

CATIONS AND ACTIVATED SLUDGE FLOC STRUCTURE

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ABSTRACT

This research was designed to investigate the effect of cations on activated sludge characteristics and also to determine their influence on digestion performance. For this purpose, cations in solution and in floc were evaluated along with various activated sludge characteristics and the collected waste activated sludge underwent both anaerobic and aerobic digestion. It was found that large amounts of biopolymer (protein + polysaccharide) remained in the effluent of WWTP that received high influent sodium but had low iron and aluminum in floc. However, sludges from plants with high sodium and high iron and aluminum dewatered well and produced high quality effluents, suggesting that iron and aluminum have significant positive effects on floc properties. Following anaerobic digestion, a significant increase in solution protein occurred and correlations between solution protein, ammonium production, percentile volatile solids reduction and iron in floc were obtained. These data indicate that iron-linked protein is released to solution when iron is reduced and its degradation is responsible for volatile solids reduction in anaerobic digestion. In aerobic digestion, polysaccharide in solution increased along with calcium, magnesium and inorganic nitrogen. This implies that divalent cation-bound biopolymer might be the primary organic fraction that is degraded under aerobic digestion. Combined (anaerobic/aerobic) digestion was performed and produced further volatile solids destruction with discrete cation and biopolymer response during each phase of digestion. These results support the theory that two types of organic matter with different cation bindings are present in floc and each type is degraded under different digestion processes.

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LITERATURE REVIEW

INTRODUCTION

Both anaerobic and aerobic digestion have been widely used to stabilize wastewater sludge prior to ultimate solids disposal. Reduction of volatile solids and odor potential and destruction of pathogens are the primary objectives of both processes. Each digestion process uses different microbiological and biochemical reactions and the major difference of two digestion processes is whether digestion proceeds in the presence or absence of molecular oxygen.

Historically, a great deal of study has been conducted to describe digestion kinetics and to assess factors that affect digestion performance. However, the fundamental question as to whether a specific sludge is better treated by anaerobic or aerobic digestion has not been evaluated. At present, there are no specific criteria for choosing a digestion method but secondary factors such as the scale or size of wastewater treatment plant, complexity of the process, and economic interests are commonly considered for digestion options. As a consequence, solids that do not meet treatment goals can be produced and unsatisfactory performance could cause additional cost. In the future, regulations and costs for solids handling could become more stringent, making efficient stabilization of greater importance.

Recently, a study demonstrated that different mechanisms for floc destruction are involved in anaerobic and aerobic digestion and they account for different characteristics of digested product with regard to volatile solids reduction and subsequent dewatering properties (Novak et al., in press). The study further suggested that an analysis of activated sludge floc structure would be necessary to understand different digestion mechanisms.

The activated sludge process is the most commonly used biological process for secondary wastewater treatment. In this process, microorganisms in wastewater are grown in the aggregated and suspended form, removing organic matter from the influent wastewater. In order to separate solid biomass from sludge liquid, good bioflocculation is required and a variety of factors are known to be involved.

Microorganisms, extracellular polymeric substances or exocellular biopolymers, and cations are major components of activated sludge flocs (Bruus et al., 1992; Higgins and Novak, 1997a). It is thought that biopolymers originate from microbial activity and wastewater influent and provide a matrix in which microorganisms can be aggregated to form flocs (Urbain et al., 1993; Dignac et al., 1998). Because a majority of exocellular biopolymers is negatively charged, multivalent cations become an important component of floc structure by providing bridging to the negative sites on the biopolymer network (Bruus et al., 1992; Urbain et al., 1993; Higgins and Novak, 1997a). Therefore, without the proper cation composition, flocs would be weak and dispersed, causing settling, dewatering, and effluent quality problems. However, there is still much debate about the role of the major cations found in activated sludge and the role of iron and aluminum remains unclear.

This research was designed to investigate the effects of cations on activated sludge characteristics and also to determine their influence on digestion performance. In order to assess the overall impact of cations on the activated sludge system, a field study rather than a lab-scale reactor study was performed and both the cations in solution and solid floc were evaluated along with various activated sludge characteristics. Subsequently, the collected waste activated sludge underwent both anaerobic and aerobic digestion for 30 days and chemical characteristics of each sludge were analyzed before and after digestion. The results were expected to be useful in determining how cations may account for many differences between wastewater sludges and further to help us develop a better understanding the influence the cation has on digestion process.

FACTORS THAT AFFECT ACTIVATED SLUDGE CHARACTERISTICS

Activated sludge is the most commonly used biological process for the secondary wastewater treatment. The primary objective is to remove organic matter from the influent wastewater using a flocculent slurry of microorganisms. In this process, microorganisms are grown, aggregated and suspended, in wastewater converting organic matter into carbon dioxide, ammonia-N, and new biomass. The separation of solid biomass from sludge liquid, a key performance of activated sludge process, can be achieved by gravity settling in a final clarifier and good bioflocculation is necessary for good settling to occur. Various factors such as the proper population of microbes and interactions between exocellular biopolymers and cations are known to be involved in

bioflocculation. In addition, operational factors such as solids retention time are known to influence the activated sludge process performance.

Solids retention time

Solids retention time (SRT), the age of sludge biomass in a bioreactor, is an important factor that can affect activated sludge characteristics. At lower SRTs, biomass will not flocculate effectively and many dispersed organic matter will be washed out of the system, causing deterioration in effluent quality (Grady et al., 1998). Therefore, selecting a proper SRT at wastewater treatment plant (WWTP) is required to produce proper activated sludge process performance. The SRT at which biomass washout occurs is referred to be minimum SRT and the design SRT should have a sludge age greater than this range (Grady et al., 1998).

Bisogni and Lawrence (1971) studied the effect of various SRTs on laboratory-grown activated sludge characteristics and showed that SRT influences settling properties of activated sludge as indicated by sludge volume index, zone settling velocity, and the extent of sludge dispersion. The authors also found that soluble chemical oxygen demand (COD) in the effluent increased with an accumulation of polysaccharide above a certain time of SRT. This organic matter in effluent is thought to be primarily nonbiodegradable material (Grady et al., 1998). Based on their results, Bisogni and Lawrence (1971) concluded that an SRT in the range from 4 to 9 days would be best. At shorter SRTs, settling problems occur and at longer SRTs, effluent quality would become problematic.

Murthy (1998) also obtained similar results from a study that showed the effect of SRT on activated sludge effluent quality. In his study, soluble polysaccharide (< 3K) and colloidal protein (0.45 μ m - 30K) increased in the effluent as the SRT increased above 10 days but the effluent biological oxygen demand (BOD) remained constant, implying that increased effluent COD was not easily degradable material.

If the organic matter recalcitrant to biodegradation increases as SRT increases, SRT might also have a significant effect on digestibility of sludge. Reece et al. (1979) found that nonbiodegradable material in activated sludge increases as SRT increases, affecting aerobic digestibility of sludge. Bruus et al. (1993) also showed that activated sludges processed at different SRTs experience different changes under anaerobic conditions. The authors reported that when activated sludges were anaerobically stored, more dramatic changes in conditioning

and dewatering properties took place in activated sludges with short SRT compared to those from higher SRT processes.

Extracellular polymeric substances

Extracellular polymeric substances (EPS) or exocellular biopolymers are a major component of activated sludge floc. Microorganisms can be aggregated in this extracellular matrix so its importance in bioflocculation can not be overstated. Consequently, a number of studies have focused on understanding the composition of EPS and its effect on settling property of activated sludge. However, a variety of extraction procedures and variation in sludges investigated have often brought controversy in defining its characteristics.

Studies using extraction methods such as cation exchange resin treatment (CER) (Frølund et al., 1996; Nielsen et al., 1996), sonication (Urbain et al., 1993) and a combination of sonication and CER (Dignac et al., 1998) have yielded highly efficient extraction and the data shows that activated sludge EPS are mainly composed of protein, polysaccharide, humic acid and nucleic acids. These studies also agreed that protein is the major constituent of EPS in activated sludge floc.

Activated sludge EPS are negatively charged due to functional groups such as carboxyl groups in EPS. Higgins and Novak (1997a) and Dignac et al. (1998) measured amino acids of protein in activated sludges and found that carboxyl containing groups such as aspartic and glutamic acids accounted for a large portion in their protein samples. As a consequence, multivalent cations play a significant role in binding negatively charged biopolymer to enhance bioflocculation. More details about the role of cations in activated sludge are discussed in the following section.

Hydrophilic and hydrophobic properties of EPS are another important factors affecting the flocculation mechanism. Hydrophobicity is of specific importance since activated sludges are highly hydrated structures (Urbain et al., 1993). Urbain et al. (1993) obtained empirical results that sludge settleability was improved at a low concentration of EPS when internal hydrophobicity of flocs increased. Higgins and Novak (1997a) showed that hydrophobic amino acids of protein were significant constituents in activated sludge and suggested that biopolymers can also bind through hydrophobic interactions. Later, Dignac et al. (1998) compared the extraction efficiency of sonication and combination of sonication and CER. The authors found that hydrophobic amino acids were also better extracted by CER, indicating that not only

negatively charged hydrophilic proteins but also hydrophobic proteins are associated with cation binding in the floc structure.

The source of EPS is also of great interest. Exocellular biopolymers can originate from two possible sources, microbial activity such as metabolism and lysis of cells and wastewater influent (Urbain et al., 1993; Dignac et al., 1998). However, it is not clear how these materials are distributed within the floc structure and how this distribution affects floc properties and the extraction of biopolymers.

Lately, some studies have suggested that EPS from different origins will also have different or unique characteristics. Nielsen and Keiding (1998) proposed a floc model in which the outer EPS for wastewater originated-EPS is bound by weak forces to a rigid backbone of floc matrix and therefore is sensitive to changes in the stability of floc. They drew this result from the observations of different degrees of floc disintegration in field activated sludge and lab-grown cultures induced by iron extraction following addition of sulfide. Novak et al. (in press) reported very different cation and biopolymer responses from anaerobic and aerobic digestion studies. Significant amounts of calcium and magnesium ions were released to solution during aerobic digestion, but this did not occur during anaerobic digestion. They attributed these results to a proposed model in which two types of biopolymer with different cation bindings exist in flocs. These are (1) lectin-like biopolymer, generated by microorganism and linked to divalent cations and (2) iron and/or aluminum bound biopolymer, trapped from incoming wastewater and cell lysis product.

Cations

Since biopolymers are negatively charged, cations become a significant structural component. As one step of flocculation, cations provide binding between negatively charged biopolymers where microorganisms can be embedded (Bruus et al., 1992; Urbain et al., 1993; Higgins and Novak, 1997a). Therefore, without the proper cation composition, flocs would be weak and dispersed, causing settling, dewatering, and effluent quality problems. Sodium, potassium, ammonium, calcium, magnesium, iron and aluminum are the major cations found in activated sludge. Although there is some debate about the specific role of these cations, it has been generally accepted that divalent and trivalent cations are better flocculants than monovalent cations. The significance of cations has been dealt in many studies using a variety of

experimental approaches or with different types of sludge and these have led to several different models of floc structure.

(1) Divalent cations

The role of divalent cations in the floc structure has been of great interest in understanding the mechanism involved in bioflocculation. Calcium removal from flocs using cation exchange resin (Keiding and Nielsen, 1997), EDTA (Kakii et al., 1985), or EGTA (Bruus et al., 1992) resulted in the desorption of organic matter, deteriorating settling and dewatering properties of sludge. While these studies stressed the effect of calcium on the stability of floc, some other works demonstrated that magnesium as well as calcium is important for the floc stability. Sobeck and Higgins (2002) reported that the sludge fed with either magnesium or calcium improved in a similar manner as to settling and dewatering properties. In their study, the authors examined three different cation induced-floc models and from this observation they suggested that divalent cation bridging theory in which both calcium and magnesium equally bind negatively charged biopolymers best explain the role of cations in the floc structure. Novak et al. (in press) reported that there was substantial increase in both magnesium and calcium in solution during aerobic digestion and ascribed this result to the degradation of floc segment that was associated with both the divalent cations. However, activated sludges investigated by Kakii et al. (1985), Keiding and Nielsen (1997), and Bruus et al. (1992) contained much more calcium than magnesium so the relative importance of calcium ion could be due to low magnesium in their wastewater.

Interactions between specific EPS and divalent cations have also been studied in depth. Since protein and polysaccharide are major constituents of EPS, their relationship with calcium and magnesium is important for understanding the floc structure. Bruus et al. (1992) suggested that polysaccharides in biopolymers are alginates and these biopolymers bind to calcium ion to form a gel-like floc structure. Urbain et al. (1993) and Dignac et al. (1998) reported that proteins are more strongly associated with calcium and magnesium than polysaccharides. Urbain et al. (1993) also found that protein has higher affinity for calcium while DNA has higher affinity for magnesium. Unlike the models which treat protein and polysaccharide separately, Higgins and Novak (1997a) proposed a floc model where proteins with lectin activity are cross-linked with polysaccharides and both magnesium and calcium provide bridging to this biopolymer network. They concluded this from the observation that polysaccharide was released when pronase was added to sludge and bound protein increased when sludge was fed with magnesium and calcium.

(2) Monovalent cations

In contrast to the divalent cations, high concentration of monovalent cations, especially sodium, has been known to be detrimental to the floc stability (Bruus et al., 1992; Higgins and Novak, 1997a; Novak et al., 1998; Sobeck and Higgins, 2002). All these studies showed that sludges that received additional sodium deteriorated in settling and/or dewatering properties. It was also reported that WWTPs treating industrial wastewater where sodium hydroxide is used for pH control generally produce sludges with poor settling and dewatering properties (Murthy et al., 1998). It is theorized that monovalent cations displace divalent cations within the cation bridged floc structure by ion exchange, making flocs weak and sensitive to any physiochemical changes on the floc structure.

Murthy and Novak (1998) studied the effect of potassium on activated sludge characteristics and found that high potassium improved the floc strength and settling property of sludge while it caused deterioration in dewatering property and effluent quality. Consequently, the authors concluded that the role of potassium in activated sludge floc is not the same as that of sodium that exacerbates all the activated sludge characteristics.

Ammonium is another monovalent cation that can affect floc structure. According to Novak (2001), the ammonium ion also replaces divalent cations in a same manner as the sodium, deteriorating floc properties. In his study, settleability and dewaterability of sludge became worse when ammonium concentration increased following the addition of nitrification inhibitor. The author suggested that poor activated sludge characteristics usually reported at short SRT or during the winter are partly because of the loss of nitrification at these conditions.

(3) M/D

By examining the effects of monovalent and divalent cations on the activated sludge characteristics, Higgins and Novak (1997b) suggested that the ratio of monovalent to divalent cations on a charge equivalent basis could be served as a rapid indicator of sludge characteristics. The authors investigated several full and lab-scale activated sludges and found that the M/D ratio was positively correlated with sludge filterability (specific resistance to filtration). They also observed that the ratio exceeding 2 resulted in the large decrease in the sludge filtering rate so that they used this number as an optimal ratio of M/D. The conceptual basis of M/D was that monovalent cations displace divalent cations within the cation bridged-floc structure by ion

exchange and deteriorate floc properties. Later, a study by Murthy (1998) showed that the M/D ratio was also an efficient parameter for describing activated sludge effluent quality.

(4) Trivalent cations

While the role of mono and divalent cations in the floc structure has been studied extensively, the role of trivalent cations such as iron and aluminum has not been studied in depth although these cations are often found at high concentrations in activated sludge (Kakii et al., 1985). Since they have higher charge valence and lower solubility than divalent cations their contributions to the floc stability should be understood better. According to Kakii et al. (1985), iron and aluminum were not affected by the acid treatment of activated sludge at pH 3 while most of calcium and magnesium were extracted, indicating that iron and aluminum are more strongly associated with sludge matrix than divalent cations.

In studies of the role of iron in activated sludge, iron (III) reduction to iron (II) either by iron reducing bacteria (Caccavo et al., 1996) or sulfate reduction to sulfide (Nielsen and Keiding, 1998) resulted in the turbidity increase in bulk water of sludge, which suggested that oxidized iron is an important constituent for floc stabilization. Rasmussen and Nielsen (1996) reported that about 70 to 90% of iron was present as ferric iron in the fresh activated sludges they investigated. However, they also admitted difficulties in identifying the species or chemical forms of ferric iron; whether they are present in floc as crystalline, amorphous or organically bound iron. Later, Nielsen and Keiding (1998) hypothesized that either the reaction of ferric ion with EPS or the precipitation as ferric hydroxides or both are involved in flocculation. In addition, both species were considered to be stronger flocculants than calcium ion.

It has been reported that ferric iron has high affinity for protein (Murthy et al., 2000; Muller, 2001; Novak et al., in press). Murthy et al. (2000) demonstrated that iron (III) salts selectively coagulated solution protein produced from autothermal thermophilic aerobic digesters. Muller (2001) compared the efficiencies of iron (III) and iron (II) salts for coagulation of solution protein and found that the former worked more effectively than the latter. Later, Novak et al. (in press) postulated that large protein release during anaerobic digestion is due to the loss of selective binding between protein and ferric iron induced by iron reduction in anaerobic conditions.

There is little information available for the specific role of aluminum in activated sludge floc. Some investigations about aluminum were found in the field of water treatment since aluminum salts are often used as coagulants for removing natural organic matters in water treatment plants. Two studies showed that polysaccharide found in lake and river water was preferentially coagulated by aluminum polychlorosulfate (Vilge-Ritter et al., 1999; Masion et al., 2000). The earlier study of these also tested aluminum sulfate (alum) but such a high selectivity for polysaccharide was not found and it was not also clear why two chemicals worked differently. Another study reported that humic substances separated from swamp water were well removed by alum at pH 5 to pH 7 (Lu et al., 1999). Since both polysaccharide and humic substances are major organic components of activated sludge EPS, there seem to be some interactions between aluminum and biopolymers in the floc structure.

PROCESS OVERVIEW OF ANAEROBIC AND AEROBIC DIGESTION

Both anaerobic and aerobic digestion has been widely used to stabilize the wastewater sludge prior to ultimate solids disposal. Reduction of volatile solids and destruction of pathogens are the primary objectives of both processes. Each digestion is processed through very different microbiological and biochemical reactions and the major difference of two digestion processes is whether digestion proceeds in the presence or absence of molecular oxygen (Metcalf and Eddy, 1991).

Aerobic digestion

The basis of aerobic digestion process is similar with activated sludge process. In the presence of molecular oxygen and nitrate, microorganisms convert organic matter into carbon dioxide, ammonia-N, water and new biomass. As available substrate is depleted, endogenous respiration, auto-oxidation of cellular protoplasm, takes place, accounting for the destruction of volatile solids (Metcalf and Eddy, 1991).

Simplicity of process and lower capital cost are the advantages of aerobic digestion compared to anaerobic process and because of these merits, aerobic digestion has been a popular option for the small scale WWTPs. However, high energy cost and lower pathogen inactivation could be the disadvantages of aerobic digestion (Grady et al., 1998).

The percentage of volatile suspended solids (VSS) reduction (38%) and specific oxygen uptake rate ($1-5 \text{ mgO}_2/\text{gVSS}\cdot\text{hr}$) are considered as indicators of sludge stabilization (Grady et al., 1998). Some previous studies demonstrated that mineralization of organic nitrogen during aerobic digestion could also be an indicator of the extent of the aerobic digestion process. Bishop and Farmer (1978) and Mavinic and Koers (1982) found that the percentage of organic nitrogen in sludge solids for both the feed and aerobically digested sludge was constant during aerobic digestion, indicating that % organic nitrogen mineralized is equivalent to % VSS destroyed. With regard to the fate of nitrogen in aerobic digestion, both the two studies also reported that significant rates of nitrification occurred during aerobic digestion. Mavinic and Koers (1982) also observed that pH of sludge decreased even below 4 due to the alkalinity consumption during nitrification

Anaerobic digestion

With comparison to aerobic digestion, anaerobic digestion is a very complex process and various groups of microorganisms in the absence of oxygen and nitrate are involved in reciprocal relationship. Conversion of organic matter into methane after several steps of biochemical reactions accounts for removing COD of feed sludge in anaerobic digestion (Metcalf and Eddy, 1991).

The anaerobic process is known to occur in 3 steps: hydrolysis, acidogenesis, and methanogenesis. In the first step, hydrolysis, insoluble organic matter and large molecular organic compounds are hydrolyzed to soluble and smaller size of organic compounds. In acidogenesis, anaerobic microorganisms break down the products of first step into hydrogen molecule and simple organic acids such as volatile fatty acids and acetic acid. In the final step of anaerobic digestion, known as methanogenesis, methanogenic bacteria convert acetic acid and hydrogen into methane and carbon dioxide. It is also believed that one third of methane is produced from the pathway of using hydrogen and the rest of methane is from the acetic acid. Methanogens are strict anaerobes and have very slow growth rate. Consequently, their metabolism is usually considered rate-limiting and long detention time is required for slow growth (Metcalf and Eddy, 1991).

The production of methane, useful end product, is the great advantage that aerobic digestion does not possess. Relatively higher pathogen inactivation can also be accomplished due to the harsh condition in anaerobic process than in aerobic digestion (Grady et al., 1998).

Different digestion mechanisms between anaerobic and aerobic digestion

Recently, a study by Novak et al. (in press) concentrated on the changes in floc properties that occur during anaerobic and aerobic digestion. The authors monitored very different cation and biopolymer response from each digestion process so they suggested that there are different digestion mechanisms between anaerobic and aerobic digestion.

Based on the observation by Novak et al. (in press), substantial amount of calcium and magnesium was released into solution during aerobic digestion. Divalent cation release was accompanied with an accumulation of solution polysaccharide. The accumulation of polysaccharide was shown to be due to the loss of enzyme activity during aerobic digestion. As indicated earlier, lectin-like biopolymer is comprised of proteins with lectin activity, polysaccharide cross-linked with proteins, and divalent cations. From the results, the authors suggested that lectin-like biopolymer in the floc structure is degraded during aerobic digestion.

Novak et al. (in press) also performed anaerobic digestion using the same waste activated sludge that underwent aerobic digestion. Unlike aerobic digestion, large amounts of protein were released into solution during anaerobic digestion and the release of divalent cations did not take place. These results made the authors suggest that different digestion mechanisms are involved in each digestion process. Novak et al. (in press) stressed the role of iron in anaerobic digestion. They hypothesized that the large release of protein is due to the rupture of preferential binding between ferric iron and protein when ferric iron is reduced to ferrous iron in anaerobic conditions. Released protein was thought to undergo degradation, accounting for volatile solids reduction under anaerobic digestion. Since divalent cations were not released during anaerobic digestion, the authors also suggested that biopolymers degraded during anaerobic process are not lectin-like biopolymers but mainly proteins coagulated by ferric iron from the wastewater influent and cell lysis material.

Since each digestion process accounts for the degradation of a specific type of biopolymer, Novak et al. (in press) also predicted that high degree of sludge stabilization could be accomplished by the combination of two digestion processes.

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CHAPTER I
The Effect of Cations on Activated Sludge Characteristics:
With an Analysis of Iron and Aluminum in Floc

THE EFFECT OF CATIONS ON ACTIVATED SLUDGE CHARACTERISTICS: WITH AN ANALYSIS OF IRON AND ALUMINUM IN FLOC

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ABSTRACT

Iron, aluminum and selected mono (sodium, potassium, and ammonium) and divalent (calcium and magnesium) cations in wastewater were analyzed in order to study the effect of these cations on settling, dewatering, and effluent quality of activated sludge. Samples of influent, mixed liquor, waste activated sludge and effluent were collected from eight different wastewater treatment plants and all these cations were measured in the solution phase of each sample. In addition, iron and aluminum associated with dried waste activated sludge was quantified. Unlike mono and divalent cations, iron and aluminum were not detected in most influents to the aeration basin, indicating that these trivalent cations are most likely associated with solids in the wastewater. It was also found that a significant amount of biopolymers (protein + polysaccharide) remained in the solution of waste activated sludge and secondary effluent of wastewater treatment plants that received high influent sodium but had low concentrations of iron and aluminum in the floc. High soluble biopolymer concentrations corresponded to poor dewatering rates of sludge, high conditioning chemical requirements, and high chemical oxygen demand in effluent. However, sludges from plants with high sodium and high iron and aluminum dewatered well and produced high quality effluents, suggesting that iron and aluminum have significant positive effects on the floc properties. These results indicated that not only concentrations and/or ratio of monovalent and divalent cations but also these floc trivalent cations could be used to predict and evaluate sludge characteristics. A modified cation ratio that included iron and aluminum was examined and it appeared to be useful in predicting sludge characteristics and effluent quality of activated sludge.

KEY WORDS

Activated sludge, floc, effluent, dewatering, conditioning, cations, iron, aluminum, biopolymer, protein, polysaccharide

INTRODUCTION

Microorganisms, extracellular polymeric substances (EPS) or exocellular biopolymers, and cations are major components of activated sludge flocs (Bruus et al., 1992; Higgins and Novak, 1997a). It is thought that biopolymers originate from microbial activity and wastewater influent and provide a matrix in which microorganisms can be aggregated to form flocs (Urbain et al., 1993; Dignac et al. 1998). Because a majority of exocellular biopolymers are negatively charged, multivalent cations become an important component of the floc structure by providing bridging to the negative sites within the biopolymer network (Bruus et al., 1992; Urbain et al., 1993; Higgins and Novak, 1997a). Therefore, without the proper cations, flocs would be weak and dispersed and this would result in poor settling, dewatering and effluent quality.

Historically, divalent cations such as calcium and magnesium have been of great interest in understanding bioflocculation mechanisms. Calcium removal from flocs using a cation exchange resin (Keiding and Nielsen, 1997), EDTA (Kakii et al., 1985), or EGTA (Bruus et al., 1992) resulted in the desorption of organic matter and poor settling and dewatering properties. While these studies stressed the effect of calcium on the stability of floc, other studies have demonstrated that magnesium as well as calcium is important for floc stability. Sobeck and Higgins (2002) reported that the sludge fed with either magnesium or calcium improved in a similar manner as to settling and dewatering properties. In their study, the authors examined three different cation induced-floc models and from this observation they suggested that divalent cation bridging theory in which both calcium and magnesium equally bind negatively charged biopolymers best explain the role of cations in the floc structure. Novak et al. (in press) reported that there was substantial increase in both magnesium and calcium in solution during aerobic digestion and ascribed this result to the degradation of floc material associated with divalent cations. However, activated sludges investigated by Kakii et al. (1985), Keiding and Nielsen (1997), and Bruus et al. (1992) were dominated by calcium ion so the relative importance of calcium in the floc structure could have reflected the lack of magnesium in their wastewater.

While divalent cations are beneficial to the floc properties, high concentration of monovalent cations, especially sodium, has been known to be detrimental (Bruus et al., 1992; Higgins and Novak, 1997b; Novak et al., 1998; Sobeck and Higgins, 2002). All these studies showed that sludges that received additional sodium deteriorated in settling and/or dewatering properties. It was also reported that wastewater treatment plant (WWTP) treating industrial wastewater where sodium hydroxide is used for pH control generally produced sludges with poor settling and dewatering properties (Murthy et al., 1998). It is theorized that ion exchange mechanisms result in displacement of divalent cations with monovalents, making flocs weak and sensitive to any physiochemical changes on the floc structure. By examining the effect of mono and divalent cations on the floc properties, Higgins and Novak (1997b, c) proposed that the ratio of monovalent to divalent cations (M/D) on a charge equivalent basis can be used to determine if the cation content is likely to cause sludge problems. According to the authors, sludge with high M/D ratio, especially greater than 2, would possess problems in settling and dewatering due to the weak floc structure induced by high monovalent cations.

While the role of mono and divalent cations in the floc structure has been studied extensively, the role of trivalent cations such as iron and aluminum has not been studied in depth although these cations are often found at high concentrations in activated sludge (Kakii et al., 1985). Since they have higher charge valence and lower solubility than divalent cations their contributions to the floc stability should be understood better. According to Kakii et al. (1985), iron and aluminum were not affected by the acid treatment of activated sludge at pH 3 while most of calcium and magnesium were extracted, indicating that iron and aluminum are more strongly associated with sludge matrix than divalent cations.

In studies of the role of iron in activated sludge, iron (III) reduction to iron (II) either by iron reducing bacteria (Caccavo et al., 1996) or by addition of sulfide (Nielsen and Keiding, 1998) resulted in a turbidity increase in bulk sludge water, which suggests that oxidized iron is an important constituent of the floc structure. Rasmussen and Nielsen (1996) reported that about 70 to 90% of iron was present as ferric iron in the fresh activated sludges they investigated. However, they also admitted difficulties in identifying the species or chemical forms of iron (III) in activated sludge. Later, Nielsen and Keiding (1998) hypothesized that either the reaction of ferric ion with EPS or the precipitation as ferric hydroxides or both are involved in flocculation and both the species were considered to be better flocculants than calcium ion. It has been recently reported that ferric iron has high affinity for protein (Murthy et al., 2000; Novak et al.,

in press). Murthy et al. (2000) demonstrated that iron (III) salts selectively coagulated solution protein produced from autothermal thermophilic aerobic digesters. Later, Novak et al. (in press) postulated that large protein release during anaerobic digestion is due to the loss of selective binding between protein and ferric iron induced by iron reduction in anaerobic conditions.

There is little information available for the specific role of aluminum in activated sludge floc. Some investigations dealing with aluminum in sludge were found in the field of drinking water treatment since aluminum salts are often used as coagulants removing natural organic matters in water treatment plants. Two studies showed that polysaccharide found in lake and river water was preferentially coagulated by aluminum polychlorosulfate (Vilge-Ritter et al., 1999; Masion et al., 2000). The earlier study of these studies also tested aluminum sulfate (alum) but such a high selectivity for polysaccharide was not found and it was not clear why two species of aluminum chemicals worked differently. Another study reported that humic substances separated from swamp water were well removed by alum at pH 5 to pH 7 (Lu et al., 1999). Since both polysaccharide and humic substances are major organic components of activated sludge EPS, there are likely to be some interactions between aluminum and biopolymers in the floc structure.

RESEARCH OBJECTIVES

An examination of the literature revealed that poor bioflocculation due to the improper cation composition might cause settling and dewatering problems. Simultaneously, critical amounts of unflocculated materials might be washed out of the system, causing deterioration in effluent quality. Previous studies also indicated that both divalent and trivalent cations are extremely important in well-flocculated activated sludge although the specific role of each of the cations is not clearly understood. In order to assess the overall impact of cations on the activated sludge systems, a field study was performed and both the solution and floc cations were evaluated along with various activated sludge characteristics. The data were used to determine how cations account for many differences between characteristics of WWTPs and sludges they produce. The hypothesis of this study was that both divalent and trivalent cations positively affect the floc stability and that each has a unique role in floc formation, sludge properties and effluent quality.

METHODS AND MATERIALS

Experimental approach

Wastewater samples were collected from eight different full-scale WWTPs. The plants were selected jointly by US Filter Inc. and the Virginia Tech research team. Some plants were sampled twice and the first and second sampling are designated with 1 and 2. The samples consisted of plant's influent (primary effluent), mixed liquor, waste activated sludge, and plant's effluent (secondary effluent). For all the samples, cations in solution were measured and dried waste activated sludge (WAS) was prepared for analysis of iron and aluminum in floc. In this study, the term 'soluble' or 'solution' refers to be the material that can be filtered through 0.45 μ m filter. However, iron and aluminum were designated as 'floc' cations since these cations were measured in the floc structure. The sludge settling and dewatering properties were measured using mixed liquor and WAS, respectively. Shear tests and conditioning tests were also conducted using WAS. The consecutive filtering processes with different sizes of filters was performed using WAS in order to analyze molecular weight distributions and biopolymer content in each of the molecular weight fractions. For this analysis, all the WAS samples were centrifuged at 9,460g for 15 minutes and the supernatant was subsequently filtered through 1.5 μ m, 0.45 μ m filters and through 30,000 Dalton (30K) and 1,000 Dalton (1K) ultrafiltration membranes (Amicon YM30 and YM1). The soluble biopolymer (protein + polysaccharide) and soluble chemical oxygen demand (COD) in secondary effluent were also analyzed.

Analysis

Solution cations, sodium, potassium, ammonium, magnesium, and calcium were analyzed using a Dionex Ion Chromatograph. Floc cations, total iron and aluminum, in dry sludge were measured using EPA method 3050B (Acid digestion for metals analysis of soils, sediments, and sludges). For this experiment, WAS was dried at 105°C and the known amount of dried sludge was removed and underwent acid digestion. The prepared samples were quantified using Atomic Absorption Spectrometer. Iron and aluminum in sludge solution were also measured using Atomic Absorption Spceterometer.

Total solids (TS), total suspended solids (TSS), mixed liquor suspended solids (MLSS), total volatile solids (VS), and volatile suspended solids (VSS) were measured according to Standard Methods (APHA, 1995). Sludge volume index (SVI) and soluble COD were measured based on methods 2710D and 5220C of Standard Methods, respectively (APHA, 1995). The protein

concentration was determined by the Hartree (1972) modification of the Lowry et al. (1951) method using bovine serum albumin as the standard. Polysaccharide was measured by the Dubois et al. (1956) method utilizing glucose as the standard.

Both ferric chloride and alum at concentrations of 100g/L and a high charge cationic polymer, BC650 (Stockhausen), at 0.5% (w/w) were used as sludge conditioners in this study. Optimum doses of each conditioner were determined using capillary suction time (CST) test by obtaining the lowest CST reading. CST and specific resistance to filtration (SRF) were used as measures of the sludge dewatering rate according to method 2710G of Standard Methods (APHA, 1995) and method described by Christensen and Dick (1985), respectively.

The shear test using a flat paddle mixer at 700 rpm was conducted to evaluate the floc strength of each WAS sample. 500mL of WAS was placed into a 2L baffled cylinder and CST values at every 2 minute upto 8 minutes were measured.

RESULTS AND DISCUSSION

Composition of the WAS samples including mono and divalent cations in sludge solutions and iron and aluminum in the dried sludges are listed in Table 1. A summary of sludge settling and dewatering properties, conditioning chemical requirements, and solution biopolymers (protein + polysaccharide) in the WAS and secondary effluent are listed in Table 2.

Table 1. Solution mono and divalent cations and floc trivalent cations in WAS

Plant	SRT (day)	TS (g/L)	VS (g/L)	Na ⁺ (mg/L)	K ⁺ (mg/L)	NH ₄ ⁺ (mg/L)	Mg ²⁺ (mg/L)	Ca ²⁺ (mg/L)	M/D (eq/eq)	Ca/Mg (eq/eq)	Al (mg/g TS)	Fe (mg/g TS)
A1	18	15.0	10.2	296	29.7	2.95	19.2	35.1	4.1	1.1	15	25
A2	28	18.7	13.9	579	27.2	ND	20.1	39.4	7.2	1.2	9.2	16
B1	11	6.57	3.18	1087	92.6	ND	18.4	23.7	18	0.8	0.7	2.9
B2	11	8.47	4.99	977	116	ND	17.9	37.2	14	1.3	0.7	3.8
C	4	8.32	6.69	67.6	71.7	49.3	25.0	24.8	2.3	0.6	13	13
D	23	6.77	5.49	93.8	11.4	ND	13.5	34.2	1.6	1.5	5.8	5.8
E	7	8.87	4.87	324	32.8	51.6	44.5	339	0.9	4.6	2.0	40
F1	3	5.57	4.57	71.2	10.1	2.4	7.7	26.0	1.8	2.0	14	5.8
F2	3	5.22	3.85	89.3	10.4	0.2	6.1	25.9	2.3	2.6	27	9.5
G	5	6.54	5.18	129	48.4	39.5	14.9	29.3	3.4	1.2	9.3	15
H	1	3.90	2.94	45.4	21.0	33.3	15.5	36.2	1.4	1.4	4.2	80

ND: Not detected

Table 2. Characteristics of activated sludge process in eight full-scale WWTPs

Plant	SVI (mL/g)	CST (sec)	SRF (Tm/Kg)	Optimum	Optimum	Optimum	Solution*	Solution*	Effluent	Effluent
				polymer (g/Kg TS)	FeCl ₃ (g/Kg TS)	Alum (g/Kg TS)	protein (mg/L)	poly- saccharide (mg/L)	protein (mg/L)	poly- saccharide (mg/L)
A1	85	60.0	2.0	2.64	80	94	3.60	2.16	3.18	2.82
A2	110	71.9	1.7	2.45	60	96	5.88	3.92	3.93	3.62
B1	373	61.6	4.7	3.57	487	164	66.8	13.8	57.0	12.4
B2	149	27.4	0.6	1.41	283	118	32.2	7.21	29.4	8.00
C	251	394	250	4.78	144	240	22.1	12.9	7.71	5.19
D	55	18.5	0.5	0.59	106	148	1.22	4.30	3.48	3.29
E	117	57.8	13	3.14	217	248	16.6	13.0	NA	NA
F1	249	44.9	5.7	1.96	72	122	3.10	2.50	1.93	3.06
F2	199	26.5	1.1	1.22	77	153	0.74	2.39	1.78	4.18
G	268	92.2	120	5.38	202	184	17.8	10.6	7.60	6.88
H	484	211	120	7.52	308	328	20.8	14.6	0.50	1.83

* Protein and polysaccharide in WAS solution

NA: Samples were not available.

Potassium release in activated sludge

It can be seen that the dewatering rates, described by CST and SRF, of the WAS samples from plants C, G, and H were quite different from those from other plants. The floc strength of these sludges were measured by conducting shear tests and evaluating the changes in CST over time. Figure 1 shows that WAS C and G were very sensitive to shear while others, except for WAS H, were shear-resistant under the same condition. Surprisingly, the dewatering rate of WAS H improved as a result of sludge shearing. Plant H was only the plant that received external iron for phosphorous removal but the reason for these unusual characteristics of WAS H was not clarified. However, low solution biopolymer in the effluent in spite of high solution biopolymer in the WAS implies that some changes might have occurred during sludge handling, which led to the poor dewatering characteristics of WAS H.

Weak floc strength of WAS C and G indicates that these sludges would dewater poorly if high shear device such as a centrifuge is used. Cation ratios previously suggested to describe sludge characteristics could not explain poor dewatering rates and weak floc strength of WAS C and G. As shown in Table 1, the ratios of M/D and Ca/Mg (Higgins and Novak, 1997c) of these sludges were close to respective optimum values 2 and 1 that are likely to bring good sludge properties. The only noticeable difference in cation contents for these sludges was that abnormally high

potassium concentration was found in the solution of WAS compared to its concentration in plant's influent. Table 3 shows potassium concentrations across each facility. Compared to the potassium concentrations in each influent, approximately five and four times greater potassium was found for WAS C and G, respectively. Since potassium is required by microorganisms for cell synthesis and is more likely an intracellular cation than extracellular (Bakker, 1993; Madigan et al., 1997), it can be thought that some stress to microbes might have been present in the influents to these two facilities.

Table 3. Potassium concentrations across a WWTP

Plant	Influent (mg/L)	ML (mg/L)	WAS (mg/L)	Effluent (mg/L)	K ⁺ Ratio (WAS/INF) (mg/mg)
A1	18.8	22.3	29.7	22.0	1.58
A2	18.1	22.0	27.2	20.5	1.51
B1	NA	94.0	92.6	97.2	-
B2	NA	111	116	117	-
C	15.6	28.0	71.7	15.7	4.59
D	7.20	9.10	11.4	8.60	1.58
E	19.2	32.0	32.8	NA	1.70
F1	9.60	10.8	10.1	7.70	1.06
F2	9.70	11.1	10.4	9.37	1.07
G	12.9	31.4	48.4	26.5	3.78
H	10.9	10.5	21.0	9.40	1.92

NA: Samples were not available

Bott and Love (in press) reported that when the toxins of electrophilic or thiol reactive chemicals were added to activated sludges, bacteria released K⁺ as specific protective stress response, causing sludge deflocculation. Rasmussen et al. (1994) observed K⁺ release during short term-anaerobic storage of activated sludge and hypothesized that the release took place because the strict aerobes lost their ability to keep internal K⁺ under anaerobic conditions. Figure 2 shows that the ratio of K⁺ in WAS to that in influent produces a useful indicator of poor dewatering rates of WAS C and G. Although the data is limited, it appears that the changes in potassium across a plant might be indicators of both toxicity and poor sludge dewatering.

Solution biopolymer in the effluent from these two facilities, as shown in Figures 3 to 6, was much higher than those from the other plants with similar cation compositions. These results also suggest that sludge characteristics associated with K⁺ release across the treatment plant might be

somewhat different from problems caused by other influent properties. As a result, data points representing wastewater from these two facilities were treated differently in the figures shown in this study and trend lines between some variables were obtained without using those data.

The effect of cations on activated sludge effluent quality

In this study, secondary effluent quality was considered to be the best indicator for activated sludge performance at each WWTP. Unwanted reactions such as deflocculation of sludge during the transfer or storage (Bruus et al., 1993; Rasmussen et al., 1994) can occur for mixed liquor samples but will not apply to effluent. Therefore, the comparisons of cations with effluent biopolymer were used to assess the impact of influent cations on activated sludge effluent quality.

(1) M/D

Higgins and Novak (1997b, c) examined several full and lab-scale activated sludges and found that the ratio of monovalent cations to divalent cations on a charge equivalent basis was positively correlated with the filtration rate (SRF) of activated sludges. The authors also observed that at a ratio exceeding 2, the dewatering rate decreased so that they used this number as an optimal ratio of M/D. The conceptual basis of M/D was that monovalent cations displace divalent cations within the cation bridged-floc structure by ion exchange, deteriorating floc properties. Later, a study by Murthy (1998) showed that the M/D ratio was also an efficient parameter for describing activated sludge effluent quality.

Solution protein and polysaccharide in each plant's effluent are shown in Table 2. Large amounts of biopolymer were found in effluents from plant B, implying that substantial fraction of biopolymer in activated sludge was deflocculated and washed out in the effluent. The solids retention time (SRT) for plant B was 11 days so SRT was not likely to contribute the high solution biopolymer in the effluent of plant B (Bisogni and Lawrence, 1971; Grady et al., 1998).

The influents to plant B contained extremely high sodium concentrations. However, the lowest iron and aluminum in the floc were detected from WAS samples. The M/D ratios of WAS B1 and B2 were 18.4 and 13.7, respectively. Poor sludge characteristics at these high M/Ds are consistent with the concept described by Higgins and Novak (1997b, c). The M/D ratios of WAS were compared to effluent biopolymer and are shown in Figure 3. In general, as the M/D ratio increased, more biopolymer was found in the effluent. Moreover, it is important to note that

effluent properties of B1 and B2, even from the same WWTP, were different over time as the cation composition varied. Data in Table 2 also shows that the settling and dewatering properties of sludge B2 also improved at a reduced M/D ratio.

However, the M/D ratio alone could not explain all the differences in characteristics of wastewater from the different WWTPs. An example was wastewater sampled from plant A. High concentrations of sodium were also found in this facility, which corresponded to WAS M/D ratios of A1 and A2 at 4.1 and 7.1, respectively. Although both the M/D ratios are much higher than optimum, settling and dewatering properties of both sludges could be considered to be reflective of good sludges. Twice as much as sodium was found in WAS A2 compared to WAS A1, but the overall sludge characteristics were not very different, implying that plant A did not seem to experience such operational problems despite high M/D or high sodium. High concentrations of iron and aluminum in floc was found in WAS A1 and A2. While both the plants A and B had high sodium in the influent, the sludge characteristics of plant A in which high floc trivalent cations was found were much better than of plant B.

(2) Trivalent cations

Dried WAS was used to measure the floc trivalent cations, iron and aluminum. Filtrate passing a 1.5-micron filter for each WAS was also prepared to measure iron and aluminum in solution, but these cations were usually less than the detection limit. Concentrations of iron and aluminum in floc are listed in Table 1. Widely varying amounts were found between the WWTPs and even for activated sludges taken from the same WWTP at different times. Maeda and Azumi (1982) and Kakii et al. (1985) investigated series of activated sludges from a particular WWTP over a year and found seasonal variations in the heavy metals such as iron and aluminum and ash content in the MLSS. These two studies reported increases in both the metals and ash content during the summer but decreases during the winter. However, they also reported that the ratio of the total metal content to ash content in the MLSS was constant throughout a year. It was speculated that the summer increase was not because of actual increase in inflow of these materials but more likely because of decrease in volatile fractions in the mixed liquor. They ascribed low VSS or high ash content in summer to the possible auto-oxidation of biomass at the higher summer temperatures.

For this study, three WWTPs were sampled twice. The sampling time, the percentage of ash in WAS, and the sum and ratio of iron and aluminum are listed in Table 4. As previous studies

indicated, the sum of iron and aluminum (except for plant B) and ash content were greater in sludges collected during the warmer period. The ratio of aluminum to iron from the same facility was also quite constant in spite of different total concentrations, which might support the previous discussion that inflows of metal content at different sampling times were likely similar. The mass of metals in sludge is usually expressed on a TS basis (Table 1). If increased iron and aluminum were not really due to the increased inflow of these materials, different units might possibly be used to express their concentrations. Since inorganic cations are more likely associated with ash content in the mixed liquor, a unit based on one gram ash was used by dividing 'mg-cation/g-TS' by 'g-ash/g-TS'. The sum of iron and aluminum based on these units are also listed in Table 4. The concentrations on these units still show differences between WAS from the same site but the percentile difference between two concentrations were reduced. Figure 4 also shows that the sum of iron and aluminum based on one gram ash explains better the relationship between trivalent cations and effluent biopolymer.

Table 4. Variations in floc cations and ash content in WAS at different times

sludge	Ash/TS (%)	Al+Fe (mg/g TS)	Al/Fe (mg/mg)	Al+Fe (mg/g ash)	Month of Sampling
A1	31.7	39.0	0.59	123	June
A2	25.8	25.0	0.58	97.0	January
B1	51.6	3.60	0.24	6.90	July
B2	41.1	4.50	0.17	10.8	November
F1	18.0	19.3	2.32	108	January
F2	26.2	36.3	2.83	138	September

More importantly, Figure 4 illustrates that as iron and aluminum increased in an activated sludge process, less solution biopolymer was found in plant's effluent. It has been speculated that both iron and aluminum might have higher flocculating ability than divalent cations, building strong and compact flocs. Keiding and Nielsen (1997) also predicted that when sludge is deficient of iron and aluminum, much of biopolymer would remain unflocculated and wash out of the system. This is clearly shown in Figure 4. Activated sludges with low iron and aluminum such as WAS B1 and B2 yielded high solution biopolymer in the effluent. A similar trend was also found between the sum of iron and aluminum and effluent COD, although the correlation was not as good as effluent biopolymer.

The linear correlations between the logarithmic values of variables were examined and strong correlations were obtained between the effluent biopolymer with the sum of iron and aluminum

in flocs ($r^2 = -0.99$) and the ratio of M/D ($r^2 = 0.90$). It could not be determined which cation content had the most significant effect, but the data suggest that both the floc trivalent cations and the M/D ratio should be considered together to describe activated sludge and effluent characteristics. The effect of cations on effluent quality might be of specific importance in wastewater treatment in Europe and Asia where effluent COD is often used for design and effluent regulations.

(3) Modified M/D

From the data above, both the M/D ratio and trivalent cations appear to be important for determining the activated sludge effluent quality. Therefore, if they can be combined, their impact on sludge characteristics might be better described. Before unifying the M/D ratio and trivalent cations, the ratio of monovalent cations to the sum of iron and aluminum in floc (M/T) was evaluated along with effluent biopolymer data. The analytical approach was similar with the M/D ratio that monovalent cations negatively affect the floc stability while trivalent cations, iron and aluminum, positively contribute to the floc properties. Figure 5 shows that the M/T ratio can also be used for describing activated sludge effluent quality. This might be because of the low variation in divalent cations in sludges, except for WAS E, examined so that the M/D ratio is mainly determined by the monovalent cation concentration. However, if sludges contain widely varying divalent cation concentrations, this ratio might become less useful.

A modification of M/D ratio with floc trivalent cations has been tried. This ratio was obtained by dividing the M/D ratio by the sum of iron and aluminum so the units of this ratio were [(eq/eq)/(mg/g-ash)]. The relationship between modified M/D ratio (mM/D) and effluent biopolymer is presented in Figure 6. As the sum of iron and aluminum and the sum of calcium and magnesium increase, the ratio decreases, so a low value would be associated with good sludge properties. Although this ratio was useful in describing sludge properties in this study, this ratio might not be the best way to describe the overall effects of mono-, di-, and trivalent cations on sludge characteristics since while the M/D ratio is based on competitions for ion exchange sites between mono and divalent cations, adsorptive reactions with biopolymers are more likely involved with iron and aluminum in activated sludge. Clearly, this concept needs more work. If divalent cations coagulate organics that differ from those coagulated by iron and aluminum, their effect might be additive.

Effect of biopolymer on sludge conditioning

The study demonstrated that when sludges contained high monovalent cations and low iron and aluminum, large amounts of biopolymers remained unflocculated, accounting for poor effluent quality. Sludges that experienced poor flocculation will not settle and dewater well. In addition, more conditioning chemicals will be required to achieve efficient sludge dewatering. Figure 7 clearly shows how various sludge characteristics are inter-related for activated sludge performance.

The effect of solution biopolymer on the performance of sludge conditioning was further investigated during this study. Novak et al. (1988) proposed that the particle size distribution is one of the most important parameter affecting the dewatering properties of sludge. In their study, the particles less than $40\mu\text{m}$ were found to be associated with the filter blinding, causing poor dewaterability of sludge. Bivins and Novak (2001) showed that colloidal biopolymer, the sum of protein and polysaccharide between $1.5\mu\text{m}$ and 30K , was strongly related with dewatering rates of anaerobically digested sludges.

As can be seen in Figure 8, the optimum conditioning dose of ferric chloride for each WAS was mainly determined by the concentrations of solution ($< 0.45\mu\text{m}$) biopolymer. Biopolymer passing 1.5-micron filter, the size also often used for separation between solids and solution, was also well coagulated by ferric chloride (data not shown). However, from Figure 9, optimum doses of alum and cationic polymer appeared to be influenced by specific size of solution biopolymer, colloidal ($1.5\mu\text{m} - 30\text{K}$) fraction. The different results between iron and aluminum salts might be because of different atomic size of each cation. Iron is larger ion so the iron conditioner might have coagulated a broader range of sizes. The different coagulating capabilities of chemical conditioners were also observed by Murthy et al. (2000) who demonstrated that iron (III) salts removed protein and polysaccharide from a wide size range while cationic polymer was effective in removing only colloidal ($1.5\mu\text{m} - 30\text{K}$) biopolymer, leaving the material less than 30K in solution

The results from conditioning tests using ferric chloride and alum might also be useful in analyzing the relationships between biopolymers and iron and aluminum in activated sludge flocs. Figure 10 implies that optimum alum dose for sludge conditioning was more likely affected by colloidal polysaccharide rather than colloidal protein. Vilge-Ritter et al. (1999) and Mason et al. (2000) also observed the high affinity of polysaccharide in natural water for

aluminum salts. However, the chemical used in two studies was aluminum polychlorosulfate and the former study did not find polysaccharide affinity for alum. Further study is needed to better understand a certain role of aluminum in activated sludge flocs. Figure 11 clearly shows that iron (III) has strong affinity for protein. In addition, the comparison between Figures 8 and 11 reveals that optimum ferric chloride dose was determined by the concentration of solution protein. This strong affinity between ferric iron and protein was also suggested by Novak et al. (in press). They hypothesized that protein release during anaerobic digestion is result of iron reduction under anaerobic conditions. While a high concentration of iron in activated sludge floc is beneficial for making strong and dense flocs by strongly attaching protein, it might also play a negative role in sludge dewatering if iron rich-sludge is kept in anaerobic conditions. Unlike iron, aluminum does not undergo reduction and therefore may provide the benefits of iron without the problems associated with reduced conditions.

Implications

Higgins and Novak (1997c) and Murthy et al. (1998) showed from their field trials that the addition of magnesium salt to the feed of the industrial WWTP resulted in dramatic improvements in sludge settling and dewatering properties. These two studies also showed that such improvements were achieved following one or two cycles of SRT. The data obtained from this research suggest that addition of iron and aluminum to activated sludge process might be beneficial for improving effluent quality. The addition of these cations contributes to strong and dense flocs, improving dewatering properties and reducing polymer conditioning costs.

Iron salts are often added to the mixed liquor for the removal of phosphorous from wastewater. As indicated earlier, plant H added iron for the same objective and it corresponded to the highest iron concentration among sludges investigated. Low biopolymer in this plant's effluent indicate that added iron might have worked as an efficient coagulant or flocculant in the mixed liquor but the high solution biopolymer in the WAS and its poor dewatering rates were not consistent with this explanation. The unusual characteristics of wastewater from this facility can also be seen in Figure 7c. In this figure, FeCl_3 conditioning data for WAS H was only the data not showing good relationship with effluent biopolymer. This is likely because iron was directly added to the mixed liquor so that iron might have formed weak hydroxy compound rather than being incorporated into the internal of bioflocs. Consequently, sludge might have easily deteriorated when external forces were introduced on this sludge.

CONCLUSION

Cations in activated sludge had significant effects on activated sludge characteristics, specifically on activated sludge effluent quality. The study also showed that flocc trivalent cations, iron and aluminum, are important constituents of activated sludge. The specific role of each cation in flocculation should be better understood. The following specific conclusions are drawn from this study:

- Measurement of potassium changes across an activated sludge facility may be useful for indicating toxicity and predicting dewatering rates of sludge.
- Sludges with high monovalent cations and low iron and aluminum are not well flocculated. As a result, large amounts of biopolymers remained in the sludge solution, deteriorating effluent quality and causing poor sludge dewatering and high conditioning chemical requirements.
- A modified cation ratio which includes iron and aluminum was useful in evaluating overall effects of common mono-, di-, and trivalent cations on activated sludge characteristics.
- Optimum iron (III) salts was determined by the concentrations of solution ($< 0.45\mu\text{m}$) biopolymer while aluminum salts reacted primarily with colloidal ($1.5\mu\text{m} - 30\text{K}$) fraction of solution biopolymer.
- Conditioning data suggested that ferric iron has strong affinity for activated sludge protein while aluminum might have higher affinity for polysaccharide in activated sludge EPS.

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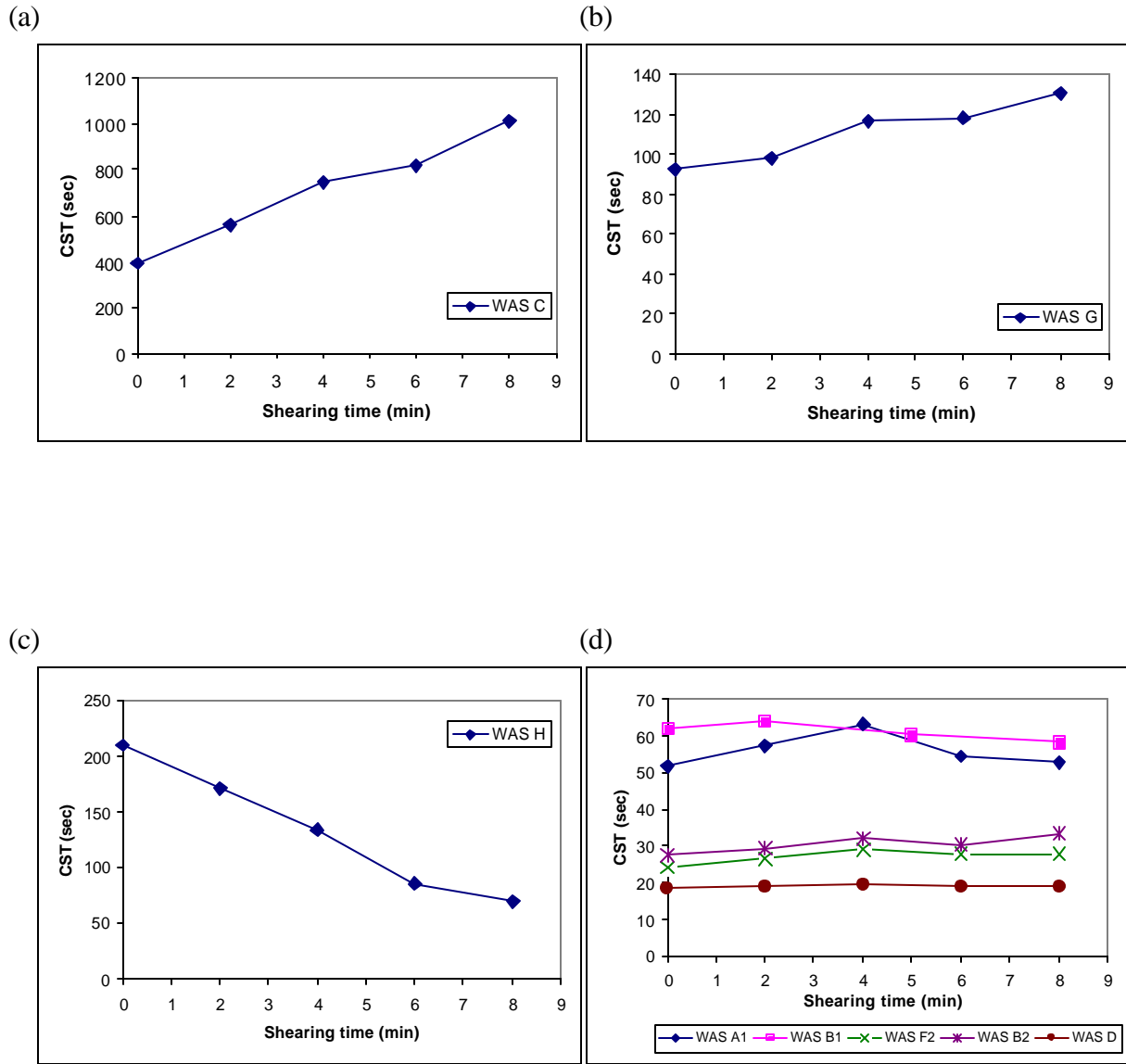


Figure 1. CST changes in (a) WAS C (b) WAS G (c) WAS H and (d) the rest WASs following the shear tests at 700 rpm

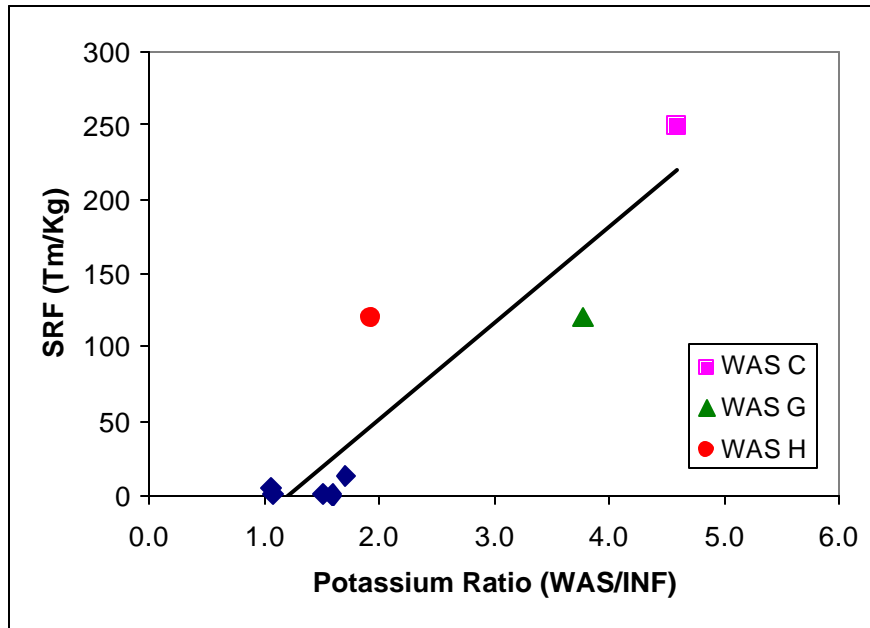


Figure 2. Effect of potassium release in the WAS on sludge dewatering characterized by SRF

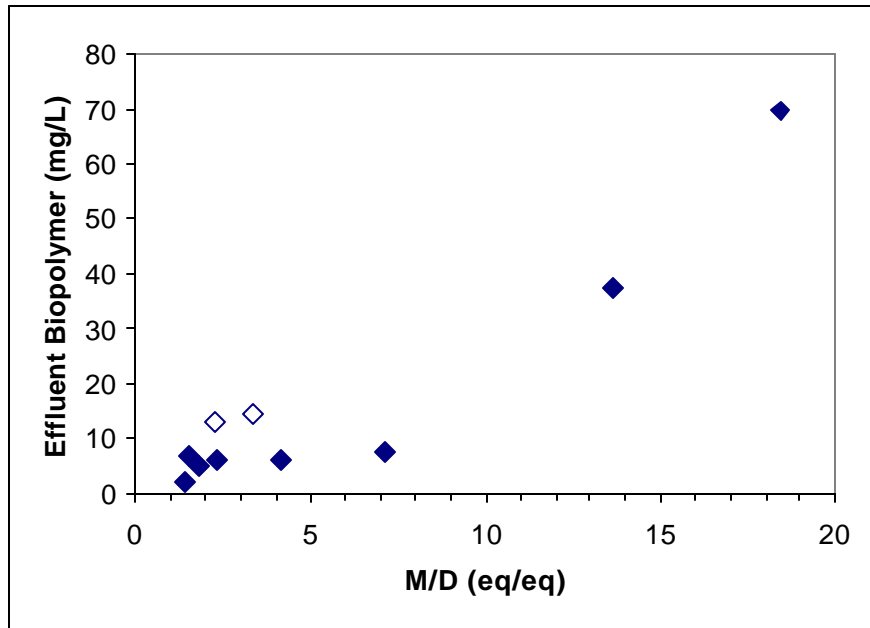


Figure 3. Effect of M/D on soluble biopolymer in the effluent

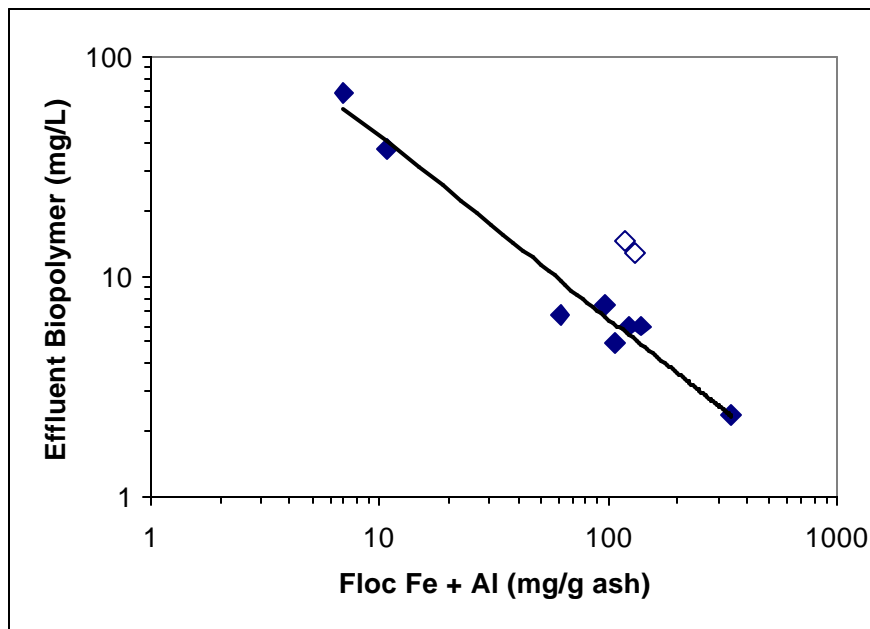
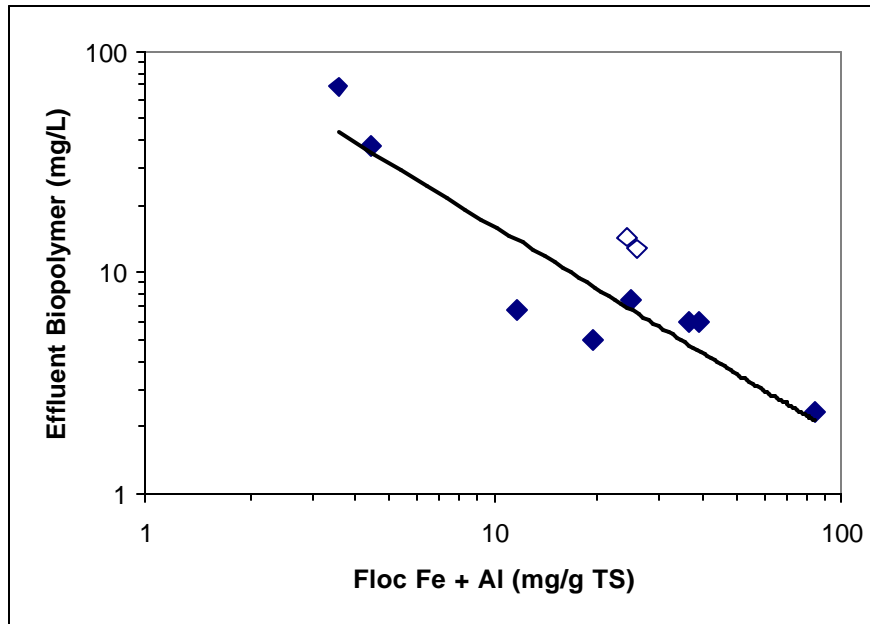


Figure 4. Effect of the sum of floc iron and aluminum on effluent biopolymer; the units of floc cations were based on (a) one gram TS ($R^2 = 0.86$) and (b) one gram ash ($R^2 = 0.97$).

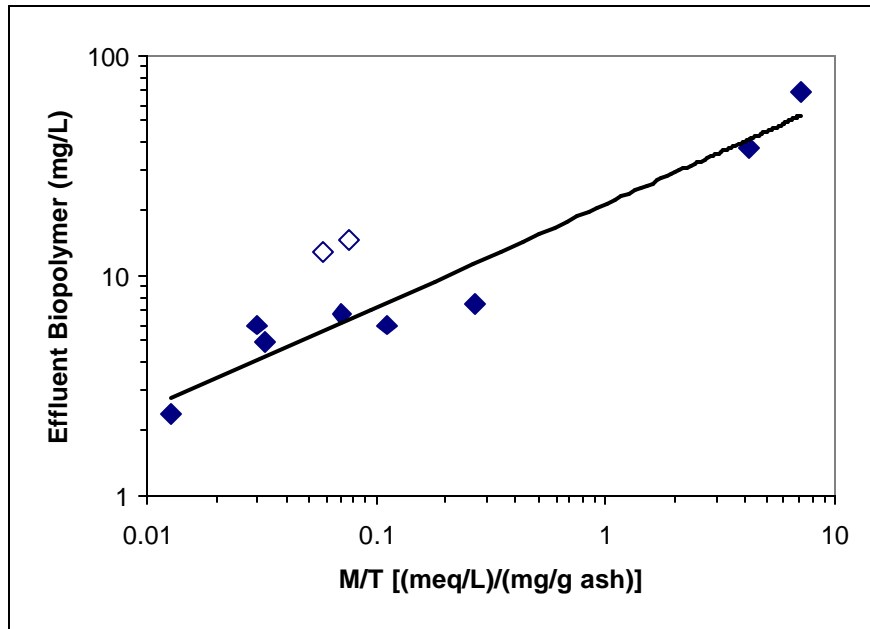


Figure 5. Effect of the ratio of monovalent cations to the sum of iron and aluminum on effluent biopolymer ($R^2 = 0.94$)

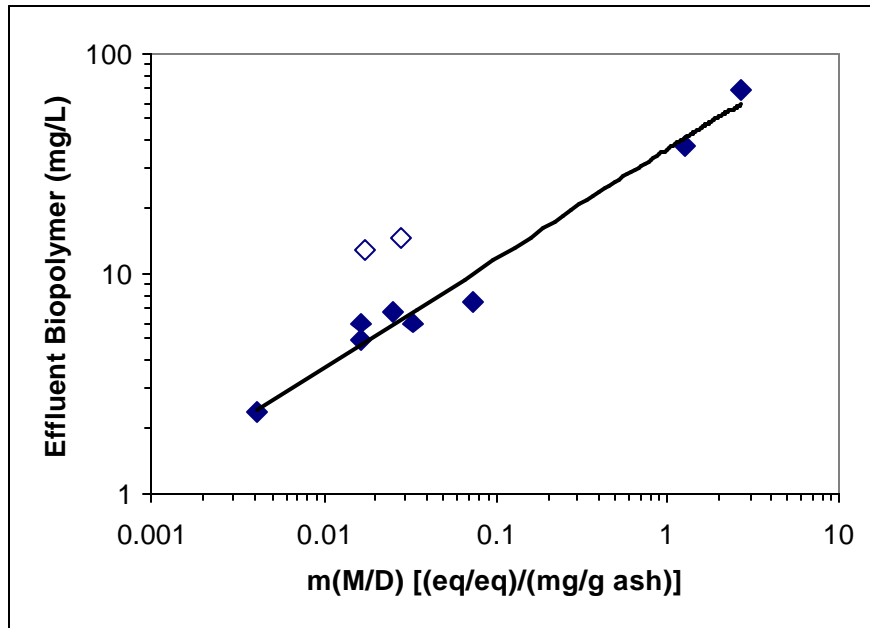


Figure 6. Effect of the modified M/D ratio on effluent biopolymer ($R^2 = 0.98$)

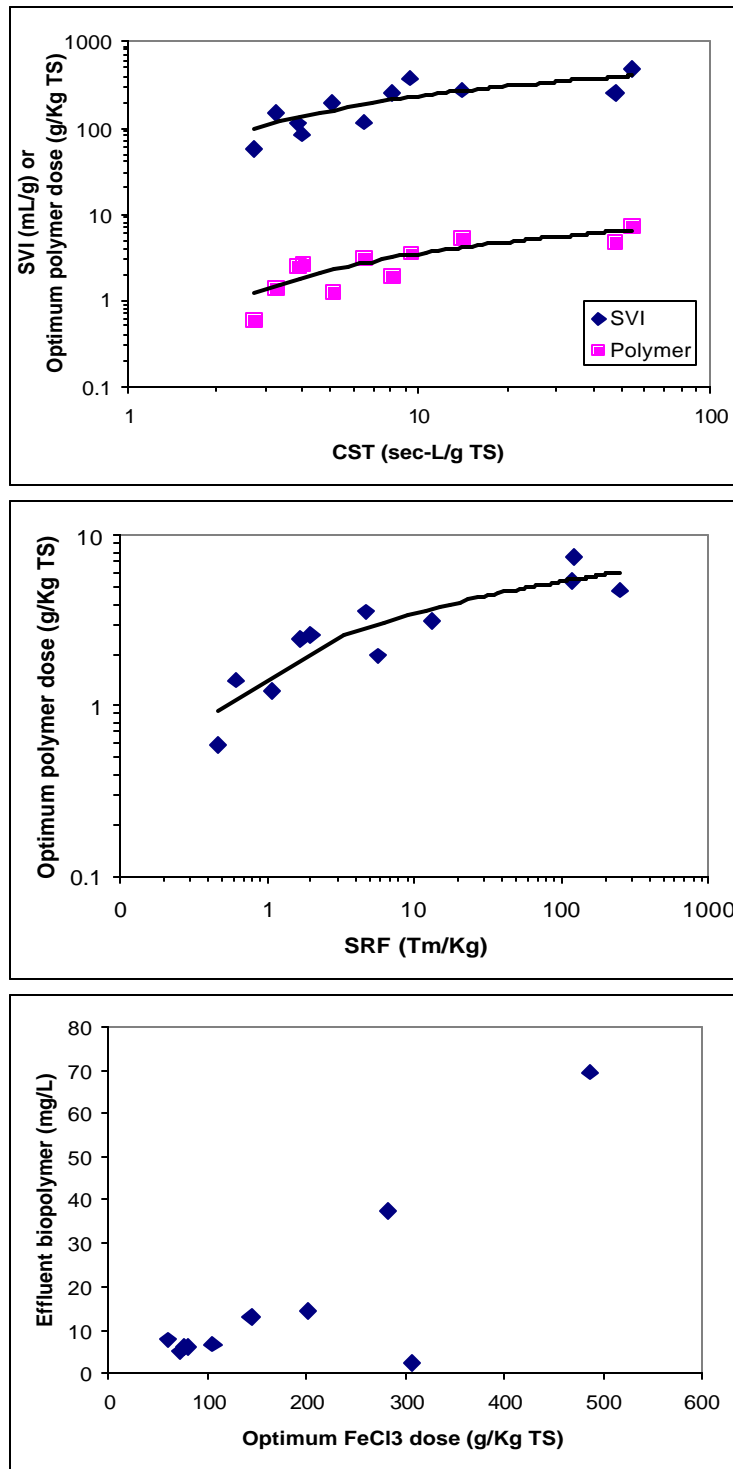


Figure 7. Relationships between (a) settling, dewatering, and conditioning characteristics (b) dewatering and conditioning and (c) conditioning and effluent biopolymer

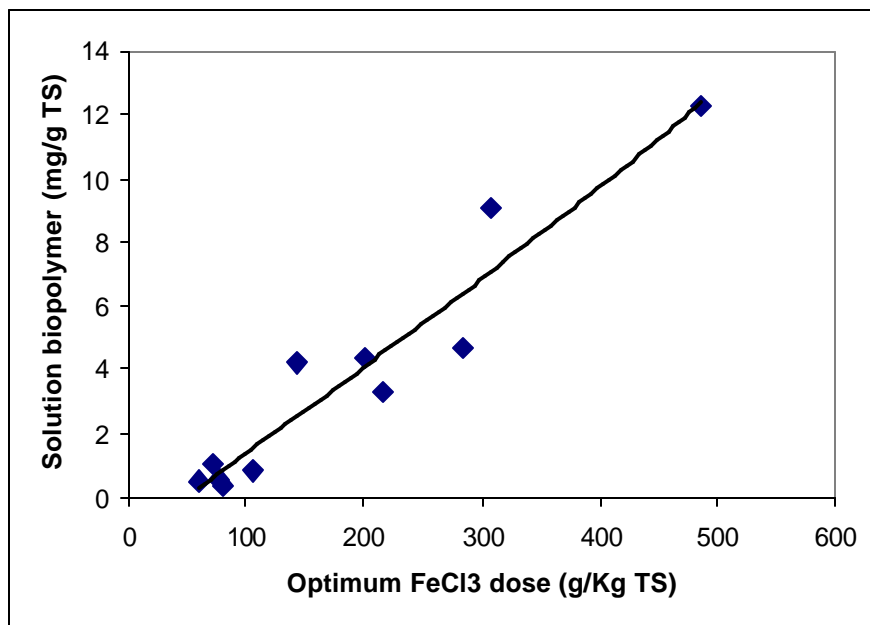


Figure 8. Relationship between optimum FeCl_3 dose and solution biopolymer in the WAS

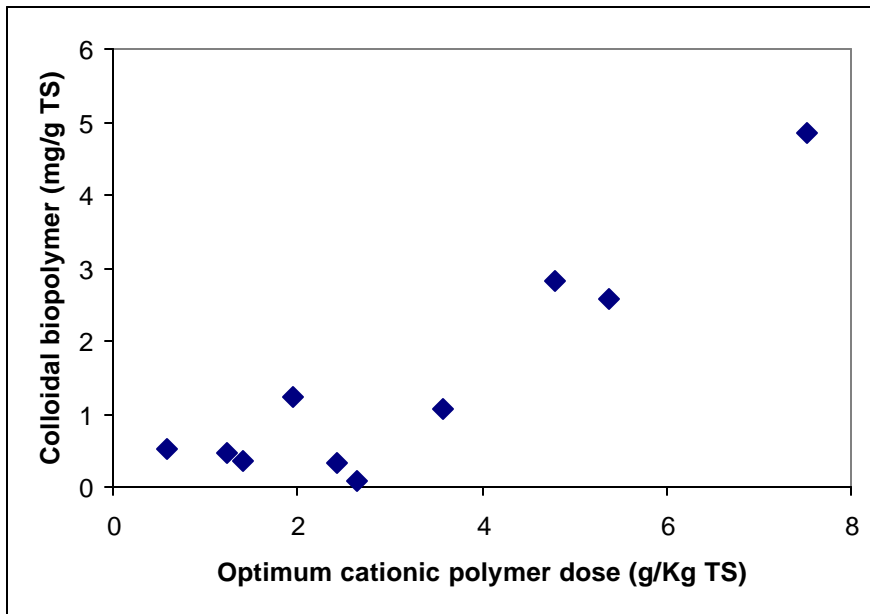
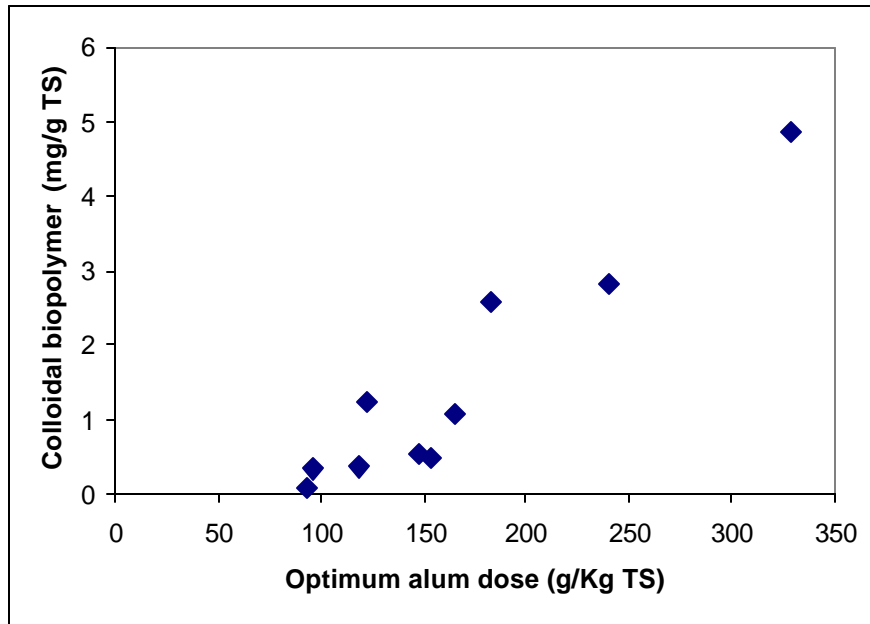


Figure 9. Relationships between colloidal (1.5 μ m – 30K) biopolymer in the WAS with (a) optimum Alum dose and (b) optimum cationic polymer dose

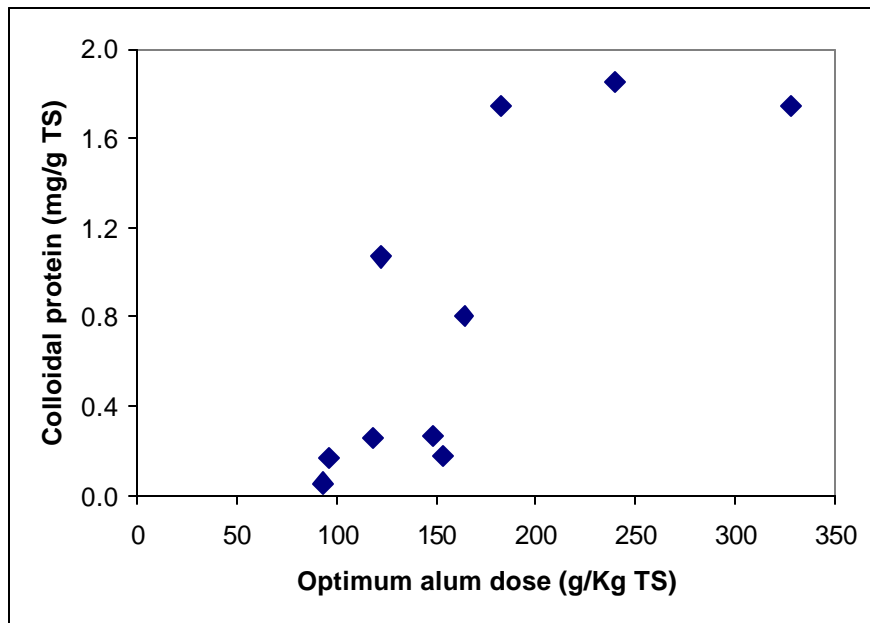
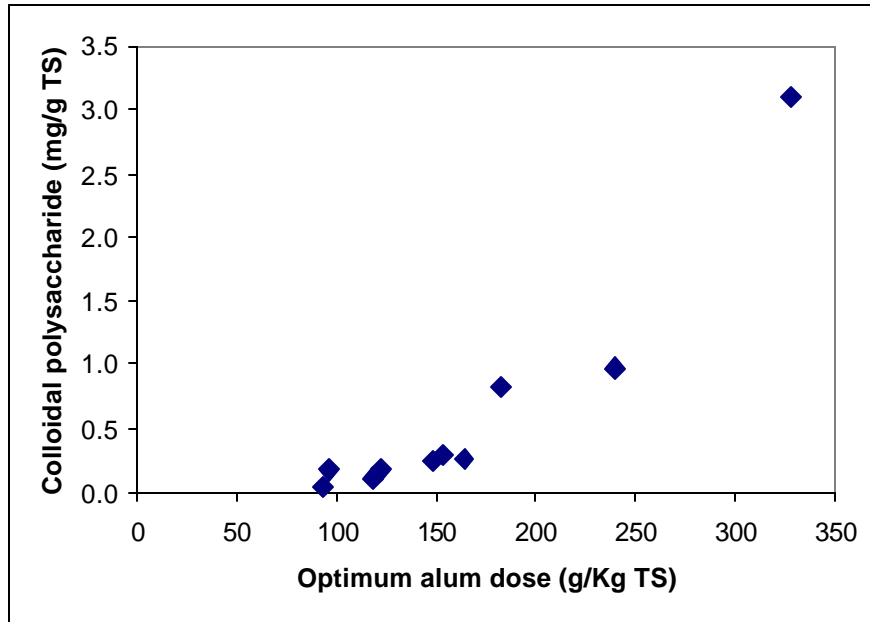


Figure 10. Alum conditioning for WASs that contained various amounts of colloidal (1.5 μ m - 30K) (a) polysaccharide and (b) protein

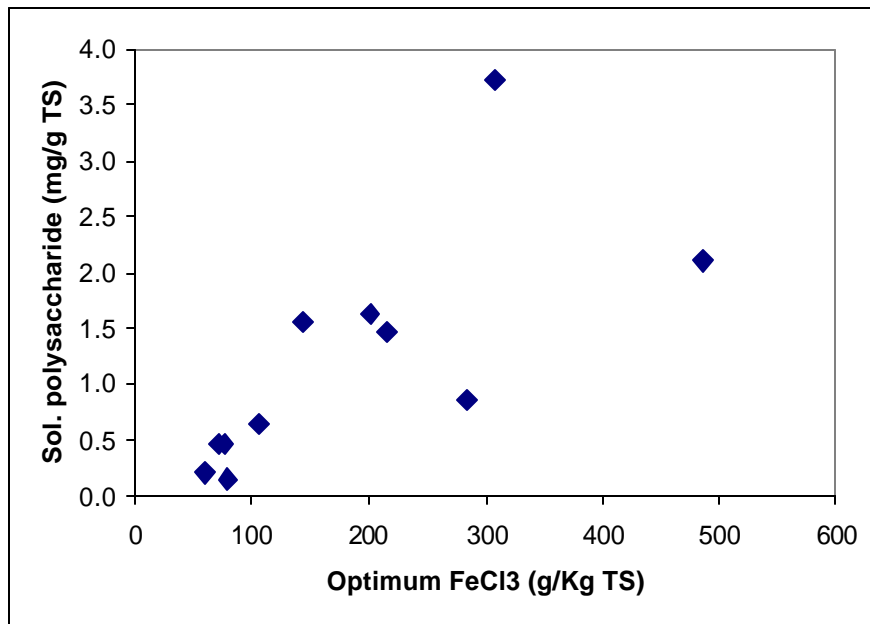
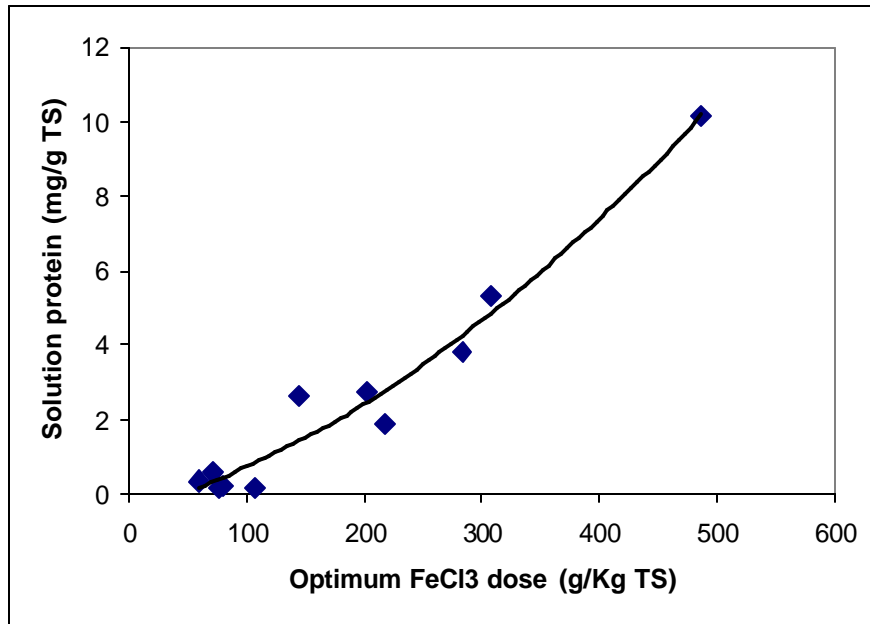


Figure 11. FeCl₃ conditioning for WASs that contained various amounts of (a) solution protein and (b) solution polysaccharide

CHAPTER II
Predicting the Digestibility of Waste Activated Sludges

PREDICTING THE DIGESTIBILITY OF WASTE ACTIVATED SLUDGES

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ABSTRACT

Laboratory digestion studies were carried out in order to characterize the changes that occur when waste activated sludge undergoes anaerobic and aerobic digestion. For this purpose, waste activated sludge collected from various wastewater treatment plants was digested separately under anaerobic and aerobic conditions for 30 days at room temperature (approximately 22°C). Before and after digestion, cations in solution and in floc, soluble anions, and soluble biopolymers (protein and polysaccharide) were measured. Following anaerobic digestion, a significant increase in solution protein took place and correlations between solution protein, ammonium production, percentile volatile solids reduction and iron concentration in activated sludge floc were obtained. These data indicate that iron-linked protein is released to solution when iron is reduced and its degradation is responsible for volatile solids reduction in anaerobic digestion. In aerobic digestion, the polysaccharide concentration in solution increased along with calcium, magnesium and inorganic nitrogen. This implies that divalent cation-bound biopolymer might be the primary organic fraction that is degraded under aerobic digestion. Combined (anaerobic/aerobic) digestion was performed using one waste activated sludge. The sludge that underwent anaerobic digestion for 30 days was then digested aerobically. The sludge that was initially digested under aerobic conditions then further degraded anaerobically. Combined digestion resulted in further volatile solids destruction with discrete cation and biopolymer response during each phase of digestion. These results support the theory that two types of organic matter with different cation bindings are present in the floc structure and each type is degraded under different digestion environments.

KEY WORDS

Anaerobic digestion, aerobic digestion, iron, soluble cations, two types of biopolymer, EPS, protein, polysaccharide, volatile solids reduction, inorganic nitrogen

INTRODUCTION

Both anaerobic and aerobic digestion have been widely used to stabilize the wastewater sludge prior to land application. Reduction of volatile solids and destruction of pathogens are the primary objectives of both processes. However, each digestion process undergoes different microbiological and biochemical reactions and the main difference is whether digestion proceeds in the presence or absence of molecular oxygen. Historically, a great deal of study has been conducted to describe digestion kinetics and to assess factors affecting digestion performance. However, the fundamental question as to whether a specific sludge is better treated by anaerobic or aerobic digestion has not been evaluated. At present, there are no specific criteria for choosing a digestion method. Rather, secondary factors such as the scale or size of wastewater treatment plant, complexity of the process, and economic interests are commonly considered for digestion option (Metcalf and Eddy, 1991; Grady et al., 1998). As a result, biosolids that do not meet treatment goals can be produced and this can compromise ultimate disposal, especially if odors are produced. In the future, regulations and costs pertaining to solids handling could become more stringent, making efficient stabilization of greater importance.

Recently, Novak et al. (in press) proposed that different mechanisms for floc destruction are involved in anaerobic and aerobic digestion and they account for different characteristics of digested sludges with regard to volatile solids reduction, subsequent dewatering properties, and odor potential. As Novak et al. (in press) also suggested, an understanding of floc structure would appear to be useful in predicting the performance of different digestion processes. Although there is much debate as to the interaction between extracellular polymeric substances (EPS) and cations in activated sludge floc, this interaction may also play a pivotal role in the efficiency of digestion. Studies using extraction methods such as cation exchange resin treatment (CER) (Frølund et al., 1996; Nielsen et al., 1996), sonication (Urbain et al., 1993) and a combination of sonication and CER (Dignac et al., 1998) minimize cell lysis and reveal that activated sludge EPS are mainly composed of protein, polysaccharide, humic acid and nucleic acids. These studies also agree that protein is the major constituent of EPS in activated sludge

floc. Exocellular biopolymers can originate from microbial activity such as metabolism and lysis of cells and wastewater influent (Urbain et al., 1993; Dignac et al., 1998). However, it is not clear how these materials are distributed within the floc structure and how this distribution affects floc properties and the extraction of biopolymer.

Lately, some studies have suggested that EPS from different origins will also have different or unique characteristics. Nielsen and Keiding (1998) proposed a floc model in which the outer EPS for wastewater originated biopolymer is bound by weak forces to a rigid backbone of floc matrix and therefore is sensitive to changes in stability of floc. They concluded this from the observations of different degrees of floc disintegration in field activated sludge and lab-grown cultures induced by iron extraction following the addition of sulfide. Novak et al. (in press) reported very different cation and biopolymer responses from anaerobic and aerobic digestion studies. Significant amounts of calcium and magnesium ions were released to solution during aerobic digestion, but this did not occur during anaerobic digestion. Based on these results, they proposed a floc model that contained two types of biopolymer with different cation bindings. These are: (1) lectin-like biopolymer, generated by microorganisms and linked to divalent cations and (2) iron and/or aluminum bound biopolymer, trapped from incoming wastewater and cell lysis product. The former was thought to be degraded primarily under aerobic conditions while the iron bound material is degraded under anaerobic conditions. The aforementioned studies indicated possibly different compositions and characteristics of two types of EPS and importance of cations in the floc structure as well.

Divalent cations stabilize floc by providing bridging to negatively charged biopolymers (Kakii et al., 1985; Bruus et al., 1992; Higgins and Novak, 1997a; Sobeck and Higgins, 2002). The relative significance of calcium and magnesium and affinity of each cation for specific EPS are not fully known. Bruus et al. (1992) suggested that polysaccharides in biopolymers are alginates and these biopolymers bind to calcium ion to form a gel-like floc structure. Urbain et al. (1993) and Dignac et al. (1998) reported that proteins are more strongly associated with calcium and magnesium than polysaccharides. Urbain et al. (1993) also found that protein has higher affinity for calcium while DNA has higher affinity for magnesium. Higgins and Novak (1997a) proposed a floc model that lectin-like proteins are cross-linked with polysaccharides and both magnesium and calcium provide bridging to this biopolymer network. The importance of both calcium and magnesium was again seen in the recent study that showed the sludge added with either cation improved in a similar manner with regard to settling and dewatering properties (Sobeck and

Higgins, 2002). In contrast to the divalents, monovalents, especially sodium, are believed to be problematic in sludge treatment. They displace divalent cations within the cation bridged floc structure by ion exchange and weaken floc strength, causing deteriorated settleability and dewaterability of sludge (Higgins and Novak, 1997b; Novak et al., 1998).

Trivalent cations such as iron and aluminum are often found at high concentrations in activated sludge (Kakii et al., 1985). Although the specific role of iron and aluminum in flocculation is not well understood, their contributions to the floc stability, especially iron, are of interest since these cations have higher charge valence and lower solubility than divalent cations. Iron (III) can be reduced to iron (II) either by iron reducing bacteria (Caccavo et al., 1996) or by addition of sulfide (Nielsen and Keiding, 1998). A significant increase in turbidity during iron reduction was observed by both research teams, indicating that deflocculation occurred when iron was reduced.

The importance of cations was also demonstrated in an aerobic digestion study. Murthy and Novak (1999) observed that an activated sludge with lower concentrations of divalent cations resulted in poorer dewatering and higher conditioning requirement during aerobic digestion. However, the release of calcium and magnesium was not monitored in this study.

RESEARCH OBJECTIVES

The characteristics of feed sludge are often neglected when choosing between anaerobic and aerobic digestion. Selecting a type of digestion without considering sludge characteristics may result in a failure to meet treatment goals. Since the type and concentration of cations strongly affect activated sludge characteristics, they might also impact the performance of digestion processes. Based on this hypothesis, changes in cations and soluble biopolymer were carefully monitored before and after both anaerobic and aerobic digestion. The study was expected to provide a better understanding of interactions between EPS and cations in the floc structure and lead to guidelines for selecting a type of digestion.

METHODS AND MATERIALS

Experimental approach

Samples of waste activated sludge (WAS) and secondary effluent were collected from eight different wastewater treatment plants (WWTPs). The plants are alphabetically numbered from A to H. Several WWTPs were sampled twice and the first and second sampling are designated with 1 and 2. Analyses of cations, anions, and biopolymer and tests of conditioning and dewatering were conducted using WAS and both anaerobically and aerobically digested sludges.

For the digestion studies, three liters of WAS was placed in four-liter batch anaerobic and aerobic digesters. Digestion was performed for 30 days at room temperature and mixing was continuously provided by a stir plate. Eight sets of WAS (A2-F2) were utilized for anaerobic and aerobic digestion. However, no anaerobic digestion data is available for WAS F2 because adhesive spilled into the reactor when replacing the stopper following sampling. The solids concentration of WAS used for the digestion studies varied from 5.22 g/L to 8.87 g/L (within +/- 20 % of mean concentration) except for WAS A2 (18.74g/L). The possible effect of different mass loadings on digestion performance was not studied in this research.

The air stones were placed at the bottom of the aerobic digester and aeration was provided through a humidifier to minimize evaporation. When make-up water was needed, the distilled water, approximately 200mL over 30 days, was added. For the anaerobic digestion systems, a rubber stopper that was pierced with a glass tube to serve as a gas collector was placed on the anaerobic digester and sealed tightly to avoid gas leakage. The gas generated during digestion was transferred to a gas collector system.

The consecutive filtering processes with different sizes of filters was performed using WAS and digested sludge in order to analyze molecular weight distributions and the biopolymer content in each of the molecular weight fractions. For this analysis, all the sludge samples were centrifuged at 9,460g for 15 minutes and the supernatant was continuously filtered through 1.5 μ m, 0.45 μ m filters and through 30,000 Dalton (30K) and 1,000 Dalton (1K) ultrafiltration membranes (Amicon YM30 and YM1). In this study, the term 'soluble' or 'solution' refers to be the material that can be filtered through 0.45 μ m filter.

Analysis

Free solution cations, sodium, potassium, ammonium, magnesium, and calcium were analyzed using a Dionex Ion Chromatograph (IC). Magnesium and calcium in solution were also quantified using an Atomic Absorption Spectrometer (AA). An AA uses a combustion process so the cations in solution associated with dissolved organic matter are measured along with free (unassociated) cations. Therefore, the difference in concentrations between by IC and AA would be the organically associated or bound cations in solution.

Total iron and aluminum in dry sludge were measured using EPA method 3050B (Acid digestion for metals analysis of soils, sediments, and sludges). For this experiment, WAS was dried at 105°C and the known amount of dried sludge was removed and underwent acid digestion. The prepared samples were quantified using an AA. Metal concentrations were expressed as mg-metal/g-ash after normalizing mg-metal/g-TS, a unit generally reported, by g-ash/g-TS (Park et al., 2002). Iron and aluminum in solution were measured using an AA. However, these cations were not detected in most of WAS solution so that iron and aluminum were designated as 'floc cations' in this study.

Soluble anions, nitrite, nitrate, phosphate, and sulfate, were determined using a Dionex Ion Chromatograph. Total solids (TS), total suspended solids (TSS), total volatile solids (VS), and volatile suspended solids (VSS) were measured according to Standard Methods (APHA, 1995). The protein concentration was determined by the Hartree (1972) modification of the Lowry et al. (1951) method using bovine serum albumin as the standard. Polysaccharide was measured by the Dubois et al. (1956) method utilizing glucose as the standard.

Both ferric chloride and alum at concentrations of 100g/L and a high charge cationic polymer, BC650 (Stockhausen), at 0.5% (w/w) were used as sludge conditioners in this study. Optimum doses of each conditioner were determined using the capillary suction time (CST) test by obtaining the lowest CST reading. CST and specific resistance to filtration (SRF) were used as measures of the sludge dewatering rate according to method 2710G of Standard Methods (APHA, 1995) and method described by Christensen and Dick (1985), respectively.

For the statistical analysis, the error bars were used in some figures representing +/- one standard deviation.

RESULTS AND DISCUSSION

Cations and activated sludge

The characteristics of WAS and secondary effluent from each facility were examined before initiating digestion studies (Table 1). Solution biopolymer in the WAS and in the effluent were of specific interest since the sludges that had poor solid/liquid separation and lost substantial amounts of organics to the effluent might digest differently from sludges that retained most of the biopolymer in the floc. The relationship between solution biopolymer in the WAS and in the effluent is shown in Figure 1. It would be expected that the solution biopolymer in the WAS and effluent biopolymer should be nearly the same. This trend can be seen in Figure 1 except for wastewater samples from three facilities. Although the observation is limited, these three WAS samples might have experienced some changes during the shipping and storage that resulted in high WAS biopolymer measurements compared to effluent biopolymer. An increase in WAS solution biopolymer could have occurred if the samples were allowed to become septic. Consequently, effluent biopolymer was considered as a better indicator for the performance of activated sludge process at each WWTP for this study since effluent biopolymer is unlikely to be affected by shipping and handling.

Solids retention time (SRT) or sludge age is thought to be an important operational factor that can affect activated sludge characteristics (Bisogni and Lawrence, 1971; Grady et al., 1998). However, an analysis of the data in Table 1 indicates no relationship between SRT and effluent properties (data not shown). Nonetheless, SRT may have a particular effect on the characteristics of digested sludge or digestion performance. Bruus et al. (1993) showed that activated sludges processed at different SRTs experienced different changes under anaerobic conditions. The authors reported that when activated sludges were anaerobically stored, more dramatic changes in conditioning and dewatering properties took place in activated sludges with short SRT compared to those from higher SRT processes. Reece et al. (1979) found that nonbiodegradable material in activated sludge increases as SRT increases, affecting aerobic digestibility of sludge.

Higgins and Novak (1997c) reported that the ratio of monovalent cations to divalent cations in activated sludge influent can be used to evaluate effluent and sludge properties. According to Higgins and Novak (1997c), sludges with an M/D ratio greater than two will not settle or dewater as well as sludge with an M/D of two or less. Murthy (1998) demonstrated that activated sludge solution biopolymer and effluent COD were also strongly influenced by the M/D ratio.

Recently, the floc cations, iron and aluminum, were also found to have significant effects on the activated sludge effluent quality (Park et al., 2002). With regard to the cation content, plant B would be most likely to have poor activated sludge properties due to its high sodium and low iron and aluminum content (Table 1). Effluent biopolymer versus M/D and the sum of iron and aluminum are shown in Figure 2. As expected, far more biopolymer was found in the secondary effluent from plant B. It can not be determined whether the M/D ratio or floc iron and aluminum had the more significant effect on effluent quality from this data, but it is clear that an improper cation composition resulted in poor binding of biopolymer to the floc structure. The inability to retain a large fraction of biopolymer might be expected to influence the digestibility of WAS B, since much less biopolymer is retained within the floc.

Table 1. Characteristics of WAS and secondary effluent of WWTPs

Plant	SRT (day)	TS (g/L)	VS (g/L)	Na ⁺ (mg/L)	K ⁺ (mg/L)	NH ₄ ⁺ (mg/L)	Mg ²⁺ (mg/L)	Ca ²⁺ (mg/L)	M/D (eq/eq)	Fe (mg/g ash)	Al (mg/g ash)	Solution*		Effluent	
												Soluton* protein (mg/L)	polysa-ccharide (mg/L)	Effluent protein (mg/L)	polysa-ccharide (mg/L)
A1	18	15.0	10.2	296	29.7	2.95	19.2	35.1	4.1	77.4	45.7	3.60	2.16	3.18	2.82
A2	28	18.7	13.9	579	27.2	ND	20.1	39.4	7.2	61.4	35.6	5.88	3.92	3.93	3.62
B1	11	6.57	3.18	1087	92.6	ND	18.4	23.7	18	5.6	1.3	66.8	13.8	57.0	12.4
B2	11	8.47	4.99	977	116	ND	17.9	37.2	14	9.2	1.6	32.2	7.21	29.4	8.00
C	4	8.32	6.69	67.6	71.7	49.3	25.0	24.8	2.3	65.9	65.6	22.1	12.9	7.71	5.19
D	23	6.77	5.49	93.8	11.4	ND	13.5	34.2	1.6	30.7	30.9	1.22	4.30	3.48	3.29
E	7	8.87	4.87	324	32.8	51.6	44.5	339	0.9	88.1	4.3	16.6	13.0	NA	NA
F1	3	5.57	4.57	71.2	10.1	2.4	7.7	26.0	1.8	32.5	75.4	3.10	2.50	1.93	3.06
F2	3	5.22	3.85	89.3	10.4	0.2	6.1	25.9	2.3	36.1	102	0.74	2.39	1.78	4.18
G	5	6.54	5.18	129	48.4	39.5	14.9	29.3	3.4	72.5	45.0	17.8	10.6	7.60	6.88
H	1	3.90	2.94	45.4	21.0	33.3	15.5	36.2	1.4	326	16.9	20.8	14.6	0.50	1.83

ND: Not detected

NA: Samples were not available

*Solution biopolymer in WAS

Iron and anaerobic digestion

Batch anaerobic digestion was conducted for 30 days for each of the WAS samples. The solids concentration, solution cations, and solution biopolymer after anaerobic digestion are listed in Table 2. As Novak et al. (in press) also observed, a large amount of protein was always found in the solution phase of anaerobically digested sludges. An analysis of molecular weight distribution revealed that this protein was composed primarily of protein less than 30K (data not shown). Novak et al. (in press) hypothesized that substantial production of protein in solution is

due to the strong affinity of protein for ferric iron in activated sludge floc so when iron is reduced during anaerobic conditions, protein bound to ferric iron is released. The high affinity of protein for iron (III) can be seen in the conditioning data in Figure 3 Each data point represents the optimum conditioning dose of FeCl_3 for individual activated sludges that contained various concentrations of protein and polysaccharide in solution. This result is consistent with the data reported by Murthy et al. (2000) who also observed that protein was selectively removed by iron (III) salts.

Studies have shown that iron can be reduced under anaerobic conditions either by iron reducing bacteria (Caccavo et al., 1996) or by addition of sulfide (Nielsen and Keiding, 1998). These two studies showed that sludge was deflocculated when iron was reduced, indicating that iron (III) is an important constituent for the stability of activated sludge floc. Muller (2001) compared the efficiencies of iron (III) and iron (II) salts for coagulation of solution protein and found that the former worked more effectively than the latter. Therefore, iron reduction under anaerobic digestion may be detrimental in terms of sludge conditioning and dewatering. However, iron reduction may be necessary for solubilization of floc-associated proteins so that they can be degraded by microorganisms during anaerobic digestion. In addition, Rasmussen and Nielsen (1996) reported that iron reduction was immediately initiated when activated sludge was stored under anaerobic conditions. Therefore, iron reduction may precede the microbial first step of anaerobic digestion, hydrolysis.

Table 2. Chemical content of sludge following anaerobic digestion for 30 days

Sludge	TS (g/L)	VS (g/L)	Na^+ (mg/L)	K^+ (mg/L)	NH_4^+ (mg/L)	Mg^{2+} (mg/L)	Ca^{2+} (mg/L)	Solution protein (mg/L)	Solution poly saccharide (mg/L)
A2	15.3	10.9	601	104	359	4.50	23.9	250	29.3
B1	6.49	3.14	1120	110	22.4	16.8	48.4	85.8	12.4
B2	7.96	4.61	1003	135	52.5	14.9	55.4	91.5	14.2
C	4.96	3.48	69.4	86.2	367	6.90	22.7	181	14.0
D	5.24	4.04	95.8	45.4	189	16.9	45.6	115	14.2
E	6.52	2.65	332	67.5	313	50.1	167	154	8.78
F1	4.17	3.13	72.7	44.8	193	11.4	28.2	109	9.93

Since VS reduction was of interest, it was carefully examined along with various other parameters during the study. As shown in Figure 4, the concentration of solution protein in anaerobically digested sludge and the percent VS reduction were strongly affected by the iron

content of activated sludge floc. In general, as floc iron increased, more protein was released into solution and more VS reduction occurred during anaerobic digestion. This shows that floc iron is an important factor in the anaerobic digestion process. The increase in the ammonium ion (Figure 5) serves as an indicator of the degradation of nitrogen containing organic matter, primarily protein. Ammonia-N in anaerobic digestion has been studied extensively because of its buffering capacity and toxic effect. However, Figure 5 shows that ammonium production is also strongly associated with VS reduction in anaerobic digestion. While some of the released protein remains in solution, influencing dewatering behavior and conditioning requirement for sludge dewatering, most of protein is degraded, accounting for much of the VS destruction in anaerobic digestion. The significance of protein in anaerobic digestion is in accordance with the work of Nielsen et al. (1996) who showed that protein was the major organic compound degraded during anaerobic storage.

There are several possible reasons why Sludge A2 does not fit the pattern of the other sludges in Figure 4. Firstly, as Table 1 shows, the SRT under which WAS A2 was generated was longer than any other SRT for the sludges that underwent anaerobic digestion. With the reasons discussed earlier, therefore, WAS A2 was not likely as susceptible to anaerobic digestion as other sludges processed at shorter SRTs. Secondly, plant A treats a mix of industrial and domestic wastewater and receives high concentration of influent sodium. As can be seen in Table 1 and Figure 2 when sodium or the M/D ratio is high, protein is not retained as effectively and is discharged into the effluent. This protein is, therefore, not available for degradation during anaerobic digestion.

Since both iron and sodium seemed to be important components for anaerobic digestion, the combination of the two cations was compared to the previous VS reduction data to see if it could explain the difference in VS reduction. The relationship between Na/Fe and %VS reduction is shown in Figure 6 and it appears that digestibility under anaerobic conditions is influenced by both iron and sodium.

As expected, characteristics of sludges B1 and B2, which initially contained low floc iron and aluminum and a high M/D, were very different from other sludges following digestion. These sludges were not as odorous as others and the color was between dark brown to gray rather than black. The soluble biopolymer content changed little and the VS destruction was significantly lower than others. No nitrate or sulfate was present in solution, showing that the sludge was

anaerobic. Toxicity was also considered but these two sludges digested well aerobically (Tables 1 and 3). These data suggest that the lack of iron-bound protein due to low floc iron or significant loss of organics into effluent due to the high sodium accounted for the poor anaerobic digestion performance. Novak et al. (in press) hypothesized that biopolymer from wastewater influent and cell lysis material would be linked with iron and/or aluminum in floc while biopolymer produced from microbes' growth for flocculation such as lectin-like biopolymer would be associated with divalent cations, calcium and magnesium. Since these two sludges contained the lowest iron and aluminum of any of the samples, the large amount of biopolymer found in the activated sludge effluent might be the wastewater related and/or cell lysed biopolymer.

Soluble cations were analyzed following anaerobic digestion. There was not any noticeable change in sodium but significantly increased potassium ion was found in all digested sludges. This implied that cells were lysed during digestion, since K^+ is present at high concentrations in cells (Bakker, 1993). However, no observable relationship between the release of potassium and any other parameters of digestibility described above was established, which implies that the effect of cell lysis on digestion is of less importance for VS destruction than is the degradation of biopolymer. Unlike large increases in divalent cations during aerobic digestion as reported by Novak et al. (in press), small decreases in magnesium and calcium occurred in some of the sludges following anaerobic digestion. The use of calcium and magnesium by microbes degrading some of the released biopolymer for growth might be a possible explanation for this uptake, which also suggests that anaerobic flocculation might occur in a similar manner to activated sludge flocculation.

Cations and aerobic digestion

The changes in solution biopolymer and cations in aerobic digestion were very different from those in anaerobic digestion. Differences in the chemical content between anaerobically and aerobically digested sludges can be contrasted by comparing the data in Tables 2 and 3. Under aerobic conditions, more polysaccharide was found in solution and a substantial increase in calcium and magnesium (Figure 7) was detected in all aerobically digested sludges. The accumulation of polysaccharide in aerobically digested sludges was shown to be due to the loss of enzyme activity during aerobic digestion (Novak et al., in press). According to Novak et al. (in press), glucosidase, a polysaccharide degrading enzyme, dramatically decreased during aerobic

digestion while peptidase, a protein degrading enzyme, remained active throughout aerobic digestion. Similar data were also obtained by Murthy and Novak (1999).

Table 3. Chemical content of sludge following aerobic digestion for 30 days

Sludge	TS (g/L)	VS (g/L)	Na ⁺ (mg/L)	K ⁺ (mg/L)	NH ₄ ⁺ (mg/L)	NO ₃ ⁻ (mg/L)	Mg ²⁺ (mg/L)	Ca ²⁺ (mg/L)	Soluiton protein (mg/L)	Solution poly saccharide (mg/L)
A2	14.0	9.24	549	76.5	194	320	61.0	184	42.8	62.1
B1	5.14	1.97	1191	123	ND	88.9	20.3	72.6	34.5	15.4
B2	7.14	3.77	979	134	ND	154	28.4	113	30.3	31.2
C	5.06	3.27	68.0	72.8	116	181	44.7	112	30.1	37.3
D	4.59	3.27	95.1	41.0	128	226	34.7	155	24.7	58.7
E	6.30	2.46	329	52.0	44.2	191	53.6	455	35.5	57.6
F1	3.67	2.66	75.1	52.3	134	205	36.3	116	19.6	33.5
F2	4.75	3.22	95.1	22.7	22.8	157	16.5	117	6.74	7.25

ND: Not detected

The increase in free calcium and magnesium ions in solution, measured by IC, indicated that organic matter associated with these cations in floc was degraded during aerobic digestion. The greatest release occurred for Sludge F2 (3.11meq Ca+Mg/g VSS reduced) while the least was for Sludge C (1.41meq/g VSS reduced). Moreover, total soluble cation measurement by AA for Sludge A2 showed that some of the calcium and magnesium (0.97meq Ca+Mg/L) was associated with organics in solution. Although the concentration difference between by IC and AA is smaller in this study, this result is consistent with the previous observation made by Novak et al. (in press) who found that significant amount of calcium was bound to dissolved organics during aerobic digestion.

If the release of calcium and magnesium is caused by the floc degradation, it should be related to parameters of sludge digestibility such as % VS or VSS reduction and inorganic nitrogen production. Previous studies reported that the percentage of organic nitrogen in sludge solids for both the feed and aerobically digested sludge was constant during aerobic digestion, indicating that % organic nitrogen mineralized is equivalent to % VSS destroyed (Bishop and Farmer, 1978; Mavinic and Koers, 1982). Figure 8 shows that sum of two divalent cations released was well correlated with inorganic nitrogen (sum of ammonium and nitrate) production, meaning that the release of free calcium and magnesium was accompanied by the degradation of nitrogenous organic matter. Figure 8 also implies that the alginate model is not sufficient to explain the release of magnesium with the production of inorganic nitrogen in aerobic digestion. Both

calcium and magnesium appear to play a role in binding negatively charged biopolymer and contribute the stability of floc. Two important observations can be made from these results. First, the portion of floc degraded during aerobic digestion is different from that under anaerobic digestion. That is, different processes or mechanisms of digestion are involved in anaerobic and aerobic digestion. Second, the accumulation of polysaccharide, the release of calcium and magnesium and degradation of nitrogen containing organic matter indicate that the major fraction of floc degraded during aerobic digestion is the lectin-like protein. Higgins and Novak (1997a) proposed that proteins with lectin activity are cross-linked with polysaccharides and both magnesium and calcium provide bridging to this biopolymer network.

Interestingly, no relationship between % VSS reduction and either of inorganic nitrogen production or cation release could be obtained in this study. Murthy and Novak (1999) also noted similar results and they described this due to the differential degradation between protein and polysaccharide associated with divalent cations during aerobic digestion. That is, while both the protein and polysaccharide are released into solution during aerobic digestion, only protein appears to undergo nearly complete degradation, while polysaccharide degradation is highly variable. The reason for the variability in polysaccharide degradation appears to be related to a decrease in specific enzyme activity (Novak et al., in press).

Inorganic nitrogen data in Table 3 illustrate that considerable nitrification occurred during aerobic digestion. Mavinic and Koers (1982) also observed nitrification and found that pH continuously dropped to below 4 due to the alkalinity consumption during nitrification. In one study, calcium and magnesium were quite readily extracted from floc at this low pH while iron and aluminum were not affected (Kakii et al., 1985). Therefore, some of the total divalent cation release into solution could also be the result of weakly acidic conditions that result from nitrification and this might also affect polysaccharide degradation.

While anaerobic digestion data indicate that divalent cation-associated biopolymer was not degraded under anaerobic conditions since no calcium and magnesium release was detected, it could not be determined whether iron or aluminum bound biopolymer was degraded during aerobic digestion.

Combined digestion

To further investigate the specific components degraded under anaerobic and aerobic digestion, a combined sequential digestion was performed using WAS E. When each of the anaerobic and aerobic digesters had been operated for 30 days, digested sludges were switched, anaerobic to aerobic and aerobic to anaerobic. If a specific type of biopolymer is degraded only under one digestion condition, further VS destruction would occur during the second phase of digestion. Simultaneously, the release of specific biopolymers and cations would also be expected to take place in a manner similar to the single digestion conditions. Tables 4 and 5 illustrate how chemical contents of interest changed after the initial and during the second stage of digestion.

(1) Anaerobic to aerobic digestion

As expected, large increases in soluble protein and NH_4^+ were detected after 30 days of anaerobic digestion. Increased K^+ in solution was also found. A small increase in free Mg^{2+} (4.9mg/L) but large decrease in free Ca^{2+} (172mg/L) was found from the sludge solution following the initial digestion. Compared to other WASs, WAS E contained about 10 times greater Ca^{2+} in solution and that might be a reason why unusually high amount of Ca^{2+} was taken up during anaerobic digestion.

After changing the process to aerobic digestion, the black color gradually disappeared, indicating that $\text{FeS}_{(s)}$ was reoxidized to Fe^{3+} and SO_4^{2-} . Free divalent cations in solution increased and calcium in excess of the initial solution concentration was measured at day 40. (There was no significant change in magnesium through the combined digestion process and this might be because this wastewater was dominated by calcium.) As shown in Figure 9, divalent cations were released and reached steady state at day 50. This divalent cation release is consistent with the previous observations that divalent cation-bound biopolymer is degraded during aerobic digestion but not during anaerobic digestion. Moreover, the sudden increase of polysaccharide in solution simultaneously with divalent cation release and the similarity between changes in the two materials from day 0 to day 60 strongly suggest that some portions digested during aerobic digestion are lectin-like biopolymers. The solution polysaccharide decreased after day 60 to a concentration less than 20mg/L. This reduction accounted for significant improvement in the dewatering rate as CST readings indicate in Table 4.

The solution protein concentration dropped as soon as aerobic digestion started. Ammonium also decreased but nitrate and nitrite increased (Figure 10), indicating that nitrification occurred

during the second phase of aerobic digestion. However, approximately 200mg/L-N of inorganic nitrogen could not be accounted for following aerobic digestion. The consumption of inorganic nitrogen could be caused by two possible reactions. Bishop and Farmer (1978) demonstrated that significant denitrification occurred in a batch aerobic digester when dissolved oxygen (DO) was below 1mg/L. Although DO data are not available, there was strong likelihood that DO was low in the reactor when the process changed from anaerobic to aerobic digestion. Also, some NH_4^+ might have been used for growth of aerobic heterotrophs. Since substrate (released biopolymer) was available, there might have been substantial growth of microbes under initial aerobic conditions. Decreased K^+ data tends to support this explanation since K^+ is required by all organisms including some enzymes involved in protein synthesis (Madigan et al., 1997). Due to the consumption of inorganic nitrogen, it could not be verified whether the reduction of solution protein was from the degradation or from the reflocculation as a result of iron reoxidation. However, the large decrease in inorganic nitrogen, coupled with a quick decrease in solution protein indicates that protein was more likely reflocculated than degraded during initial aerobic conditions.

Table 4. Sludge characteristics during combined digestion I (anaerobic to aerobic digestion)

		WAS	Anaerobic	Aerobic					
		Day 0	Day 30	Day 40	Day 50	Day 60	Day 70	Day 80	Day 87
Free Ca^{2+}	(meq/L)	16.9	8.34	19.1	22.6	22.1	22.6	22.5	22.3
Free Mg^{2+}	(meq/L)	3.66	4.12	4.02	4.21	4.13	4.30	4.15	4.13
Free $\text{Ca}^{2+}+\text{Mg}^{2+}$	(meq/L)	20.6	12.5	23.2	26.8	26.3	26.9	26.6	26.4
K^+	(meq/L)	0.84	1.73	1.36	1.27	1.23	1.22	1.17	1.17
Na^+	(meq/L)	13.0	13.6	13.4	13.4	13.1	12.8	12.7	12.6
NH_4^+	(mg/L-N)	51.6	313	40.9	4.54	ND	2.05	ND	ND
NO_3^-	(mg/L-N)	2.30	ND	49.5	44.1	43.7	63.6	93.0	97.8
NO_2^-	(mg/L-N)	ND	ND	37.4	54.0	54.3	32.3	ND	ND
S. protein	(mg/L)	16.6	154	29.7	20.1	23.1	20.5	18.4	15.6
S. polysaccharide	(mg/L)	13.0	8.78	99.5	132	124	54.2	19.1	16.5
% VS reduction	(%)	0	45.6	51.1	54.0	56.5	62.2	56.9	63.1
CST	(sec)	49.3	155	224	182	164	31.1	37.8	43.7

ND: Not detected

Table 5. Sludge characteristics during combined digestion II (aerobic to anaerobic digestion)

		WAS	Aerobic	Anaerobic	
		Day 0	Day 30	Day 70	Day 87
Free Ca ²⁺	(meq/L)	16.9	22.7	17.8	18.2
Free Mg ²⁺	(meq/L)	3.66	4.41	4.04	4.17
Free Ca ²⁺ +Mg ²⁺	(meq/L)	20.6	27.1	21.8	22.4
K ⁺	(meq/L)	0.84	1.33	1.39	1.50
Na ⁺	(meq/L)	13.0	13.8	13.6	13.7
NH ₄ ⁺	(mg/L-N)	51.6	44.2	90.9	99.7
NO ₃ ⁻	(mg/L-N)	2.30	191	ND	ND
NO ₂ ⁻	(mg/L-N)	ND	17.4	ND	ND
S. protein	(mg/L)	16.6	35.5	35.1	29.2
S. polysaccharide	(mg/L)	13.0	57.6	24.5	19.2
%VS reduction	(%)	0	49.5	62.2	62.6
CST	(sec)	49.3	517	423	167

ND: Not detected

(2) Aerobic to anaerobic digestion

Little gas production and color change was observed for the first 32 days after changing the system from aerobic to anaerobic process. Nitrate (191mg/L-N) produced during the prior aerobic digestion likely impeded the development of anaerobic conditions. Denitrification was needed and glucose was added on a stoichiometric basis to consume the nitrate. The sludge turned black 6 days after adding the external organic source. This observation suggests that the performance of anaerobic digestion using aerobically digested sludge is not very practical. However, the data can still be used to analyze how the different cations are associated with different biopolymer in the floc structure.

Figure 11 shows changes in the sum of Ca²⁺ and Mg²⁺, solution biopolymer and ammonium during the aerobic to anaerobic combined digestion. Both free divalent cations and solution polysaccharide increased during aerobic digestion but decreased during anaerobic digestion. Again, these results are the same as the previously observed in separate digestion phases. The concentration of protein remained relatively constant throughout digestion, but the increase in NH₄⁺ indicates that protein was released and degraded during anaerobic conditions. Degradation of protein during the second phase anaerobic digestion is not from the lectin-like biopolymer since divalent cations were not released. Rather, these cations were taken up. Calcium decreased from 22.7meq/L to 18.2meq/L and magnesium from 4.41meq/L to 4.17meq/L. It was likely from the degradation of protein bound to iron in floc.

(3) VS destruction in combined digestion

Distinct responses of solution biopolymer and divalent cations, similar to those in individual digestion process, were seen in the combined digestion, indicating that some portion of flocs can only be digested under one digestion condition and not the other. If each digestion process only consumes one type of organic material, total VS destruction in two series of combined digestion might provide information about how much of each portion occupies flocs. However, it should be noted that these two types of biopolymer do not take into account cell biomass and other types of EPS such as humic substances and nucleic acids. Therefore, each % VS reduction does not necessarily indicate the degradation of distinct biopolymer. VS reduction in the initial anaerobic digestion was 45.6% and 49.5% for aerobic digestion. Additionally, 17.5% and 13.1% of VS was consumed in the second aerobic and anaerobic digestion phases, respectively. These numbers tend to reflect that the degradable divalent cation-bound biopolymer in WAS E might have been smaller than iron-linked biopolymer based on the assumption that the former was not degraded under the anaerobic digestion.

Figure 12 shows that both series of combined digestion resulted in the same percentile volatile solids reduction of WAS E, 67%. This high destruction of volatile solids is most likely to meet the requirement for production of class A biosolids defined by US EPA 40CFR Part 503 (1993). As predicted by Novak et al. (in press), stabilization of activated sludge will be best accomplished by combined digestion. Anaerobic digestion followed by aerobic digestion would be a good option. Improvement in dewatering properties might be another benefit of this type of combined digestion, but only if the second stage was long enough to consume the polysaccharides released early in the second stage.

Conditioning characteristics of digested sludges

The importance of colloidal material in dewatering properties was also seen in this study. As shown in Figure 13, biopolymer between 1.5 μ m and 30K, which are defined in this study as colloidal biopolymer, were responsible for the specific resistance to filtration. Considering very different characteristics of anaerobic and aerobic sludge, this size fraction seems to be universal in determining dewaterability of sludge. Bivins and Novak (2001) reported that biopolymer in this size was also very resistant to degradation in temperature phased anaerobic digestion. They showed that the colloidal fraction produced in thermophilic anaerobic digestion did not change during mesophilic digestion and as a result, the dewatering rate and polymer conditioning dose

did not change, even though most biopolymer less than 30K was degraded during subsequent mesophilic digestion.

However, particle size alone does not explain all the conditioning and dewatering properties of digested sludges. It was observed that inorganic conditioners worked quite differently on anaerobically and aerobically digested sludges. In order to investigate which type and size of biopolymer is coagulated by chemical conditioners (cationic polymer, FeCl_3 , and alum), conditioning test followed by molecular weight separation utilizing anaerobic and aerobic sludge (Sludge D) was performed. Cationic polymer worked in a similar fashion for both anaerobic and aerobic sludges. In contrast, alum was not able to effectively condition most aerobically digested sludges, but it was effective for anaerobically digested sludges and was comparable to iron conditioner in its effectiveness (data not shown).

During the conditioning tests with iron or aluminum salts, the pH was not controlled but a decreased pH might have affected the efficiency of alum for aerobically digested sludge. As a consequence, the results from iron conditioning might be more useful for analyzing the difference between anaerobic and aerobic sludges, since iron is more insoluble than aluminum and iron conditioner is efficient over a wider pH range. Figure 14 shows how the colloidal fraction in digested sludge was conditioned by two different (optimum and about half of optimum) doses of FeCl_3 . For anaerobically digested sludge, over 50% of colloidal protein and polysaccharide was coagulated by underdosed iron salts and about 99% of both colloidal biopolymer was removed by optimum conditioning dose. In contrast, different doses of iron salts brought little difference in coagulation of colloidal biopolymer produced from aerobic digestion. Besides, only about 80% of colloidal protein and 50% of colloidal polysaccharide was removed by either optimum or underdosed FeCl_3 . These results indicate that aerobically digested sludges are relatively hard to condition with inorganic chemicals. The data also imply that biopolymers that were originally bound with different cations might also have different physiological characteristics.

CONCLUSIONS

The digestion performance for waste activated sludge was strongly influenced by the cation content in the sludge. Specifically, iron in floc turned out to be crucial in determining digestibility of sludge in anaerobic digestion. The data associated with cation and biopolymer from single and combined digestion processes suggest that two different biopolymer networks are present in activated sludge floc and each type is mainly degraded under different conditions of digestion. Therefore, the evaluation of feed sludge characteristics, especially cation content, should be beneficial for predicting digestibility of sludge. Specific conclusions of this study can be summarized as follows:

- Both the M/D ratio and floc cations (iron and aluminum) were important for determining sludge characteristics.
- Protein release induced by iron reduction and its degradation were important mechanisms accounting for volatiles solids reduction in anaerobic digestion. Consequently, sludge deficient of iron did not degrade effectively under anaerobic digestion due to the lack of iron bound protein in activated sludge.
- Sodium also appeared to affect sludge digestibility.
- The accumulation of solution polysaccharide with the release of divalent cations and production of inorganic nitrogen in aerobic digestion indicated that lectin-like biopolymer was the primary organic matter that was degraded during aerobic digestion.
- Combined digestion produced further VS destruction with discrete cation and biopolymer response during each phase of digestion, suggesting that the best sludge destruction would be achieved by combination of anaerobic and aerobic digestion.
- The performances of inorganic conditioners for anaerobically and aerobically sludges were different, implying that biopolymers released during each type of digestion might also have different physiological characteristics.

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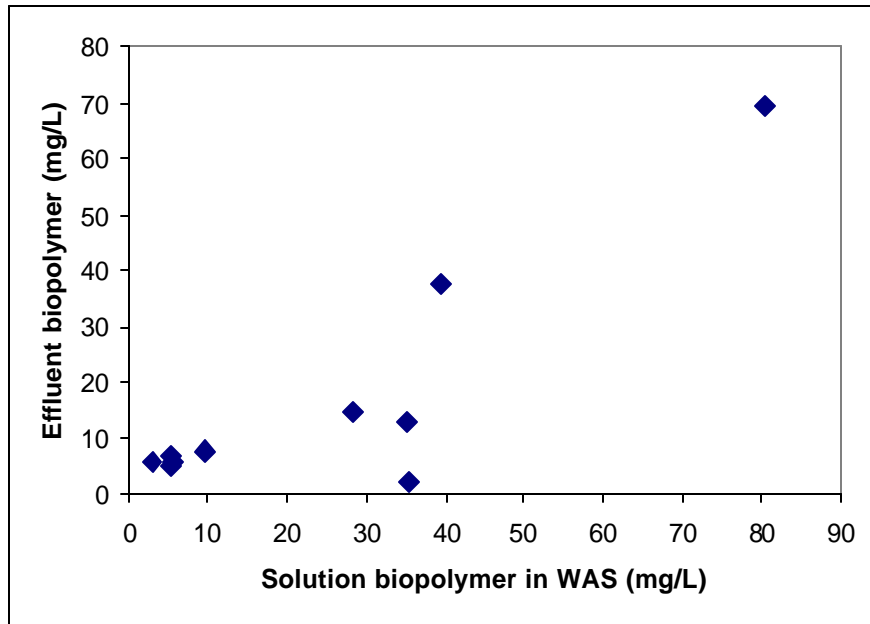


Figure 1. Relationship between solution biopolymer in the WAS and in the effluent

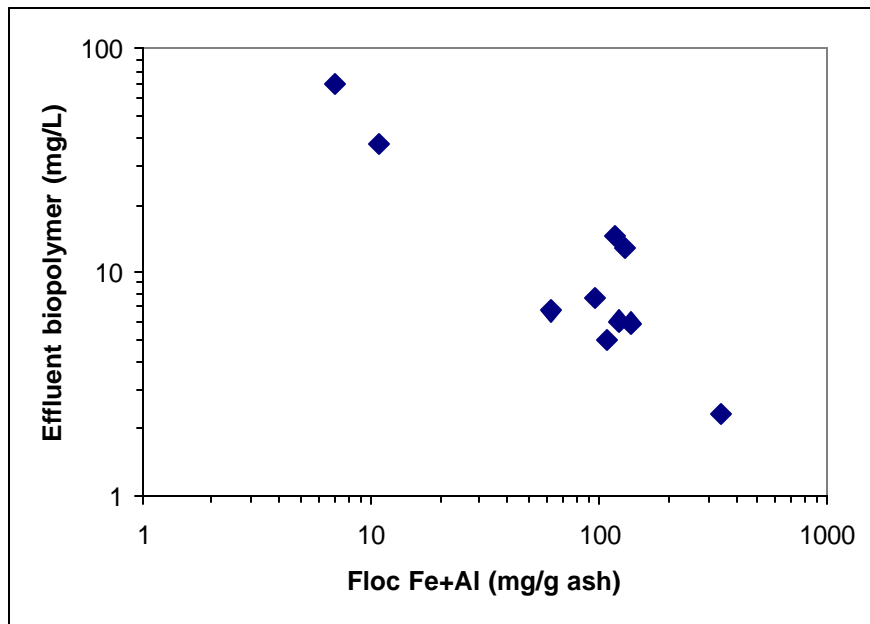
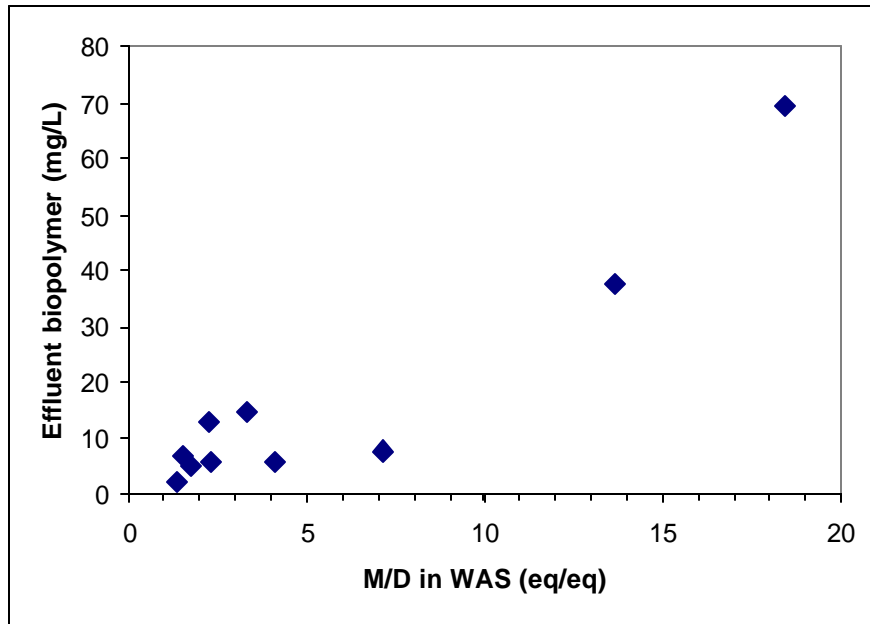


Figure 2. Effects of (a) M/D and (b) the sum of iron and aluminum in floc on soluble biopolymer in the effluent

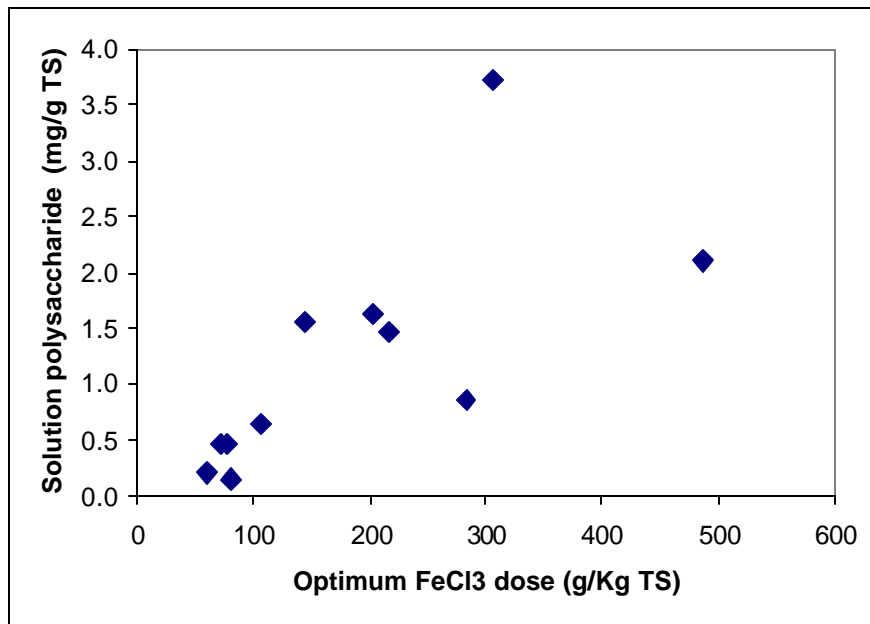
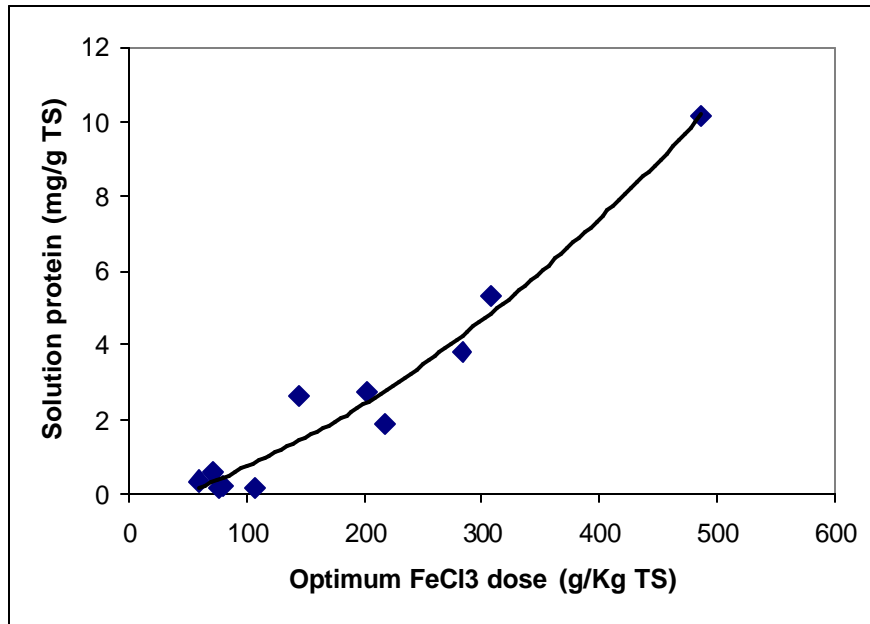


Figure 3. Optimum conditioning doses of FeCl₃ for WASs that contained various amounts of (a) solution protein and (b) solution polysaccharide

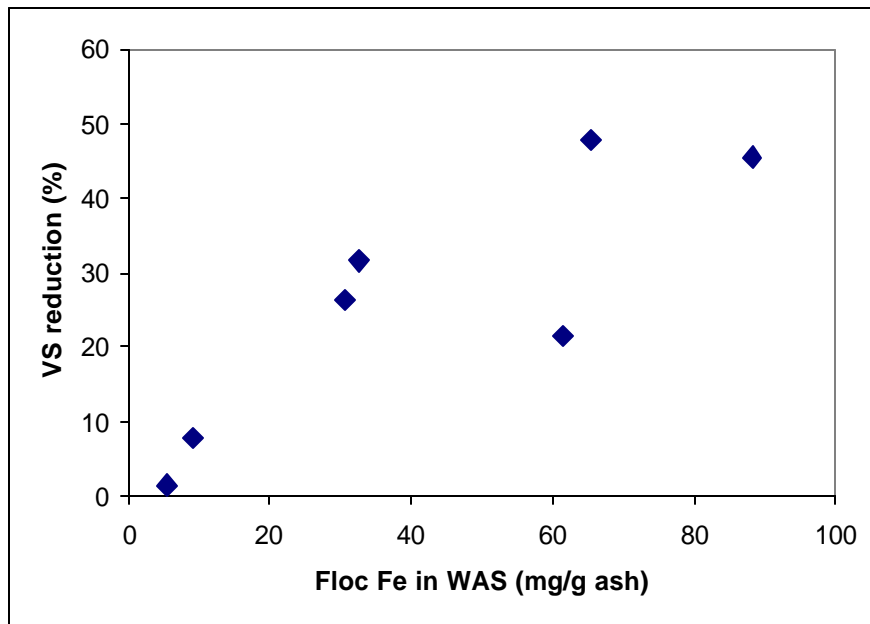
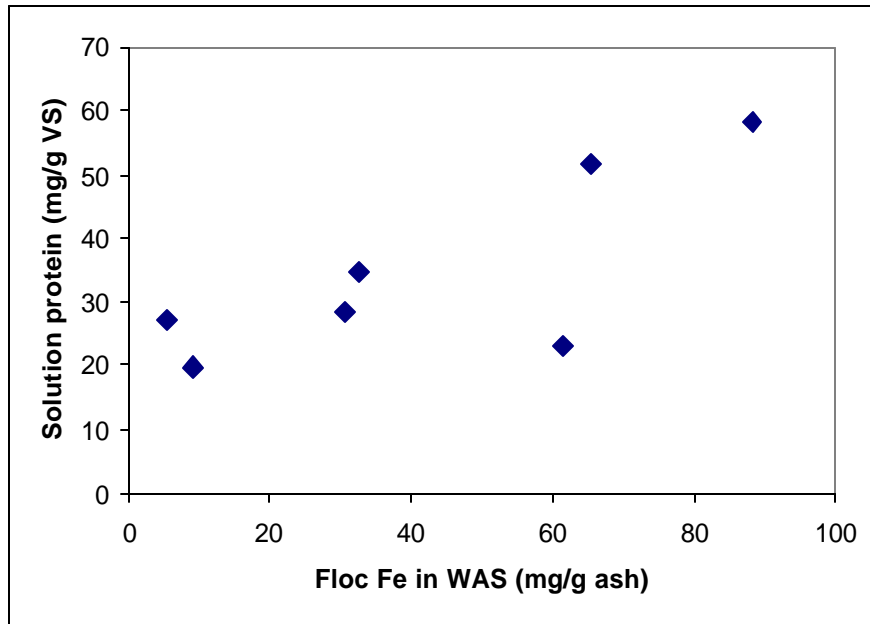


Figure 4. Effect of iron on (a) protein release into solution and (b) percent volatile solids reduction under anaerobic digestion

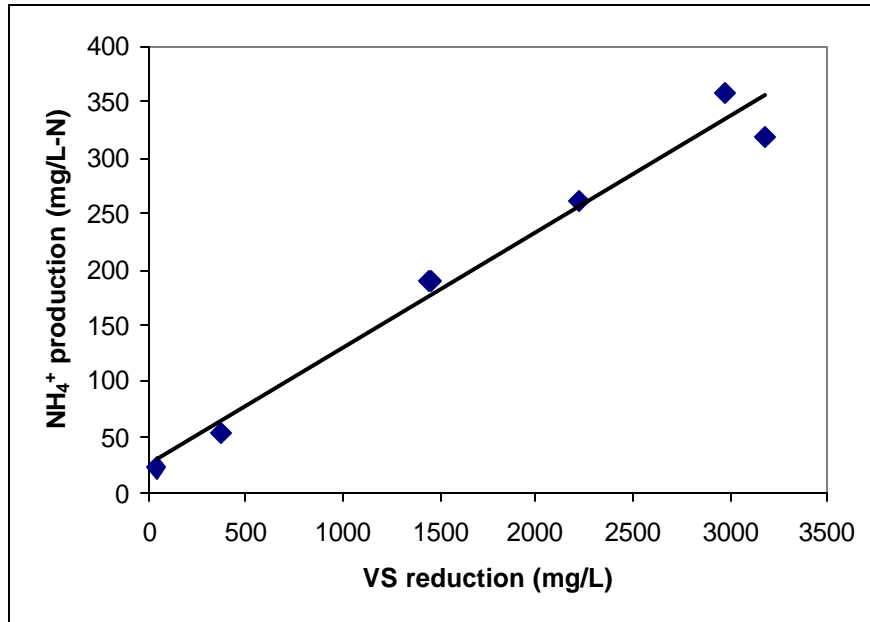


Figure 5. Relationship between ammonium production and volatile solids reduction in anaerobic digestion ($R^2 = 0.97$)

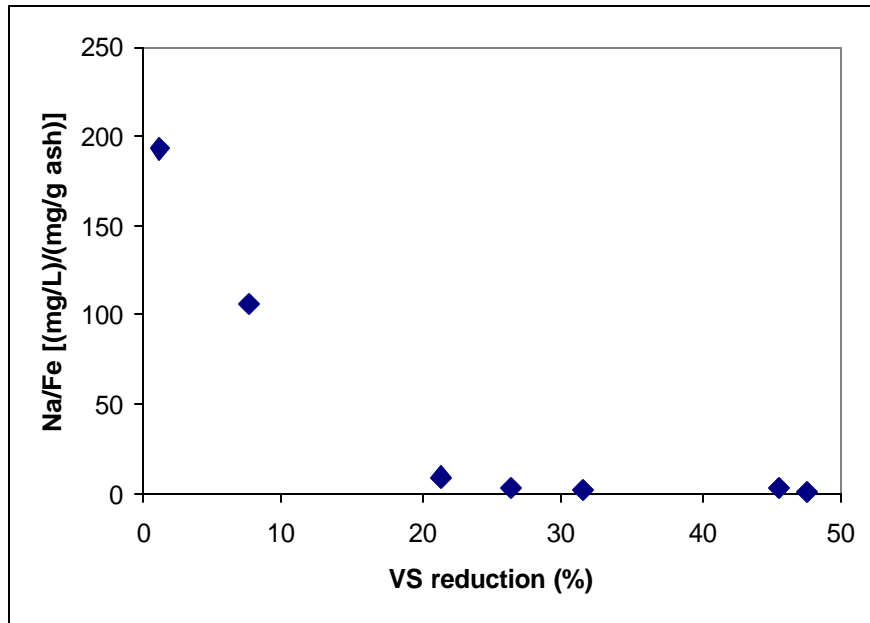


Figure 6. Effect of Na/Fe on percent volatile solids reduction in anaerobic digestion

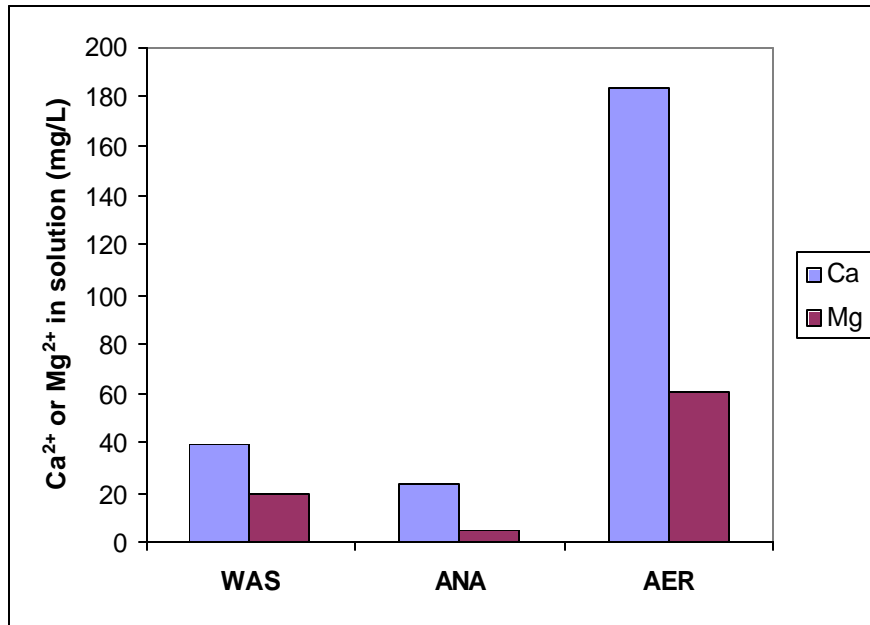


Figure 7. Comparison of calcium and magnesium in solution before and after digestion, Sludge A2 (ANA: anaerobically digested sludge; AER: aerobically digested sludge)

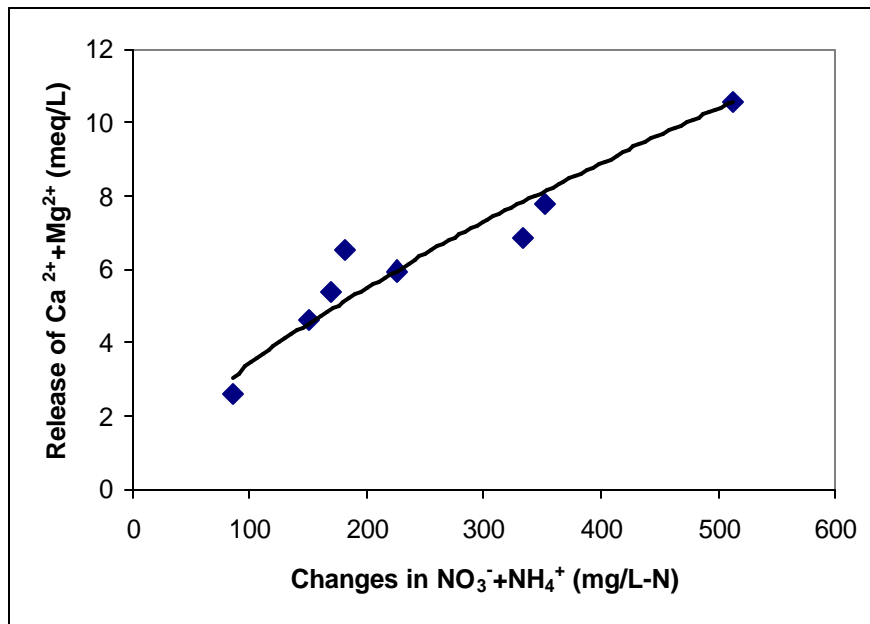
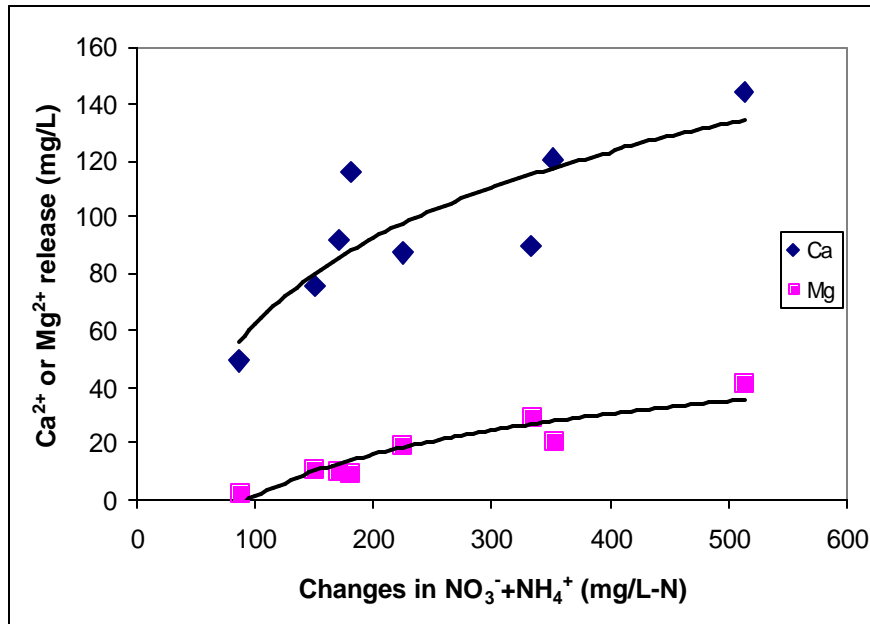


Figure 8. Relationships between (a) calcium and magnesium release and inorganic nitrogen production and (b) the sum of divalent cation release and inorganic nitrogen production in aerobic digestion

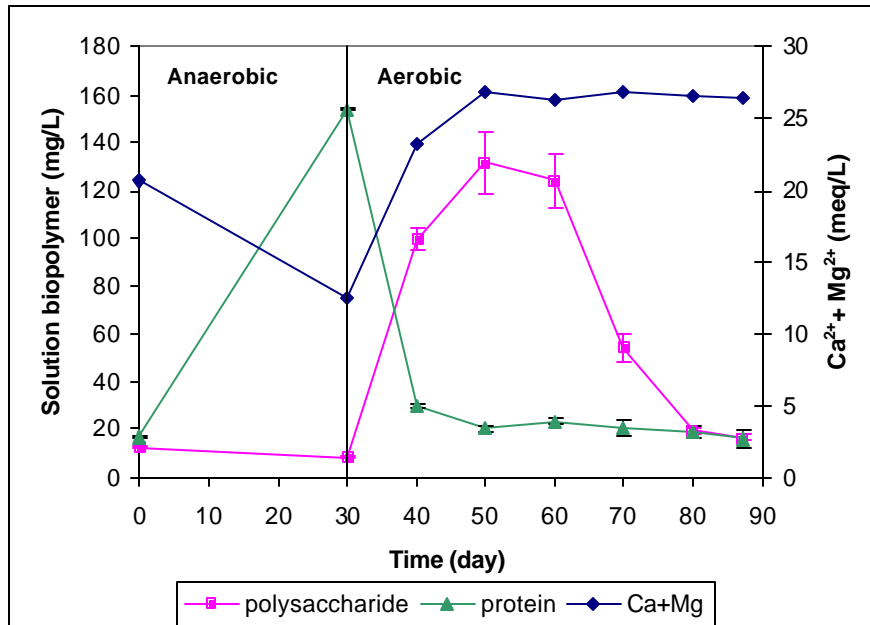


Figure 9. Changes in solution protein and polysaccharide and the sum of calcium and magnesium during anaerobic to aerobic combined digestion (protein and polysaccharide; n = 3)

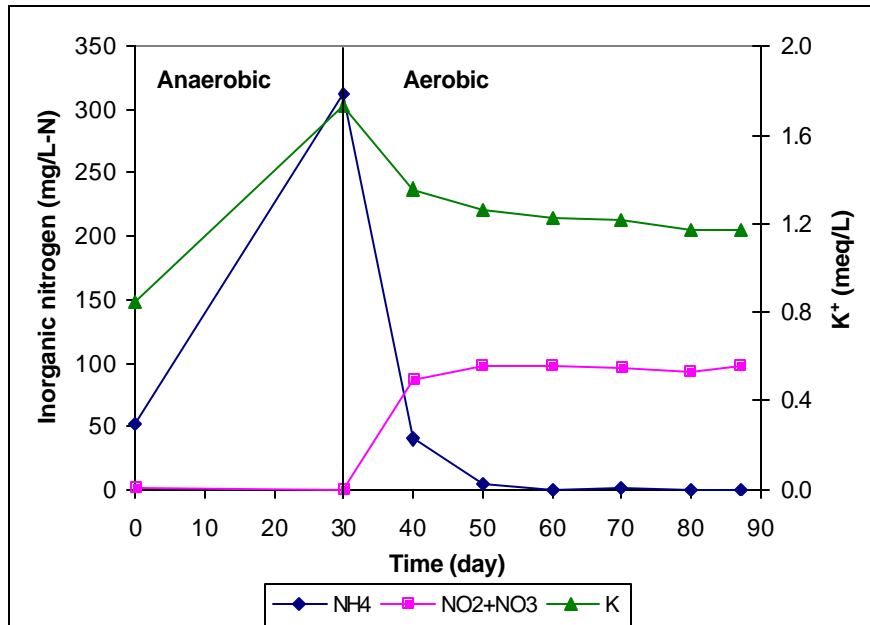


Figure 10. Changes in inorganic nitrogen and potassium during anaerobic to aerobic combined digestion

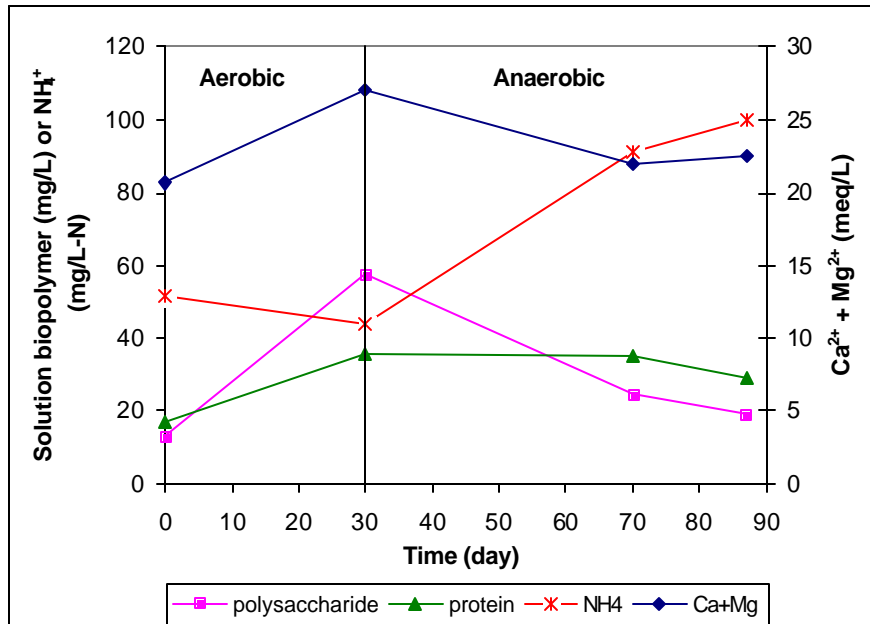


Figure 11. Changes in solution protein and polysaccharide, ammonium, and the sum of calcium and magnesium during aerobic to anaerobic combined digestion (protein and polysaccharide; n = 3)

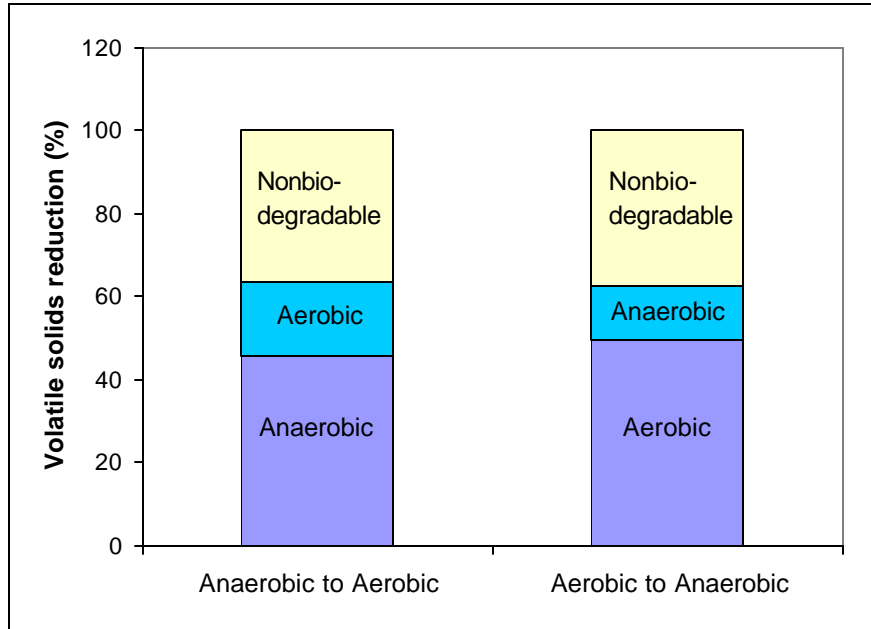


Figure 12. Percent volatile solids reduction in two series of combined digestion, Sludge E

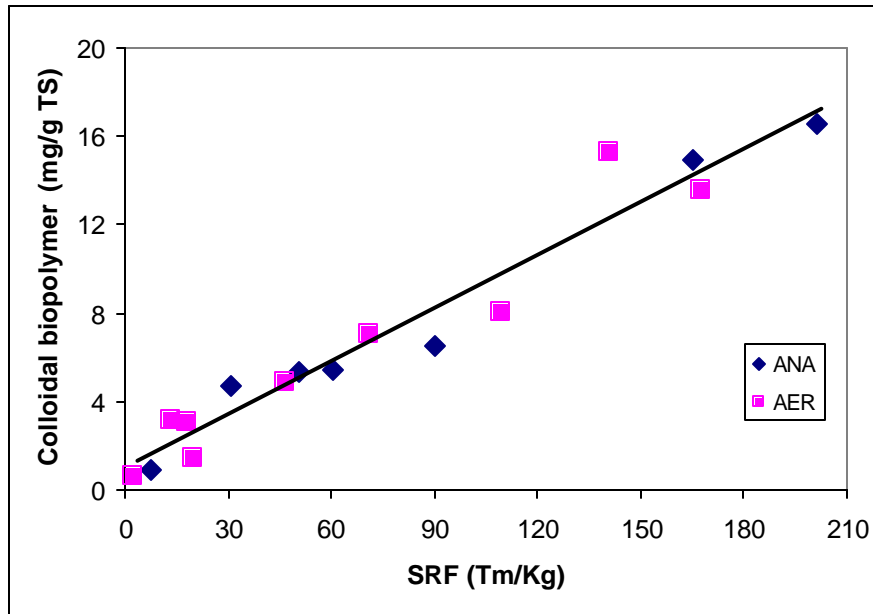


Figure 13. Relationship between SRF and colloidal (1.5 μ m - 30K) biopolymer in digested sludges (ANA: anaerobically digested sludge; AER: aerobically digested sludge)

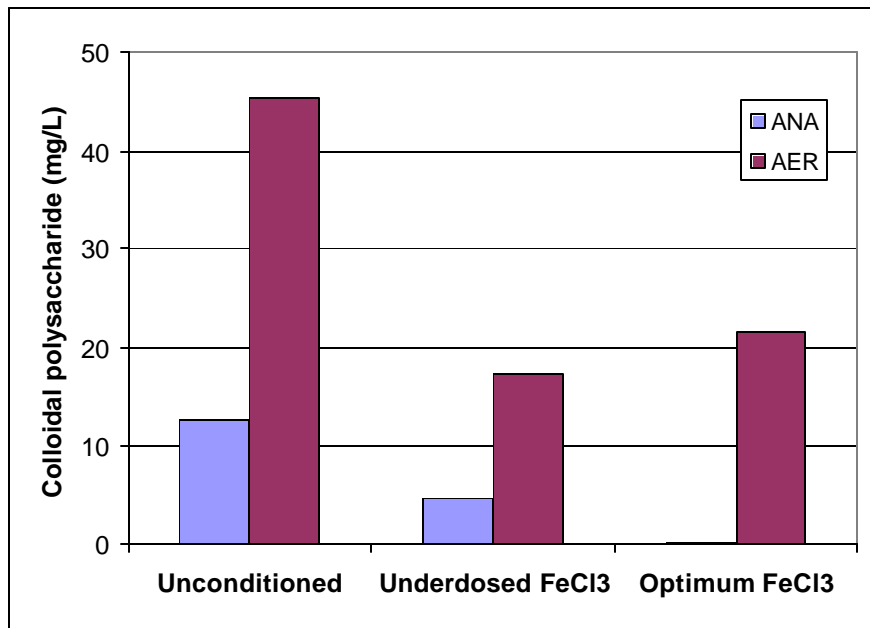
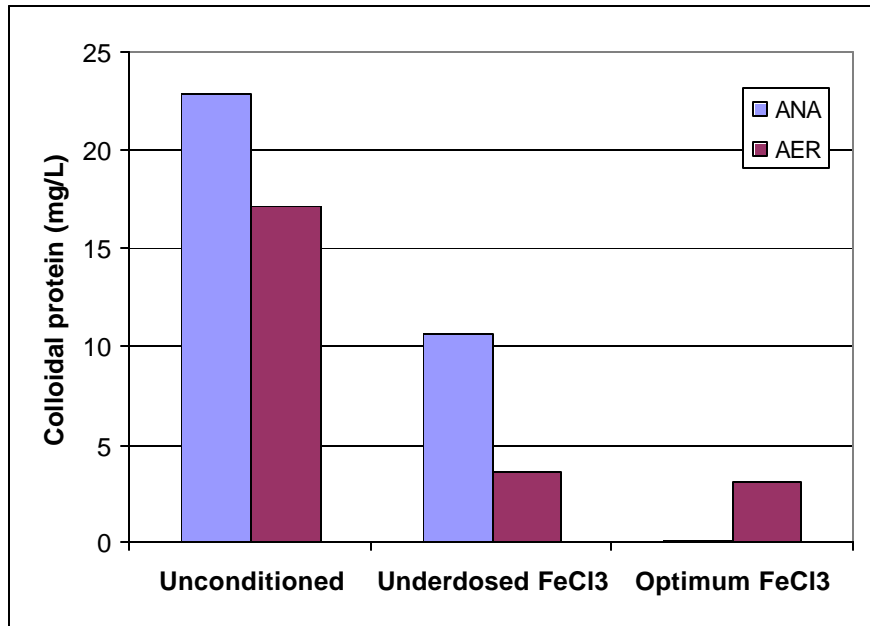


Figure 14. Coagulation of colloidal (1.5 μ m – 30K) (a) protein and (b) polysaccharide in anaerobically and aerobically digested sludges by FeCl₃, Sludge D (ANA: anaerobically digested sludge; AER: aerobically digested sludge)

APPENDIX

Table 1. Characteristics of mixed liquor

Plant	MLSS (mg/L)	MLVSS (mg/L)	SVI (mL/g)	ZSV (cm/hr)	M/D (eq/eq)
A1	5,525	3,913	85	67.5	4.96
A2	5,843	4,640	110	24.1	6.76
B1	2,630	2,290	373	1.57	20.2
B2	3,420	2,913	149	66.3	13.9
C	2,187	1,933	251	45.8	1.38
D	2,080	1,764	55	426	1.33
E	2,057	N	117	N	0.68
F1	5,250	4,478	249	24.2	1.53
F2	N	N	199	12.6	1.74
G	1,717	1,510	268	81.9	1.92
H	961	702	484	75.9	0.79

N: not measured

Table 2. Characteristics of secondary effluent

Plant	Effluent TSS	Effluent* COD	COD Std. dev	Effluent* Protein	Protein Std. dev	Effluent* Poly- saccharide	Poly- saccharide Std. dev	Effluent* Biopolymer
A1	10.0	N	N	3.18	0.13	2.82	0.47	6.00
A2	31.0	32.0	1.9	3.93	0.20	3.62	0.48	7.55
B1	37.0	305	49.4	57.0	6.30	12.4	0.16	69.4
B2	14.7	296	24.4	29.4	0.08	8.00	0.32	37.4
C	1.8	62.4	7.7	7.71	0.12	5.19	0.79	12.9
D	7.8	29.6	2.8	3.48	0.55	3.29	0.00	6.77
E	NA	NA	NA	NA	NA	NA	NA	NA
F1	39.1	3.8	3.7	1.93	0.11	3.06	0.32	4.99
F2	27.8	23.8	N	1.78	N	4.18	0.63	2.41
G	6.7	96.0	11.3	7.60	0.06	6.88	0.63	14.5
H	1.0	26.4	5.8	0.50	0.19	1.83	0.48	2.33

* Soluble effluent data

NA: samples were not available

Table 3. Characteristics of WAS and anaerobically (ANA) and aerobically digested sludge (AER)

	Plant	SRT (day)	TS (mg/L)	VS (mg/L)	M/D (eq/eq)	CST (sec)	SRF (Tm/Kg)	Cake solids (%)	Polymer (g/Kg TS)	FeCl3 (g/Kg TS)	Alum (g/Kg TS)	Soluble protein (mg/L)	Soluble polysa- ccharide (mg/L)	Soluble bio- polymer (mg/L)	1.5µm- 30K protein (mg/L)	1.5µm- 30K polysa- ccharide (mg/L)	1.5µm- 30K bio- polymer (mg/L)
WAS	A1	18	14,980	10,230	4.14	60.0	1.97	13.2	2.64	80	93	3.60	2.16	5.76	0.76	0.66	1.42
	A2	28	18,740	13,910	7.16	71.9	1.70	11.5	2.45	60	97	5.88	3.92	9.80	3.09	3.18	6.27
	B1	11	6,570	3,180	18.4	61.6	4.68	13.2	3.57	487	164	66.8	13.8	80.5	5.28	1.67	6.95
	B2	11	8,470	4,990	13.7	27.4	0.61	12.2	1.41	283	118	32.2	7.21	39.4	2.16	0.90	3.06
	C	4	8,320	6,680	2.27	394	250	NQ	4.78	144	240	22.1	12.9	35.0	15.4	8.09	23.5
	D	23	6,770	5,490	1.55	18.5	0.46	11.0	0.59	106	148	1.22	4.30	5.52	1.79	1.67	3.46
	E	7	8,870	4,870	0.81	57.8	13.3	6.46	3.14	216	248	16.6	13.0	29.6	N	N	N
	F1	3	5,570	4,570	1.80	44.9	5.70	6.56	1.96	72	122	3.10	2.50	5.60	5.93	1.01	6.94
	F2	3	5,220	3,850	2.32	26.5	1.09	9.19	1.22	77	153	0.74	2.39	3.13	0.90	1.46	2.36
G	5	6,540	5,180	3.35	92.2	120	2.87	5.38	202	183	17.8	10.6	28.4	11.4	5.44	16.8	
H	1	3,900	2,940	1.41	211	120	3.67	7.52	308	328	20.8	14.6	35.4	6.81	12.1	18.9	
ANA	A1		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	A2		15,340	10,940	31.0	1727	N	N	8.69	352	443	250	29.3	280	179	44.2	223
	B1		6,490	3,140	13.9	76.6	7.50	11.1	3.61	616	210	85.8	12.4	98.2	1.63	4.33	5.96
	B2		7,960	4,610	12.5	66.0	30.6	4.49	4.50	804	412	91.5	14.2	106	29.4	8.08	37.5
	C		4,960	3,500	15.1	1499	201	NQ	10.4	403	NQ	181	14.0	195	66.3	15.7	82.0
	D		5,240	4,040	4.31	37.6	60.2	2.96	4.18	210	305	115	14.2	129	16.0	12.5	28.5
	E		6,520	2,650	2.62	186	21.5	14.0	N	N	917	154	8.78	163	N	N	N
	F1		4,170	3,130	6.38	121	165	NQ	8.59	269	624	109	9.93	120	48.3	14.0	62.4
	F2		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
G		4,860	3,240	9.27	419	90.3	5.14	7.24	658	988	119	11.6	130	20.9	10.9	31.7	
H		3,010	1,700	3.90	193	50.7	11.1	4.30	332	664	89.0	10.7	99.7	10.7	5.23	16.0	
AER	A1		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	A2		13,960	9,240	2.58	403	46.7	NQ	5.70	143	NQ	42.8	62.1	105	22.2	46.0	68.2
	B1		5,140	1,970	10.4	53.9	17.9	4.30	5.32	78	62	34.5	15.4	49.9	10.9	4.89	15.8
	B2		7,140	3,770	5.79	102	13.3	15.6	3.62	95	NQ	30.3	31.2	61.5	10.2	12.3	22.6
	C		5,060	3,270	1.21	1040	141	NQ	10.2	126	NQ	30.1	37.3	67.4	43.7	33.6	77.3
	D		4,590	3,270	1.16	618	168	NQ	6.50	131	NQ	24.7	56.7	81.4	17.0	45.3	62.3
	E		6,300	2,460	0.65	761	94.4	NQ	N	N	NQ	35.5	57.6	93.1	N	N	N
	F1		3,670	2,660	1.37	182	71.1	11.8	3.52	87	NQ	19.6	33.5	53.1	6.63	19.3	25.9
	F2		4,750	3,220	0.83	35.5	2.37	11.6	2.51	101	101	6.74	11.3	18.0	0.42	2.76	3.18
G		5,320	3,390	1.03	562	109	NQ	8.08	83	NQ	16.5	40.6	57.1	12.5	30.1	42.6	
H		3,580	2,110	0.53	107	20.0	14.4	2.22	67	NQ	14.1	18.6	32.7	1.91	3.34	5.25	

NQ: desired parameters could not be quantified

* no digestion performance

Table 4. Solution sodium (Na⁺) in activated sludge process and in digested sludges

Plant	influent (mg/L)	ML (mg/L)	Effluent (mg/L)	WAS (mg/L)	ANA (mg/L)	AER (mg/L)
A1	402	311	295	296	NA	NA
A2	482	555	537	579	601	549
B1	NA	1165	974	1087	1120	1191
B2	NA	1041	1041	977	1003	979
C	63.7	61.9	63.0	67.6	69.4	67.9
D	78.1	93.2	91.1	93.8	95.8	95.1
E	418	318	NA	300	313	318
F1	113	75.4	97.6	71.2	72.7	75.1
F2	54.5	57.1	88.0	89.3	NA	95.1
G	115	133	129	129	125	121
H	45.9	45.5	48.7	45.4	46.6	48.4

Table 5. Solution potassium (K⁺) in activated sludge process and in digested sludges

Plant	influent (mg/L)	ML (mg/L)	Effluent (mg/L)	K ⁺ Ratio (WAS/Inf)	WAS (mg/L)	ANA (mg/L)	AER (mg/L)
A1	18.8	22.3	22.0	1.58	29.7	NA	NA
A2	18.1	22.0	20.5	1.51	27.2	104	76.5
B1	NA	94.0	97.2	-	92.6	110	123
B2	NA	111	117	-	115.6	135	134
C	15.6	28.0	15.7	4.59	71.7	86.2	72.8
D	7.20	9.05	8.59	1.58	11.4	45.4	41.0
E	19.2	32.0	NA	1.70	32.8	67.5	52.0
F1	9.60	10.8	7.73	1.06	10.1	44.8	52.3
F2	9.73	11.1	9.37	1.07	10.4	NA	22.7
G	12.8	31.4	26.5	3.78	48.4	72.2	59.3
H	10.9	10.5	9.43	1.92	21.0	32.6	26.5

Table 6. Soluble ammonium and nitrate before and after digestion

Plant	NH ₄ ⁺			NO ₃ ⁻		
	WAS (mg/L)	ANA (mg/L)	AER (mg/L)	WAS (mg/L)	ANA (mg/L)	AER (mg/L)
A1	2.95	NA	NA	2.24	NA	NA
A2	ND	359	194	1.95	ND	320
B1	ND	22.4	ND	2.43	ND	88.9
B2	ND	52.5	ND	2.23	ND	154
C	49.3	367	116	22.8	ND	181
D	ND	189	128	2.60	ND	226
E	51.6	313	44.2	2.30	ND	191
F1	2.36	193	134	2.24	ND	205
F2	0.19	NA	22.8	9.55	NA	157
G	39.5	253	119	1.99	ND	274
H	33.3	237	55.7	2.02	ND	173

ND: not detected

Table 7. Solution magnesium (Mg²⁺) in activated sludge process and in digested sludges

Plant	influent (mg/L)	ML (mg/L)	Effluent (mg/L)	WAS (mg/L)	ANA (mg/L)	AER (mg/L)
A1	21.2	14.5	18.2	19.2	NA	NA
A2	29.8	18.3	18.7	20.1	4.54	61.0
B1	19.4	17.9	17.8	18.4	16.8	20.3
B2	12.8	18.2	17.3	17.9	14.9	28.4
C	6.38	13.7	6.96	25.0	6.85	44.7
D	11.8	13.8	13.01	13.5	16.9	34.7
E	36.0	36.9	NA	44.5	50.1	53.6
F1	12.8	10.2	8.08	7.72	11.4	36.3
F2	5.19	6.02	5.66	6.10	NA	16.5
G	15.8	18.8	16.9	14.9	7.40	44.7
H	12.1	12.0	12.2	15.5	19.9	20.4

Table 8. Solution calcium (Ca²⁺) in activated sludge process and in digested sludges

Plant	influent (mg/L)	ML (mg/L)	Effluent (mg/L)	WAS (mg/L)	ANA (mg/L)	AER (mg/L)
A1	40.0	33.3	40.1	35.1	NA	NA
A2	46.0	44.2	46.1	39.4	23.9	184
B1	27.3	24.5	22.7	23.7	48.4	72.6
B2	25.6	41.8	22.3	37.2	55.4	113
C	25.2	26.6	28.8	24.8	22.7	112
D	27.5	42.7	27.8	34.2	45.6	154
E	265	373	NA	339	167	455
F1	29.9	30.0	31.7	26.0	28.2	116
F2	20.4	22.6	23.5	25.9	NA	117
G	33.9	39.7	43.1	29.3	33.8	185
H	39.4	37.4	44.7	36.2	49.1	190

Table 9. Iron and aluminum in dried WAS

Plant	Al (mg/TS)	Fe (mg/TS)	Al+Fe (mg/TS)	Al (mg/ash)	Fe (mg/ash)	Al+Fe (mg/ash)	Al/Fe (mg/mg)
A1	14.5	24.5	39.0	45.7	77.4	123	0.59
A2	9.17	15.8	25.0	35.6	61.4	97.0	0.58
B1	0.69	2.90	3.59	1.34	5.62	6.96	0.24
B2	0.66	3.79	4.45	1.61	9.22	10.8	0.17
C	12.9	12.9	25.8	65.2	65.5	131	1.00
D	5.84	5.80	11.6	30.9	30.7	61.6	1.01
E	1.96	39.7	41.7	4.35	88.1	92.4	0.05
F1	13.5	5.84	19.4	75.4	32.5	108	2.32
F2	26.8	9.47	36.2	102	36.1	138	2.83
G	9.28	15.1	24.4	44.6	72.5	117	0.62
H	4.16	80.2	84.3	16.9	326	343	0.05

Table 10. Cation ratios of WAS

Plant	Na/Fe (mg/L)/(mg/g ash)	M/T (meq/L)/(mg/g ash)	(M/D)/T (eq/eq)/(mg/g ash)
A1	3.827	0.112	0.034
A2	9.431	0.267	0.074
B1	193.4	7.135	2.648
B2	105.9	4.198	1.261
C	1.032	0.057	0.017
D	3.057	0.071	0.025
E	3.400	0.181	0.009
F1	2.189	0.032	0.017
F2	2.475	0.030	0.017
G	1.772	0.077	0.029
H	0.140	0.013	0.004

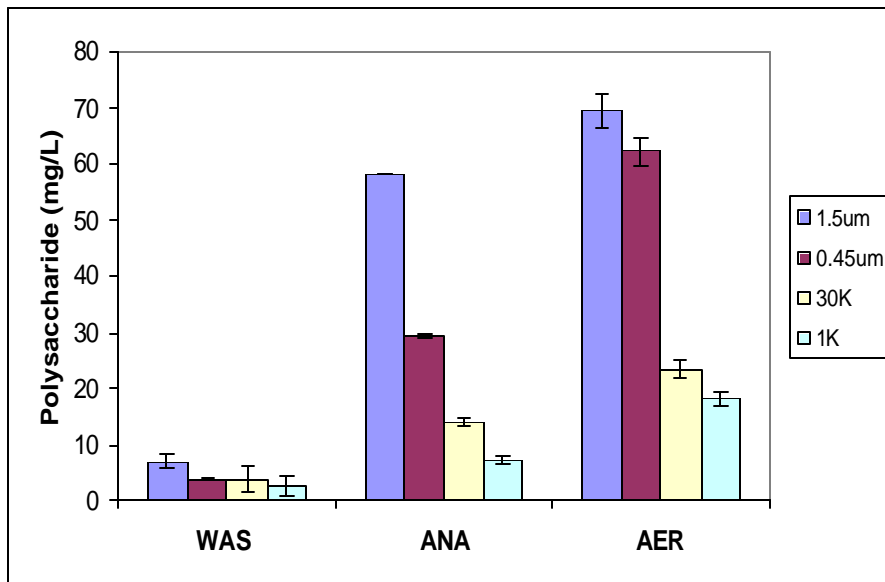
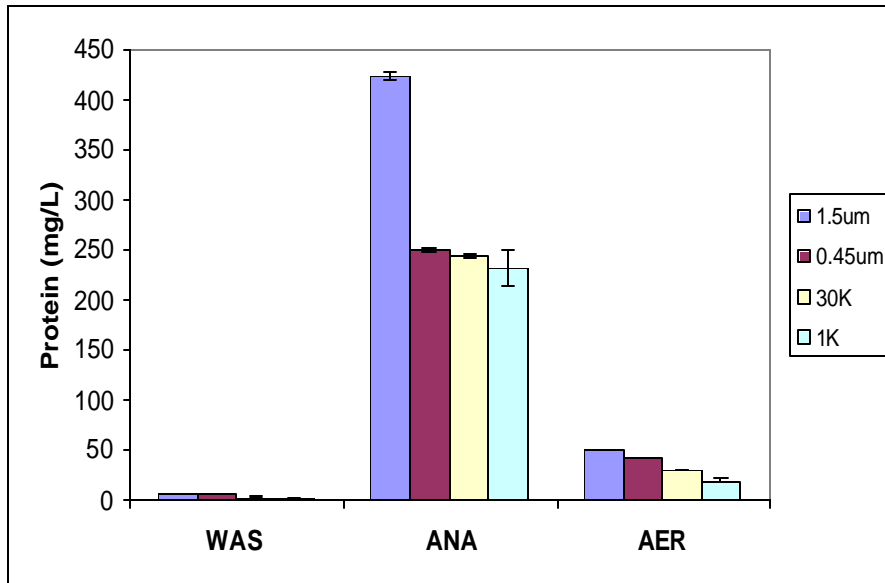


Figure 1. Changes in molecular weight distributions of (a) protein and (b) polysaccharide before and after digestion for Sludge A2

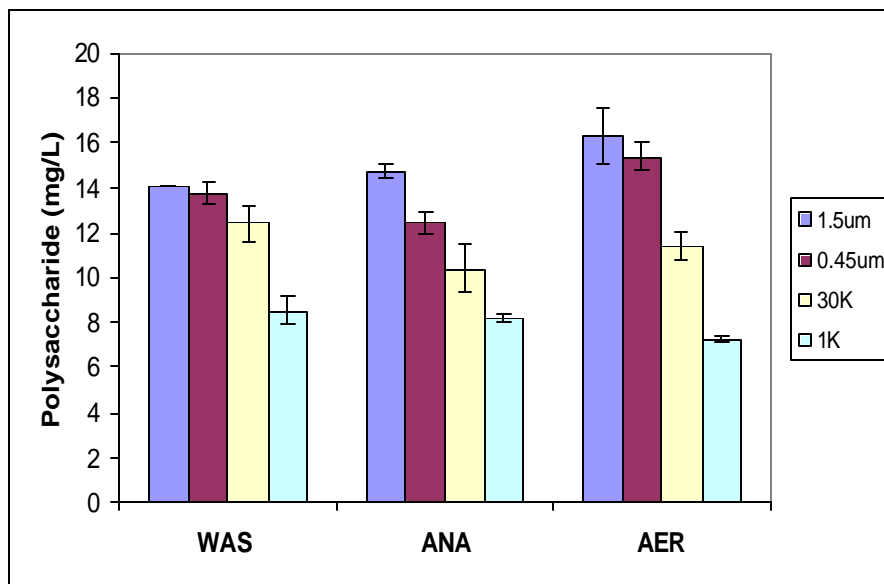
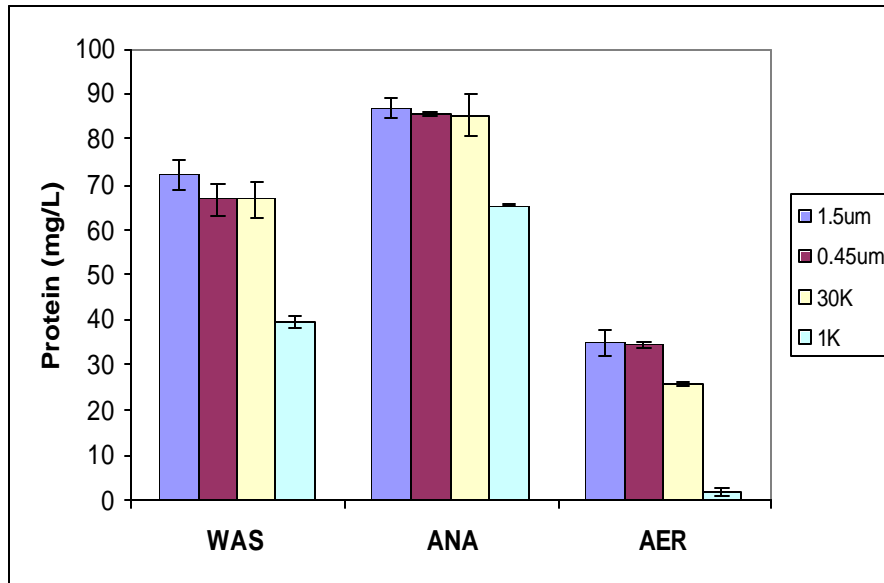


Figure 2. Changes in molecular weight distributions of (a) protein and (b) polysaccharide before and after digestion for Sludge B1

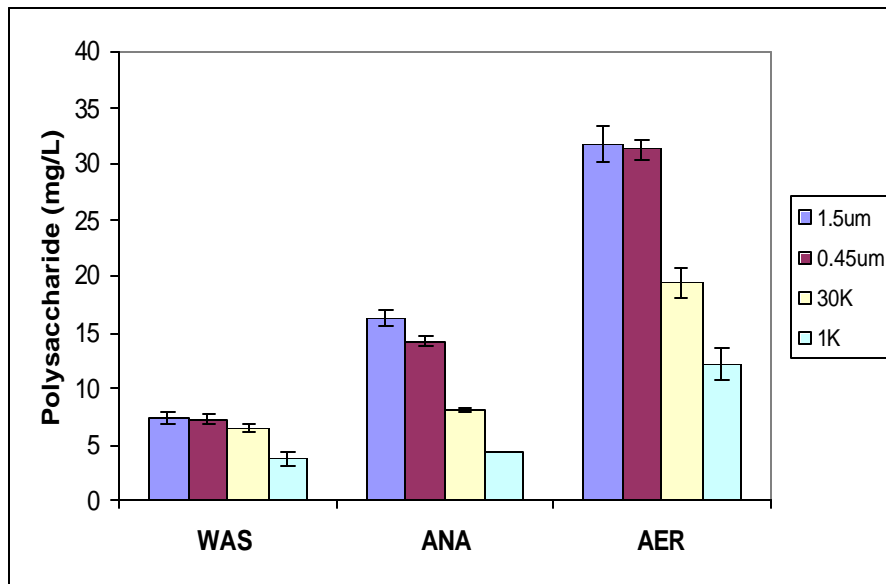
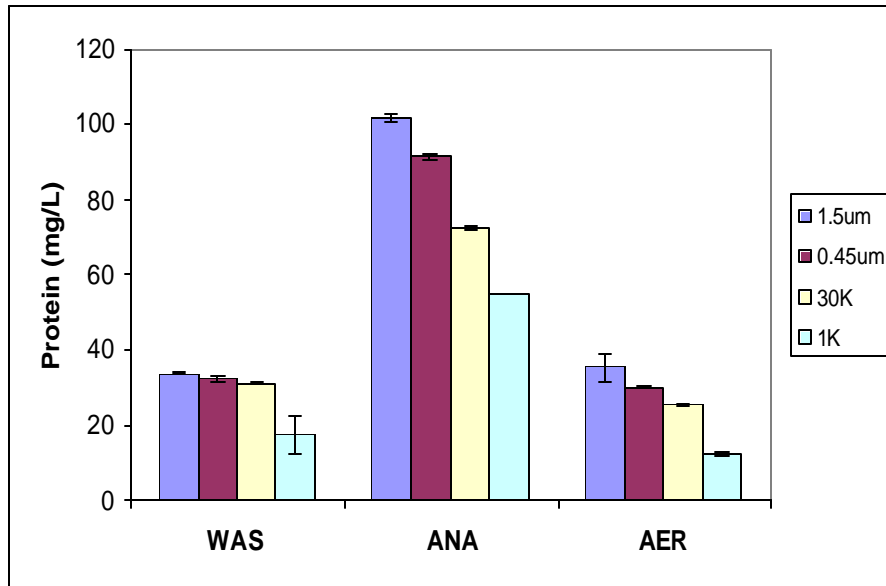


Figure 3. Changes in molecular weight distributions of (a) protein and (b) polysaccharide before and after digestion for Sludge B2

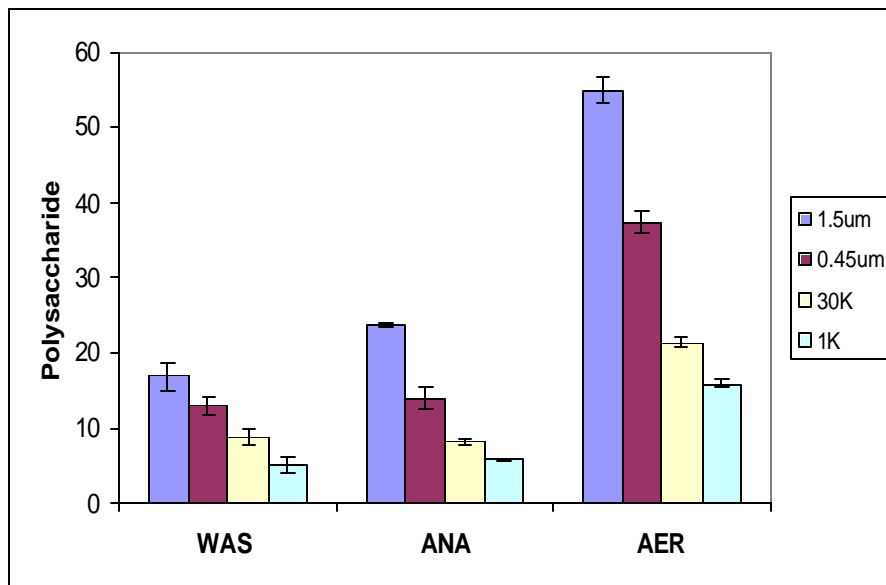
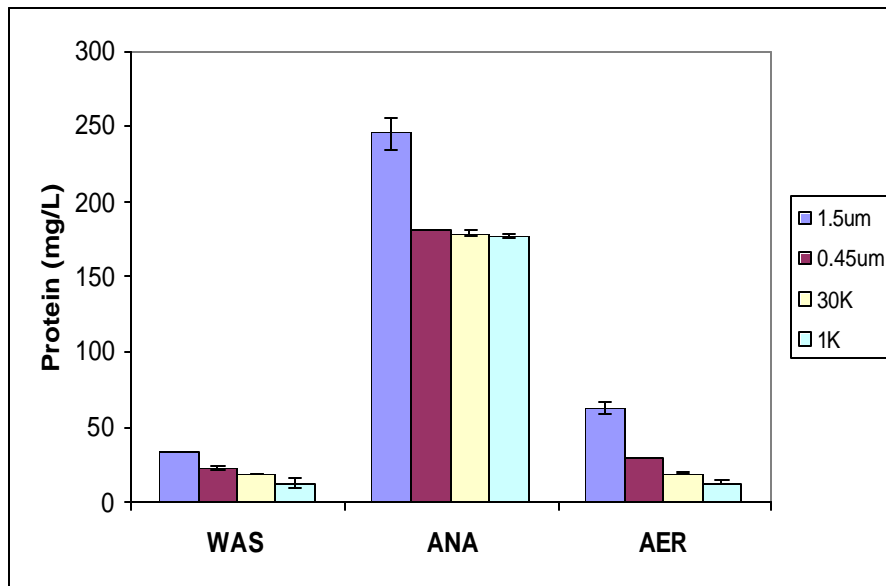


Figure 4. Changes in molecular weight distributions of (a) protein and (b) polysaccharide before and after digestion for Sludge C

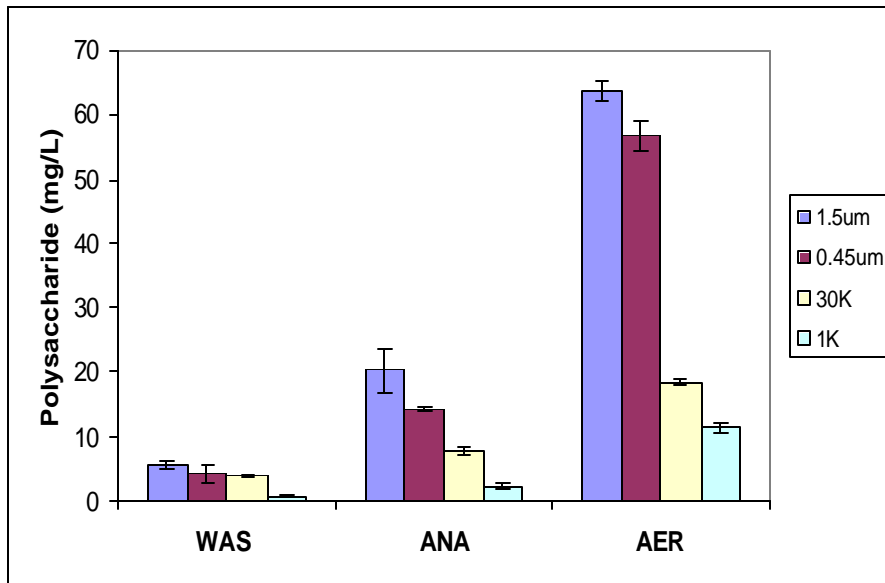
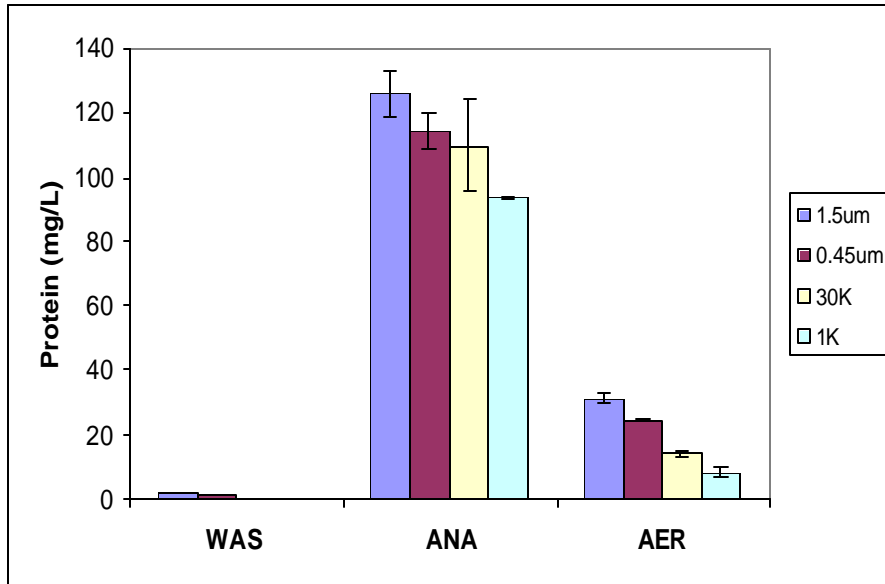


Figure 5. Changes in molecular weight distributions of (a) protein and (b) polysaccharide before and after digestion for Sludge D

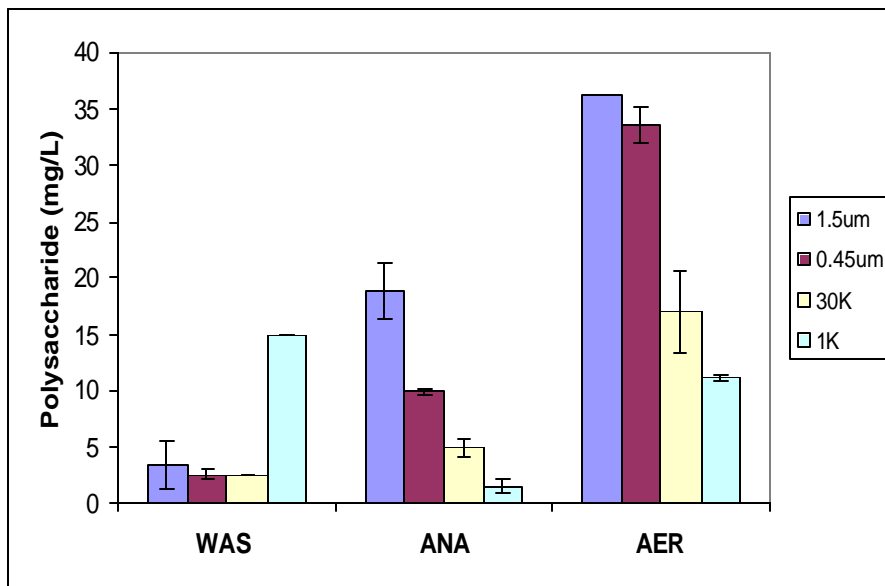
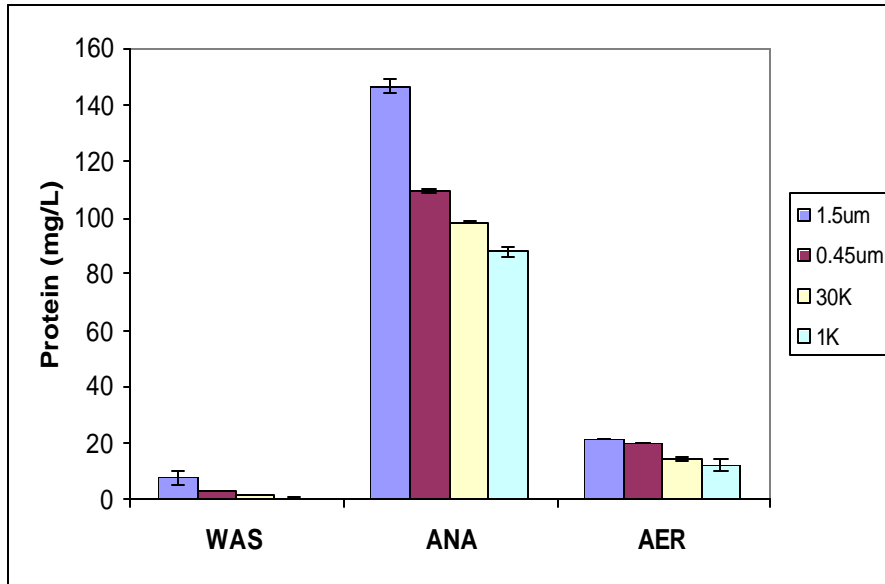


Figure 6. Changes in molecular weight distributions of (a) protein and (b) polysaccharide before and after digestion for Sludge F1

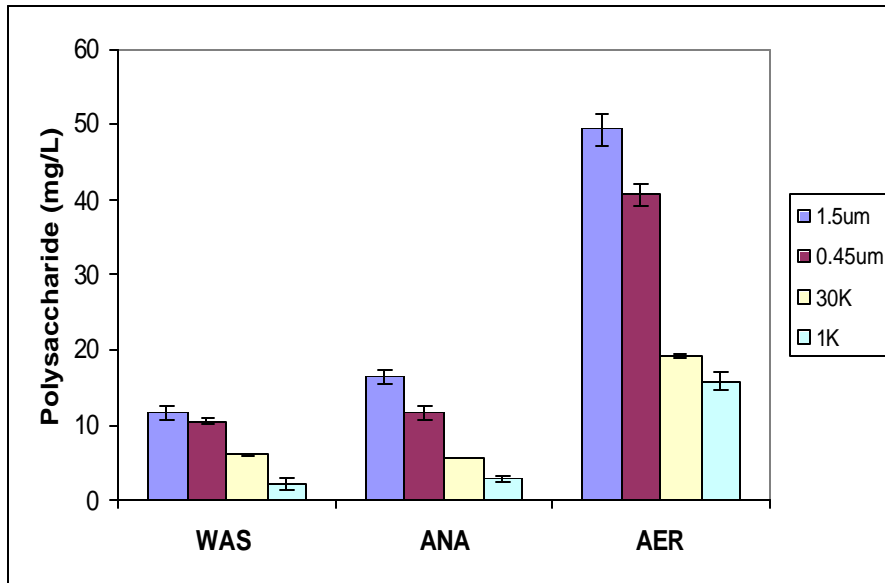
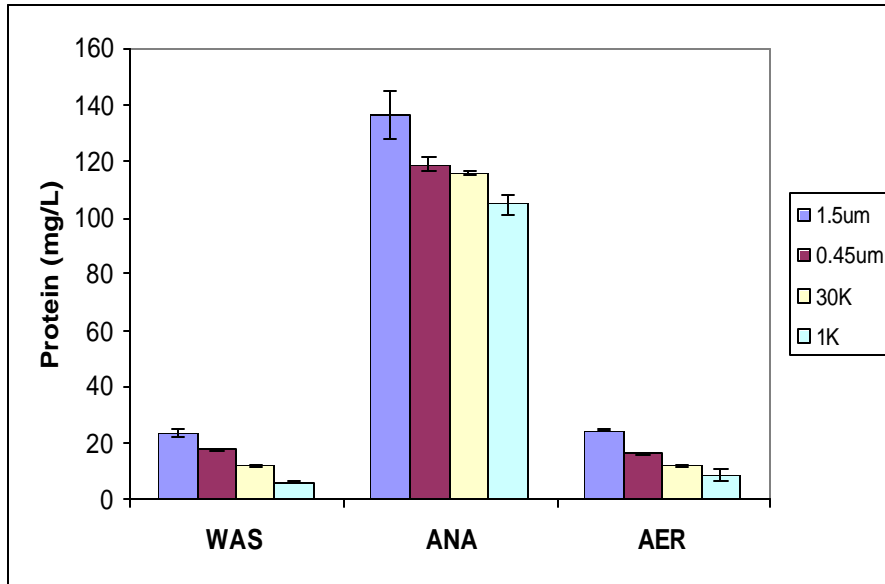


Figure 7. Changes in molecular weight distributions of (a) protein and (b) polysaccharide before and after digestion for Sludge G

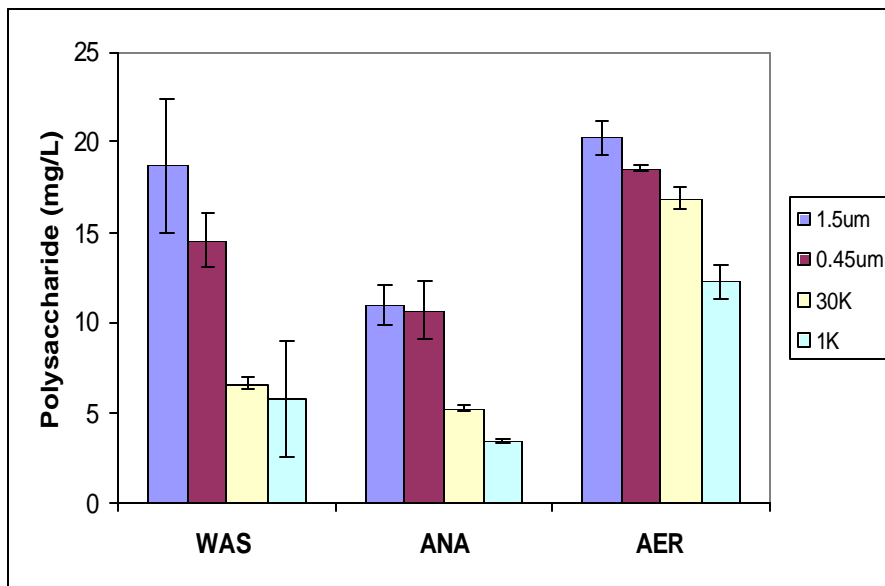
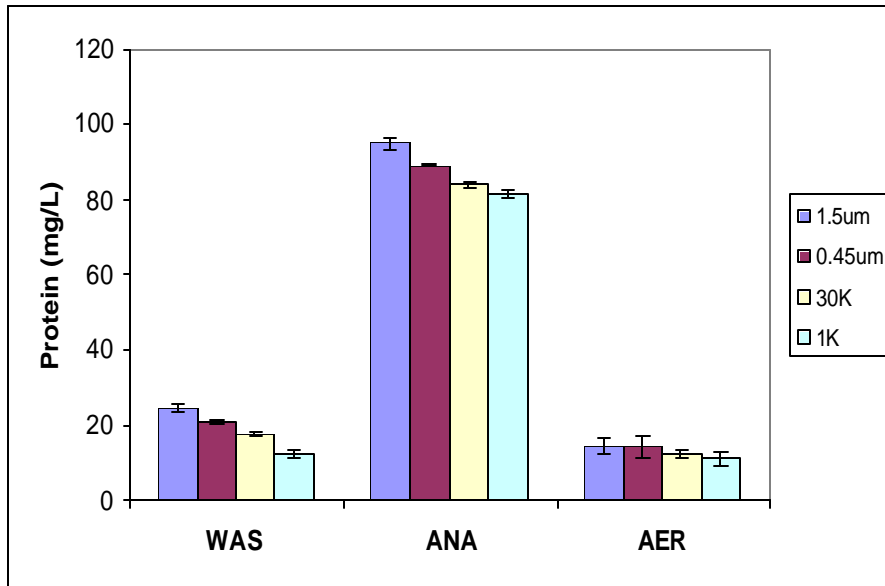


Figure 8. Changes in molecular weight distributions of (a) protein and (b) polysaccharide before and after digestion for Sludge H

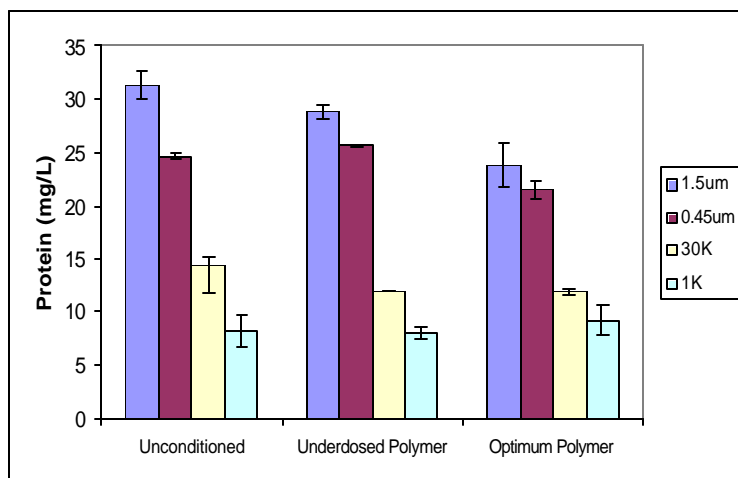
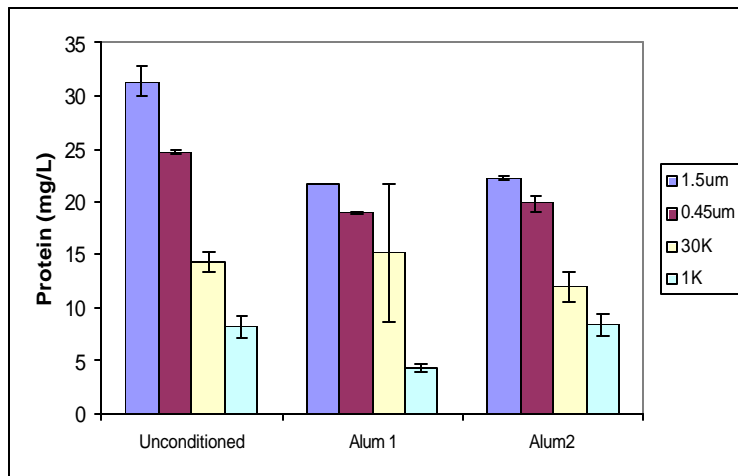
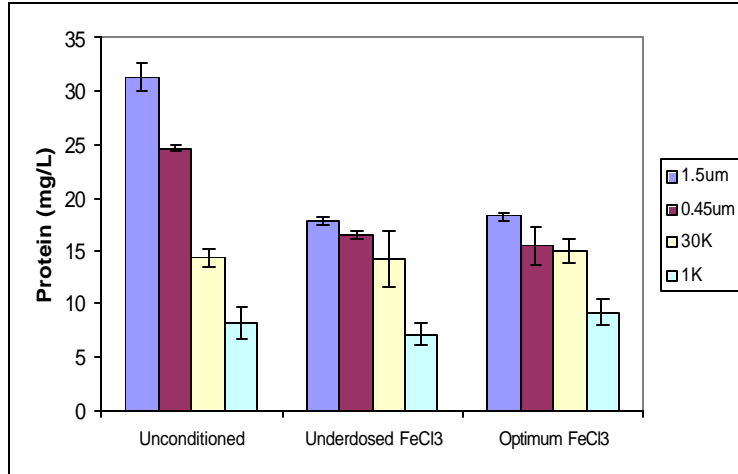


Figure 9. Coagulation of protein in aerobically digested sludge (Sludge D) by (a) FeCl₃ (b) alum and (c) cationic polymer

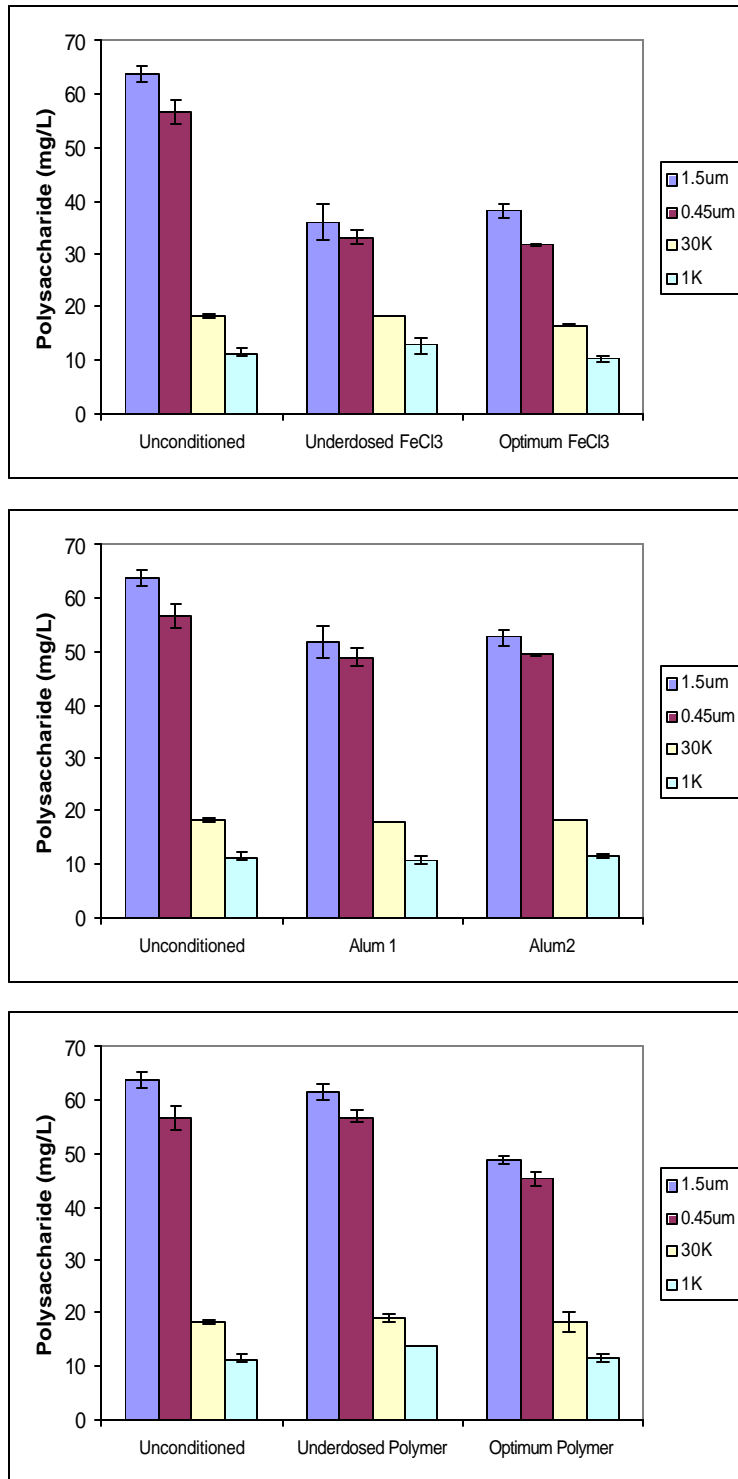


Figure 10. Coagulation of polysaccharide in aerobically digested sludge (Sludge D) by (a) FeCl₃, (b) alum and (c) cationic polymer

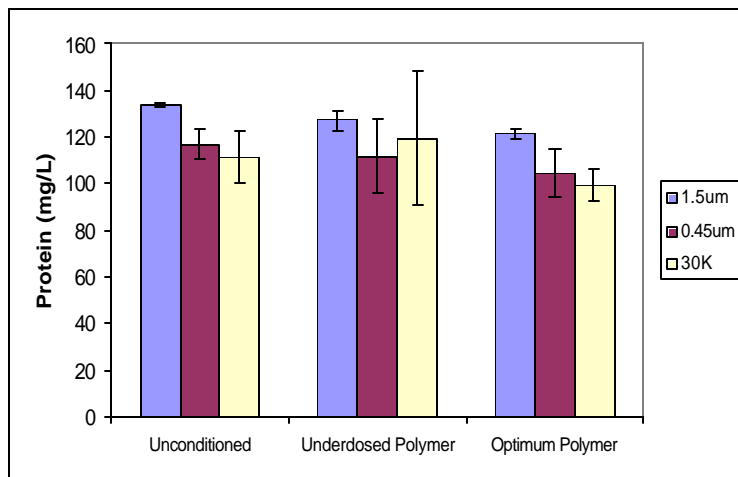
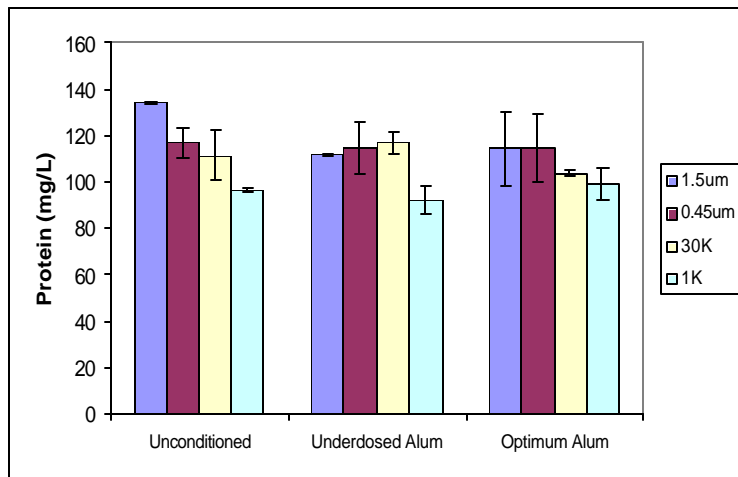
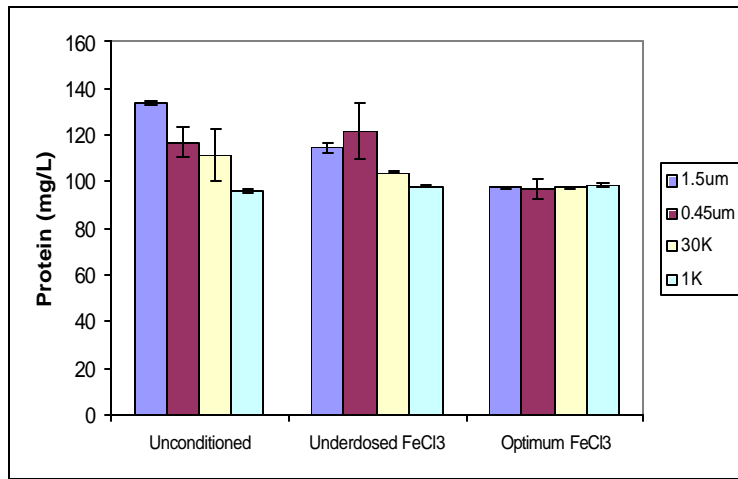


Figure 11. Coagulation of protein in anaerobically digested sludge (Sludge D) by (a) FeCl₃, (b) alum and (c) cationic polymer

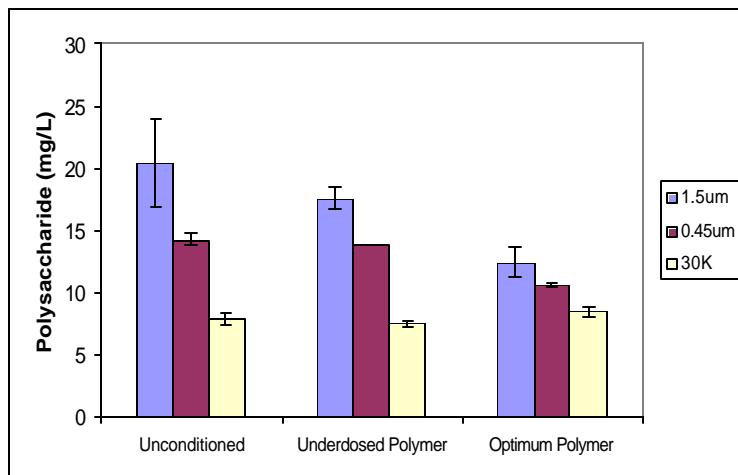
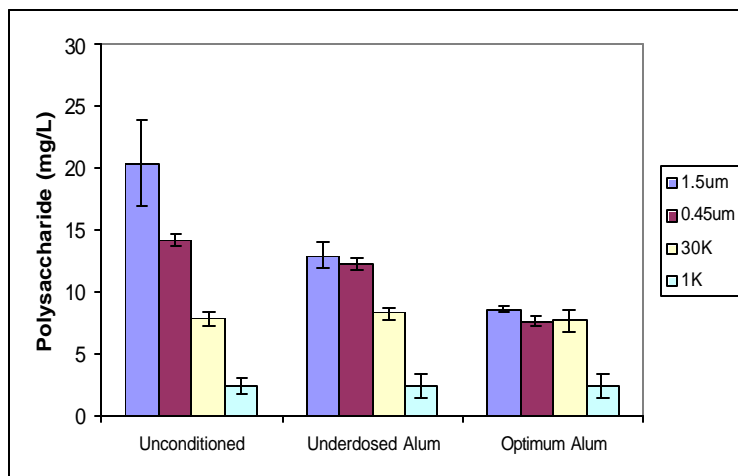
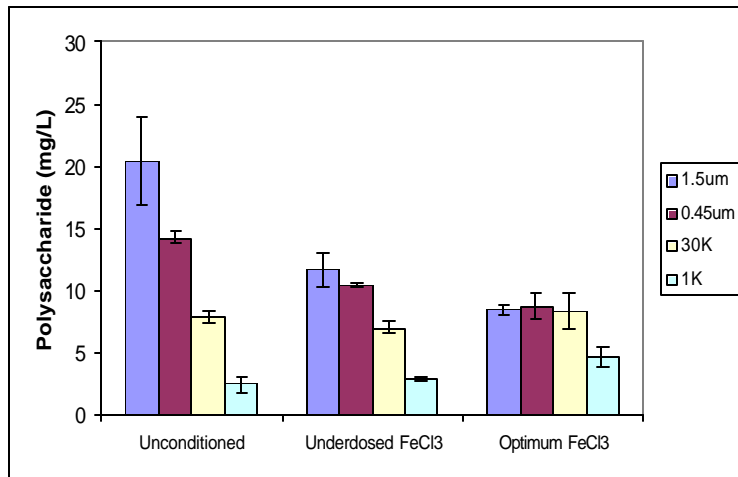


Figure 12. Coagulation of polysaccharide in anaerobically digested sludge (Sludge D) by (a) FeCl_3 , (b) alum and (c) cationic polymer

VITA

Chul Park was born on September 25, 1973 in Daegu, Korea. He attended Youngnam University, Korea, in 1992 and received a Bachelors of Science in Environmental Engineering in February of 2000. During his undergraduate work, he served military duty in Korean Army from April of 1993 to June of 1995. In 1998 he was awarded the scholarship of overseas program at Youngnam University and he attended Washington State University for the following year as an exchange student. In August of 2000, he started his masters work in Environmental Engineering at Virginia Polytechnic Institute and State university, Blacksburg, VA. Following masters work, he plans to stay and pursue a doctorate in the same filed.