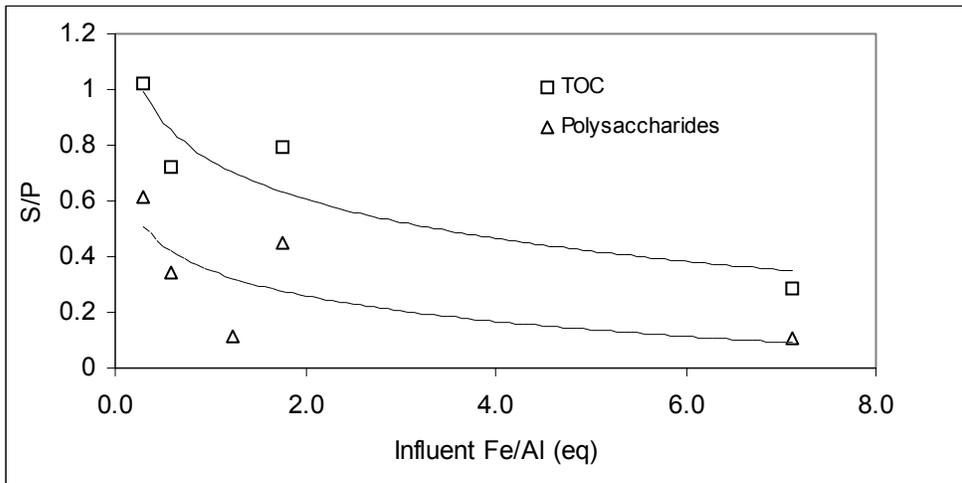
Figure 10. Effects of the influent iron to aluminum ratio (molar equivalent basis) on the S/P ratios of TOC and polysaccharides.

Discussion

Influent size distribution

Most of the influent TKN, 68 percent, was present as ammonia. The remaining 32 percent was organic nitrogen with 78 percent of it in material larger than 0.45μm and 41 percent of it in material larger than 1.5μm. The average protein distribution closely mirrored the organic nitrogen distribution, with 73 percent of the protein present in material larger than 0.45μm, and 58 percent of it in material larger than 1.5μm. The similarity of these distributions is logical because protein is the major constituent of EPS, and thus should be a major contributor to organic nitrogen.

The TOC and polysaccharide distributions were similar to the organic nitrogen and protein distributions. An average of 61 percent of the TOC was present in material larger than 0.45μm, with 51 percent of the TOC in material larger than 1.5μm. An average of 74 percent of the influent polysaccharides was present in material larger than 1.5μm. The average humic acids distribution was much more evenly divided than the other constituents. The material larger than 1.5μm contained 27 percent of the total humic acids and the material smaller than 1kDa contained 28 percent. The other fractions contained between 11 and 18 percent of the total.

These distributions illustrate the importance of the largest size fractions on the organic nitrogen and carbon loading to the activated sludge basin. The performance of the primary treatment can play a large role in carbon removal, but may not play as large a role in TKN removal since most of the TKN is in the form of ammonia. Optimization of primary treatment, such as a coagulation step prior to a primary clarifier, longer settling time in a primary clarifier, or a coarse filter may yield large reductions in the carbon loading and some reduction in the nitrogen loading to the activated sludge basin. This has the potential to reduce the size of the activated sludge basin or the volume of sludge produced in the activated sludge process.

When the EPS constituents are converted into nitrogen and carbon equivalent concentrations, the results did not exactly match those in the size characterization of the organic nitrogen and organic carbon. The size distribution of the nitrogen equivalent concentration of protein closely matched the organic nitrogen size distribution and protein was the largest contributor to the total organic nitrogen in the mass balance analysis. This shows good agreement. However, while the size distribution of the carbon equivalent concentration of most closely matched the TOC size distribution, humic acid was found to be the largest contributor to the total TOC. Polysaccharides contributed the same amount of carbon to the total as protein.

S/P Cation Analysis

The discrepancies between the size distribution and the mass balance do not become any clearer when the S/P cation analysis is considered. There was no trend observed for the protein S/P ratio with the Fe/Al ratio, while the organic nitrogen, TOC, humic acid, and polysaccharide S/P

ratios were all negatively correlated with the Fe/Al ratio. This indicates that it may not be protein that is causing the decrease in organic nitrogen concentration with increasing Fe/Al, but it may be humic acid instead. Humic acid and polysaccharides both decreased with the Fe/Al ratio, so the TOC trend could be caused by either of these constituents.

The data presented here indicate that the S/P ratio varies widely with the influent Fe/Al ratio. This ratio could be quickly used to adjust the S/P ratio of material in the influent to increase the accuracy of the ASM models with respect to the SRT needed for hydrolysis and the response to a diurnal flow pattern. Part of the improved accuracy that could be achieved in the rapid characterization of wastewater for the ASM models is dependent upon determining whether the assumption about inert material used in this analysis is correct. It was assumed that inert material was evenly distributed among the size fractions for each constituent studied. This assumption requires further study to confirm its validity.

Analysis Overview
The trends noted above are limited by the narrow range of cation concentrations observed. While most treatment plants would likely fall into the range presented, this range may be too narrow to fully discern the roles of the cations studied. The size of the data set is also restrictive. Further study with more treatment plant samples is needed.

Since the data from the filtered samples were subtracted from each other to produce the individual size fractions, adjusting one data point affected two size fractions. A total of 21 percent of the activated sludge influent data was affected by data adjustment.

Although there are limitations to the data, the results and conclusions that can be drawn are considerable. To our knowledge, this is the first study to examine the size distribution of activated sludge influent constituents and the effects of cation concentration on this distribution.

Conclusions
Most of the activated sludge influent TKN is present as ammonia, with the majority of the organic nitrogen present in material larger than $0.45\mu m$. The majority of the TOC, protein, and polysaccharides were present in material larger than $0.45\mu m$, while the majority of the humics were present in material smaller than $0.45\mu m$. Proteins were the major contributor to organic nitrogen and humic acids were the major contributor to organic carbon.

Using the division of 0.45μm between particulate and soluble material, the S/P ratio of the influent material was dependent upon the influent Fe/Al ratio. The S/P ratios of organic nitrogen, organic carbon, humic acids and polysaccharides decreased as the Fe/Al ratio increased.

Improved primary treatment through particulate removal can play a large role in carbon removal, but may not play as large a role in TKN removal, since most of the TKN is in the form of ammonia. However, the removal of particulates needs to be considered relative to the role particulate material plays in reducing the fluctuations in the activated sludge system caused by a diurnal flow pattern.

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Distribution of Colloidal Organic Nitrogen and Organic Carbon
in Activated Sludge Effluent as Influenced by Cations

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Abstract

Five activated sludge wastewater treatment plants were examined to determine the size distribution of organic nitrogen and carbon in the effluent and the effects of aluminum, iron, and the monovalent to divalent cation ratio on this distribution. Material larger than 0.45μm contained over half of the effluent TKN. Of the size fractions considered, the largest percentage of the effluent TKN was in material between 1.5μm and 0.45μm. This size fraction accounted for an average of 36 percent of the effluent TKN. The two depth filters studied removed all organic nitrogen in material larger than 0.45μm. By contrast, most of the TOC was found in the smallest size fractions. The material between 30kD and 1kDa accounted for 51 percent of the total TOC, with an additional 30 percent of the TOC in material smaller than 1kDa. Influent aluminum concentration had no observable effect on the concentration or distribution of organic nitrogen or organic carbon. Influent iron appeared to play a role in the flocculation of organic nitrogen containing material between 0.45μm and 1kDa in size. A high monovalent to divalent cation ratio appeared to play a role in deflocculating organic nitrogen containing material larger than 1.5μm and increased the concentration of TOC smaller than 1kDa.

Introduction

Efficient flocculation and settling of activated sludge biomass is important to remove organic matter and the associated nutrients, contaminants, and contaminant formation potential from wastewater treatment plant effluent. However, even when a floc does settle well, some organic matter remains in the effluent as a result of the properties of the particles that remain and the operation of the clarifier (Hilligardt and Hoffmann, 1997). The particles that remain have low settling velocities due to particle size, density, porosity, concentration (Hilligardt and Hoffmann, 1997), or microbial population (Wilén et al., 2003). The efficient operation of a clarifier is also a function of operational and design parameters, such as the sludge blanket depth and thickness, solids loading, overflow rate, and clarifier shape (Tchobanoglous et al., 2003). These parameters can decrease the particle settling velocity, or induce mixing of settled floc particles into the clarified liquid.

The small particles that remain after clarification contain a significant percentage of the oxygen demand and nutrients that are released from the treatment plant. Neis and Tiehm (1997) found that an average of 74 percent of the chemical oxygen demand (COD) and 36 percent of the phosphorus in the secondary effluent that they sampled was associated with particles smaller than 0.1μm. Holbrook et al. (in press), found an average of 8.3mg/L organic carbon smaller than 1.5μm, 5.7mg/L organic carbon smaller than 30kDa, and 2.5 mg/L organic carbon smaller than 1kDa in secondary clarifier effluent. Oxygen demand and nutrients released from a wastewater treatment plant can cause deterioration of the receiving water body.

In addition to nutrients, there are contaminants and contaminant formation potential associated with effluent organic matter. Endocrine disrupting chemicals (EDCs) bind to effluent particles and are released from the treatment plant along with the small organic particles (Holbrook et al., in press). The carcinogen N-nitrosodimethylamine (NDMA) is formed when chloramine interacts with nitrogen-containing compounds, including dimethylamine (DMA) and some types of cationic polymer, during disinfection (Najm and Trussel, 2001). Although the formation of NDMA from DMA has been heavily studied, this reaction fails to account for the majority of the NDMA formation potential in activated sludge (Mitch et al., 2003). Other nitrogen-containing compounds must react with chloramine to produce NDMA. It is important to understand the distribution of nitrogen-containing material in the effluent in order to improve process design to facilitate its removal and reduce the NDMA formation potential.

One option for small organic matter removal is to flocculate it so that it can be removed more easily via settling or filtration. Cations play a large role in how an activated sludge biomass flocculates. This occurs because cations interact with extracellular polymeric substances (EPS) that are present in influent wastewater, produced during biomass growth, and released during cell lysis. Extracellular polymeric substances are comprised of proteins, polysaccharides, and humic acids, with protein being the dominant constituent (Urbain et al., 1993, Frølund et al., 1996, Nielsen et al., 1996, Dignac et al., 1998, Wilén et al., 2003, and Leppard et al., 2003).

Since the carboxyl and amino functional groups in EPS are negatively charged, these functional groups interact with cations in solution. Divalent cations form a bridge between negatively charged EPS, helping to flocculate the biomass. Murthy et al. (1998) observed an improvement in activated sludge settling after the addition of magnesium in full-scale field trials. The role of calcium was supported by Biggs et al. (2001), who observed an increase in floc size when calcium was added to an activated sludge solution. When the activated sludge mixed liquor has a high monovalent to divalent cation (M/D) ratio, a weak floc structure and poor settling can result (Higgins and Novak, 1997b). A high M/D ratio has been correlated with high activated sludge

effluent COD and biopolymer (Murthy and Novak, 2001). It is hypothesized that EPS may make up a large amount of the organic matter that remains in wastewater effluent.

Aluminum and iron are both trivalent cations that are present in wastewater and play a significant role in bioflocculation (Park et al., in press). Novak et al. (2003) proposed a model in which cations bind to EPS depending on whether the EPS was present in the activated sludge influent or was produced during the activated sludge process. They proposed that the EPS produced by microbial growth is linked to divalent cations and EPS from the influent and cell lysis products is linked to trivalent cations. However, not much is known about the role of trivalent cations in activated sludge flocculation other than the above studies.

As nitrogen, NDMA, and EDC contamination in wastewater receiving water bodies becomes a larger environmental and public health issue, it is becoming more important to advance the state of the art in their removal. It is important to better understand flocculation mechanisms to enhance the removal of organic matter from effluent. Although further removal is possible with some forms of tertiary treatment, including depth filtration, improvements in flocculation may be a more cost-effective removal option. By examining the relationships between cations and the size distribution of organic nitrogen and organic carbon in activated sludge effluent, we hope to gain further insight into flocculation mechanisms.

Materials and Methods

Primary effluent and secondary effluent were obtained from five wastewater treatment plants and transported to the laboratory either directly (Blacksburg and Peppers Ferry samples) or shipped overnight (Blue Plains, Piscataway, and Parkway samples). The Blue Plains and Piscataway wastewater treatment plants are provided with depth filtration following their activated sludge clarifiers and effluent samples from these filters were also obtained. The samples that were shipped were transported in coolers containing ice packs to maintain a temperature of approximately 4°C. The samples that were transported directly to the laboratory were stored overnight at 4°C. All samples were transported in plastic containers.

After an unfiltered sample was removed for characterization (whole fraction), the remainder of the sample was centrifuged for 15 minutes at 7,000 RPM (8,700xg) before being filtered by a 1.5μm glass fiber filter, a 0.45μm nitrocellulose membrane filter, a 30kDa ultrafilter, and a 1kDa ultrafilter. All samples were stored in acid-washed glass containers at 4°C at a pH less than 2. The pH was adjusted to this value with concentrated sulfuric acid.

Total Kjeldahl Nitrogen (TKN) measurements were conducted according to method 4500-N_{org} C (APHA, 1998) and ammonia measurements were conducted according to method 4500-NH_3 C (APHA, 1998). Total organic carbon was analyzed with a Sievers 800 TOC Analyzer for fractions smaller than 0.45μm and with a Dohrmann Carbon Analyzer for samples larger than 0.45μm. The whole influent samples were digested according to method 3030 E (APHA, 1998) and analyzed by ICP (Ultima) for sodium, potassium, magnesium, calcium, aluminum and iron. Organic nitrogen is defined as the TKN of a size fraction less the ammonia concentration for the sample.

Results

Sample Key:

Whole – unfiltered sample

>1.5 – sample fraction larger than 1.5μm

1.5-0.45 – sample fraction between 0.45μm and 1.5 μm

0.45-30k – sample fraction between 0.45 μm and 30kDa

30k-1k – sample fraction between 1kDa and 30kDa

<1k – sample fraction smaller than 1kDa

Size Fractionation

The distribution of TKN in the five treatment plants studied is shown in Figure 1. All five plants had effluent TKN concentrations less than 1.2mg-N/L. This indicates that the nitrification and clarification processes are performing very well.
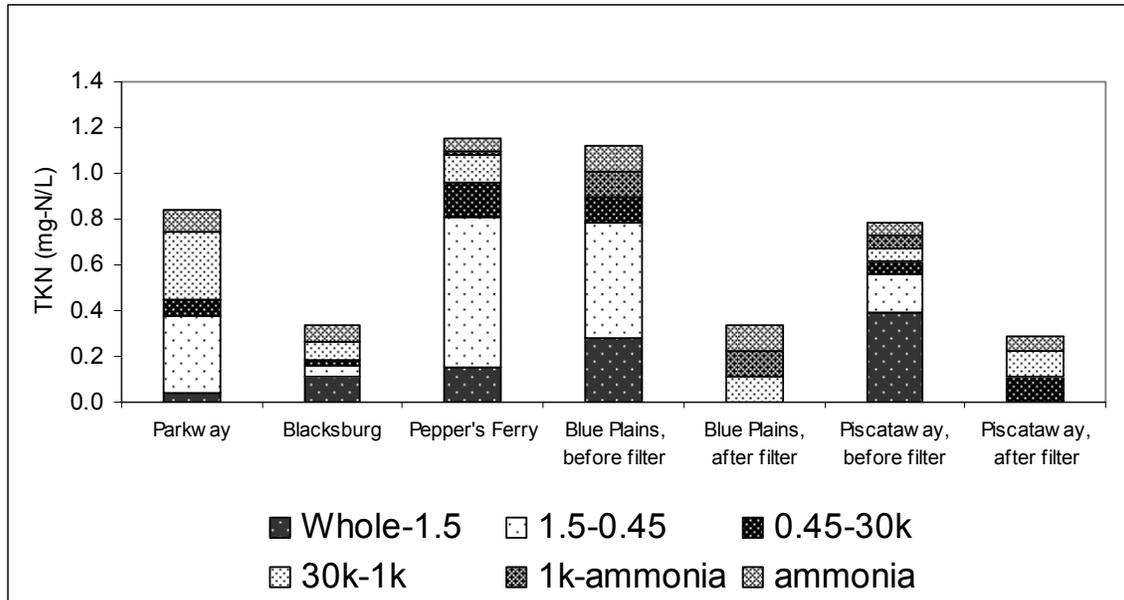
Figure 1. Distribution of TKN in sample effluent.

On average, 61 percent of the secondary effluent TKN was contained in material larger than 0.45μm with 36 percent of the total TKN contained in particles between 1.5μm and 0.45μm. The material between 0.45mm and 1kDa accounted for an average of 24 percent of the effluent TKN, with the remaining 15 percent consisting of soluble organic nitrogen (<1kDa) and ammonia. This size distribution shows that a significant amount of the total effluent organic nitrogen is contained in small particles, but the 1.5μm-0.45μm fraction was the single largest contributor to effluent TKN.

The total effluent TOC was lowest at Piscataway, which had 4.3mg-C/L, and highest at Parkway, which had 6.8mg-C/L (Fig. 2). The distribution of TOC was very consistent among the treatment plants, with the fraction between 30kDa and 1kDa accounting for between 41 percent and 68 percent of the total TOC and the fraction smaller than 1kDa accounting for between 27 percent and 69 percent of the total TOC. None of the other size fractions contained more than 10 percent of the total TOC. The Blue Plains TOC data was too inconsistent to be included in this analysis. The TOC was not measured for the size fractions larger than 0.45μm at Pepper's Ferry.
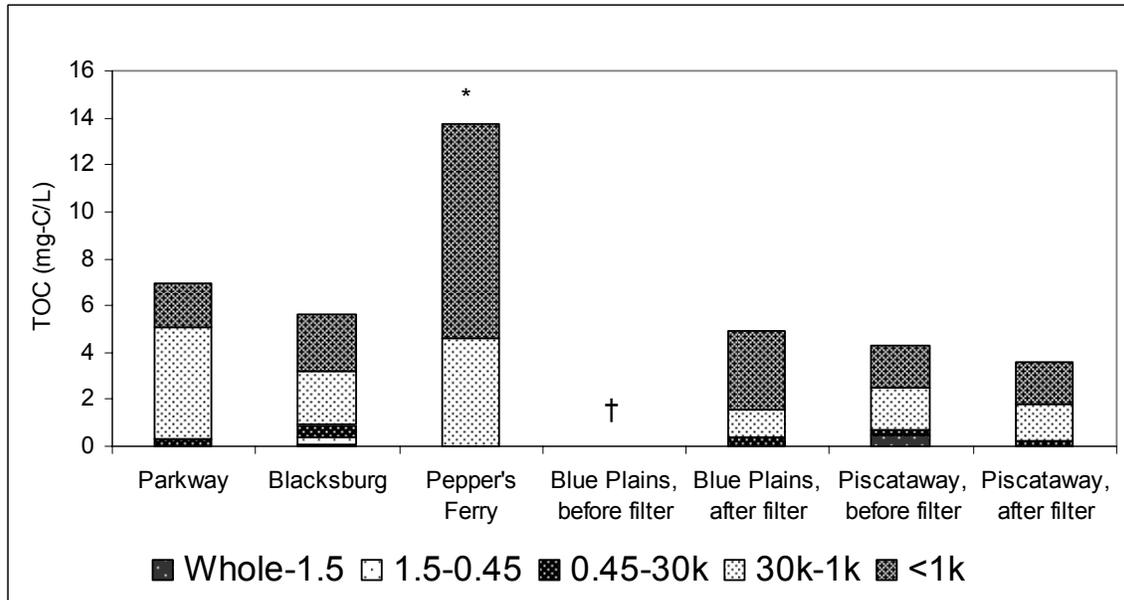
Figure 2. Distribution of TOC in the field sample effluents. *The TOC for material larger than 0.45μm was not measured for Pepper's Ferry. † The Blue Plains, before filter sample was too inconsistent for analysis.

Filtration Performance

As indicated in Figure 1, the depth filter at Blue Plains removed 70 percent of the secondary effluent TKN, all of which was larger than 30kDa. The depth filter at Piscataway removed 64 percent of the secondary effluent TKN, all of which was larger than 0.45μm. Due to the inconsistencies in the TOC data from the Blue Plains secondary effluent, no analysis of size fraction removal was possible. The TOC removal at Piscataway was low at 16 percent, but this removal clearly occurred in the concentration of TOC in material larger than 1.5μm (Fig. 2).

The removal of particles by tertiary filtration was not simply a size exclusion process. While we found that all of the TKN-containing material larger than 0.45μm was removed from both filters, the size distribution of TKN-containing particles smaller than 0.45μm after the filters was different than before the filters. The Blue Plains filter shifted to smaller particles, while the Piscataway filter shifted to larger particles. These transformations could be due to biological activity within the filters or breakthrough of certain particle sizes. Although only one sample was able to be analyzed for TOC removal, that removal occurred in the material larger than 1.5μm.

Effects of Cations

The cation data from the treatment plants is shown in Table 1.

Table 1. Treatment plant influent cation concentrations in mg/L and ratios on an equivalent basis.

	Al	Fe	Na	K	Ca	Mg	Fe/Al	M/D
Parkway	1.83	1.14	51.75	11.83	52.23	7.33	0.30	0.79
Blacksburg	0.28	0.34	59.12	12.02	42.02	13.63	0.59	0.89
Pepper's Ferry	1.04	2.70	306.57	11.31	72.42	16.95	1.25	2.72
Blue Plains	0.48	7.21	57.89	12.41	50.74	10.08	7.18	0.89
Piscataway	0.72	2.65	48.61	8.73	78.61	6.13	1.77	0.53

The influent aluminum concentration did not appear to have a strong effect on the total effluent TKN or the size distribution of organic nitrogen containing particles (Fig. 3). There was a slight increase in organic nitrogen concentration in the $1.5\mu m$-$0.45\mu m$ fraction, accompanied by a slight decrease in the Whole-$1.5\mu m$ fraction, although the magnitudes of the changes were small and the trends were weak. There were no trends noted in the size fractions smaller than $0.45\mu m$ and this data was omitted from Figure 3.
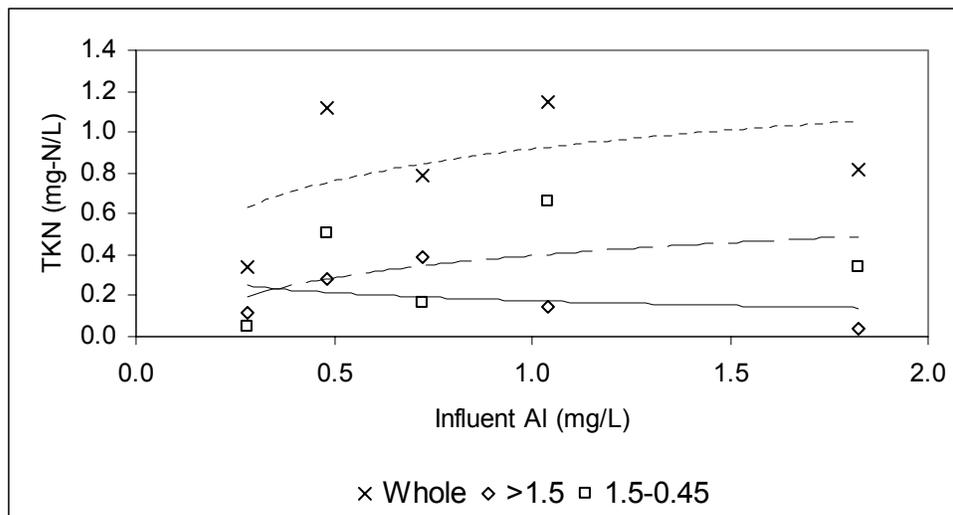
Figure 3. TKN concentration for three size fractions as a function of the influent aluminum concentration.

The TOC concentrations were independent of the influent aluminum concentration (Fig. 4). There was a slight increase in the 30kDa-1kDa fraction, but this was not correlated with a decrease in concentration in any size fraction or an increase in the total TOC. The fractions larger than $1.5\mu m$ and between $1.5\mu m$ and $0.45\mu m$ were omitted from Figure 4 for clarity.
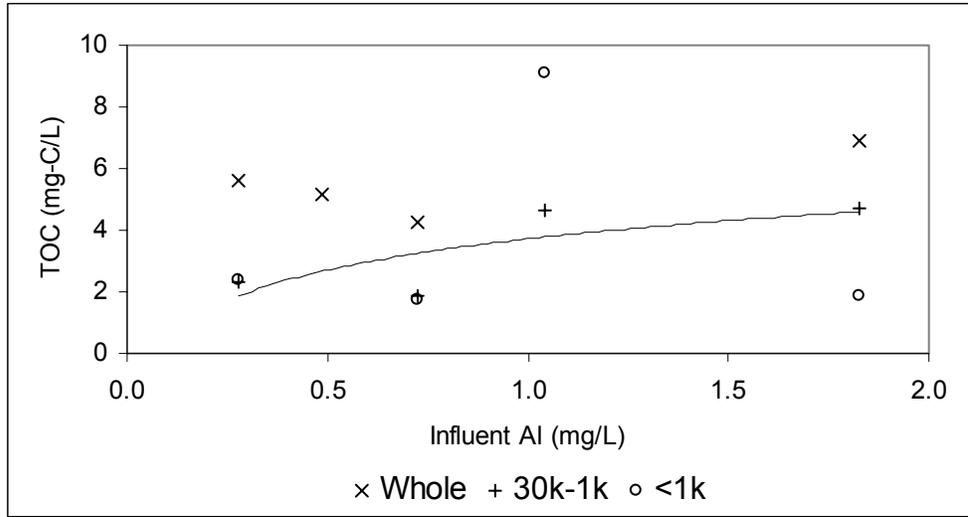
Figure 4. Effluent TOC distribution of field samples with respect to influent aluminum concentration.

The total effluent TKN was positively correlated with the influent iron concentration (Fig. 5). The majority of this increase occurred in the 1.5μm-0.45μm fraction. There was a small decrease in the organic nitrogen concentration in the 30kDa-1kDa fraction with an increase in iron concentration. There were no trends noted in the ammonia concentration and this data was omitted from Figure 5.
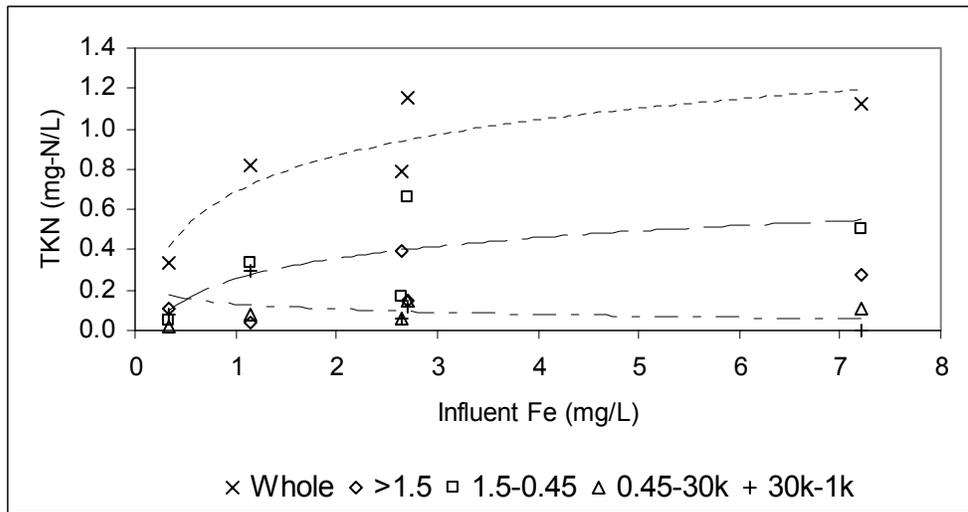
Figure 5. TKN concentration for five size fractions as a function of the influent iron concentration.

There were no trends noted for the concentration of TOC in the size fractions with respect to iron (Fig. 6). The Blue Plains plant had the highest amount of iron, but the data was too inconsistent

to report the concentration in each size fraction. Only the concentration of the whole sample from Blue Plains is presented in Figure 6.
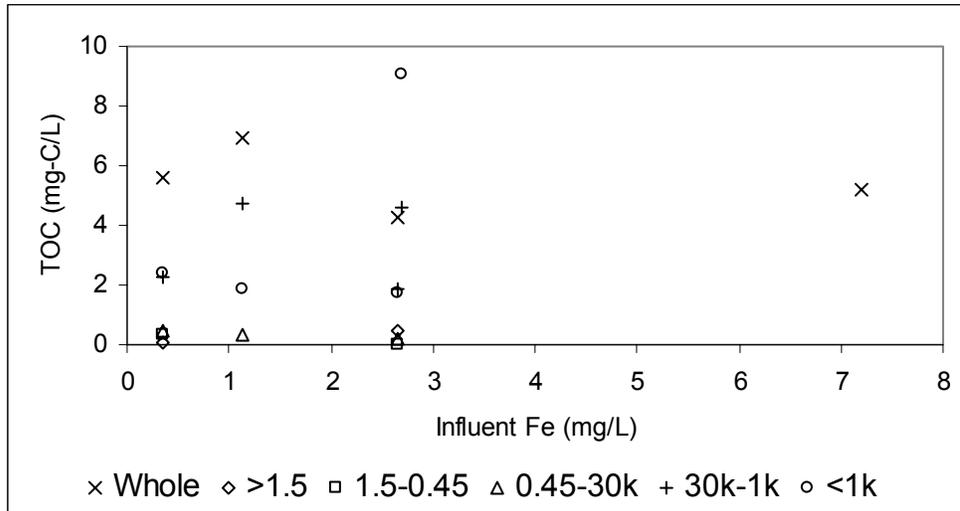
Figure 6. Effluent TOC distribution of field samples with respect to influent iron concentration.

As the M/D ratio increased, the total effluent TKN increased slightly (Fig. 7). There was an increase in the 1.5μm-0.45μm size fraction that is accompanied by a small decrease in the Whole-1.5μm fraction. There were no trends noted in the size fractions smaller than 0.45μm and this data was omitted from Figure 7.
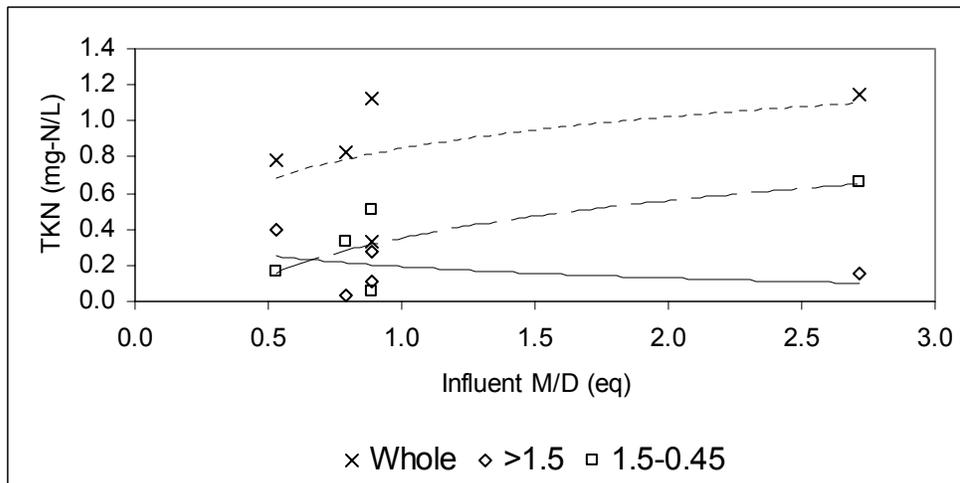
Figure 7. TKN concentrations for three size fractions as a function of the influent M/D ratio.

Since the TOC was not measured for fractions larger than 0.45μm for the Pepper's Ferry sample and this sample had the highest M/D ratio, trends cannot be fully determined for the whole TOC

concentration with respect to the M/D ratio (Fig. 8). However, there was a notable increase in the concentration of TOC in the fraction smaller than 1kDa, driven up by the Pepper's Ferry sample, up even higher than the total TOC concentration of the other treatment plants.
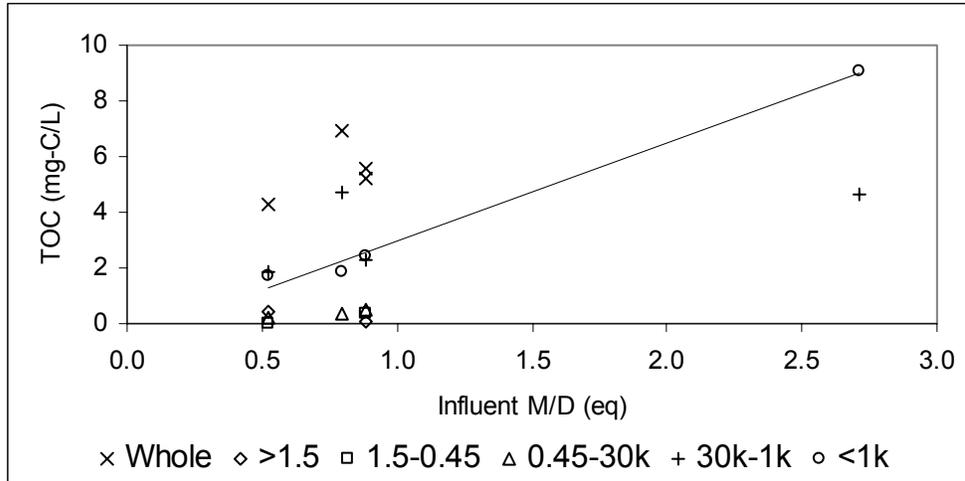
Figure 8. Effluent TOC distribution of field samples with respect to influent M/D ratio.

Discussion

The trends noted above are limited by the narrow range of cation concentrations observed. While most treatment plants would likely fall into the range presented here, the range may be too narrow to determine the roles of the cations studied independent of process factors like SRT, clarifier performance, and process type. Further study on more treatment plants is needed.

Although the data set was limited, the results and conclusions that can be drawn are significant. To our knowledge, this is the first study to examine the size distribution of TKN and TOC in activated sludge effluent to such small size fractions. The size distribution analysis showed that over half of the effluent TKN was contained in material between $1.5\mu m$ and $0.45\mu m$ in size, with the majority of this material smaller than 1.5mm. The material between $1.5\mu m$ and $0.45\mu m$ contained an average of 36 percent of the effluent TKN. By contrast, most of the TOC was found in the smallest size fractions. The material between 30kD and 1kDa accounted for 51 percent of the total TOC, with an additional 30 percent of the TOC in material smaller than 1kDa.

There were no trends in the TKN analysis associated with aluminum. The influent iron concentration was correlated with an increase in total TKN, and increase in the concentration of organic nitrogen in the $1.5\mu m$ to $0.45\mu m$ fraction and a decrease in the concentration of organic

nitrogen in the 30kDa to 1kDa size fraction. There was no net change in the concentration of organic nitrogen in the 0.45μm to 30kDa size fraction. This suggests that iron plays a role in flocculating material between 0.45μm and 1kDa into material between 1.5μm and 0.45μm. The increase in total TKN suggests that more TKN is not settling out in the clarifier and making it through to the effluent. This could be due to iron forming larger and less dense floc with a reduced settling velocity.

The M/D ratio was correlated with a slight increase in the total TKN, driven by an increase in the 1.5μm-0.45μm fraction. This increase was accompanied by a decrease in the organic nitrogen concentration of the fraction larger than 1.5μm. This suggests that a high M/D ratio may cause deflocculation of material larger than 1.5μm.

As with TKN, the influent aluminum concentration did not greatly effect the TOC distribution. An increase in the M/D ratio appears to increase the concentration of TOC in material smaller than 1kDa, but no other increases or decreases were seen. Because of inconsistencies in data, no TOC trends could be found with respect to iron.

These results are especially significant when the tertiary filter performance is considered. The filters studied removed an average of 67 percent of the effluent TKN from the secondary clarifier. Most significant is that all of the detectable organic nitrogen larger than 0.45μm was removed from both clarifier effluent samples.

The removal of TOC by depth filtration was not as successful as organic nitrogen. The TOC removal at the Piscataway filter was only 10 percent and the Blue Plains data was too inconsistent for analysis. The correlations between TOC size distribution and cation concentration were only observed in fractions smaller than the size removal cutoff that was observed for TKN. Therefore, the influent cation concentration is not expected to affect the TOC removal efficiency in depth filtration.

The efficient removal of organic nitrogen containing particles by depth filtration is surprising considering that the removal efficiency reported for particles between 2μm and 10μm in depth filters has been reported to be between -20 percent (more particles in filter effluent than influent) and 50 percent (Kaminski et al., 1997 and van der Graff, 2001). This range was a function of the coagulant dose, filter particle size, and breakthrough stage of the filter. These studies measured effluent particle count, and so 2-5μm particles were at the bottom end of their detection limit. The data presented in this paper is focused on the removal of organic nitrogen containing particles and removal is measured by TKN removal, not by particle count. It is possible that the efficient

performance of the filters in this study may be accounted for by a preferential removal of organic nitrogen containing particles.

The filtration data underlies the importance of the 1.5μm-0.45μm fraction in effluent TKN. While raising the influent iron concentration may increase the total TKN concentration in secondary effluent, the increase will be largest in the material between 1.5μm and 0.45μm, all of which is removable by depth filtration. Although high M/D values were correlated with a slight increase in total TKN, the most significant change was a shift in the particle size of organic nitrogen containing particles from particles larger than 1.5μm to particles between 1.5μm and 0.45μm. All detectable material larger than 0.45μm was removed in tertiary filtration, so the M/D parameter would not be expected to have as large an impact on the effluent quality of a treatment plant outfitted with tertiary depth filtration.

Conclusions

While the most effective means for achieving low organic nitrogen and carbon concentrations in activated sludge effluent will be unique for each treatment plant the cation concentration has been shown to impact both the concentration and size distribution of material in the effluent that contains organic nitrogen and carbon. The impacts of the cation concentrations should be considered in process design.

To this end, this study found that although the role of aluminum in flocculating activated sludge remains unclear, iron appears to play a role in flocculating nitrogen containing material in the size range of 0.45μm-1kDa. Neither the concentration nor distribution of TOC was related to the iron concentration. Monovalent cations appear to deflocculate organic nitrogen containing material larger than 1.5μm into material between 0.45μm and 1.5μm and increase the concentration of TOC smaller than 1kDa. While tertiary depth filtration was found to be more effective at TKN removal than expected, with particle removal down to 0.45μm being observed, removal of TOC was poor. The cation concentration appears to affect the concentration and distribution of organic nitrogen much more than organic carbon.

Further inquiry is needed to verify the results obtained in this study and to better elucidate the role of aluminum in activated sludge treatment. While the results presented here show the potential for better organic nitrogen removal by the addition of cations during the activated sludge process, tertiary treatment is a very valuable tool for achieving low organic nitrogen concentrations in effluent.

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Distribution of Colloidal EPS in Activated Sludge Effluent
as Influenced by Cations

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Abstract

Five activated sludge wastewater treatment plants were examined to determine the size distribution of extracellular polymeric substances (EPS) in the effluent and the effects of aluminum, iron, and the monovalent to divalent cation ratio on this distribution. Protein, humic acid, and polysaccharide were the EPS constituents that were examined. Effluent protein was mostly contained in material larger than $0.45\mu m$, while most of the humic acid and polysaccharide was contained in material smaller than 30kDa. Iron appeared to be flocculating protein-containing material in the $1.5\mu m$ to $0.45\mu m$ size range into material larger than $1.5\mu m$ and causing an increase in the total protein concentration. The M/D ratio was correlated with an increase in the total polysaccharide concentration as a result of increases in the larger than $1.5\mu m$, $1.5\mu m$ to $0.45\mu m$, and the $0.45\mu m$ to 30kDa size fractions. Depth filtration removed protein containing material larger than $0.45\mu m$ but did not do a consistent job of removing polysaccharide.

Introduction

Cations affect activated sludge flocculation because the extracellular polymeric substances (EPS) that are present in wastewater influent, produced and excreted by cells during growth, and released from cells during death and lysis, have a negative surface charge. This negative surface charge comes from the amino and carboxyl functional groups of protein (Higgins and Novak, 1997a), the most common EPS constituent (Urbain et al., 1993, Frølund et al., 1996, Nielsen et al., 1996, Dignac et al., 1998, Wilén et al., 2003, and Leppard et al., 2003).

Divalent cations form a cation bridge between negatively charged EPS, helping to flocculate the biomass. Murthy et al. (1998) observed an improvement in activated sludge settling after the addition of magnesium in full-scale field trials. The role of calcium was supported by Biggs et al. (2001), who observed an increase in floc size when calcium was added to an activated sludge solution. When the activated sludge mixed liquor has a high monovalent to divalent cation (M/D) ratio, a weak floc structure and poor settling can result (Higgins and Novak, 1997b). A high M/D ratio has been correlated with high activated sludge effluent COD and biopolymer (Murthy and

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Novak, 2001). It is hypothesized that small pieces of EPS may make up a large amount of the organic matter that remains in wastewater effluent.

Efficient flocculation and settling of an activated sludge biomass is important to remove organic matter and the associated nutrients, contaminants, and contaminant formation potential from wastewater treatment plant effluent. The smallest particles that remain after clarification contain a significant percentage of the oxygen demand and nutrients that are released from the treatment plant. Neis and Tiehm (1997) found that an average of 74 percent of the chemical oxygen demand (COD) and 36 percent of the phosphorus in the secondary effluent that they sampled is associated with particles smaller than 0.1μm. Holbrook et al. (in press), found an average of 8.3mg/L organic carbon smaller than 1.5μm, 5.7mg/L organic carbon smaller than 30kDa, and 2.5 mg/L organic carbon smaller than 1kDa in secondary clarifier effluent. Oxygen demand and nutrients released from a wastewater treatment plant can cause a deterioration of the receiving water body.

In addition to nutrients, there are contaminants and contaminant formation potential associated with effluent organic matter. Endocrine disrupting compounds (EDCs) bind to effluent particles and are released from the treatment plant in this along with the organic matter (Holbrook et al., in press).   The carcinogen N-nitrosodimethylamine (NDMA) is formed when chloramine interacts with nitrogen-containing compounds, including DMA and some types of cationic polymer, during disinfection (Najm and Trussel, 2001). Although the formation of NDMA from DMA has been heavily studied, this reaction fails to account for the majority of the NDMA formation potential in activated sludge (Mitch et al., 2003). Other nitrogen-containing compounds must react with chloramine to produce NDMA. It is important to learn more about effluent particle size distributions in order to develop better removal options.

Aluminum and iron are both trivalent cations that are present in wastewater and play a significant role in bioflocculation (Park et al., in press).   Novak et al. (2003) proposed a model in which cations bind to EPS depending on the source of the material. Based on cation release during aerobic and anaerobic digestion, they hypothesized that the EPS produced by microbial growth is linked to divalent cations, and EPS from the influent and cell lysis products is linked to trivalent cations. However, not much is known about the role of trivalent cations in activated sludge flocculation other than the above studies.

As nitrogen, NDMA, and EDC contamination in wastewater receiving water bodies becomes a larger environmental and public health issue, it is becoming more important to advance the state of the art in their removal. Since small particulate matter that does not settle out in secondary

clarification contains nitrogen, with its associated NDMA formation potential, and EDCs, it is important to better understand flocculation mechanisms to enhance their removal.

This study examined the makeup of the EPS material contained in activated sludge wastewater effluent and the effects of cations on this material. The performance of tertiary depth filtration on the removal of the EPS constituents is also examined. The data in this paper is in conjunction with Robbins et al. (in press).

Methods

Activated sludge effluent grab samples were obtained from five wastewater treatment plants and transported to the laboratory either directly (Blacksburg and Peppers Ferry samples) or shipped overnight (Blue Plains, Piscataway, and Parkway samples). The Blue Plains and Piscataway wastewater treatment plants are equipped with depth filtration following their activated sludge clarifiers, and effluent samples from these filters were also obtained. The samples that were shipped were transported in coolers containing ice packs to maintain a temperature of approximately 4°C. The samples that were transported directly to the laboratory were stored overnight at 4°C. All samples were transported in plastic containers.

Sample treatment and analysis

After an unfiltered sample was taken (whole fraction), the remainder of the sample was centrifuged for 15 minutes at 7,000 RPM (8,700xg) before filtration by a 1.5μm glass fiber filter, a 0.45μm nitrocellulose membrane filter, a 30,000 Dalton (30kDa) ultrafilter, or a 1,000 Dalton (1kDa) ultrafilter. Samples to be analyzed for TOC and metals were stored in acid-washed glass containers at 4°C and at a pH less than two. The pH was adjusted to this value with concentrated sulfuric acid. Samples for protein, humic acid, and polysaccharide measurements were stored at -15°C in sterile polypropylene vials.

Total organic carbon was analyzed with a Sievers 800 TOC Analyzer for fractions smaller than 0.45μm and with a Dohrmann Carbon Analyzer for samples larger than 0.45μm. The whole influent samples were digested according to method 3030 E. (APHA, 1998) and analyzed by ICP (Ultima) for sodium, potassium, magnesium, calcium, aluminum and iron. Protein and humic acid measurements were performed according to Frølund et al. (1996) and polysaccharides were performed according to Dubois et al. (1956)*.* Spectroscopic measurements were made using a Beckmann DU640 spectrometer.

Data Analysis

In order to examine the effects of cations on the influent characteristics, the results for the samples of adjoining sizes were subtracted from each other in order to obtain the quantity of material between the two sizes. For example, the TOC for the sample filtered through a $0.45\mu m$ filter was subtracted from the TOC for the sample filtered through a $1.5\mu m$ filter to obtain the quantity of TOC that is smaller than $1.5\mu m$ but larger than $0.45\mu m$. The fractions were also analyzed to determine what percentage of the whole sample they represented. On occasion, these analyses produced a negative and obviously incorrect value. The inconsistent data points were adjusted in order to include as much data as possible into the research efforts.

Data inconsistencies occurred when a sample produced a higher value than a sample from the same source filtered through a more porous membrane; or a value lower than a sample from the same source filtered through a less porous membrane. When inconsistencies occurred, the values that were too high were adjusted to equal the next larger size fraction and the values that were too low were adjusted to equal the next smaller size fraction. For example, if a sample filtered through a 30kDa filter produced a value larger than the sample from the same source filtered through a $0.45\mu m$ filter, the data from the 30kDa filter would be adjusted to equal that from the $0.45\mu m$ filter. Likewise, if a sample filtered through a 30kDa filter produced a value smaller than the sample from the same source filtered through a 1kDa filter, the data from the 30kDa filter would be adjusted to equal that from the 1kDa filter. This is illustrated in in Figure 1. The value for the $0.45\mu m$ filter sample did not fit the general trend. Since this value was too low and the values around it confirm that this data point is incongruous; the fraction between $1.5\mu m$ and $0.45\mu m$ would be too large and the fraction between $0.45\mu m$ and 30kDa would be negative. Figure 2 shows how that data point was treated to bring it into line with the other samples from the same source. It was adjusted upwards to equal the 30kDa value.
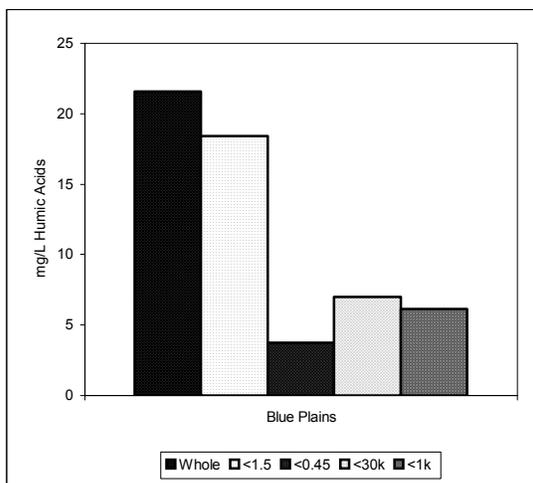

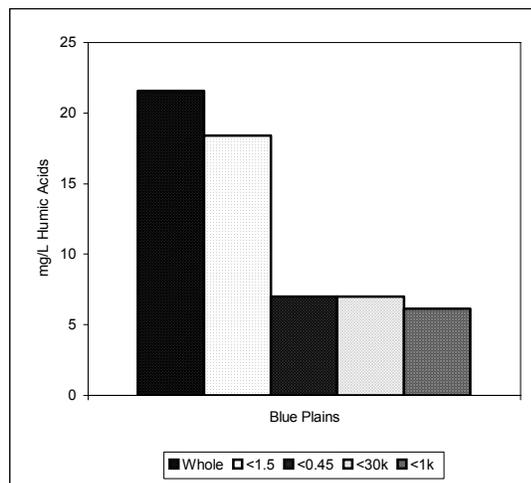Figure 1. Before data correction.   Figure 2. After data correction.

These changes only occurred if it was obvious that one data point did not fit with the other filtered samples. If a general trend could not be determined from the set of filtered data, generally there were multiple inconsistencies and all of the data from that analysis was disregarded.

In addition to the magnitude of the concentration for each size fraction, the concentration relative to the total concentration was considered for the size fractions of each constituent. This information was only included here if it provided additional information.

Results
Sample Key:
Whole – unfiltered sample
Whole-1.5 – sample fraction larger than 1.5μm
1.5-0.45 – sample fraction between 0.45μm and 1.5 μm
0.45-30k – sample fraction between 0.45 μm and 30kDa
30k-1k – sample fraction between 1kDa and 30kDa
<1k – sample fraction smaller than 1kDa

Constituent Distribution
The effluent protein was lower than 5mg/L at all of the treatment plants except for Blue Plains, which contained 29.4mg/L protein (Fig. 3). The fraction larger than 1.5μm accounted for 96 percent of the protein at Blue Plains. This fraction was also significant at Piscataway, Pepper's Ferry, and Parkway, where it accounted for 43 percent, 36 percent, and 70 percent, respectively. On average, the 1.5μm to 0.45μm size fraction contained 32 percent of the protein, although the values ranged from 4 percent to 73 percent. Material larger than 0.45μm contained 81 percent of the effluent protein on average. The fraction between 0.45μm and 30kDa accounted for 17 percent of the protein on average, ranging from 0 percent to 34 percent. With the exception of the fraction larger than 1.5μm at Blue Plains, none of the size fractions contained more than 1.6mg/L of protein. The filter effluent from Blue Plains was too inconsistent for analysis.
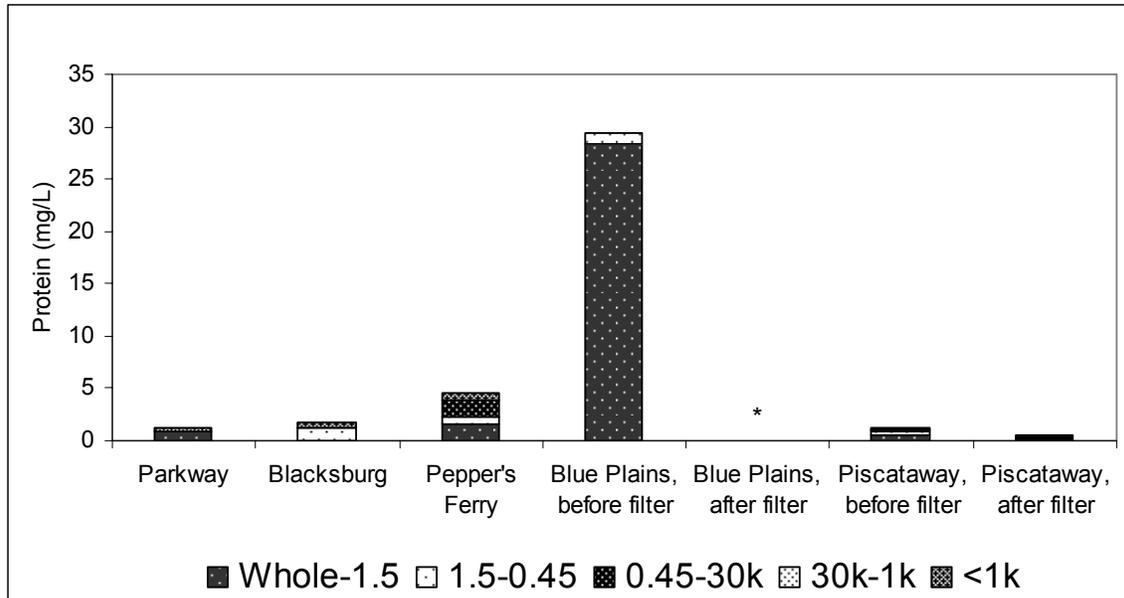
Figure 3. Distribution of protein in the field sample effluents. *The Blue Plains tertiary filter was too inconsistent for analysis.

The total humic acid concentration ranged from 6.3mg/L at Blue Plains to 13.1mg/L at Parkway (Fig. 4). The material between 30kDa and 1kDa was the most significant contributor to the total, with an average of 6.0mg/L, or 58 percent, of the total. The material smaller than 1kDa was the next most significant contributor to the total, with an average of 2.0mg/L, or 17 percent. The Piscataway clarifier effluent and the Blue Plains sample after the teritiary depth filter were too inconsistent for analysis.
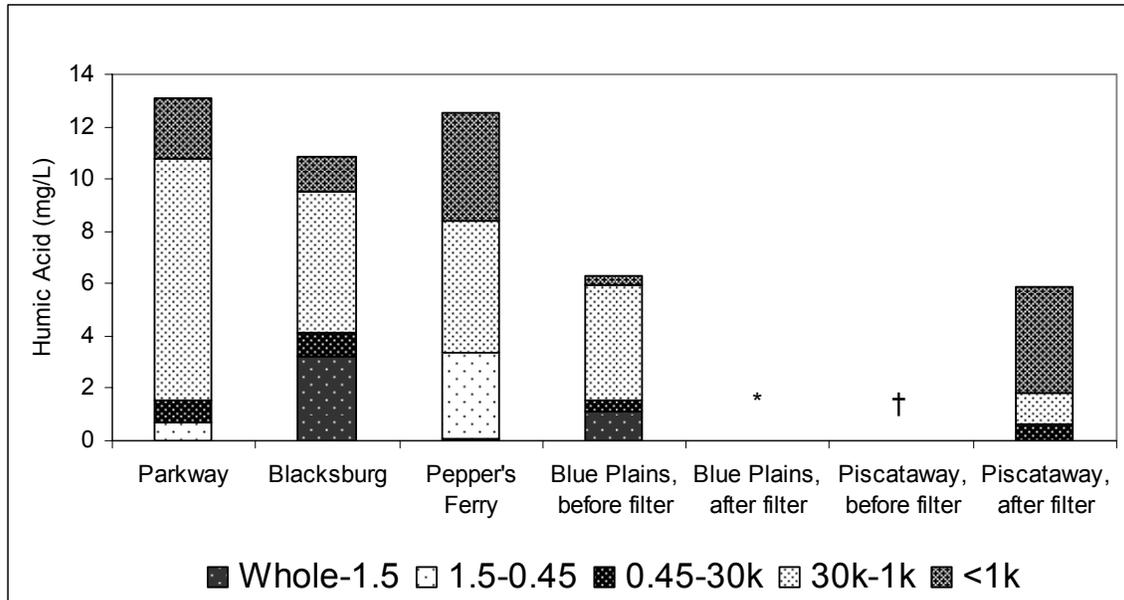
Figure 4. Distribution of humic acid in the field sample effluents. * and † The Blue Plains after filter and Piscataway before filter data was too inconsistent for analysis.

The effluent polysaccharides concentration ranged from 1.3mg/L at Piscataway to 6.3mg/L at Parkway (Fig. 5). The polysaccharides were largely contained in the smallest two size fractions, which combined accounted for an average of 71 percent of the total concentration. On average, the fraction smaller than 1kDa contained the most polysaccharide, with 45 percent. The fraction between 1.5mm and 0.45mm was the next most significant, accounting for an average of 16 percent of the total polysaccharide concentration.
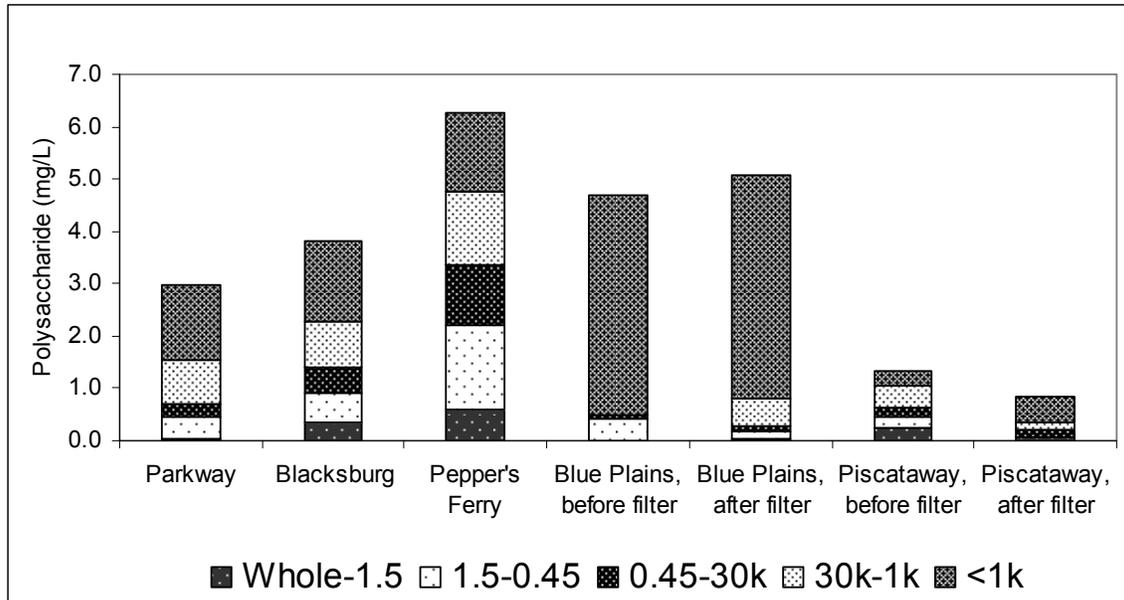
Figure 5. Distribution of polysaccharide in the field sample effluents.

Filter Performance

The tertiary filter effluent protein and humic acid concentrations were too inconsistent at Blue Plains to be analyzed. At Piscataway, the activated sludge effluent humic acid data was too inconsistent for analysis, so a tertiary filter removal percentage for humic acid cannot be calculated. The Piscataway filter removed 60 percent of the activated sludge effluent protein, but there was no clear size cutoff. The polysaccharide removal was inconsistent for the two filters, with an increase in polysaccharide concentration at Blue Plains to 4.8mg/L from 4.2mg/L, and a total removal of 37 percent at Piscataway. While the polysaccharide concentration in material larger than 1.5μm was reduced by 0.3mg/L at Blue Plains, 0.5mg/L was detected in the 30kDa to 1kDa size fraction that was not detected in the clarifier effluent. At Piscataway, polysaccharide containing material larger than 0.45μm was removed, along with half of the polysaccharide containing material between 30kDa and 1kDa.

Cation Comparisons

The protein concentrations were very close to the lower end of the method's linear range and no correlations with the influent aluminum concentration were noted (Fig. 6).
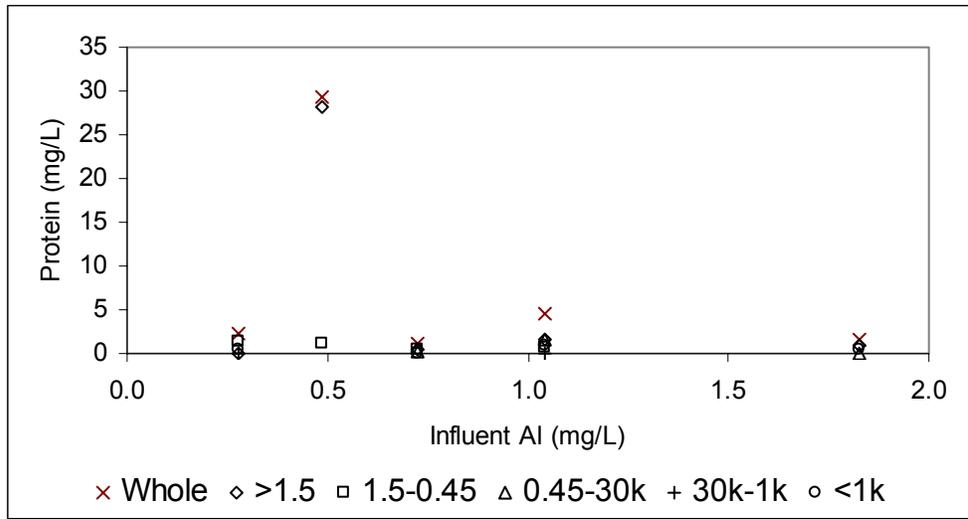
Figure 6. Effluent protein distribution of field samples with respect to influent aluminum concentration.

The effluent humic acid concentration showed a general increase as the influent aluminum concentration increased (Fig. 7). The strongest suggestion for this increase was in the 30kDa-1kDa size fraction, but this connection was weak at best.

Figure 7. Effluent humic acid distribution of field samples with respect to influent aluminum concentration.

There were no trends observed in the polysaccharides data presented in Figure 8. The magnitudes of the polysaccharides changed greatly but were not correlated with the aluminum concentration.
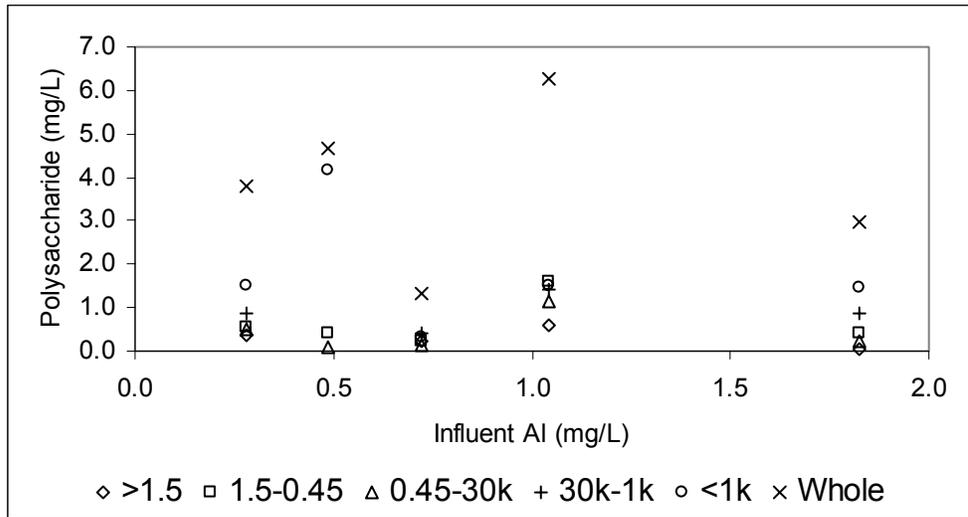
Figure 8. Effluent polysaccharide distribution of field samples with respect to influent aluminum concentration.

The total protein concentration increased as the influent iron concentration increased (Fig. 9). The Blue Plains treatment plant had a much larger amount of material larger than 1.5μm than the other treatment plants and influenced the increasing trend. When the relative fractions were considered in Figure 10, a positive correlation was seen in protein in the percentage of protein in the fraction larger than 1.5μm. This was accompanied by a negative correlation in the percentage of protein between 1.5μm and 0.45μm.
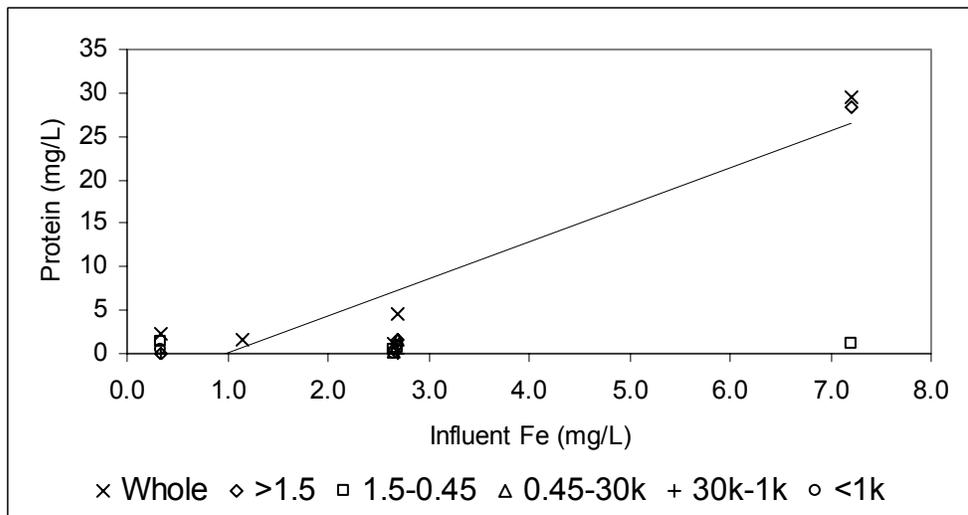
Figure 9. Effluent protein distribution of field samples with respect to influent iron concentration.
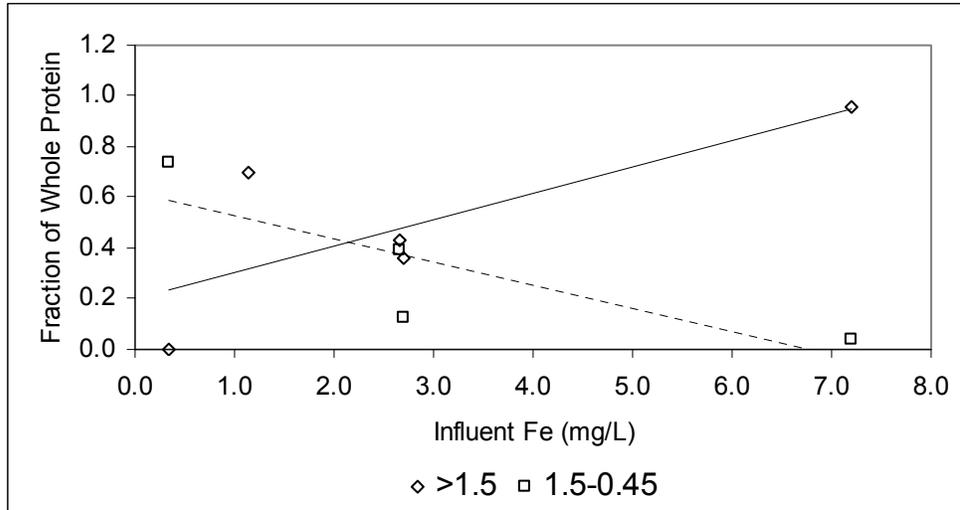
Figure 10. Percentage of the whole effluent protein in each size fraction of the field sample effluents with respect to influent iron concentration.

The concentration of humic acid in the whole effluent sample decreased as the influent iron concentration increased, but there were no strong trends that suggest what size fractions this reduction was occurring in (Fig. 11).
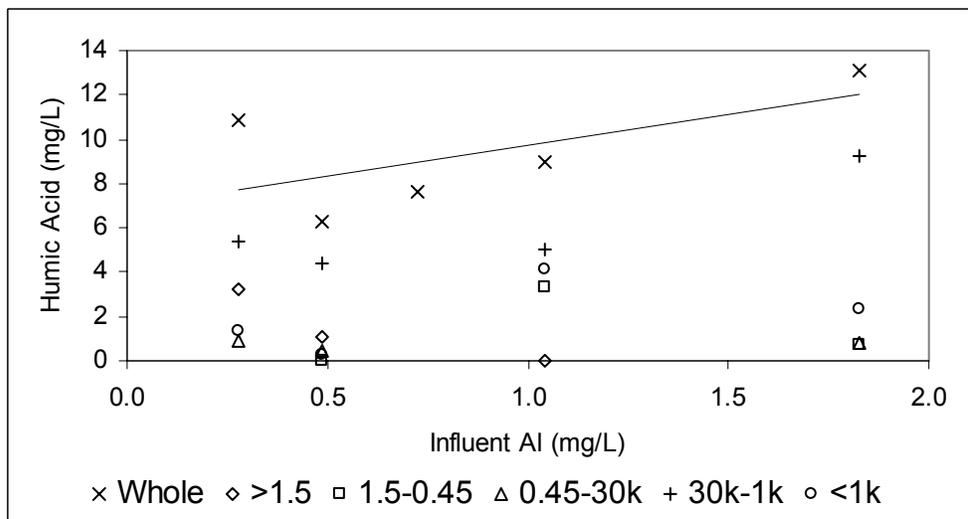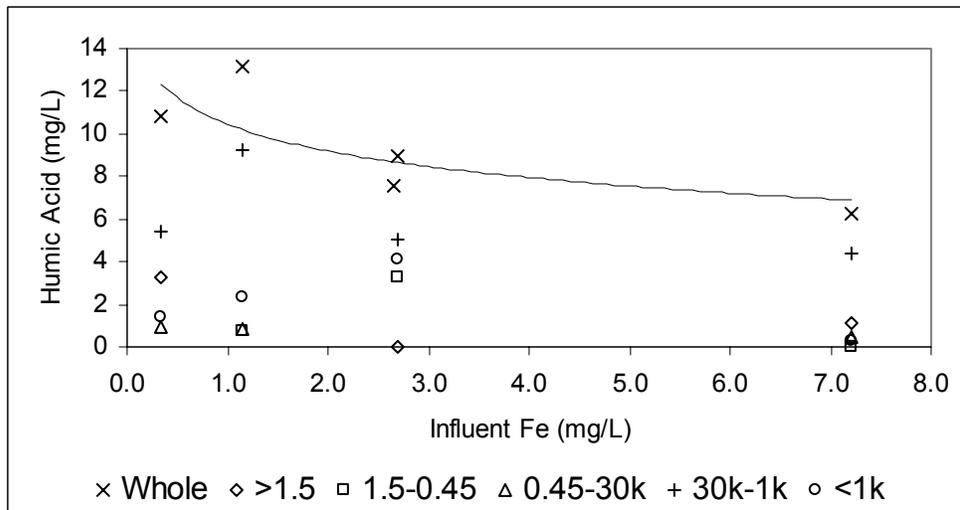

Figure 11. Effluent humic acid distribution of field samples with respect to influent iron concentration.

There were no trends noted in the magnitude of the polysaccharide concentration shown in Figure 12.
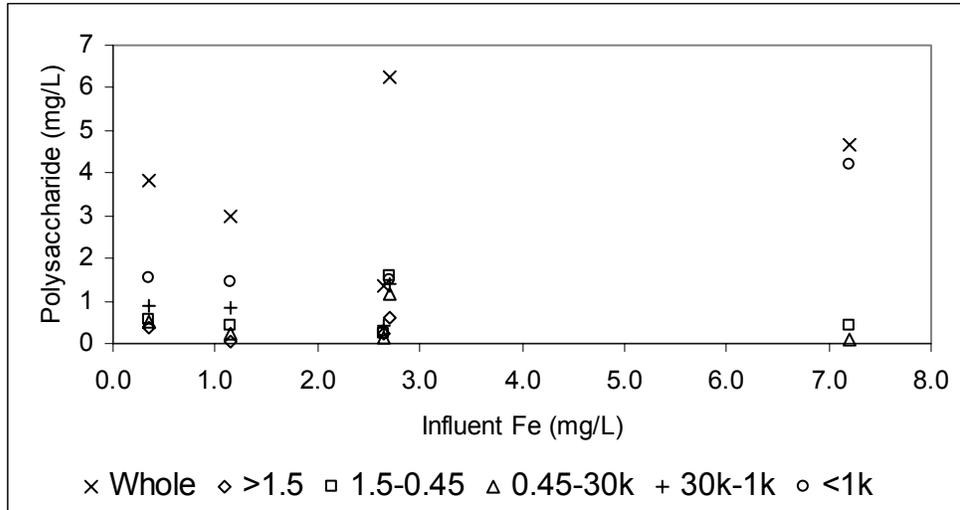
Figure 12. Effluent polysaccharide distribution of field samples with respect to influent iron concentration.

As can be observed in Figure 13, there were no trends observed for the protein concentration with respect to the M/D ratio in the influent. When the Blue Plains sample is ignored, there was an increasing trend found in the total protein concentration, ranging from 1.1mg/L at an M/D of 0.5 to a protein concentration of 4.6 at an M/D of 2.7 (Fig. 14).
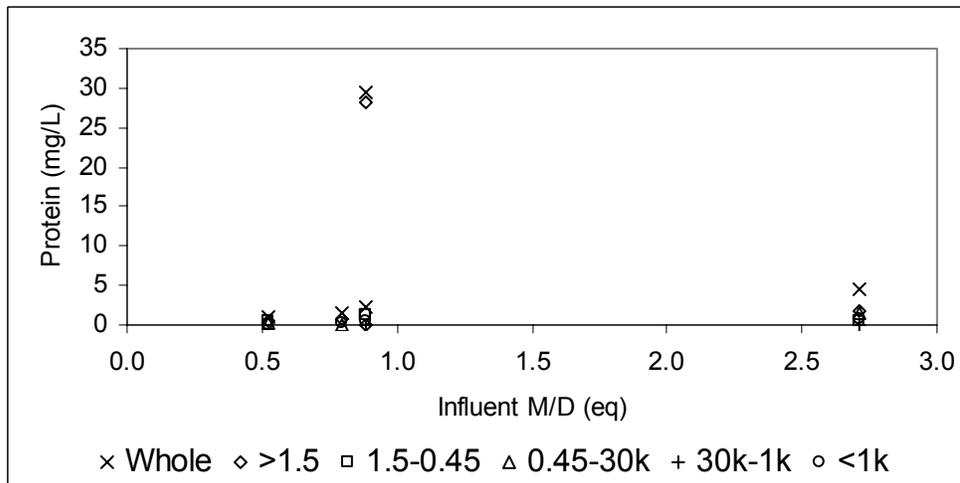
Figure 13. Effluent protein distribution of field samples with respect to influent M/D ratio.

Figure 14. Effluent protein distribution of field samples with respect to influent M/D ratio without the Blue Plains data.

The humic acids data did not show any trends with respect to the influent M/D ratio (Fig. 15).

Figure 15. Effluent humic acid distribution of field samples with respect to influent M/D ratio.

The polysaccharides data in Figure 16 shows a strong increase in total polysaccharides as the M/D ratio increases. This trend was a result of increases in the concentration of polysaccharides in the Whole-1.5μm, 1.5μm-0.45μm, and the 0.45μm-30kDa size fractions. No corresponding decreases in concentration were seen.

Figure 16. Effluent polysaccharide distribution of field samples with respect to influent M/D ratio.

Discussion

Constituent distribution

The largest size fractions were significant in the protein data, where 81 percent of the effluent protein, on average, was contained in material larger than 0.45μm, with the fraction larger than 1.5μm containing an average of 49 percent of the total effluent protein. This echoes the findings of Robbins et al. (in press) where the majority of the effluent organic nitrogen was also in material larger than 0.45μm. This is to be expected since protein is the major constituent of EPS and contains nitrogen. Humic acids also contain nitrogen, but they were largely found in the smallest size fractions, with 58 percent of them found in material between 30kD and 1kDa and an additional 17 percent in material smaller than 1kDa.

The polysaccharide distribution mirrored the humic acid distribution with 71 percent of the polysaccharides associated with material smaller than 30kDa. Only 26 percent of the total was found in the 30kDa to 1kDa fraction, with the remaining 45 percent associated with the material smaller than 1kDa.

From this data it can be concluded that protein is a significant contributor to the organic nitrogen in the samples analyzed by Robbins et al. (in press), while the humic acids do not significantly contribute to the organic nitrogen concentration. The distribution of humic acids and polysaccharides were similar. Mass balances on organic nitrogen and organic carbon could not be performed as in Robbins et al (in press) due to data inconsistencies and values close to the method detection limits.

Cation analysis

Iron appears to effect protein in a similar fashion as the organic nitrogen in Robbins et al. (in press). The total protein concentration increased as the influent iron concentration increased. When the relative fractions were considered, the trend of an increasing percentage of protein in material larger than 1.5mm was also seen, and was accompanied by a decrease in the percentage of protein between 1.5μm and 0.45μm. Iron appeared to be flocculate protein-containing material in the 1.5μm to 0.45μm size range into material larger than 1.5μm.

When the outlying point from Blue Plains is ignored as in Fig. 27, an increasing M/D ratio increases the total protein in the effluent, but no other trends were seen in the concentrations of the individual size fractions. The case for a high M/D ratio causing deflocculation is not as strong with respect to protein as it was with organic nitrogen in Robbins et al. (in press), but the increase in total protein is a significant result. The range of M/D values examined may be too small to see any trends that would elucidate the role of the M/D ratio on protein. No strong trends were found for protein with respect to the influent aluminum concentration. We conclude that protein compounds may be causing the changes in the organic nitrogen found in Robbins et al. (in press).

Contrary to the behavior of protein, the concentration of humic acid in the whole effluent sample decreased as the influent iron concentration increased, although there were no strong trends that suggest what size fractions this reduction was occurring in. The humic acid data did not show any trends with respect to the influent M/D ratio. Aluminum appears to increase the total humic acid concentration, but none of the individual size fractions had a consistently increasing trend. Humic acid did not appear to be causing the changes in organic nitrogen that have been noted above.

Because there are a significant amount of polysaccharides present in EPS, it was assumed that they might cause the changes in TOC found in Robbins et al. (in press). However, there were no trends observed in the polysaccharides data with respect to aluminum or iron. When the M/D ratio was considered, the data shows a strong increase in total polysaccharides as the M/D ratio increased. This trend was a result of increases in the concentration of polysaccharides in the greater than 1.5μm, 1.5μm to 0.45μm, and the 0.45μm to 30kDa size fractions. No corresponding decreases in total or relative concentrations were seen. The TOC data in Robbins et al. (in press) and the polysaccharide data presented here are inconsistent with each other with respect to changes in cation concentrations.

The effects of the cation concentrations on the concentration and distribution of the constituents studied in the effluent can be interpreted with respect to the filtration data. For example, iron

appeared to flocculate protein-containing material between 1.5μm and 0.45μm into material larger than 1.5μm. All protein containing material larger than 0.45μm was removed by filtration at Piscataway, so this flocculation would not be expected to alter the filter effluent quality. No significant changes were noted in the individual size fractions for humic acid with respect to aluminum, iron, or the M/D ratio, but the total concentration was negatively correlated with iron and positively correlated with aluminum. The changes in total concentration could affect how long the filter can be run before breakthrough occurs. The M/D ratio was positively correlated with an increase in polysaccharide concentration for all size fractions larger than 30kDa. This increase may also affect the filtration time before breakthrough.

Analysis Overview
The trends noted above are limited by the narrow range of cation concentrations observed. While most treatment plants would likely fall into the range presented here, the range may be too narrow to determine the roles of the cations studied independent of process factors like SRT, clarifier performance, and process type. Further study on more treatment plants is needed.

Since the data from the filtered samples were subtracted from each other to produce the individual size fractions, adjusting one data point affected two size fractions. A total of 39 percent of the data was affected by data adjustment.

Although there are limitations in the data, the results and conclusions that can be drawn are considerable. To our knowledge, this is the first study to examine the size distribution of activated sludge effluent constituents, the effects of cation concentration on this distribution, and the removal of these constituents by depth filtration.

Conclusions
Effluent protein was mostly contained in material larger than 0.45μm, while most of the humic acid and polysaccharide was contained in material smaller than 30kDa.

The distribution protein reported here mirrors that of the organic nitrogen noted in Robbins et al. (in press), but a mass balance was not possible.

Iron appeared to be flocculating protein-containing material in the 1.5μm to 0.45μm size range into material larger than 1.5μm and causing an increase in the total protein concentration.

The M/D ratio was correlated with an increase in the total polysaccharide concentration as a result of increases in the larger than 1.5μm, 1.5μm to 0.45μm, and the 0.45μm to 30kDa size fractions.

Depth filtration removed protein containing material larger than 0.45μm, but did not do a consistent job of removing polysaccharide.

More study is needed to further confirm these results.

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Lab-scale Activated Sludge Effluent Characteristics as Influenced by Cations

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Introduction

Lab reactors have been used by many researchers to control the influent characteristics and simulate a full-scale activated sludge process. This allows a controlled study of one parameter to be conducted. Recently, several researchers have used lab reactors to study the effects of cation concentration on flocculation in activated sludge (Murthy and Novak, 2001, Park et al, in press). Cations affect flocculation because the extracellular polymeric substances (EPS) that are present in wastewater influent, produced and excreted by cells during growth, and released from cells during death and lysis, have a negative surface charge. This negative surface charge comes from the amino and carboxyl functional groups of protein (Higgins and Novak, 1997a), the most common EPS constituent (Urbain et al., 1993, Frølund et al., 1996, Nielsen et al., 1996, Dignac et al., 1998, Wilén et al., 2003, and Leppard et al., 2003).

Divalent cations form a cation bridge between negatively charged EPS, helping to flocculate the biomass. Murthy et al. (1998) observed an improvement in activated sludge settling after the addition of magnesium in full-scale field trials. The role of calcium was supported by Biggs et al. (2001), who observed an increase in floc size when calcium was added to an activated sludge solution. When the activated sludge mixed liquor has a high monovalent to divalent cation (M/D) ratio, a weak floc structure and poor settling can result (Higgins and Novak, 1997b). A high M/D ratio has been correlated with high activated sludge effluent COD and biopolymer (Murthy and Novak, 2001). It is hypothesized that small pieces of EPS may make up a large amount of the organic matter that remains in wastewater effluent.

Efficient flocculation and settling of an activated sludge biomass is important to remove organic matter and the associated nutrients, contaminants, and contaminant formation potential from wastewater treatment plant effluent. The smallest particles that remain after clarification contain a significant percentage of the oxygen demand and nutrients that are released from the treatment plant. Neis and Tiehm (1997) found that an average of 74 percent of the chemical oxygen demand (COD) and 36 percent of the phosphorus in the secondary effluent that they sampled is associated with particles smaller than $0.1\mu m$. Holbrook et al. (in press), found an average of 8.3mg/L organic carbon smaller than $1.5\mu m$, 5.7mg/L organic carbon smaller than 30kDa, and 2.5 mg/L organic carbon smaller than 1kDa in secondary clarifier effluent. Oxygen demand and

nutrients released from a wastewater treatment plant can cause a deterioration of the receiving water body.

In addition to nutrients, there are contaminants and contaminant formation potential associated with effluent organic matter. Endocrine disrupting compounds (EDCs) bind to effluent particles and are released from the treatment plant in this along with the organic matter (Holbrook et al., in press). The carcinogen N-nitrosodimethylamine (NDMA) is formed when chloramine interacts with nitrogen-containing compounds, including DMA and some types of cationic polymer, during disinfection (Najm and Trussel, 2001). Although the formation of NDMA from DMA has been heavily studied, this reaction fails to account for the majority of the NDMA formation potential in activated sludge (Mitch et al., 2003). Other nitrogen-containing compounds must react with chloramine to produce NDMA. It is important to learn more about effluent particle size distributions in order to develop better removal options.

Aluminum and iron are both trivalent cations that are present in wastewater and play a significant role in bioflocculation (Park et al., in press). Novak et al. (2003) proposed a model in which cations bind to EPS depending on the source of the material. Based on cation release during aerobic and anaerobic digestion, they hypothesized that the EPS produced by microbial growth is linked to divalent cations, and EPS from the influent and cell lysis products is linked to trivalent cations. However, not much is known about the role of trivalent cations in activated sludge flocculation other than the above studies.

As nitrogen, NDMA, and EDC contamination in wastewater receiving water bodies becomes a larger environmental and public health issue, it is becoming more important to advance the state of the art in their removal. Since small particulate matter that does not settle out in secondary clarification contains nitrogen, with its associated NDMA formation potential, and EDCs, it is important to better understand flocculation mechanisms to enhance their removal.

This study uses lab reactors to examine the effects of iron and aluminum concentration on the effluent quality. Previous lab reactor studies have characterized effluent quality by bulk mixed liquor parameters, such as settling velocity, sludge volume index, or filterability. This study will characterize the effluent in terms of the individual EPS constituents and the size of the particles that they are associated with. By examining the role of iron and aluminum and their roles in flocculation, we hope to gain further understanding of the role of trivalent cations in order to improve effluent quality.

Methods

Eight lab-scale CSTRs were operated in order to isolate the effects of iron and aluminum on the effluent characteristics. Each reactor had a total volume of 10L and had a main activated sludge chamber and a clarifier separated by a baffle. The effluent was discharged from a tube on the top of the clarifier and stored in a bucket. Synthetic feed was supplied via a drip tube to the activated sludge chamber. Paddles stirred the activated sludge chamber and an air stone provided intermittent aeration. A diagram is shown in Figure 1.
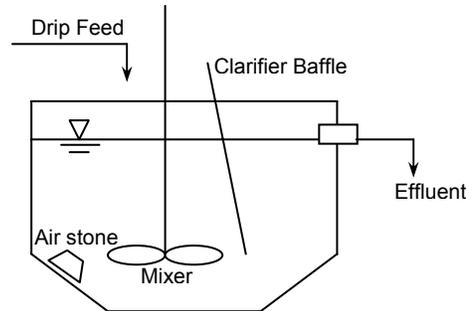

Figure 1. Cross section of a lab reactor, not to scale.

The reactors were run twice in sets of four reactors. The first set of reactors had an influent iron concentration of 0.2mg/L (low iron) and the second set had an influent iron concentration of 2mg/L (high iron). Within each set, the influent aluminum concentration was 0.1mg/L for reactor 1 (R1), 0.5mg/L for reactor 2 (R2), 1.5mg/L for reactor 3 (R3), and 2.5mg/L for reactor 4 (R4). The first set had an SRT of 12 days and an HRT of 1 day. The second set had an SRT of 8 days and an HRT of 0.8 days. The two sets were operated under different conditions because of a need to control the presence of filamentous organisms. The feed characteristics are shown in Table 1. The reactors were started with mixed liquor from the Blacksburg municipal wastewater treatment plant. Effluent samples were taken from the effluent storage bucket and stored at 4°C overnight before processing. No influent samples were taken.

Table 1. Lab reactor influent information and operation data. Cation concentrations in mg/L, ratios on an charge equivalent basis, SRT and HRT in days.

	Na	K	Ca	Mg	Al	Fe	M/D	COD	SRT	HRT
Low Iron	64.4	25.5	38.0	23.0	0.1 – 2.5	0.2	0.97	550	12	1
High Iron	50.6	19.6	28.0	17.0	0.1 – 2.5	2.0	1.0	550	8	0.8

Sample treatment and analysis

From reactor startup, the effluent solids concentration, sludge volume index and chemical oxygen demand were monitored. When the effluent solids and COD were consistent day to day, if more than 3 SRTs had elapsed, the effluent samples were taken.

After an unfiltered sample was taken (whole fraction), the remainder of the sample was centrifuged for 15 minutes at 7,000 RPM (8,700xg) before being filtered by a 1.5μm glass fiber filter, a 0.45μm nitrocellulose membrane filter, a 30,000 Dalton (30kDa) ultrafilter, or a 1,000 Dalton (1kDa) ultrafilter. Samples to be analyzed for Total Kjeldahl Nitrogen (TKN), ammonia, total organic carbon (TOC), and metals were stored in acid-washed glass containers at 4°C at a pH <2. The pH was adjusted to this value with concentrated sulfuric acid. Samples for protein, humic acid, and polysaccharide measurements were stored at -15°C in sterile polypropylene vials.

Total Kjeldahl nitrogen (TKN) measurements were conducted according to method 4500-N_{org} C. (APHA, 1998) and ammonia measurements were conducted according to method 4500-NH_3 C. (APHA, 1998). Total organic carbon was analyzed with a Sievers 800 TOC analyzer for fractions smaller than 0.45μm and with a Dohrmann Carbon Analyzer for samples larger than 0.45μm. The whole influent samples were digested according to method 3030 E. (APHA, 1998) and analyzed by ICP (Ultima) for sodium, potassium, magnesium, calcium, aluminum and iron. Protein and humic acid measurements were performed according to Frølund et al. (1996) and polysaccharides were performed according to Dubois et al (1956). Spectroscopic measurements were made using a Beckmann DU640.

Results
Sample key:
LI – low iron (0.2mg/L)
HI – high iron (2.0mg/L)
R1 – reactor 1
R2 – reactor 2
R3 – reactor 3
R4 – reactor 4
Whole – unfiltered sample
<1.5 – sample filtered through a 1.5μm filter
<0.45 – sample filtered through a 0.45μm filter
<30k – sample filtered through a 30kDa filter
<1k – sample filtered through a 1kDa filter

As seen in Figure 2, the total TKN varied greatly between the low iron set and the high iron set. The total TKN for the low iron set varied from 1.6mg-N/L to 8.9mg-N/L, while the TKN for the high iron set varied from 0.0mg-N/L to 0.2mg-N/L. While the TKN of the filtered samples generally followed a decreasing trend with an increased level of filtration, the ammonia values for all of the reactors from both runs were almost as high if not higher than the total TKN values.
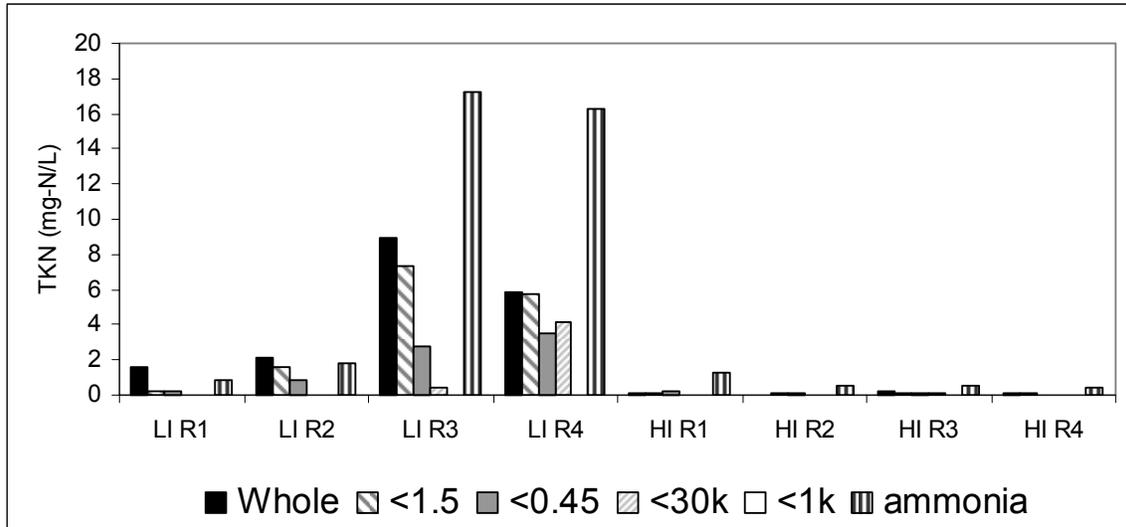

Figure 2. TKN of filtered samples from laboratory reactors.

The average total TOC for the low iron reactor set was 9.8mg-C/L with a range from 8.1mg-C/L to 13.0mg-C/L (Fig. 3). The average total TOC for the high iron set was 8.0mg-C/L with a range of 5.7mg-C/L to 11.5mg-C/L. As with the TKN distribution, the filtered samples did not always follow a decreasing trend with increasing filtration.
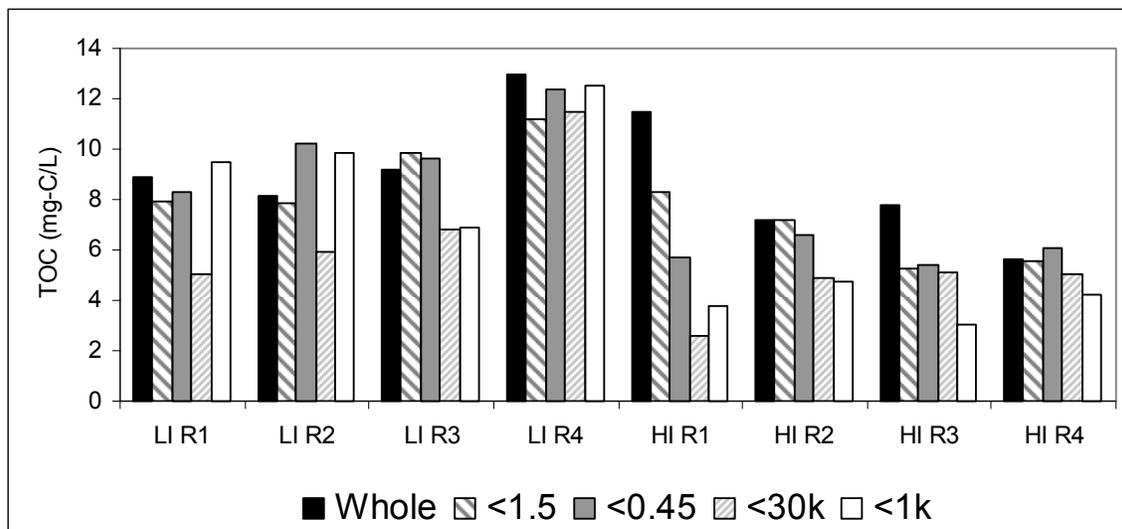
Figure 3. TOC of filtered samples from laboratory reactors.

The total effluent protein concentration for all of the reactors varied from 5.3mg/L to 11.5mg/L and is presented in Figure 4. The distributions appear similar with the exception of R4 from the high iron set, although the distributions are too inconsistent for detailed analysis. It should be noted that the protein concentrations for the high iron samples are much higher than would be expected from the TKN values. This will be discussed further in the analysis section.
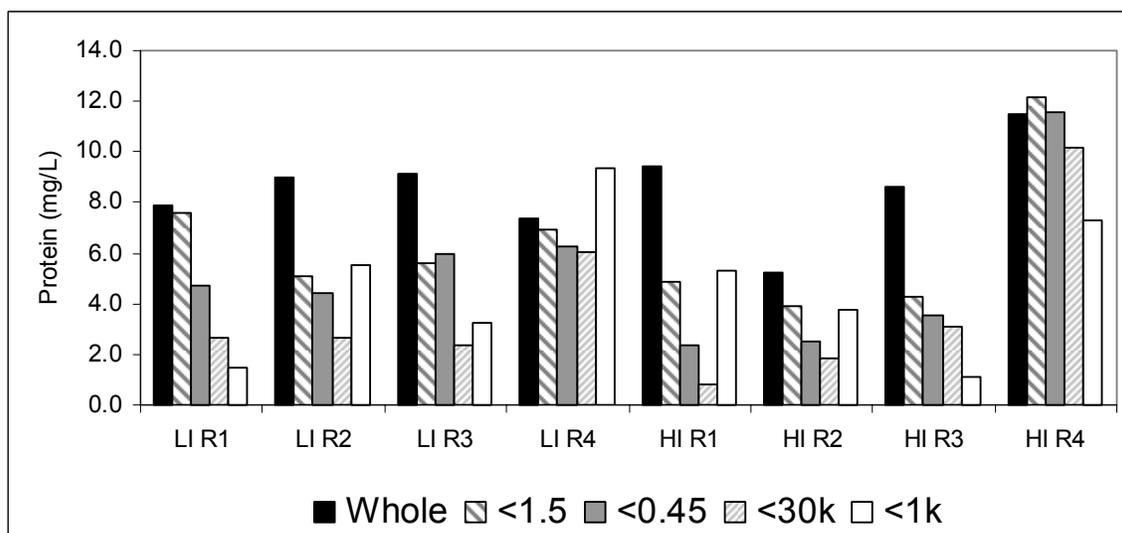

Figure 4. Protein of filtered samples from laboratory reactors.

Figure 5 shows the concentrations of humic acids in the eight lab reactors. The total humic acid concentration ranged from 11.5mg/L in R4 in the high iron set to 27.9mg/L in R4 in the low iron set. The low iron set had an average total humic acid concentration of 23.4mg/L, while the high

iron set had an average of 14.5mg/L. Especially in the high iron set, a decreasing trend is seen from R1 to R4. This decrease correlates with an increase in influent aluminum concentration across these reactors.
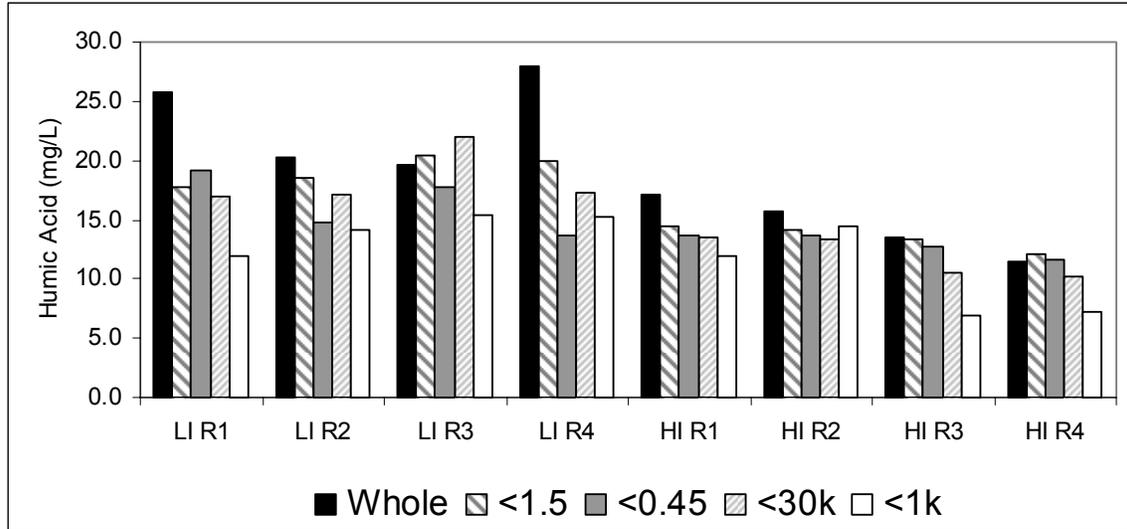

Figure 5. Humic acids of filtered samples from laboratory reactors.

The polysaccharide concentrations in the reactors are shown in Figure 6. With the exception of R1 from the high iron run, the total polysaccharide concentration varied from 3.4mg/L to 10.4mg/L, with the lowest value of 3.4mg/L in R2 from the high iron run being unrepresentative of the other samples from that reactor. Although the trends in size distribution are difficult to quantify due to data inconsistencies, a general trend can be observed that most of the polysaccharides are present in the smallest size fractions. Looking at each set of four reactors separately, a general trend of decreasing polysaccharide concentration with increasing influent aluminum concentration can be inferred.
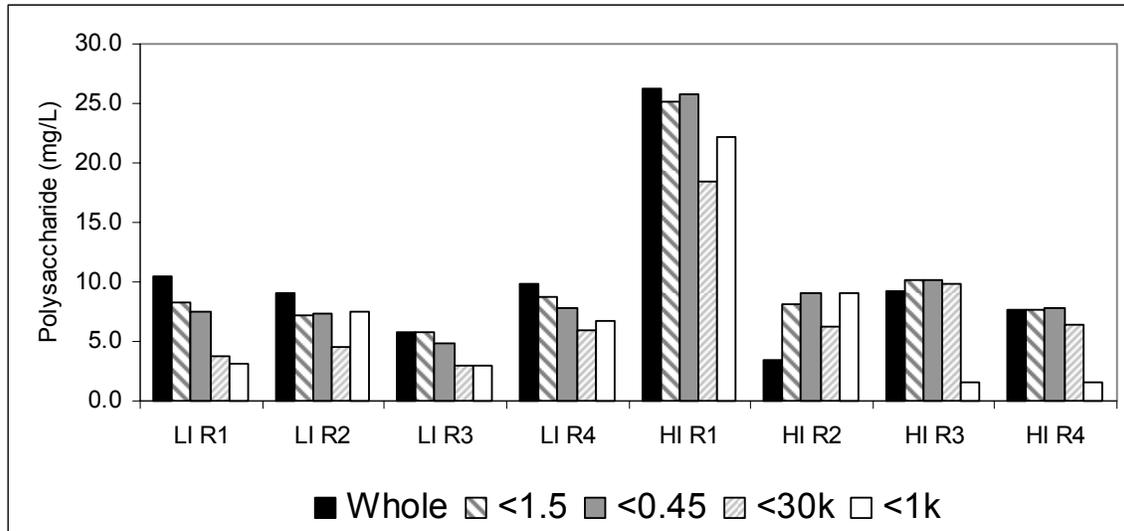
Figure 6. Polysaccharides of filtered samples from laboratory reactors.

Discussion

These reactor runs were intended to be compared to the effluents from field samples collected (Robbins et al., in press). Despite using the same methods for sample preparation and analysis, the two sets of data cannot be analyzed together for several reasons. The field effluent data in Robbins et al. (in press) had about half of the concentration of TKN, proteins, humic acids, and polysaccharides that were observed in the lab reactors. This could be due to the less efficient performance of the clarifiers in the lab reactors and the difference in influent characteristics between the field and lab samples. In addition, the analyses from the lab reactors often did not show a consistent decreasing trend in concentration with an increasing level of filtration. These inconsistencies in the data prevented a detailed analysis of the individual size fractions as was done in Robbins et al. (in press).

Despite the differences between the lab reactor samples and field samples in Robbins et al (in press), there are some similarities that should be noted. A quantitative analysis is not possible with the lab reactors, but the protein, humic acid, and polysaccharide distributions were similar between the lab and field samples. The protein was found mostly in the largest size fractions, while humic acids and polysaccharides were found mostly in the smallest size fractions.

Although the two reactor sets were not operated under the same conditions, some similarities between the two sets can be drawn with respect to the role of aluminum in the activated sludge process. In both the humic acids and polysaccharides data, a general decreasing trend with increasing aluminum concentration was observed. Aluminum may play a role in the flocculation of these materials, allowing them to settle more efficiently in the clarifier section of the reactor.

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With respect to the influent iron concentration, the humic acids and TOC data show that the average whole concentration was lower in the high iron run than the low iron run. The dramatic decrease in TKN between the two runs is likely due to nitrate interference in the TKN analysis. None of these iron trends can be separated from the differences in the operation of the reactors.

Some general observations can be made about the analyses presented here. The TKN values for both reactor runs did not correlate well with the protein or humic acid values. This is surprising, because protein is the major constituent of EPS and both proteins and humic acids contain nitrogen. The TKN values for the first run varied widely between the low R1 and R2 values to the much higher R3 and R4 values. The protein and humic acid values for this run were much more equal across the reactors. In the second run, the TKN values were much lower than would be expected, considering the protein and humic acid values. This, plus the ammonia values that were higher than the TKN tests, indicates that there was additional TKN in the samples that was not detected by the TKN analysis. This could be due to interferences in the TKN test. The most likely interfering compound is nitrate, which can cause oxidation and loss of ammonia during the TKN acid digestion (APHA). Another source of interference could by pyrolytic loss of nitrogen if the temperature of the acid digestion was too high. Of these two interferences, the presence of nitrate is most plausible, since a large number of TKNs have been run at this burner setting, and this interference has not been seen. The reactors did have intermittent aeration, but it may not have been sufficient to cause enough denitrification of the nitrate to bring the nitrate level down low enough to prevent an interference with the TKN analysis. The nitrate level was not measured for any of the reactors.

The TOC and polysaccharide tests showed much better agreement across the reactors in both runs, with the exception of R1 in the second reactor run. Although the R1 TOC from the second reactor run was higher than the values for the other three reactors in that run, it was not higher by the factor observed in the polysaccharide analysis results. Since all of the reactors received the same feed and the filtered sample fractions were stored in separate containers, if an interfering compound caused the increase in polysaccharides, it must have been introduced in the effluent storage bucket.

Conclusions

The reactor runs differed enough in effluent quality and data consistency from the field sample effluent in Robbins et al (in press) to prevent combining the lab and field sample analyses. However, the distributions of protein, humic acids and polysaccharides were similar between the lab and field samples, with most of the protein present in the largest size fractions and most of the humic acids and polysaccharides present in the smallest size fractions.

From these reactor runs, a general conclusion can be made that aluminum may play a role in flocculating humic acids and polysaccharides. The role of iron in flocculation was not clear.

The nitrate concentration of the lab reactors is an important factor in the ability to measure effluent quality and should be monitored during future reactor runs.

When these reactors have been used in the past, they have been used to measure properties of the mixed liquor. Their usefulness in studies dealing with effluent quality may be limited by the clarifier design. The results are not necessarily directly comparable with samples from full-scale clarifiers.

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