Literature Review

The digestion process plays an important role in the solids handling system of a wastewater treatment process. Waste sludge is digested for destruction of organic matter and reduction of pathogens. Digestion may occur either in the presence or absence of molecular oxygen. Historically, a majority of the work on digestion focused on process performance and volatile solids reduction, and not on the change in flocs and the impact this has on sludge dewatering properties.

The objective of this study was to examine the digestion process from the perspective of floc structure, biopolymer and cation release from the floc, and the resulting impact on sludge dewatering properties. Both aerobic and anaerobic digestion were studied using activated sludge from two different sources. The primary data was obtained at a temperature of 20°C. While this temperature is lower than that normally used for anaerobic digestion, it allowed for direct comparisons to be made between aerobic and anaerobic digestion from a mechanistic perspective. However, anaerobic storage at 20°C has been studied extensively. Activated sludge is often stored prior to dewatering in full scale systems (Bruus *et al*., 1993). Rasmussen *et al*. (1994) also researched the implications of dewaterability, and the physical/chemical changes of anaerobically stored sludge in a nutrient removal plant.

Overview of the anaerobic and aerobic digestion process

The breakdown of extracellular material (biopolymer) originating from biological and lytic activity of cells can be considered one of the predominate functions in digestion. This extracellular material can be generally quantified as high molecular weight compounds (M_w > 10,000) produced by microorganisms under various environmental conditions (Morgan, 1990).

The anaerobic digestion process can be divided into three main categories. The first step, hydrolysis, converts larger molecules into smaller units including converting particulate organic matter into dissolved organic matter. Dissolved organic matter is then

broken down into volatile fatty acids, a process commonly referred to as acidogenesis. The final step, methanogenesis, converts intermediate acid compounds (primarily acetic acid and hydrogen) into methane and carbon dioxide. Methanogenesis is the rate limiting step in anaerobic digestion due to the slow growth rates of methanogens; most digestor failures are due to methanogenic upset (Metcalf and Eddy, 1991).

Aerobic digestion is another alternative method for the stabilization of waste sludge. Waste activated sludge, primary and waste activated sludge, trickling filter secondary sludge, and primary and trickling filter secondary sludge are commonly treated by applications for aerobic digestion (Reynolds and Richards, 1996). Most of the microorganisms involved in aerobic digestion are facultative with the exception of nitrifiers, which are obligate aerobes. Nitrification is a frequently occurring process in aerobic digestion (Reynolds and Richards, 1996). As available substrates are depleted, microorganisms respire endogenously, whereby cells consume protoplasm and other internal parts to maintain cell function. The end products consist of carbon dioxide, ammonia, and water (Metcalf and Eddy, 1991). Advantages of aerobic digestion are fewer operational problems, lower capital costs, less laboratory control and daily maintenance, and much lower biological oxygen demand present in the supernatant. Disadvantages are high energy requirements due to mixing and aeration, no useable by-product generated, and a lower solids content. Therefore, the volume of sludge to be dewatered in aerobic digestion is larger (Reynolds and Richards, 1996).

Characterization of activated and digested sludge material

There is much debate as to the quantity and type of extracellular polymers associated with activated sludge flocs. In general, extracellular polymers in activated sludge originate from two different sources, cell lysis and the wastewater influent (Urbain *et al*., 1993). Frolund *et al*. (1996) quantified activated sludge extracellular polymeric substances (EPS) using a cation exchange resin extraction technique. The authors found proteins, humics, and carbohydrates present in activated sludge with percentages of 46-52%, 18-23%, and 17% of volatile solids, respectively. DNA, and uronic acids were also found in the EPS. Urbain *et al*. (1993) also observed more protein than polysaccharides in

activated sludge; conversely, other authors (Horan and Eccles, 1986; Morgan *et al*., 1990; Forster, 1983) found polysaccharides to be the major constituent in activated sludge.

The biopolymer content of anaerobically digested sludge is also of interest. Forster (1983) found proteins and lipo-polysaccharides to be the major components of the extracellular material in anaerobically digested sludge. Morgan *et al*. (1990) confirmed; a study of extractable carbohydrate and protein of various activated and digested sludges found higher yields of protein than carbohydrates in anaerobically digested sludge. However, Morgan *et al*. (1990) also found that the anaerobically digested and granular sludges yielded significantly less extracellular polymer than the activated sludges studied. This conclusion was drawn on data based on the authors' experiments, as well as comparisons to data from other authors. Morgan *et al*. (1990) hypothesized that the differences in the amounts of extracted exocellular polymers between anaerobically digested biosolids and activated sludge might be explained by biopolymer degradation under anaerobic conditions, specifically by methanogenic bacteria.

Murthy and Novak (in press) investigated biopolymer release in aerobic digestion. The authors measured an increase in soluble polysaccharides in the supernatant. As the digestion time increased, the concentration of soluble polysaccharide increased while the soluble protein concentration remained fairly low. Murthy and Novak intimated that the low protein concentration was due to peptidase activity and thus degradation in the floc. The degradation of protein resulted in a weaker floc and indicated that proteins play a major role in floc formation and floc strength. The authors also suggested that the high polysaccharide content in solution led to a high polymer demand and poor dewatering properties.

It is clear from the aforementioned authors' data that many differences exist in the determination of the biopolymer content of activated sludges and digested biosolids. The experimental extraction technique used to quantify biopolymer concentration in sludge is a fundamental tool, yet different extraction techniques yield different results (Novak and Haugan, 1981). Poxon and Darby (1997) attempted to improve quantification of extracellular polyanions using a dye-adsorption method with sodium alginate as a standard, however the authors acknowledged limitations based on the method

assumptions. Overall, a simple definitive biopolymer quantification method has not been found.

Impact of biopolymer on sludge dewaterability

The influence of extracellular polymer on sludge settleability and dewatering properties has been reported in the literature (Wu *et al*., 1982, 1985; Novak *et al*., 1977). Novak *et al*. (1977) speculated that extracellular polymer, specifically anionic biopolymers, influenced dewatering and thickening of activated sludge. Polysaccharides have been implicated in settling characteristics due to the net anionic charge associated with this species. More recently, Higgins and Novak (1997a) intimated that the protein content of an activated sludge may also play a significant role in bioflocculation. The addition of pronase resulted in a degradation of proteins that resulted in decreased dewatering properties; the addition of a polysaccharide degrading enzyme resulted in no substantial change in dewatering properties.

Rasmussen *et al*. (1994) observed a decrease in filterability with a concomitant increase turbidity and dissolved organic carbon in the supernatant in anaerobically stored biosolids. The authors proposed that the turbidity was composed of bacterial colloids released from the floc and the dissolved organic carbon release due to bacterial degradation of organic matter and hydrolysis of biopolymers from the floc. Bruus *et al*. (1993) found similar results with increases in the total dissolved carbon and turbidity in the supernatant, as well as a linear increase in turbidity with respect to specific resistance to filtration.

Detailed work performed by Poxon and Darby (1997) found conflicting evidence between dewaterability and total polysaccharide concentration between two sludges with different feed compositions. The authors concluded that a simple relationship could not be found between total polyanion concentration and filterability; in one sludge the filterability decreased and in the other sludge the filterability increased. Poxon and Darby concluded that the specific extracellular polyanion biochemical characteristics are more important than the total polyanion concentration. Colloidal polysaccharide release was instead examined and the authors found an increase in filterability of both sludges with an increase

in colloidal polysaccharide. The magnitude of the increases in filterability of these two sludges were not significant when compared to the increases in dewaterability required for full scale conditioning, and thus Poxon and Darby suggested that polyanions are not a major contributor to the poor dewaterability of a sludge.

Nielsen and Keiding (1998) observed that when sulfide was added to activated sludge under anaerobic conditions and stirred vigorously, a disintegration of the flocs became apparent with an increase in turbidity. The increase in turbidity reflected a decrease in floc strength. The specific resistance to filtration was found to increase with a linear increase in turbidity, coinciding with data from Bruus *et al*. The same experiment was performed with laboratory cultures grown in strong, dense flocs; addition of sulfide resulted in very little floc disintegration. The authors concluded that a strong, dense floc will be less susceptible to sulfide effects because the exocellular polymers did not break apart by an Fe(III) reduction. The differences between the activated sludge properties versus the laboratory grown sludge are attributed to initial floc formation (presence of filamentous organisms, microcolony formation), and the amount and type of adsorbed extracellular polymer (macromolecules, bacteria, fibers).

Current models for floc structure

Jorand *et al*. (1995) evaluated the nature of floc structure using sonication time as a measure of floc dispersion. An increased release of protein, DNA, and carbohydrates with a concomitant reduction in volatile solids was observed as a function of sonication time. Proteins were the most abundant exocellular polymers released; carbohydrates and DNA released were similar and approximately one-third of the protein released. A silver proteinate staining method for α(1-4) and β(1-4) linkages in polysaccharide chains performed on activated sludge flocs revealed that a polysaccharide gel matrix linked bacterial cells together to form the floc. Nielsen and Keiding (1998) also confirmed the presence of polysaccharides within the floc matrix by staining with Ruthenium Red.

Higgins and Novak (1997a) proposed a separate floc model in which the role of a lectin-like protein is an important component. The authors suggested that a majority of the bound protein may be classified as a lectin, a nonenzymatic protein that is selective for

anion sugar residues. The authors hypothesized that proteins and polysaccharides are linked within the biopolymer framework; the addition of a proteolytic enzyme cause a release of bound polysaccharide. Furthermore, the model by Higgins and Novak suggests that divalent cations characterize an important part of floc structure by cross-linking polysaccharides, or cation bridging, and stabilizing binding activity and structural stability of the lectin-like proteins. Bruus *et al*. (1992) intimated calcium also played an important role in floc structure by cross-linking exopolymers; sludge deteriorated with the removal of calcium by EGTA with consequent increases in turbidity and decreases in dewaterability.

Role of calcium and magnesium in floc disintegration

Calcium has been implicated as a major component of floc structure because of its ability to bridge between electronegative carboxyl and phosphate groups on bacterial surfaces (Morgan *et al*., 1990; Urbain *et al*., 1993; Higgins and Novak, 1997a). Higgins and Novak (1997a) evaluated the relationship between exocellular biopolymer content, cations, and settling and dewatering properties of laboratory scale activated sludge systems. The authors found that an increase of bound protein was associated with a displacement of divalent cations by sodium in the floc matrix. This phenomena was associated with a deterioration in settling properties and clarification.

Bruus *et al*. (1992) showed that through ion exchange reactions, the extraction of calcium from the floc was followed by an increase in the supernatant turbidity and a decrease in dewaterability. The addition of magnesium to activated sludge showed the most immediate effect on the amount of calcium released; the addition of calcium to the floc showed an increase in magnesium approximately a third of the aforementioned calcium release. Addition of EGTA, a calcium specific chelating agent, was effective in replacing calcium and releasing small particles; a decrease in dewaterability was witnessed. However, no specific data from this study pointed to the type of biopolymer that is selective for calcium or magnesium; the authors simply proposed that approximately half of the total amount of calcium in the activated sludge is linked to exocellular polymers.

The role of magnesium in polymeric bridging with respect to calcium is not as clearly understood. Urbain *et al*. (1993) observed a higher calcium than magnesium content in activated sludge. The authors suggested that this difference in affinity for calcium over magnesium in EPS might be due to the 'larger' atomic size of calcium over magnesium. Using full-scale plant activated sludge data, Higgins and Novak (1997b) stressed that the calcium to magnesium ratio was as equally important as the monovalent to divalent ratio in assessing flocculation, settling, and dewatering problems; ratios near 1.0 were considered optimum. Therefore, the role of magnesium in activated sludge should not be ignored, but the exact relationship between magnesium and calcium requires further study.

Role of iron in floc disintegration

The role of iron in binding biopolymer in activated sludge and digested biosolids is a current issue in the wastewater treatment field. Rasmussen and Nielsen (1996) demonstrated that almost all iron(II) and iron(III) was associated with the sludge floc matrix, and a large percentage (70-90%) of iron found in fresh activated sludge existed in the oxidized form of Fe(III). Most of the iron(III) is historically assumed to be present as ferric hydroxide, however actual separation of iron species in the floc has not been achieved due to difficult extraction procedures (Rasmussen and Nielsen, 1996). The authors intimate that a majority of the iron may be organically bound because of the high organic content in activated sludge, or the natural affinity of extracellular polymers for iron.

Enzymatic activities of anaerobically and aerobically digested biosolids

The measurement of active enzymes important to oxidative substrate removal is a relatively recent alternative method to evaluate microbial biomass and activity over more traditional methods, such as the determination of volatile suspended solids or ATP content (Nybroe *et al*., 1992). Enzymes are actively involved in the conversion of complex particulate and dissolved organic matter into lower molecular weight compounds by extracellular hydrolytic enzymes, and these lower molecular weight compounds are taken

up by cells for use as energy and carbon sources. According to Nybroe *et al*. (1992), these enzymes reflect extracellular hydrolysis or cellular oxidative metabolism. The two enzymatic assays used in this study are glucosidase and alanine-aminopeptidase. Glucosidase is an enzyme involved in the degradation of starch; alanine-aminopeptidase is involved in protein degradation.

Nybroe *et al*. (1992) states that since complex organic compounds are broken down into simple low-molecular weight compounds, then it can be expected that digested sludge would have a characteristic enzymatic profile, and enzymatic activities can be used as a control parameter for the digestion process (1992). These authors found glucosidase activity to be three times higher in digested sludge than in activated sludge, and the peptidase activity to be significantly lower in anaerobically digested sludge than activated sludge. The author also stated that a higher peptidase activity should be expected in hydrolyzed sludge given that a significant portion of nitrogenous material is solublized during hydrolysis (Nybroe *et al*., 1992).

Nielsen *et al*. (1996) found a decrease in the protein biopolymer fraction over a six day anaerobic storage time and a minor reduction in the carbohydrate content. However, specific enzymatic activities were not measured, nor were correlations made to specific dewatering properties. Nielsen *et al*.'s (1996) data show that some protein degradation does occur; high-performance size-exclusion chromatography indicated that observed changes in biopolymer content were due to degradation of existing compounds and not the production of new EPS compounds

Sarada and Joseph (1993) performed an interesting study using batch and semicontinuous processes to evaluate enzymatic activities of tomato processing waste. β-Glucosidase and neutral protease activities were measured. The optimum pH for glucosidase activity was between 4.4 and 5.2; the protease did not show any pH optimum. In the batch process, glucosidase activity remained fairly constant at very low levels and it starts to peak toward the end of the process (70-80 days). However, in the authors' study the pH of the batch reactors fell from 6.8 to 4.0. Thus, glucosidase activity comparisons could be misconstrued if pH differences existed between studies. The neutral protease peaked early in the digestion process, showed a decrease, and then peaked again.

In the semicontinuous process, HRT strongly influenced the activities of glucosidase and protease. Protease activities were barely detectable at any HRT. An accumulation of protein during anaerobic digestion and very low peptidase activity agrees with data obtained from this study. Glucosidase activity was highest at an HRT of 8 days and when the pH was 5. In the open system, Sarada and Joseph maintain that enzymatic activity is dependent on the continuous supply and nature of the substrate. Thus, these tentative results indicate that enzymatic activity could be an important parameter in the reduction of biocolloids, although specific reasons for particular enzymatic profiles are not yet fully understood.

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Biopolymer and Cation Release in Aerobic and Anaerobic Digestion and the Consequent Impact on Sludge Dewatering and Conditioning Properties

Mary E. Rust, Sudhir N. Murthy, and John T. Novak

Sludge dewatering and chemical conditioning requirements were examined from the perspective of biopolymer and cation release from activated sludge flocs. Both aerobic and anaerobic digestion processes were considered from two different activated sludge sources at a temperature of 20°C. Polymer demand and specific resistance to filtration increased with an increase in total soluble biopolymer concentration for all temperature ranges. In anaerobic digestion, the protein release was three times greater than the polysaccharide release. Conversely, aerobic digestion of the same sludge resulted in a greater release of polysaccharides than proteins. Polymer conditioning requirements in the anaerobic digestors were an order of magnitude higher than in the aerobic digestors; proteins were considered to be the biopolymer fraction responsible for the high polymer conditioning requirements and poor dewatering properties. Biopolymer is released to the supernatant as colloids bound by divalent cations. Peptidase and glucosidase activity were used to monitor enzymatic activity relative to biopolymer release and degradation. The reasons for the increases and decreases in hydrolase activity are unknown.

Key words- protein, polysaccharide, dewatering, conditioning, cations, anaerobic digestion, aerobic digestion, iron

The digestion process plays an important role in the solids handling system of a wastewater treatment process. Waste sludge is digested for destruction of organic matter and reduction of pathogens. Digestion may occur either in the presence or absence of molecular oxygen. Historically, a majority of the work on digestion focused on process performance and volatile solids reduction, and not on the change in flocs and the impact this has on sludge dewatering properties.

The anaerobic digestion process can be divided into three main categories. The first step, hydrolysis, converts larger molecules into smaller units including converting particulate organic matter into dissolved organic matter. Dissolved organic matter is then broken down into volatile fatty acids, a process commonly referred to as acidogenesis. The final step, methanogenesis, converts intermediate acid compounds (primarily acetic acid and hydrogen) into methane and carbon dioxide. Methanogenesis is the rate limiting step in anaerobic digestion due to the slow growth rates of methanogens; most digestor failures are due to methanogenic upset (Metcalf and Eddy, 1991). Aerobic digestion is another alternative method for the stabilization of waste. As available substrates are depleted, microorganisms undergo endogenous respiration whereby cells consume protoplasm and other internal parts to maintain cell function. The end products consist of carbon dioxide, ammonia, and water (Metcalf and Eddy, 1991).

Characterization of activated and digested sludge material

There is much debate as to the quantity and type of extracellular polymers associated with activated sludge flocs. Frolund *et al*. (1996) quantified activated sludge extracellular polymeric substances (EPS) using a cation exchange resin extraction technique. The authors found proteins, humics, and carbohydrates present in activated sludge with percentages of 46-52%, 18-23%, and 17% of volatile solids, respectively. Forster (1983) found proteins and lipo-polysaccharides to be the major components of anaerobically digested sludge. Morgan *et al*. (1990) confirmed; a study of extractable carbohydrate and protein of various activated and digested sludges found higher yields of protein than carbohydrates in anaerobically digested sludge.

Murthy and Novak (in press) measured an increase in soluble polysaccharides during aerobic digestion. As the digestion time increased, the concentration of soluble polysaccharide increased while the soluble protein concentration remained fairly low due to protein degradation within the floc. The degradation of protein resulted in a weaker floc and indicated that that proteins play a major role in floc formation and floc strength. The authors also suggested that the lower protein content and high polysaccharide content in solution led to a high polymer demand and poor dewatering properties.

Impact of biopolymer on sludge dewatering

The influence of extracellular polymer on sludge settleability and dewatering properties has been reported in the literature (Wu *et al*., 1982, 1985; Novak *et al*., 1977). Novak *et al*. (1977) speculated that extracellular polymer, specifically anionic biopolymers, influenced dewatering and thickening of activated sludge. Polysaccharides have been implicated in settling characteristics due to the net anionic charge associated with this species. More recently, Higgins and Novak (1997) intimated that the protein content of an activated sludge may also play a significant role in bioflocculation. The addition of a pronase resulted in a degradation of proteins that resulted in poorer dewatering properties; the addition of a polysaccharide degrading enzyme resulted in no substantial change in dewatering properties.

Rasmussen *et al*. (1994) observed a decrease in filterability with a concomitant increase in turbidity and dissolved organic carbon in the supernatant of anaerobically stored biosolids. The authors proposed that the turbidity was composed of bacterial colloids released from the floc; the dissolved organic carbon release was hypothesized to be due to bacterial degradation of organic matter and hydrolysis of biopolymers from the floc. Bruus *et al*. (1990) found similar results with increases in the total dissolved carbon and turbidity in the supernatant.

Detailed work performed by Poxon and Darby (1997) found conflicting evidence between dewaterability and total polysaccharide concentration between two sludges with different feed compositions. The dewaterability of a sludge, or filterability, was expressed using a filterability index based on the capillary suction time and corrected for the solids concentration and viscosity of the sample. The authors concluded that a simple relationship could not be found between total polyanion concentration and filterability; in one sludge the filterability decreased and in the other sludge the filterability increased as polysaccharides increased. Poxon and Darby concluded that the specific extracellular

polyanion biochemical characteristics are more important than the total polyanion concentration. Colloidal polysaccharide release was instead examined and the authors found an increase in filterability of both sludges with an increase in colloidal polysaccharide. The magnitude of the increases in filterability of these two sludges were not significant when compared to the increases in dewaterability required for full scale conditioning, and thus Poxon and Darby suggested that soluble polysaccharides are not a major contributor to the poor dewaterability of a digested sludge.

Current models for floc structure

Jorand *et al*. (1995) evaluated the nature of floc structure using sonication time as a measure of floc dispersion. An increased release of protein, DNA, and carbohydrates with a concomitant reduction in volatile solids was observed as a function of sonication time. Proteins were the most abundant exocellular polymers released; carbohydrates and DNA released were similar and approximately one-third of the protein released. A silver proteinate staining method for α(1-4) and β(1-4) linkages in polysaccharide chains performed on activated sludge flocs revealed that a polysaccharide gel matrix linked bacterial cells together to form the floc. Nielsen and Keiding (1998) also confirmed the presence of polysaccharides within the floc matrix by staining with Ruthenium Red.

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The objective of this study was to examine the digestion process from the perspective of floc structure, biopolymer and cation release from the floc, and the resulting impact on sludge dewatering properties. Both aerobic and anaerobic digestion were studied using activated sludge from two different sources. The primary data collected from this study was obtained at a temperature of 20°C. While this temperature is lower than that normally used for anaerobic digestion, it allowed for direct comparisons to be made between aerobic and anaerobic digestion from a mechanistic perspective. However anaerobic storage at 20°C has been studied extensively. Activated sludge is often stored prior to dewatering in full scale systems (Bruus *et al*., 1993). Rasmussen *et al*. (1994) also researched the implications of dewaterability, and the physical/chemical changes of anaerobically stored sludge in a nutrient removal plant.

Methods and Materials

Experimental approach Aerobic batch reactors (20°C) and anaerobic batch reactors were maintained at different temperatures to examine differences in polymer conditioning

requirements, dewatering properties, and biopolymer and cation releases. From a comparison of the data obtained from these experiments, a model for floc structure is proposed.

Sludge matrix Two different municipal waste treatment plant sludges were used in this digestion study: Pepper's Ferry Regional Wastewater Treatment Facility and the Lower Stroubles Creek Treatment Plant of the Blacksburg-VPI Sanitation Facility. An industry discharging into the Pepper's Ferry wastewater treatment plant network utilizes sulfuric acid in their process and then neutralizes with sodium hydroxide. Consequently, Pepper's Ferry possesses high sodium (250 - 500 mg/L Na^+)and sulfate (> 450 mg/L SO_4) in their influent. Conversely, Blacksburg receives relatively low sodium and sulfate in their influent (~ 50 mg/L Na^+ and SO_4).

The Pepper's Ferry plant is a conventional plant with an aerated activated sludge process and an anaerobic digestion process. The Blacksburg plant has recently been retrofitted to function as an biological nutrient removal plant; an anaerobic zone exists in the activated sludge basin. Therefore, the activated sludge properties are very different between the two plants and make ideal comparisons for this study. All sludge used for the digestion studies were taken from the recycled activated sludge (RAS) line, and concentrated to 1.1 to 1.3% solids.

Reactor configuration The digestion study consisted of four three liter batch reactors; two each of Pepper's Ferry and Blacksburg under aerobic and anaerobic conditions kept at a constant temperature of 20°C, respectively. The anaerobic reactors were continuously purged with nitrogen gas, with samples taken from a siphon pump attached to the lid of the reactor. The reactors were continuously stirred on a stir plate in a constant temperature room at 20°C; the anaerobic temperature study at 30°C was performed in a shaking water bath in 1.5 L reactors.

Samples were centrifuged at 10,000*g* for 15 minutes. The supernatant was removed and filtered through a 1.5 um filter. The biopolymer concentration in the filtered supernatant was considered the soluble biopolymer fraction.

Biopolymer analysis Protein concentration was measured using the Hartree (1972) modification of the Lowry *et al*. (1951) method. Polysaccharide was measured using the Dubois *et al*. (1956) method. Bovine serum albumin and glucose were used as protein and polysaccharide standards, respectively.

Dewatering and conditioning properties Mixed liquor and volatile suspended solids (MLSS and MLVSS) were determined using Method 2540D and 2540E of *Standard Methods* (1995), respectively. Specific resistance to filtration (SRF) was performed as described by Christensen and Dick (1985). The capillary suction time (CST) was determined using Method 2710G of *Standard Methods* (1995). Optimum polymer conditioning tests were determined using a low molecular weight cationic polymer at 0.5%.

Ion analysis Free calcium and magnesium were measured using a Dionix (DX120) Ion Chromatograph containing a CS12 column and conductivity detector (Dionex 2010X) with a self generating suppression of the eluent. The eluent, methane sulfonic acid at 20 mM, ran at a flow rate of 1.0 mL/min. Total solution calcium and magnesium were measured using an Atomic Absorption Spectrophotometer. Ferrous iron was measured using a photometric analysis by Method 8146 of *Standard Methods* (1995).

Ultrafiltration analysis
Samples were ultrafiltered at 55 psi through Amicon® YM30 and YM3 partly hydrophilic membranes in order to assess the smaller size fractions. The YM30 and YM3 filters have molecular sizes at approximately 30,000 dalton and 3,000 dalton, respectively. Samples were filtered through 1.5 μ, 0.45 μ, 30,000 dalton (30k), and 3,000 dalton (3k) filters and analyzed for proteins and polysaccharides.

Determination of glucosidase activity and alanine-aminopeptidase activity An assay for leucine-aminopeptidase was performed using L-leucine-p-nitroanilide as described by Murthy and Novak (in press). Samples were assayed for leucine-aminopeptidase with one mL cell-free extract using the method of Prescott and Wilkes (1976). Samples were assayed for p-nitrophenyl-β-D-glucose with one mL cell-free extract using the method of Wozniak and Owens (1994). Enzyme activities are expressed on a volume basis.

Results and Discussion

The purpose of this study was to examine the aerobic and anaerobic digestion process in batch systems from the perspective of floc structure and biosolids dewatering. Biopolymer and cation release into solution and the resulting impact on sludge dewatering properties and conditioning requirements were investigated. There is little data in the literature that shows the chemical nature of anaerobic biopolymers and the consequent impact on dewatering properties. The composition of biopolymers released from the floc and the fractionation of these biopolymers during digestion needs to be better understood from a process and economic perspective. If biopolymer release exhibits a major impact on dewatering of sludge and results in an expensive polymer demand, then it follows that we should attempt to understand biopolymer release mechanistically and suggests ways to reduce biopolymer content in sludge.

Biopolymer release in aerobic and anaerobic digestion and the effect on dewatering properties

One of the first observations that were made between the aerobic and anaerobic digestors were the large differences in dewatering properties. The polymer demand and the CST over digestion time for all four reactors is illustrated in Figure 1. The polymer demand for the anaerobic biosolids is an order of magnitude higher than for the aerobic biosolids. The CST data for the anaerobic biosolids was more than an order of magnitude higher than the CST for the aerobic biosolids. These relative differences between the

aerobic and anaerobic reactors give a very clear picture of physical and chemical sludge characteristics, even though the dewatering data presented in this study may not be indicative of full scale systems. The specific nature of the biopolymers released to the supernatant was thus investigated with respect to the impact on polymer conditioning and filterability.

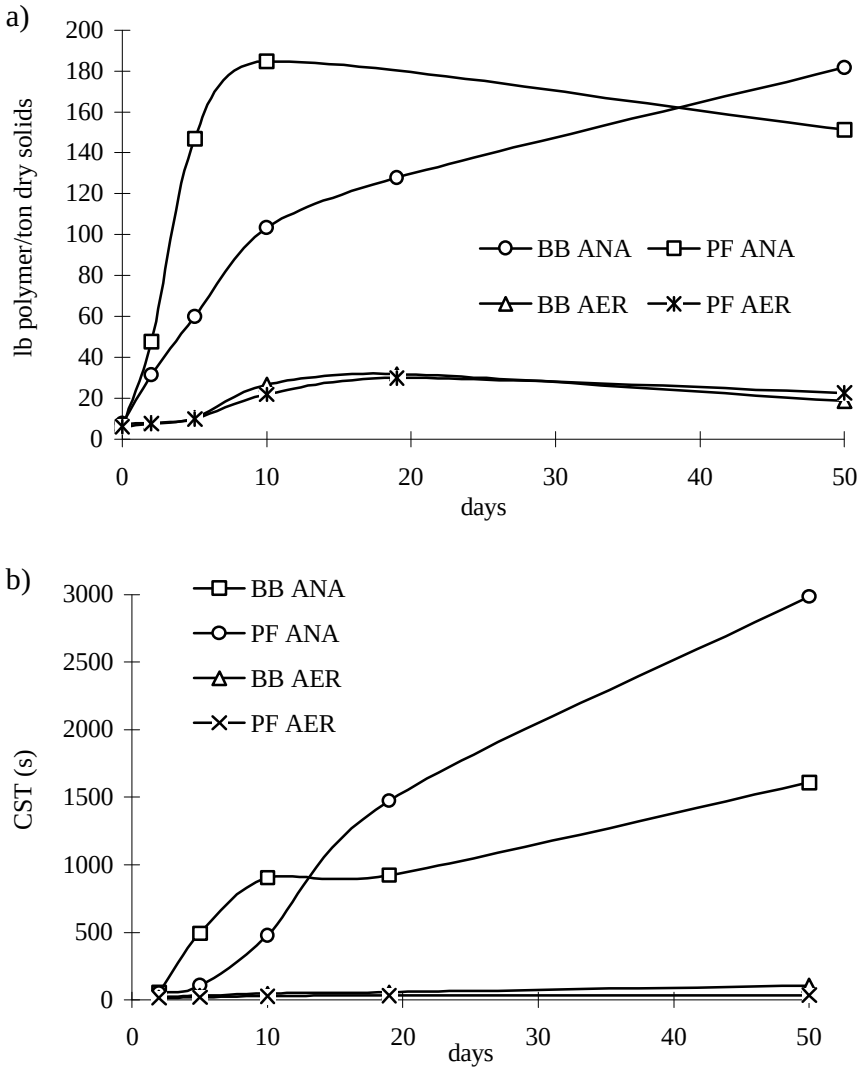


Figure 1: a) Polymer Demand, and b) CST for Blacksburg (BB) and Pepper s Ferry (PF) Aerobic (AER) and Anaerobic (ANA) Digestion Study, 20°C

Biopolymer release to the supernatant has been investigated by several researchers (Higgins and Novak, 1997; Murthy and Novak, in press; Murthy *et al*., submitted). For example, Higgins and Novak (1997) measured the effects of cation addition to activated sludge on the biopolymer content of an activated sludge. The quantity of biopolymer in the supernatant was considered the soluble, or released, fraction; the bound fraction was extracted from the remaining pellet. The amount of bound protein decreased and the soluble protein fraction released into solution increased, resulting in a deterioration in the

floc structure and poorer dewatering properties. It was thought that this was the result of an ion exchange process where sodium replaced calcium in the floc, resulting in weaker biopolymer binding and therefore poorer dewatering properties.

Obvious and large differences in dewatering properties exist for the aerobic and anaerobic digested biosolids, as shown in Figure 1. In order to illustrate the comparisons in floc deterioration between aerobic and anaerobic digestion, Figure 2 shows the soluble protein and polysaccharide released over time for Blacksburg and Pepper's Ferry aerobic and anaerobic biosolids. The anaerobic digestors released protein into solution at concentrations an order of magnitude greater than the protein released in the aerobic digestors. Conversely, the soluble polysaccharide release in both sets of digested biosolids is remarkably similar until day 30. In the anaerobic digestors the soluble polysaccharide concentration continues to increase beyond day 30, and in the aerobic digestors the soluble polysaccharide concentration decreases slightly. The soluble protein released in the aerobic reactors is less than one-half the soluble polysaccharide concentration (with the exception of the Pepper's Ferry day 50 data point). This data coincides with the work of Murthy and Novak (in press) who observed an increase in soluble polysaccharide and minor protein release during aerobic digestion. In contrast to the observations made in this study, Nielsen *et al*. (1996) found only small quantities of protein and polysaccharide in anaerobically digested biosolids.

An alternative to the graphic approach presented in Figure 2 can be seen in Table 1. Table 1 lists the protein to polysaccharide ratio for Blacksburg and Pepper's Ferry digestion studies. With the exception of the 50 day data point for the anaerobic digestors (reflecting a polysaccharide increase) the protein to polysaccharide ratios are fairly constant for both the aerobic and anaerobic digestion studies. The anaerobic digestors result in a protein to polysaccharide ratio eight to nine times higher than the protein to polysaccharide ratio for the aerobic digestors.

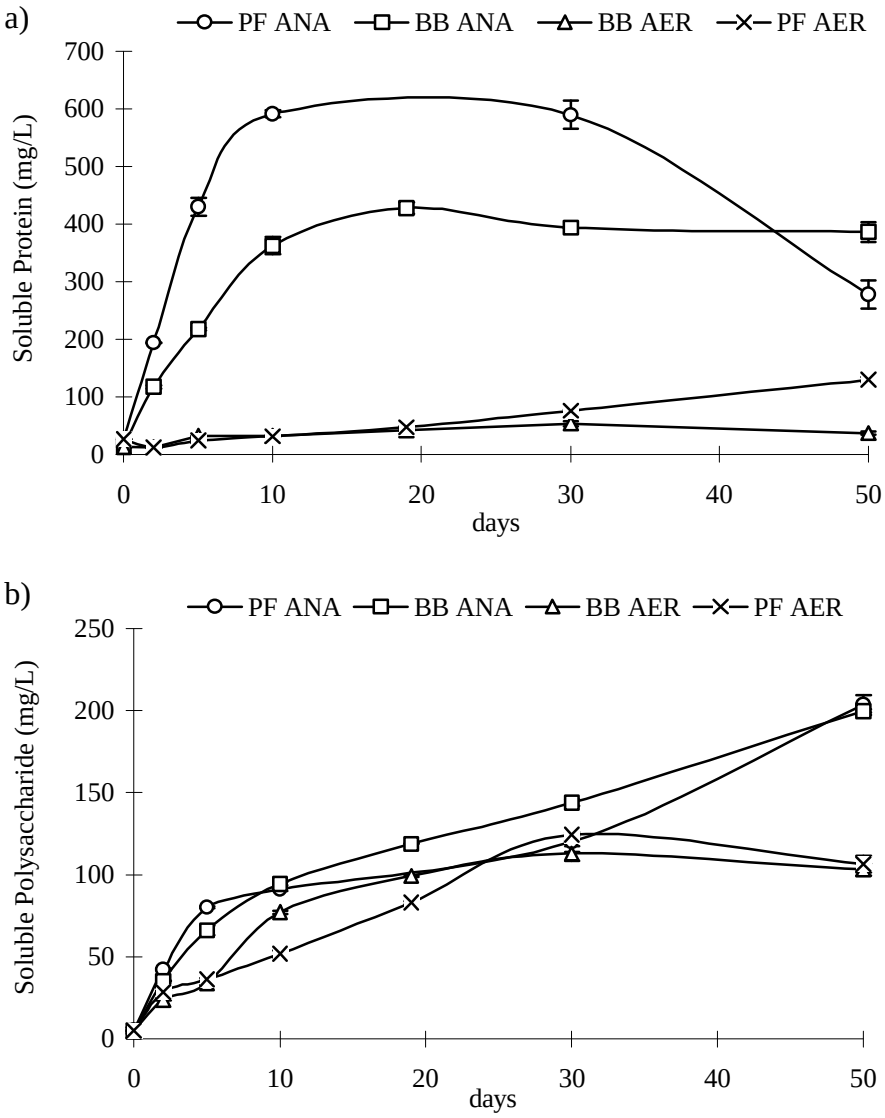


Figure 2: a) Soluble Protein, and b) Soluble Polysaccharide for Blacksburg (BB) and Pepper s Ferry (PF) Aerobic (AER) and Anaerobic (ANA) Digestion, 20°C

Table 1: Protein to Polysaccharide Ratios for Blacksburg (BB) and Pepper s Ferry (PF) Aerobic (AER) and Anaerobic (ANA) Digestion Studies

	Protein to Polysaccharide Ratio			
days	**PF AER**	**PF ANA**	**BB AER**	**BB ANA**
0	5.06	5.06	2.65	2.65
2	0.41	4.58	0.48	3.33
5	0.66	5.36	0.94	3.28
10	0.61	6.48	0.42	3.84
19	0.57	6.23	0.42	3.60
50	1.22	1.37	0.36	1.93

A relationship between the biopolymer released to the supernatant and polymer conditioning demand can be established. Figure 3(a, b, c) illustrates the polymer demand versus soluble protein, soluble polysaccharide, and total soluble biopolymer. The graphs are presented in semi-log form. In Figure 3a, as the soluble protein concentration increases the polymer demand increases substantially. A 600 mg/L soluble protein concentration yields a polymer demand of over a 100 lb. polymer/ton dry solids, whereas a 200 mg/L protein concentration yields a polymer demand of 20 lb. polymer/ton dry solids. The differences between aerobic and anaerobic digestion is again clearly shown with anaerobic digestion resulting in a higher polymer demand. Polymer demand versus soluble polysaccharide concentration is plotted in Figure 3b. The data points show no trends that link polymer demand with a given soluble polysaccharide concentration. For example, there are two soluble polysaccharide concentrations associated with the aerobic and anaerobic digestion studies that correspond to one polymer conditioning demand. An increase in the sum of the soluble protein and polysaccharide concentrations (Figure 3c) results in an increase in polymer demand. Thus, the overall soluble biopolymer demand plays a very important role in polymer conditioning requirements, however the soluble protein fraction contributes to the majority of the polymer demand.

Figure 4 illustrates specific resistance to filtration (SRF) versus soluble protein, polysaccharide, and total soluble biopolymer. The specific resistance to filtration data behave very similarly to the trends observed for polymer demand. An increase in soluble protein results in poorer dewatering properties, a phenomena reflected by the increase in the specific resistance to filtration (Figure 4a). Conversely, an increase in the soluble polysaccharide concentration (Figure 4b) results in two separate trends for the aerobic and anaerobic digestion studies that were previously observed for the polymer conditioning demand data set. This data suggests that polysaccharides do not contribute substantially to the rate of dewatering, whereas proteins are primarily responsible for the poor dewatering properties seen in the digestion process. However, the effects of the total sum of proteins and polysaccharides in solution should not be completely disregarded. The scatter in the data can be attributed to several factors. Specifically, the particle size distribution of the biopolymers released may affect the rate of dewatering, a phenomena termed blinding (Novak *et al*., 1988) and this is not accounted for in the plot. The physical instability or poor shear resistance of the digested biosolids with increased digestion time can also contribute to the scatter in both the polymer conditioning and SRF data (Phillips *et al*., 1997).

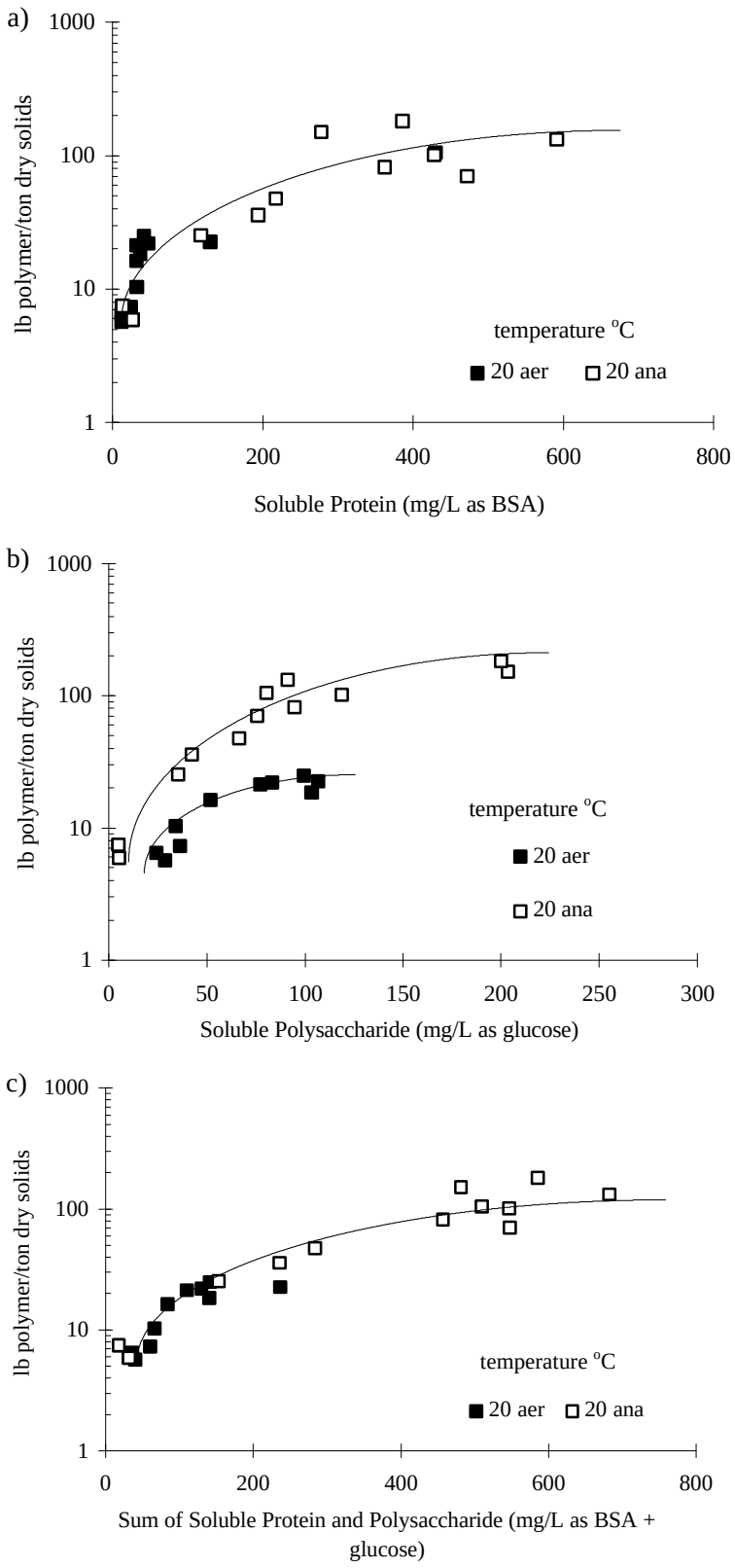


Figure 3: Polymer Demand versus a) Soluble Protein, b) Soluble Polysaccharide, and c) Sum of Soluble Proteins and Polysaccharides for Blacksburg and Pepper s Ferry Aerobic (aer) and Anaerobic (ana) Digestion Studies, 20°C

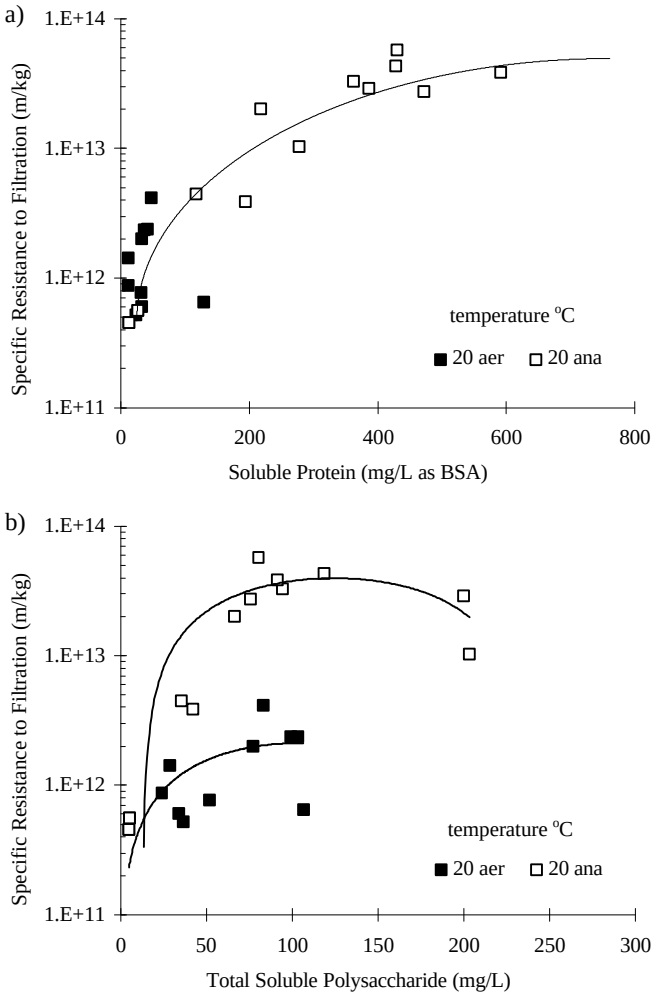


Figure 4: Specific Resistance to Filtration versus a) Soluble Protein, and b) Soluble Polysaccharide for Blacksburg and Pepper s Ferry Aerobic (aer) and Anaerobic (ana) Digestion Studies, 20°C

Role of calcium and magnesium in floc disintegration

Calcium has been implicated as a major component of floc structure because of its ability to bridge between electronegative carboxyl and phosphate groups on bacterial surfaces (Morgan *et al*., 1990; Urbain *et al*., 1993; Higgins and Novak, 1997). Higgins and Novak (1997) evaluated the relationship between exocellular biopolymer content, cations, and settling and dewatering properties of laboratory scale activated sludge systems. The authors found that an increase of bound protein was associated with a displacement of divalent cations by sodium in the floc matrix. This phenomena was associated with a deterioration in settling properties and poorer clarification.

The dissolved calcium concentrations in the aerobic digestion studies were found to be greater than 200 mg/L by day 50 in both the Blacksburg and Pepper's Ferry reactors (Figure 5). The magnesium concentrations for both aerobic reactors increased from an initial concentration of 15 mg/L to 50 mg/L by day 50. The increase in divalent cations in solution would likely be caused by two mechanisms: an ion exchange reaction in which calcium and magnesium were being replaced by another cation, or as biopolymer was

degraded, cations would be released in conjunction with volatile solids destruction. It seamed reasonable to assume that if cations are associated with biopolymer in the floc, they might also be associated with biopolymer in solution. To separate the free or dissolved cations from those bound to solution biopolymer, two measurements of calcium and magnesium were used. The ion chromatograph (IC) was used to measure dissolved cations while the total cation concentration was measured by atomic adsorption (AA). The difference between the two is the cation concentration associated with solution biopolymer.

Table 2 lists the polysaccharide concentration and the solution bound calcium and magnesium concentration in mol/L for Blacksburg and Pepper's Ferry aerobic digestion. In the Blacksburg aerobic digestion study, the bound calcium concentration increases throughout digestion time. The bound magnesium concentration exceeds the bound calcium concentration on day 10, but then decreases to non-detectable levels by day 50. For the Pepper's Ferry aerobic study, an increase in bound calcium was also observed over digestion time; the bound magnesium concentration is approximately one-quarter of the bound calcium concentration on day 10, increases to three-quarters of the bound calcium by day 19, and then finally ends at approximately one-half the bound calcium on day 50.

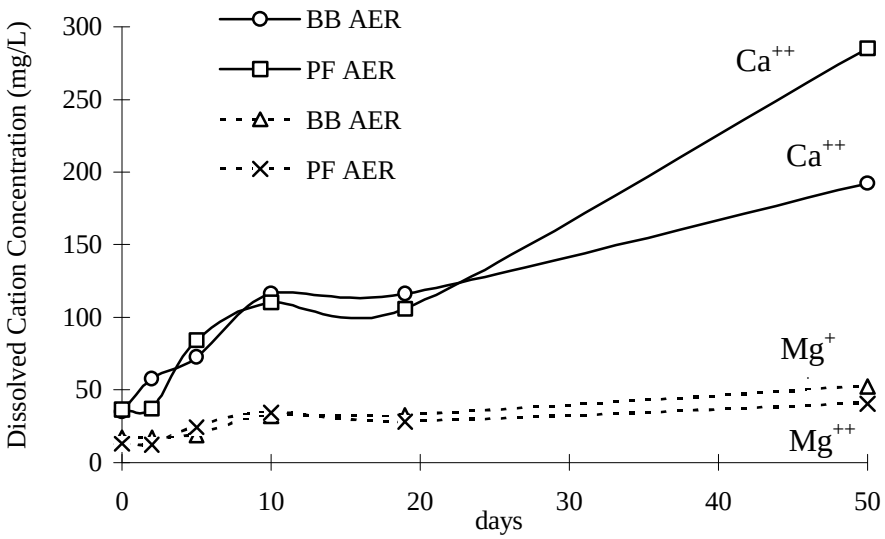


Figure 5: Dissolved Calcium and Magnesium Concentrations for Blacksburg (BB) and Pepper s Ferry (PF) Aerobic (AER) Digestion Studies, 20°C

Table 2: Solution Bound Calcium and Magnesium Relative to the Polysaccharide Concentrations for Blacksburg and Pepper s Ferry Aerobic Digestion Study, 20°C

	Blacksburg Aerobic Digestion				Pepper s Ferry Aerobic Digestion			
day	Poly. mg/L	Solution Bound Ca^{++} mol/L	Solution Bound Mg^{++} mol/L	Sum Of Divalent Cations	Poly. mg/L	Solution Bound Ca^{++} mol/L	Solution Bound Mg^{++} mol/L	Sum Of Divalent Cations
0	4.9	ND		0	5.2		ND	0
2	24.1	ND	0.09	.09	28.7	1.00	ND	1.0
5	34.0	ND	0.16	.16	36.4	0.27	ND	0.27
10	77.1	0.84	1.17	2.01	51.8	3.17	0.75	3.92
19	99.3	0.91	0.64	1.55	83.0	3.28	2.51	5.79
50	103.3	2.43	ND	2.43	106.6	3.36	1.84	5.20

(ND = Not Detectable)

The specificity of biopolymers to calcium and magnesium is unclear. The free calcium and magnesium concentrations are relatively consistent between the two sludges. The bound calcium and magnesium data do not display very clear trends, although the bound calcium concentrations are consistently higher (with the exception of one Blacksburg day 10 data point). The average influent concentrations of calcium and magnesium were investigated to assess possible reasons for the divalent cation behavior. For both activated sludge sources, the influent calcium concentration averages at .75 mol/L; the influent magnesium concentration averages at approximately half the calcium concentration. Physiological cell requirements may be partly responsible for the higher bound calcium than magnesium concentrations, as well as different organic loadings to the treatment plants.

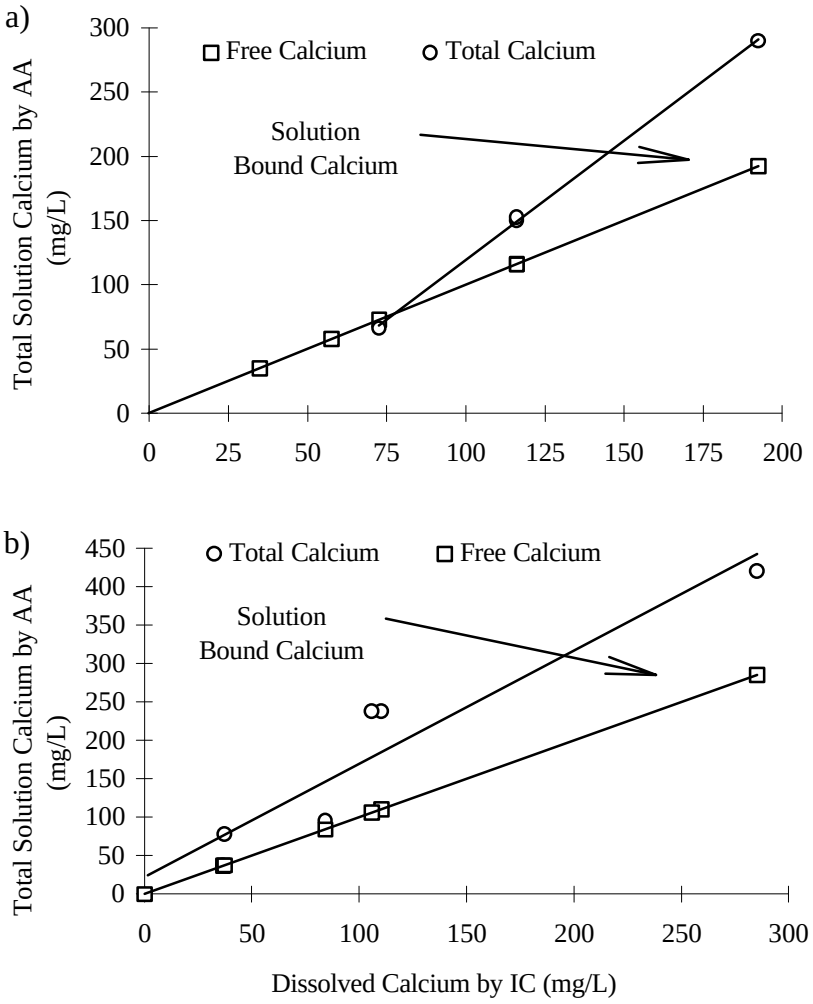


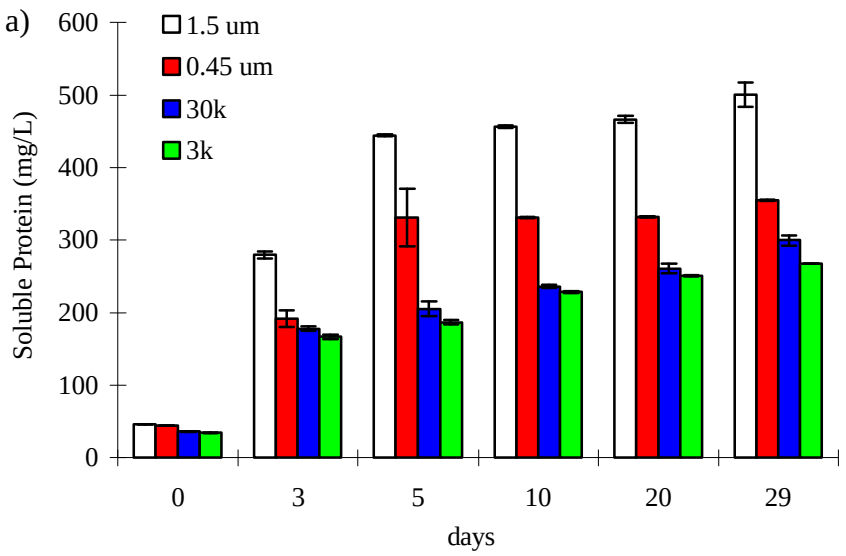
Figure 6: Partitioning of Free and Colloidal Calcium Released to the Supernatant for a) Blacksburg, and b) Pepper s Ferry Aerobic Digestion Study, 20°C

Higgins and Novak (1997) suggested that proteins and polysaccharides are linked within the biopolymer framework; they added a proteolytic enzyme and found an increase in polysaccharides in solution. The authors also suggested that a majority of the bound protein may be lectin-like which would further link protein with anionic sugar residues; this lectin-like activity might also be aided by the presence of divalent cations. Since the quantity of soluble protein in the aerobic reactors is small compared with the polysaccharide concentration, it was assumed that a majority of the calcium and magnesium would be bound to the polysaccharide biopolymer fraction, however the divalent cation bound to protein should not be disregarded. Figure 6a and b illustrates the relationship between free and bound calcium for Blacksburg and Pepper's Ferry aerobic digestion, respectively. For the Blacksburg biosolids, the trend between bound calcium and polysaccharide is evident with an increase in bound calcium and a concomitant increase in polysaccharide.

A separate anaerobic digestion study was performed to look at the molecular weight distribution of proteins and polysaccharides in solution. Referring to Figure 7a, the

molecular weight distribution of proteins in solution does not change substantially over digestion time. There is very little separation between 30k and 3k membrane pore sizes throughout the entire study. By days 20 and 29, the difference in concentration between the 0.45 µ and the 30k filters becomes smaller. This data suggests that approximately 25% of the proteins in solution are larger that 0.45 µ, and 40% of the proteins in solution have molecular weights less than 3k.

The molecular weight distribution of soluble polysaccharides is very different from the protein distribution (Figure 7b). The majority of the polysaccharides in solution at the end of the digestion study are between 0.45 µ and 30k molecular weights. This data suggests that polysaccharides in solution are much larger colloidal pieces than proteins; the smaller molecular weight fractions that are released into solution are degraded early in the digestion process. Therefore it appears that as sludge deteriorates, proteins and polysaccharides are released into solution as colloids and not as discrete monomers (Figure 8). Specifically in the aerobic study, the majority of the divalent cations are bound to polysaccharides, although the possibility of the divalent cations binding to protein should not be disregarded.



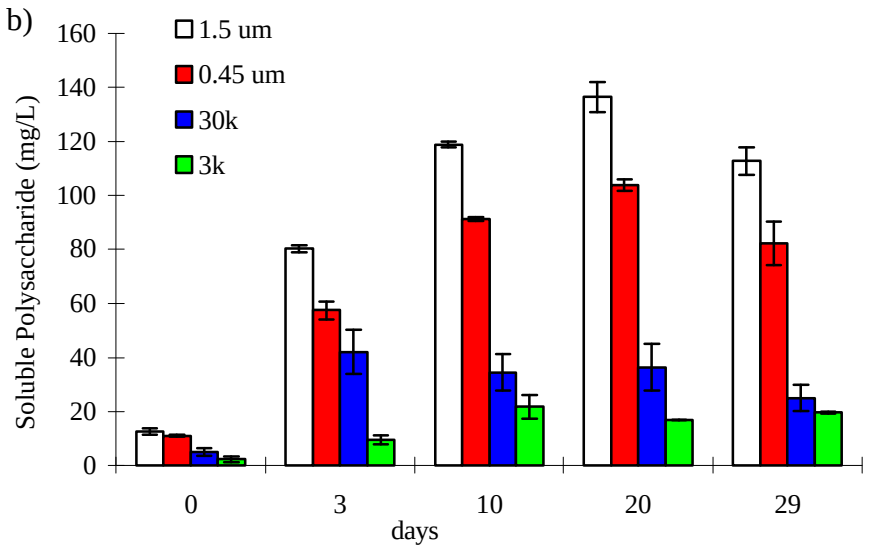


Figure 7: Molecular Weight Distributions of a) Soluble Protein and b) Soluble Polysaccharide for Pepper s Ferry Digested Biosolids, 38°C

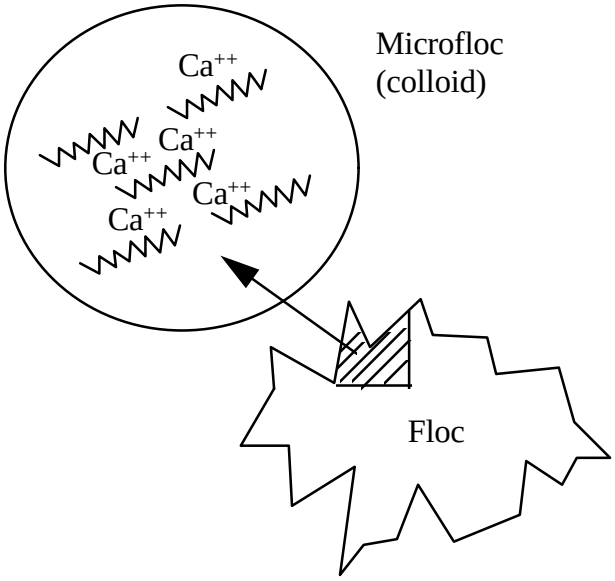


Figure 8: An illustration of floc deterioration during digestion. Proteins and polysaccharides bound by calcium are released into solution as colloids (or microflocs) and not as discrete units.

Enzymatic activity

Enzymatic activity was measured to assess any possible connection with protein and polysaccharide release. The two enzymatic assays used in this study are glucosidase and alanine-aminopeptidase. Glucosidase is an enzyme involved in the degradation of starch; alanine-aminopeptidase is involved in protein degradation. Nybroe *et al*. (1992) suggested that digested sludge should have a characteristic enzymatic profile, and enzymatic activities could be used as a control parameter for the digestion process.

Figure 9 illustrates the peptidase and glucosidase activities for Blacksburg and Pepper's Ferry aerobic digestion. For both biosolids, the enzymatic activities are similar with the peptidase activity being slightly higher; between days ten and fifty the glucosidase activities decrease close to zero. The decreased glucosidase activity helps explain the increase in soluble polysaccharide; starting at day 10 for both studies only one sample showed measurable glucosidase activity and the level was small. The decrease in Pepper's Ferry peptidase activity also reflects an increase in total soluble protein by day 50. The reasons for the drop in peptidase and glucosidase activity in aerobic digestion are unknown, but the data are consistent with results from Murthy and Novak (in press) who observed an accumulation of polysaccharide in aerobically digested sludge and little accumulation of protein over a 20 day digestion period. The enzymatic activity data of the anaerobically digested biosolids was not as consistent in reflecting the soluble biopolymer concentration.

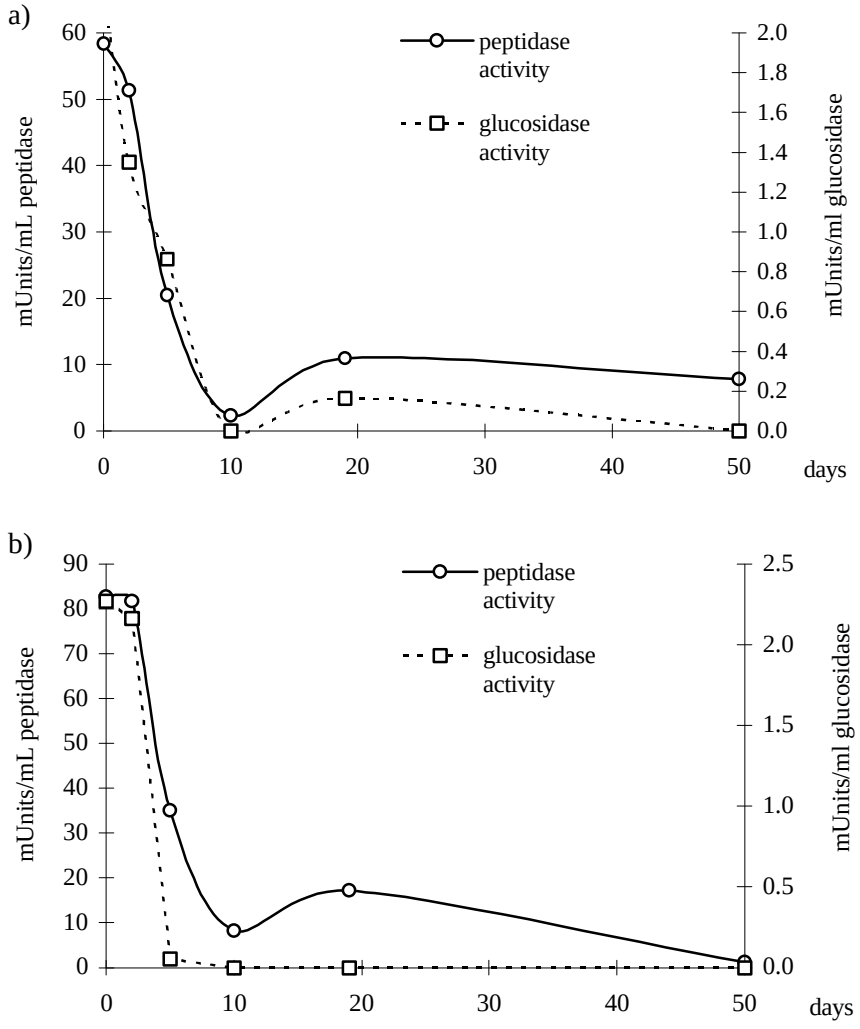


Figure 9: Peptidase and Glucosidase Activities for a) Blacksburg, and b) Pepper s Ferry Aerobically Digested Biosolids, 20°C

Proposed floc structure

A model for floc structure is proposed that accounts for changes that occur during digestion. An increase in soluble polysaccharide with a concomitant increase in bound calcium is strong evidence to suggest that calcium plays a major role in binding polyanions, with magnesium exhibiting a lesser role. Based on evidence from Higgins and Novak (1997), lectin-like proteins bind polysaccharides in the floc, with or without the stabilizing presence of divalent cations. As floc structure disintegrates under reducing conditions, biopolymer colloids are released into the supernatant bound by divalent cations. The released proteins and polysaccharides do not exist as discrete molecules, but rather as large colloids. Ammonia, generated as a by-product of cell lysis, would also act to drive divalent cations out of the floc through ion exchange reactions, thus facilitating the release of protein and polysaccharide biocolloids (Murthy *et al*., in press).

Conclusions

- Polymer demand and specific resistance to filtration increased during both anaerobic and aerobic digestion coupled with an increase in total soluble proteins and polysaccharides in solution.
- Protein release was substantially greater than the polysaccharide release under anaerobic conditions; polysaccharides were the dominant biopolymer released under aerobic conditions.
- The protein biopolymer fraction accounted for much of the polymer conditioning requirements rather than the polysaccharide biopolymer fraction.
- The proteins and polysaccharides that are released into solution are released as colloids bound by divalent cations. The relationship between calcium and magnesium specificity is unclear.

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Engineering Significance

The digestion process plays an important role in the solids handling system of a wastewater treatment process. Economical polymer conditioning requirements and ease of dewatering are two fundamental biosolid properties. High molecular weight cationic polymers and ferric chloride are common polymer conditioning agents used to condition digested biosolids. The literature suggests that the degree of mixing, the time of application in the digestion process, and the physical and chemical state of the floc affect the amount and type of conditioning agent used. Therefore, understanding floc structure and protein and polysaccharide release is an important aspect of the digestion process because of the impact these biopolymers have on the overall management of biosolids.

The type of exocellular polymer that dominates polymer conditioning and dewatering properties have long been debated in the literature. Some authors implicated proteins, some authors implicated polysaccharides, and some authors suggested humic acids are the dominant biopolymer released from the floc. In this study, comparisons between biopolymer release and polymer conditioning requirements in aerobic and anaerobic digestion reactors revealed that protein was the dominant biopolymer released in anaerobic digestion and polysaccharides were the dominant biopolymer release in aerobic digestion. In conjunction with these results, the polymer conditioning demand in the anaerobic reactor was an order of magnitude higher than in the aerobic reactor. Therefore, it was concluded that proteins function in a primary role for polymer conditioning requirements, and polysaccharides function in a secondary role. Polymer demand increased in a semi-log fashion with an increase in total soluble biopolymer.

The literature also documents the use of enzymatic profiles in monitoring and improving the digestion process. Studies on hydrolase activities in batch and semi-open systems in tomato processing studies have been performed; enzymatic activities have also been assessed for full-scale digestion processes. Our study concludes that enzymatic activity does indeed reflect the increases or decreases in soluble biopolymer release and degradation. Active or inactive enzymes may depend on the pH, the input loading into the system, or the rate of washout of the microflora. The specific reasons for the presence or

absence in enzymatic activity are unknown. From an engineering perspective, active enzymes equate to less soluble biopolymer and thus less polymer demand. If the digestion process could be designed to perform at an optimum for specific enzymes, then operating costs for digestion process would become more economical.

Cation release was also evaluated in this study. Evidence from this study suggests that calcium is more selective than magnesium in binding polysaccharides, and iron may play an important role in binding protein. The bound calcium data suggests that polysaccharides are released as colloids as the floc disintegrates. Therefore, it can be speculated that proteins are also released as colloids. The size of the biocolloids released to the supernatant influences the rate of dewatering and the polymer conditioning demand. Understanding floc structure mechanistically in wastewater treatment continues to play an important role in conceptually bridging the historical black-box approach of design to one of specific relationships between microorganisms and their environment.

Appendix A

The Effects of Temperature on Anaerobic Digestion: Implications for Conditioning and Dewatering

Mary E. Rust, Sudhir N. Murthy, and John T. Novak

The effects of temperature kinetics on biopolymer release and sludge dewatering and conditioning properties were evaluated in this study at temperatures of 30, 38, 55, and 55-36°C. Polymer demand and specific resistance to filtration increased with and increase in temperature. The polymer demand was found to be a function of the proteins and polysaccharides released to solution. Higher temperatures yielded higher biopolymer release. Protein degradation, tracked by the production of ammonia, slowed after a 3 to 5 day detention time and contributed substantially to the reduction of volatile solids. Any further volatile solids removal was most likely due to acetate degradation. The release of biopolymer was partially attributed to ammonia production and ferrous iron reduction. It was hypothesized that the solubility of iron increased with increasing temperature, and therefore might explain the similar trends in soluble COD and biopolymer.

Key words- protein, polysaccharide, dewatering, conditioning, ammonia, anaerobic, thermophilic, iron, volatile solids

The United States Environmental Protection Agency (EPA) has implemented new biosolids disposal regulations for the wastewater sludge management industry (U.S. EPA, 1989). The regulations are divided into five sections; general provisions, land application, surface disposal, pathogens and vector attraction reduction, and incineration. Class B biosolids criteria have a lower threshold for pathogen content and heavy metal content (WEF Residuals Management Committee, 1993). Class A biosolids are pathogen free and can be applied to any land application, whether it be agricultural or land with any human access (Aitken and Mullennix, 1992). The expected criteria for Class A biosolids lists specific criteria for *Salmonella* spp., viruses, protozoa, and helmiths. Criteria for these individual pathogens can be substituted for indicator organism criteria if activated sludge is treated with a high temperature process (Aitken and Mullennix, 1992). Thermophilic anaerobic digestion processes were not considered by the EPA as a feasible treatment process due to the historical instability of the process. However, any process can be used if subjected to a lengthy review for approval by the EPA (Aitken and Mullennix, 1992).

Most thermophilic digestion temperatures range from 49 to 57°C, while mesophilic temperatures range from 30 to 38°C. Thermophilic digestion is kinetically faster than mesophilic digestion; every 10°C temperature change doubles the biochemical reaction rate (Metcalf and Eddy, 1991). Higher temperatures decrease the amount of time and the size or number of digestors required to stabilize solids. Despite the advantage of a faster biochemical rate, thermophilic digestors have historically been associated with numerous

undesirable process qualities, among them high energy costs, odor, instability, and a poor quality supernatant (Metcalf and Eddy, 1991).

The current question posed to scientists and engineers becomes one of a practical importance. Many of the advantages and disadvantages of thermophilic digestion are well documented with regard to 503A regulations, however, some of the impacts on plant operations (filtrate recycle, sludge handling, etc.) are not well understood. Recent research on Temperature Phased Anaerobic Digestion (TPAD) has shown it to be a successful thermophilic process. Sludge is initially thermophilically digested and then transferred to a mesophilic digestor (Streeter *et al*., 1997). The advantages of TPAD include increased volatile solids destruction, increased biogas production, decreased coliform count, lower retention times, and less foaming (Streeter *et al*., 1997). Sturgeon Bay Utilities, Wisconsin, was required to convert their single stage mesophilic anaerobic digestion process to TPAD in order to achieve Class A pathogen reduction in their biosolids. The dry solids content of digested biosolids increased from 13.4% to 19.8%. Furthermore, the polymer demand decreased 35% (Vik and Olsen, 1997). Thus, the application of a TPAD system was a highly successful venture at Sturgeon Bay Utilities.

Autothermal thermophilic aerobic digestion (ATADs) is another high temperature process for the stabilization of biosolids. The ATAD process uses oxygen to accelerate volatile solids destruction at temperatures between 50 and 65°C. ATADs are considered autothermal because most of the heat is generated by endogenous respiration of microorganisms (Murthy *et al*., submitted). College Station, Texas, ATAD systems began operation in 1995. The polymer conditioning costs before the College Station ATAD system startup averaged at $25/ton dry solids; after startup the polymer demand increased to over $200/ton dry solids (Murthy *et al*., submitted). The TPAD and the ATAD give two examples of a thermophilic digestion process with very different solids handling issues.

The implications with regard to polymer conditioning and dewatering of different thermophilic systems depend on the reactor design and process kinetics. The objective of this study was to examine the effects of temperature on polymer conditioning and dewatering requirements of digested biosolids. As thermophilic digestion becomes more popular, it is important to understand the effects of temperature kinetics from the solids handling perspective, the reduction of overall operating costs being the primary goal. Thermophilic digestion may exceed EPA criteria for pathogen destruction, but it is the municipal wastewater treatment plants that have to ultimately pay for conditioning and dewatering of the biosolids.

Methods and Materials

Experimental approach Anaerobic batch reactors were maintained at different temperatures (30,38, 55, and 55-36°C) to examine differences in polymer conditioning requirements, dewatering properties. Biopolymer (proteins and polysaccharides), ammonia generation, and soluble COD in the supernatant were examined to provide any clues for the reasons behind the deterioration in biosolids properties.

Sludge matrix Activated sludge from Pepper's Ferry Regional Wastewater Treatment Authority was used in this study. An industry discharging into the Pepper's Ferry wastewater treatment plant network utilizes sulfuric acid in their process and then neutralizes with sodium hydroxide. Consequently, Pepper's Ferry possesses high sodium (250 - 500 mg/L Na^+) and sulfate (> 450 mg/L SO_4) in their influent. Conversely, Blacksburg receives relatively low sodium and sulfate in their influent (~ 50 mg/L Na^+ and SO_4). The Pepper's Ferry plant is a conventional plant with an aerated activated sludge process and an anaerobic digestion process. The Blacksburg plant has recently been retrofitted to function as an biological nutrient removal plant; an anaerobic zone exists in the activated sludge basin. Therefore, the activated sludge properties are very different between the two plants and make ideal comparisons for this study. All sludge used for the digestion studies were taken from the recycled activated sludge (RAS) line, and concentrated to 1.1 to 1.3% solids.

Reactor configuration and sampling Anaerobic batch reactors were operated at temperatures of 30, 38, 55, and 55-36°C. The activated sludge used for the thermophilic studies were subjected to a preliminary acclimation period for 8 days at 55°C. The reactors used for data collection were spiked with the acclimated culture. The reaction vessels, 1.5 L reactors, were placed in a shaking water bath. The reactor for the thermophilic/mesophilic study was taken out of the thermophilic water bath on day four and placed in a separate water bath at 36°C for the remainder of the experiment. Samples were spun at 10,000 *g* for 15 minutes. The supernatant was removed and filtered through a 1.5 um filter. The biopolymer concentration in the filtered supernatant was considered the soluble biopolymer fraction.

Biopolymer analysis Protein concentration was measured using the Hartree (1972) modification of the Lowry *et al*. (1951) method. Polysaccharide was measured using the Dubois *et al*. (1956) method. Bovine serum albumin and glucose were used as protein and polysaccharide standards, respectively.

Dewatering and conditioning properties Mixed liquor and volatile suspended solids (MLSS and MLVSS) were determined using Method 2540D and 2540E of *Standard Methods* (1995), respectively. Specific resistance to filtration (SRF) was performed as described by Christensen and Dick (1985). The capillary suction time (CST) was determined using Method 2710G of *Standard Methods* (1995). Optimum polymer conditioning tests were determined using a low molecular weight cationic polymer at 0.5%.

Ion analysis Free ammonia was measured using a Dionix (DX120) Ion Chromatograph containing a CS12 column and conductivity detector (Dionex 2010X) with a self generating supression of the eluent. The eluent, methane sulfonic acid at 20 mM, ran at a flow rate of 1.0 mL/min. Total soluble ferrous iron was measured using an Atomic Absorption Spectrophotometer.

COD analysis Soluble COD was analyzed using Method 5220C of *Standard Methods* (1995).

Acetate analysis Acetate was measured with a Hewlett-Packard 5880 gas chromatograph and a flame ionization detector.

Results and Discussion

The primary goal of this study was to examine the effects of temperature on polymer conditioning requirements and dewatering properties for anaerobically digested activated sludges. Biopolymer (protein and polysaccharide) release, ammonia generation, acetate release, and COD release to the supernatant were analyzed to identify specific reasons for the changes in biosolids properties. The relationship between volatile solids reduction and protein degradation was also evaluated to assess the significance of detention time and temperature.

The release of biopolymer from activated sludge flocs into solution has recently been implicated in the deterioration of sludges during digestion (Rust *et al*., submitted; Murthy *et al*., submitted). The ATAD biosolids research indicated that released biopolymers were responsible for excessive polymer conditioning costs and poor dewatering properties (Murthy *et al*., submitted). Increases in proteins and polysaccharides were observed with an increase in detention time through three ATAD reactors in series; the thermophilic (and third) ATAD reactor showed a 2080 mg/L level of protein and a 900 mg/L level of polysaccharide in the supernatant (Murthy *et al*., submitted).

Figure 1 shows the polymer demand and specific resistance to filtration (SRF) data versus total soluble biopolymer at different temperatures for anaerobically digested sludges from two sources. The graphs are presented in semi-log form. Both the polymer demand and the SRF data increase with an increase in total soluble biopolymer (total soluble biopolymer is the sum of proteins and polysaccharides released to the supernatant). The effects of temperature on polymer conditioning can clearly be seen. Referring to Figure 1a, a 400 mg/L difference in total soluble biopolymer between the 30°C and the 55°C study results in an order of magnitude increase in polymer demand. More scatter is seen in the SRF data than the polymer conditioning data (Figure 1b), probably due to a blinding phenomena that is not accounted for in the plot (Novak *et al*., 1988). The increase in soluble biopolymer and the concomitant deterioration in dewatering properties agree with the data collected by Murthy *et al*. (submitted).

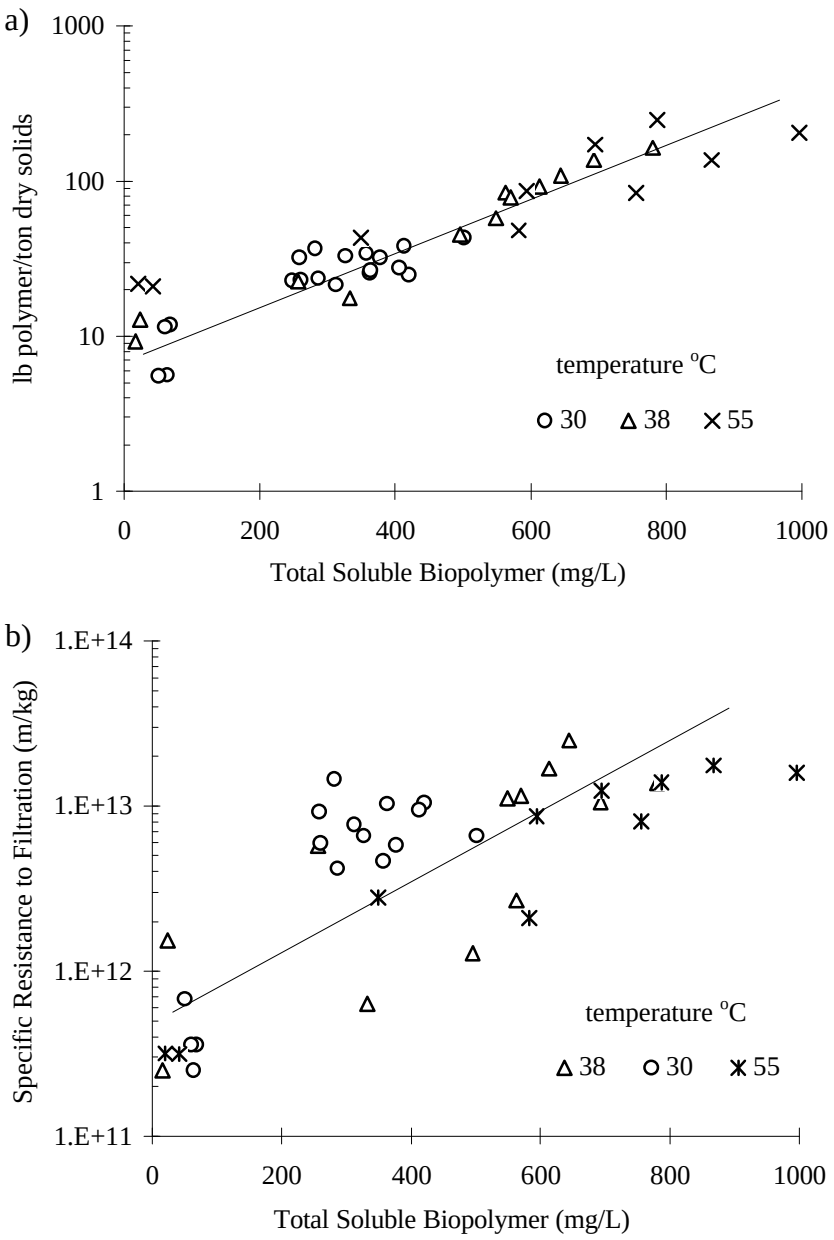

Figure A1: a) Polymer Demand, and b) Specific Resistance to Filtration versus Total Soluble Biopolymer Concentration for Blacksburg and Pepper s Ferry Anaerobic Digestion Studies at 30, 38, and 55°C

The dewatering data in Figure 1 was plotted over time in order to evaluate specific temperature trends associated with the digestion process. Figure 2 illustrates the effect of temperature on polymer demand and the specific CST for Pepper's Ferry and Blacksburg digested biosolids. In Figure 2a and b, the polymer demand is shown to increase with increasing temperature. The 30°C study shows a gradual increase in polymer demand and little deterioration in dewatering properties; in the 55°C study the increase in polymer demand and the deterioration in dewatering properties is rapid and substantial.

Specifically, the polymer demand for the 55°C study is almost twice the demand for the 38°C study and four times the demand for the 30°C study.

The thermo/meso study was an imitation of the TPAD process (Figure 2a and c). The reactor was allowed to digest for four days at 55°C, and then digested at 36°C for the remainder of the study. The polymer demand for the thermo/meso study (Figure 2a) follows the 55°C study until day 4; the temperature change induces an immediate leveling off of the polymer requirements and then a gradual increase. The CST data (Figure 2c) in the thermo/meso study also follows the 55°C trend until the temperature changes. The deterioration then slows but continues to increase.

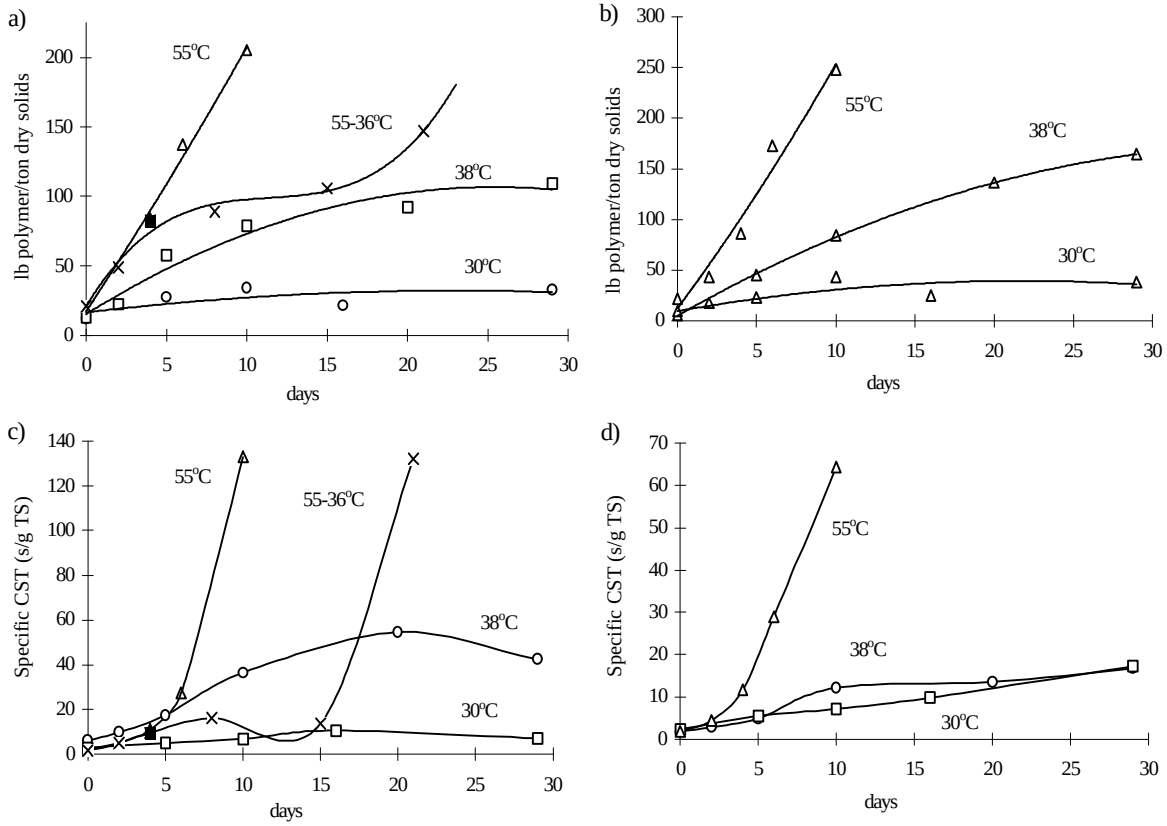

Figure A2: a) Polymer Demand, and c) CST for Pepper s Ferry Anaerobic Digestion Studies; and b) Polymer Demand, and d) CST for Blacksburg Anaerobic Digestion Studies at Temperatures of 30, 38, 55, and 55-36°C. The black square in a) and c) represents the change from thermophilic to mesophilic conditions.

An explanation for the increased polymer demand at higher temperature ranges may be found by evaluating the protein and polysaccharide releases over time. Figure 3 illustrates the protein and polysaccharide releases at different temperatures over time for Pepper's Ferry and Blacksburg digested biosolids. Both the protein and polysaccharide releases follow the same trend as the polymer demand; an increase in protein and polysaccharide release is seen with an increase in temperature. The interesting aspect of this data is that

the highest rate of protein release appears to take place within the first 5 days of digestion regardless of temperature (indicated by dotted line). The proteins and polysaccharides then level off in a manner characteristic of the increase in temperature. A decrease in the temperature of the biosolids, as shown in the thermo/meso study, results in a immediate decline in the quantity of biopolymer released to the supernatant (Figure 3a and c).

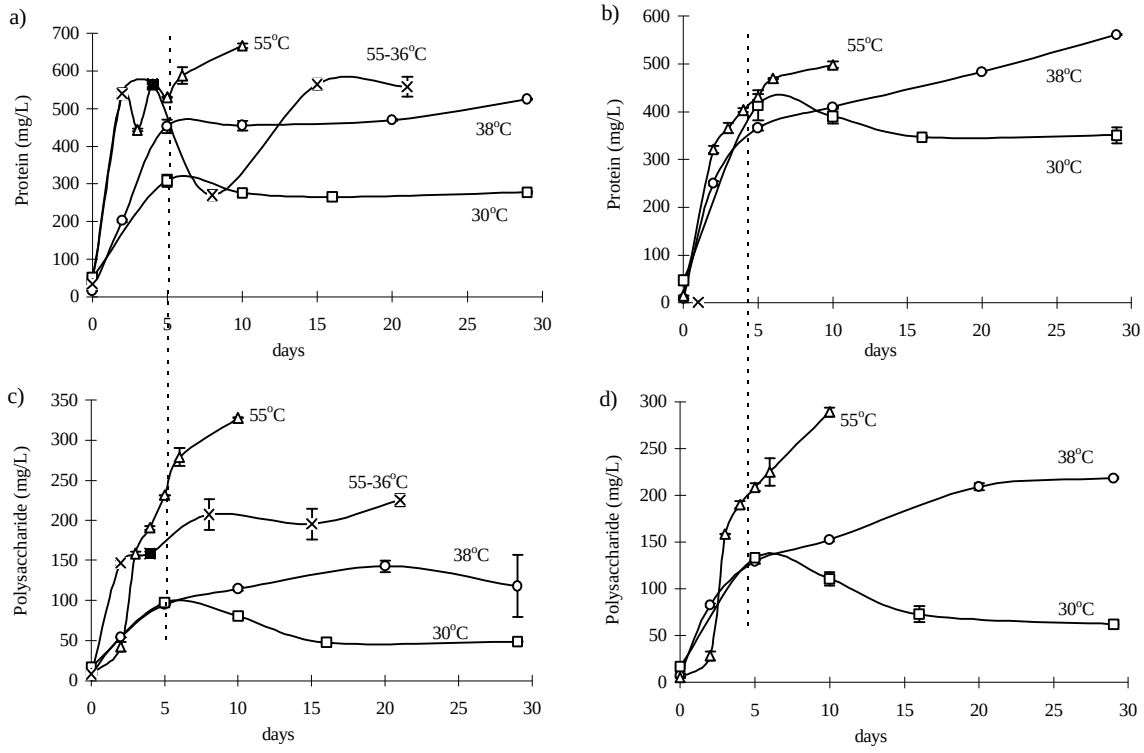

Figure A3: a) Protein, and b) Polysaccharide for Pepper s Ferry Anaerobic Digestion Studies; and c) Protein, and d) Polysaccharide for Blacksburg Anaerobic Digestion Studies at Temperatures of 30, 38, 55, and 55-36°C. The black square in a) and b) represents the change from thermophilic to mesophilic conditions.

The ammonia released to the supernatant is illustrated in Figure 4a and 4b for Pepper's Ferry and Blacksburg digested biosolids, respectively. With the exception of the 30°C study for both biosolids, the ammonia generation peaks at approximately 200 mg/L by day 5 (shown by the dotted line). Proteins contain approximately 16% nitrogen (Metcalf and Eddy, 1991). Thus, the 200 mg/L ammonia concentration represents approximately a 1250 mg/L degradation of protein. Concurrently, large releases of biopolymer from the floc is observed up to day 5, greater than 600 mg/L for the 55°C study. The ammonia generation may be partly responsible for the biopolymer release by driving divalent cations out of the floc through ion exchange reactions (Murthy *et al.*, in press).

After day 5 the ammonia generation levels off, as shown in Figure 4a and b, suggesting that no substantial protein degradation is taking place. The data in Figures 4 and 5

suggest that a majority of the protein degradation happens within the first few days of the digestion process, regardless of temperature. Therefore ammonia generation may be a good indicator for the optimum detention time of a thermophilic process, such as TPAD. Thermophilic detention times could possible be shortened, thus preventing excessive polymer conditioning demands. Furthermore, the biopolymer releases gradually increase beyond day 5 characteristic of the temperature. The releases contribute to the poor dewatering properties observed in Figure 1.

Different biopolymer data trends were observed in pilot plant data from I.D.I. in Indianapolis. The I.D.I. pilot plant is a TPAD process with an initial thermophilic digestor followed by a mesophilic digestor. The protein and polysaccharide concentrations were 705 mg/L and 357 mg/L, respectively, in the supernatant of the thermophilic digestor; the biopolymer concentrations then declined in the mesophilic digestor to 182 and 101 mg/L for proteins and polysaccharides, respectively (Table 1). An ammonia increase of 235 mg/L equates to a 1500 mg/L degradation of protein, some of which can be attributed to the decrease of biopolymer in the supernatant. Therefore, the batch data presented in this study is not entirely representative of continuous systems. A continuous system, unlike a batch system, could maintain a culture that would degrade the soluble biopolymer fraction as well as a further reduction in volatile solids.

Table A1: Biopolymer and Ammonia for the I.D.I. Pilot Plant, Indianapolis

	Protein	Polysaccharide	Ammonia
Thermophilic digestor	705 mg/L	357 mg/L	325 mg/L
Mesophilic digestor	182 mg/L	101 mg/L	560 mg/L

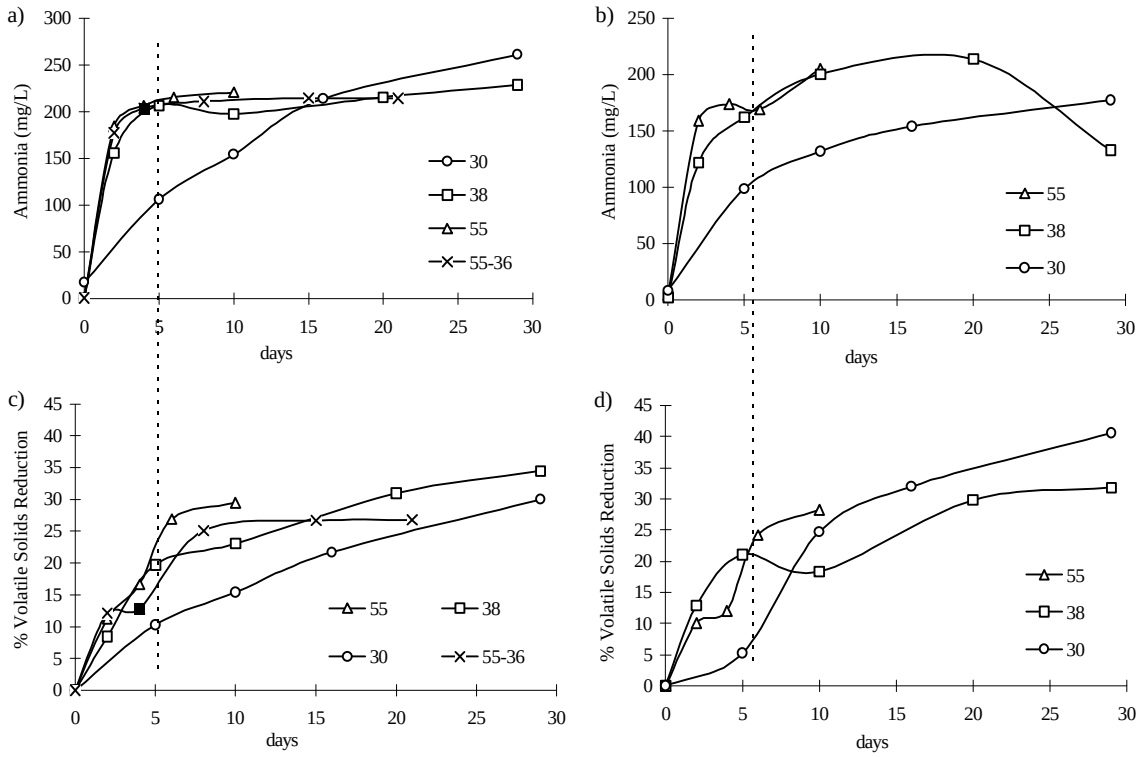

Figure A4: a) Ammonia, and c) % Volatile Solids Reduction for Pepper s Ferry Anaerobic Digestion Studies; and b) Ammonia, and d) % Volatile Solids Reduction for Blacksburg Anaerobic Digestion Studies at Temperatures of 30, 38, 55, and 55-36°C. The black square in a) and c) represents the change from thermophilic to mesophilic conditions.

Volatile solids reduction was evaluated to assess any particular trends with ammonia generation. Figure 4c and d illustrate the increase in volatile solids reduction over time for Pepper's Ferry and Blacksburg, respectively. The greatest increase in volatile solids reduction appears to coincide with ammonia generation (indicated by the dotted line), with the exception of the 30°C studies. The continued gradual increase in volatile solids degradation is most likely due to the degradation of acetate (Figure 5c and d).

Rust *et al*. (submitted) hypothesized that iron plays a substantial role in the binding of proteins and the flocculation of proteins as ferric hydroxide. The authors observed a large ferrous iron release to the supernatant with a concomitant protein release under anaerobic conditions. For these set of experiments, we hypothesize that iron release is a function of temperature; at a higher temperature, the kinetics of iron reduction increases along with the solubility of iron, therefore more protein is released into solution. Figure 6 illustrates the total soluble ferrous iron release for Pepper's Ferry digested biosolids at different temperatures. This data tentatively agrees with our hypothesis; the rate of ferrous iron release from the floc increases with an increase in temperature. The initial ferrous iron decline is most likely due to precipitation with sulfides.

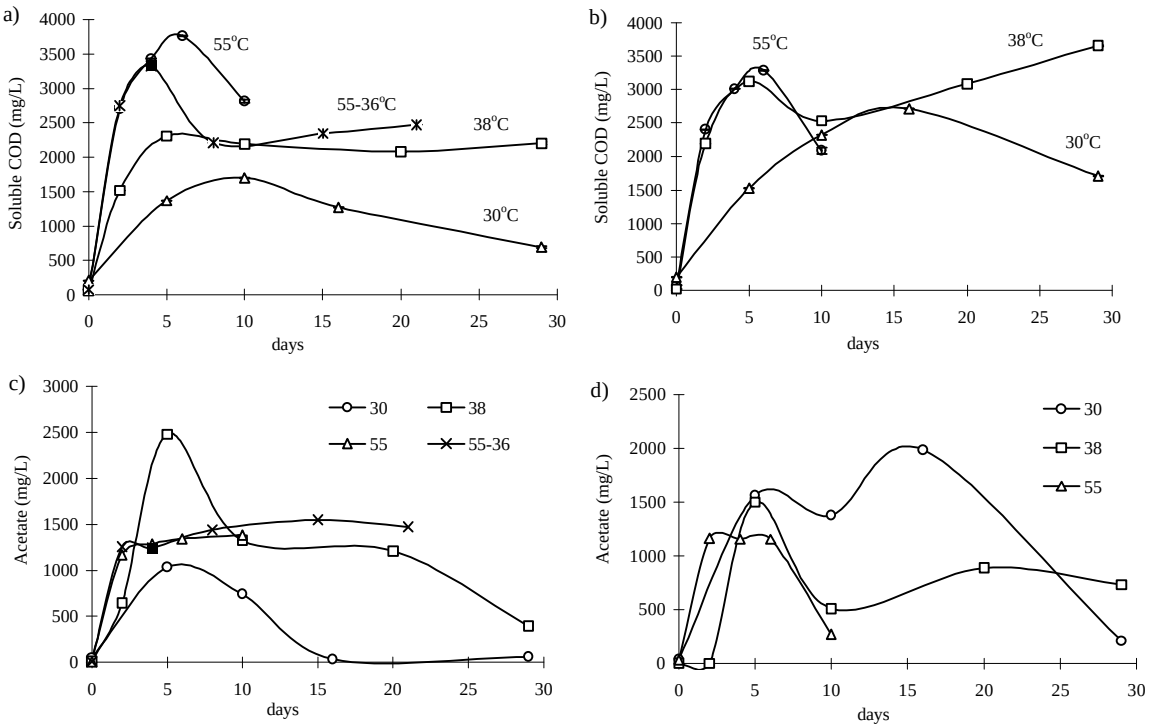

Figure A5: a) Soluble COD, and c) Acetate for Pepper s Ferry Anaerobic Digestion Studies; and b) Soluble COD, and d) Acetate for Blacksburg Anaerobic Digestion Studies at Temperatures of 30, 38, 55, and 55-36°C. The black square in a) and c) represents the change from thermophilic to mesophilic conditions.

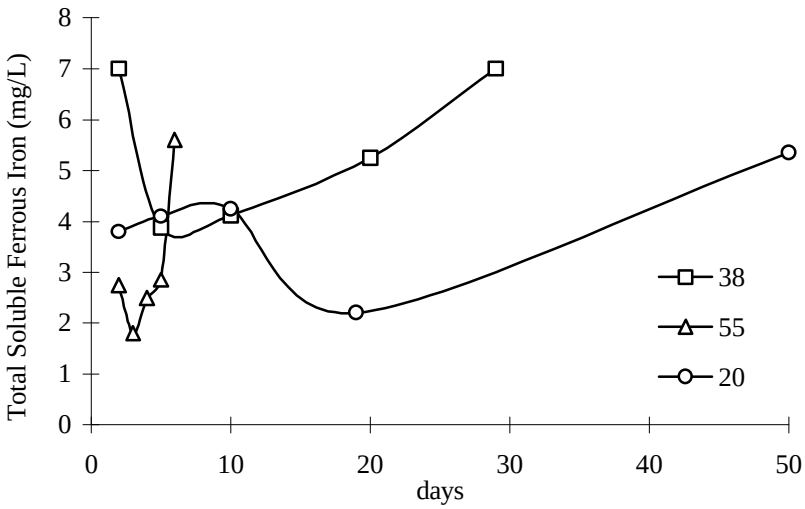

Figure A6: Total Soluble Ferrous Iron Released for Pepper s Ferry Anaerobic Digestion Studies at Temperatures of 20, 38, and 55°C.

Discussion

Protein degradation, indicated by ammonia generation, partially contributed to the volatile solids removal during the digestion process. Polysaccharide degradation also contributing to volatile solids removal, could not be tracked other than the production of acetate. Our batch data suggests that protein degradation ceases after day five of the digestion process regardless of temperature. Therefore ammonia generation may be a good indicator for the detention time required for a thermophilic process, such as TPAD, as long as the detention time meets EPA criteria for pathogen reduction at a given temperature. Furthermore, excessive polymer conditioning demands could be prevented by lowering the temperature of the biosolids earlier in the digestion process, a phenomena demonstrated by the thermo/meso study.

The soluble biopolymer fraction also continues to increase after ammonia generation stops. The soluble biopolymer fraction contributes substantially to the polymer conditioning demand and the poor dewaterability of a sludge. Our data suggests that biopolymer release is dependent on temperature; an increase in temperature resulted in an increase in the quantity of biopolymer released to the supernatant. Thus, a lower mesophilic temperature may be a viable economic alternative to a TPAD process since polymer conditioning costs would decrease substantially with a decrease in temperature.

Higgins and Novak (1997) demonstrated that the cation content in the feed significantly affects floc strength in relation to activated sludge settlability and dewaterability. A high sodium influent will result in a weaker floc and thus cause poorer settling and dewatering. Conversely, a sludge with a high divalent cation content will not release as much biopolymer from the floc into solution as a sludge with a high monovalent content. Therefore, volatile solids reduction can be misconstrued to be a volatile solids removal (Murthy *et al*. in press). Any biopolymer content in the supernatant will be filtered out in a solids test, and will render the volatile solids content of the system to be lower than the actual value. Thus, a biopolymer release into solution constitutes a volatile solids removal, and not a true volatile solids reduction. A treatment plant with a poorer sludge may find it easier to meet EPA criteria for volatile solids reduction due to the removal of volatile matter from the floc (biopolymer release), but the plant will have substantial solids handling difficulties. In contrast, an activated sludge with a high divalent cation content may not be able to achieve as high of a volatile solids “reduction” with the same detention time, but will find it easier to dewater the digested biosolids.

Mechanistically, the biopolymer release can be attributed to one of two phenomena. Ammonia, generated as a by-product of cell lysis within the floc, would act to drive out divalent cations through ion exchange reactions (Murthy *et al*., in press) and thus facilitate the release of biopolymer. Iron may also play an important role in protein release. We hypothesize that ferrous iron release is a function of temperature; at higher temperature kinetics the solubility of iron becomes greater and therefore more protein is released into solution.

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