THE ROLES AND INTERACTIONS OF CATIONS, PROTEINS, AND POLYSACCHARIDES IN THE
SETTLING AND DEWATERING OF ACTIVATED SLUDGE.

by

Matthew J. Higgins

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APPROVED:

J. T. Novak, Chairperson

C. W. Randall

J. C. Little

N. G. Love

R. E. Benoit

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(ABSTRACT)

The roles of cations and exocellular biopolymer on the settling and dewatering of activated sludge was investigated. Both laboratory and full-scale activated sludge systems were examined. The results of the study showed the settling and dewatering properties of the activated sludge were dependent on the calcium, magnesium, potassium and sodium concentrations added to the feed. A minimum of 0.72 meq/L each of calcium and magnesium in the feed was necessary for acceptable settling and dewatering. Two types of microbial cultures were observed, one required both calcium and magnesium in the feed while the other required either calcium or magnesium, but not both, for optimization of settling and dewatering. Sodium addition to the feed improved the settling of activated sludge when the ratio of sodium to calcium plus magnesium was equal to approximately one on a meq/L basis. When this ratio was greater than two, the settling and dewatering properties deteriorated, but the deterioration could be reversed by increasing the calcium and magnesium concentration in the feed. In general, the data supported the cation bridging model for bioflocculation, and the cations act to bind protein to the biofloc structure.

Results from the full-scale activated sludge plants correlated well with results from laboratory activated sludge systems and demonstrated the cation content in these systems had a direct impact on the settling and dewatering properties. Field trials in
which divalent cations were added to activated sludge systems resulted in dramatic improvements in the settling properties of these systems.

Characterization of the exocellular protein extracted from laboratory, industrial and municipal activated sludges revealed the presence of a single protein, which appears to be a lectin. The molecular weight of the protein measured by SDS PAGE was approximately 16,000 Daltons with similar amino acid composition as microbial lectins. Also, amino acid sequencing analysis indicated the N-terminal sequence of the protein was consistent with those of microbial lectins. In addition, the activated sludge cultures exhibited lectin activity as demonstrated by binding site inhibition experiments. A model of bioflocculation that includes the role of protein was proposed.
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I. INTRODUCTION

A key aspect to the efficiency of activated sludge systems is the separation of the biological solids from the liquid phase, and ultimately the dewatering of these biosolids. Separation results from the aggregation of bacteria into flocs which can then be removed by sedimentation. Problems with treatment efficiency are most frequently linked to the solid/liquid separation step. In addition, dewatering of solids from the activated sludge process can represent a significant portion of the overall operation and maintenance costs of these systems.

Despite the importance of the settling and dewatering of activated sludge, a complete understanding of the mechanisms involved in these processes is not available. Researchers have shown the interactions between exocellular biopolymers and cations are involved in the flocculation in activated sludge systems. Exocellular biopolymers are produced by bacteria, and they can remain attached to the cell surface as a capsule or excreted into the medium as a soluble fraction. These biopolymers mainly consist of protein and polysaccharides. Structural analysis of the biopolymers has shown that they contain negatively charged functional sites such as carboxyl groups which contribute to the overall negative charge of bioflocs. It is believed that divalent cations bridge between the negatively charged functional groups of biopolymers, thereby binding biopolymers and stabilizing the exocellular matrix. The divalent cations can be extracted from the floc by ion-exchange reactions such as with high concentrations of monovalent cations like sodium. Exchange of divalent cations by monovalent cations typically leads to a deterioration in floc properties such as settling and dewatering. Therefore, a cation balance likely exists that will optimize the binding of exocellular biopolymer within bioflocs which will optimize settling and dewatering properties.
However, this balance has not been defined. In addition, the relative roles of protein and polysaccharide in bioflocculation has not been determined. Most research has focused on the role of polysaccharide in bioflocculation even though most of these same studies have shown the exocellular protein concentrations are greater than the polysaccharide concentrations.

OBJECTIVES

The objectives of this research were to examine the effect of cations on the settling and dewatering properties of activated sludge. The research was aimed at defining a cation balance that optimized settling and dewatering properties, and also in defining cation balances that caused or led to poor settling and dewatering. If successful, the data could then be used as a diagnostic tool to assess the cation content of activated sludge systems and how it may be affecting the settling and dewatering properties.

In addition, the interactions between exocellular biopolymers and cations were investigated to determine the relative roles of biopolymer in bioflocculation. An emphasis was placed on the isolation and characterization of the exocellular protein in activated sludge systems.
II. THE EFFECT OF CATIONS ON THE SETTLING AND DEWATERING OF
ACTIVATED SLUDGES - LABORATORY RESULTS

ABSTRACT

The effect of cations on the settling and dewatering of activated sludge was
investigated using laboratory scale activated sludge reactors. Bactopeptone was used as
feed to the reactors due to its low cation concentration. The results of the study showed
the settling and dewatering properties of the activated sludge were dependent on the
calcium, magnesium, potassium and sodium concentrations added to the feed. A minimum
of 0.72 meq/L each of calcium and magnesium in the feed was necessary for acceptable
settling and dewatering, below this concentration, settling and dewatering properties
were very poor. Two types of microbial cultures were observed; one required both
calcium and magnesium in the feed while the other required either calcium or
magnesium, but not both, for optimization of settling and dewatering. The poor settling
and dewatering of suspensions receiving no additional cations could be improved by batch
addition of calcium and/or magnesium. However, improvements were greater when an
equal concentration of the cation was added to the feed. Sodium addition to the feed also
improved the settling of activated sludge at a ratio of sodium to calcium plus magnesium
equal to approximately one on a meq/L basis. Above a sodium to calcium plus magnesium
ratio of 2 to 1, the settling and dewatering properties deteriorated, but the deterioration
could be reversed by increasing the calcium and magnesium concentration in the feed to
reduce the ratio of sodium to calcium and magnesium. Not only were the absolute
concentrations of the cation important to settling and dewatering, but the ratios of
sodium to calcium and magnesium were important as well.
Keywords. activated sludge, bioflocculation, settling, dewatering, cations, exocellular biopolymers.

INTRODUCTION

A key aspect to the efficient operation of activated sludge systems is the separation of the biological solids from the liquid phase and the subsequent dewatering of these biosolids. Separation results from the aggregation of bacteria into flocs which can then be removed by sedimentation. Despite its obvious importance, the mechanisms involved in floc formation and the factors influencing floc strength are not well understood. Researchers have shown that cations and exocellular biopolymers produced by microbes are involved in this process, however, the mechanisms for their involvement have been disputed. For example, several researchers have suggested cations aid in flocculation by bridging negative sites on exocellular biopolymers (Tezuka, 1969, Novak and Haugan, 1978 and Bruus et al., 1992). Zita and Hermansson (1994) alternatively suggested that cation induced flocculation and deflocculation could be explained by double layer compression theory.

Most of the investigations into the role of salts in bioflocculation used batch experiments in which dilution or exchange processes were used to extract cations from the bacterial suspensions, resulting in deteriorating sludge properties. Upon replacing the cations, sludge properties improved, thereby demonstrating the physical/chemical role of cations in flocculation (Pavoni et al., 1972, Tezuka, 1969, Novak and Haugan, 1981). Only a limited number of studies have examined the effect of cations in the growth media and these studies have provided conflicting results. For example, Tezuka
(1969) showed that floculant growth of a *Flavobacterium* strain was dependent on the presence of both calcium and magnesium. However, Endo et al. (1976) reported floculant growth of a *Flavobacterium* occurred only when adequate concentrations of calcium were present in the growth media, and that magnesium was not necessary for floculation. Angelbeck and Kirsch (1969) found dispersed growth occurred with a strain of *Zoogloea ramigera* when calcium and magnesium were present in the media; floculant growth occurred when calcium and magnesium were removed from the media by addition of chelating agents. Shimizu and Odawara (1985) reported an *Agrobacterium* isolated from activated sludge flocculated well with only magnesium added to the growth media, and flocculation decreased with only calcium added to the media. These studies were conducted on several different monocultures, grown in batch mode. Although the bacteria used in these studies were cultured from activated sludge systems, the results cannot be applied to the wide variety of organisms in these systems, especially since the studies reported conflicting results. Also, these studies did not assess the effect of cations on dewatering properties.

The purpose of this research was to determine the effect of cations on floculation, settling and dewatering in the mixed populations found in activated sludge systems using continuous culture operation, and to define a cation balance that optimized settling and dewatering characteristics. The research was also used to evaluate the mechanisms involved in floc formation and resistance to shear. These data can then be used as a diagnostic tool to assess full-scale activated sludge systems to determine if settling and dewatering can be improved by adjustments in the cation balance.
METHODS AND MATERIALS

Materials. The cations used in this study, Ca++, Mg++, Na+, and K+ were added as laboratory grade chloride salts.

Laboratory Activated Sludge System Setup and Operation. Ten liter, continuous flow, bench scale reactors were used to simulate the activated sludge process. The reactor configuration is shown in Figure 2.1. The reactors consisted of a complete mix zone and a settling zone that were separated by a slanted baffle. An aeration stone provide air and mixing to the system. Bactopeptone was used as feed to the reactor at a concentration of 300 mg/L. The concentration of several cations and nutrients in the bactopeptone feed are given in Table 2.1. The low concentration of calcium and magnesium in the feed allowed these parameters to be increased to provide a range of divalent cation concentrations. The influent pH was consistently near 7.00 for all feed conditions. The hydraulic detention time for all tests was 0.5 days and the sludge age was maintained at 10 days. The reactors were seeded with mixed liquor from the Blacksburg, Virginia municipal wastewater treatment plant.

Steady State Determination. The activated sludge reactors were operated until the reactor achieved steady state, typically after 20-40 days of operation. Visual inspection of settling and dewatering parameters plotted as a function of time were used to determine the steady state period. The reactor was considered to be at steady state when the variability in the settling and dewatering characteristics was less than approximately 20% between sampling periods. The steady state values of each parameter were calculated as the average of values during the steady state period. Typically, the steady state values represent the average of at least four data points from the steady state period.
Filamentous Organism Quantification. Microscopic observations of the biological suspensions were performed periodically to assess the culture characteristics and also to quantify the filamentous organism content. Filamentous organism content was measured using the method of Jenkins et al. (1986) in which the number of filamentous organisms were rated on a scale of 0-6, where 0 corresponds to no filamentous organisms present and 6 corresponds to excessive growth of filamentous organism. All data reported represent studies with filamentous counts less than or equal to 2.

Cation Analysis. Calcium, magnesium, potassium, sodium, and ammonium were measured using a Dionex ion Chromatograph using a CS12 column and conductivity detection with self regenerating suppression of the eluent. The eluent was 20 mM methanesulfonic acid and the flow rate was 1 mL/min.

Settling and Dewatering Properties. Total suspended solids (TSS) and volatile suspended solids (VSS) were analyzed using Method 2540D and 2540E, respectively, in Standard Methods (1992). The settling properties of the biological suspension were characterized by the Sludge Volume Index (SVI) as described by Method 2710D in Standard Methods (1992). The dewatering characteristics of the biological suspension were determined by capillary suction time (CST) using Method 2710G in Standard Methods (1992) and specific resistance to filtration (SRF) according to the method of Christensen and Dick (1985). Floc density was determined using the isopycnic Percoll method described by Knocke et al. (1993).

Statistical Analysis. The pooled t-test was used to statistically compare settling and dewatering of two data sets. Analysis of variance (ANOVA) was used for statistical comparison of more than two data sets. A p-value of 0.05 was used as the cutoff for statistical significance.
Conditioning and Dewatering. Cationic polymer was used for conditioning and dewatering of thickened sludges from the reactors. Polymer solutions were made to a final concentration between 5 and 10% by mixing concentrated polymer with distilled water for 30 minutes. During conditioning and dewatering the polymer was added to a 50 mL sludge sample and mixed for 20 seconds in a square container. The mixing speed was 200 rpm. After mixing, the CST was measured and the optimum dosage was considered as the dose which resulted in the minimum CST.

Batch Settling and Dewatering Tests with Cation Addition. Batch tests were performed on 250 mL mixed liquor samples. Cations were added to the sample using the appropriate volume of a concentrated salt solution. Prior to addition of a volume of the salt solution, the suspension was allowed to settle and an equivalent volume of the supernatant was removed. This minimized differences due to volume changes. In general, the volume added was less than 5% of the total sample volume. The samples were then mixed for 15 minutes at 50 rpm on a paddle mixer and then analyzed for settling and dewatering properties.

Extracellular Protein and Polysaccharide Extraction and Analysis. Biopolymer extractions were performed to obtain a soluble and bound fraction. A 40 mL sample of the biomass was centrifuged at 8000 x g for 15 minutes. The supernatant was removed and the biopolymer in this supernatant was considered the "soluble" biopolymer fraction. The remaining pellet was then resuspended in 40 mL of 1 mM NaOH (pH=11) by mixing in a Waring blender for three seconds. Next, the sample was mixed at 90 rpm for 15 minutes on a paddle mixer and then centrifuged at 8000 x g for 15 minutes. The resultant supernatant was considered the "bound" biopolymer fraction. Protein was measured using the Hartree (1972) modification of the Lowry et al. (1951) method.
Polysaccharide was measured using the method of Dubois (1956). Bovine serum albumin and glucose were used as protein and polysaccharide standards, respectively.

![Diagram of a laboratory scale activated sludge reactor](image)

**Figure 2.1.** Profile View of Laboratory Scale Activated Sludge Reactor.

**Table 2.1.** Cation and nutrient concentration in bactopeptone feed used in laboratory activated sludge reactors. Bactopeptone concentration is 300 mg/L.

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Concentration (mg/L)</th>
<th>Concentration (meq/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺</td>
<td>20.9</td>
<td>0.91</td>
</tr>
<tr>
<td>NH₄⁺</td>
<td>0.5</td>
<td>0.03</td>
</tr>
<tr>
<td>K⁺</td>
<td>2.1</td>
<td>0.05</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>3.8</td>
<td>0.32</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>13.7</td>
<td>0.68</td>
</tr>
<tr>
<td>PO₄³⁻</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>15.9</td>
<td></td>
</tr>
<tr>
<td>TKN</td>
<td>46.5</td>
<td></td>
</tr>
<tr>
<td>COD</td>
<td>30.0</td>
<td></td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSION

Effect of calcium and magnesium ratio. The literature has shown that both calcium and magnesium are required for the flocculation or adhesion of monocultures of certain bacteria (Tezuka, 1969 and Lodeiro et al., 1995). In this study, the effect of calcium and magnesium on mixed populations in activated sludge was examined using laboratory reactors and bactopeptone feed. The bactopeptone feed (Table 2.1) was then supplemented with either calcium or magnesium salts or both. Initially, a reactor was seeded with mixed liquor from the Blacksburg, Virginia Municipal activated sludge plant and fed only bactopeptone with no additional salts added to the feed. The feed concentrations of calcium and magnesium were then changed throughout the study. The SVI and CST for this reactor along with the concentrations of calcium and magnesium are plotted in Figure 2.2. The settling and dewatering properties were best when both calcium and magnesium were added to the feed. When only one or neither of the cations were added to the feed, the SVI and CST increased. The SVI and CST typically began to increase after one sludge age from the time a cation was removed from the feed. At no time in the experiment were filamentous organisms observed in significant quantity. Therefore, this system required both calcium and magnesium for adequate settling and dewatering properties.

This experiment was repeated to further assess the effect of the calcium to magnesium ratio on activated sludge properties. The results from this experiment showed that the calcium to magnesium ratio did not affect the settling and dewatering properties of the reactor. And the presence of either calcium or magnesium, or both of
the cations resulted in good settling and dewatering. Thus, two conditions arose from reactors seeded with the same mixed liquor source at different times. One culture was sensitive to the calcium and magnesium ratio while another culture was insensitive to this ratio. The ratio sensitive microbial consortium was named Type 1, while the insensitive consortium was named Type 2. Type 1 cultures were dominated by s-shaped, motile bacteria and Type 2 cultures were predominantly short, straight rods. Another distinguishing feature of the Type 1 bacteria was the very high CST values measured during no salt addition to the feed compared to those of the Type 2 cultures. Comparison of the results from these two experiments are shown in Table 2.2. The results agree with the literature in that the sensitivity to the calcium and magnesium ratio depends on the type of bacteria being cultured (Tezuka, 1969, Endo et al., 1976, and Lodeiro et al., 1995). This may explain, in part, why different researchers obtained different results. It also suggests that some difference in the response to cations in the feed may occur at different wastewater facilities.
Figure 2.2. SVI and CST and changes in the calcium and magnesium concentrations over time of reactor run. Arrows on top graph show points where the feed was changed to only include the cation listed above the arrow.
Table 2.2. Comparison of SVI and CST for the calcium/magnesium ratio sensitive and insensitive cultures with different feed conditions.

<table>
<thead>
<tr>
<th>Feed Addition</th>
<th>Type 1 Culture</th>
<th></th>
<th>Type 2 Culture</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SVI (mL/g)</td>
<td>CST (s)</td>
<td>SVI (mL/g)</td>
<td>CST (s)</td>
</tr>
<tr>
<td>None</td>
<td>607</td>
<td>374</td>
<td>632</td>
<td>20.2</td>
</tr>
<tr>
<td>Ca = 2.5 mM</td>
<td>594</td>
<td>193</td>
<td>153</td>
<td>9.9</td>
</tr>
<tr>
<td>Mg = 2.5 mM</td>
<td>710</td>
<td>336</td>
<td>152</td>
<td>9.6</td>
</tr>
<tr>
<td>Ca=Mg = 5 mM</td>
<td>159</td>
<td>8.0</td>
<td>142</td>
<td>9.5</td>
</tr>
</tbody>
</table>

**Effect of calcium and magnesium concentration.** A separate study was performed to determine the effect of increasing calcium and magnesium concentrations on activated sludge settling and dewatering properties. An equimolar ratio of calcium and magnesium was added to the feed for all these experiments. The laboratory reactors were seeded with activated sludge from the Blacksburg, Virginia municipal activated sludge plant and operated until steady state was considered to have been attained.

The effect of calcium and magnesium concentration on SVI and effluent suspended solids is shown in Figure 2.3. When no salts were added to the feed, a non-filamentous bulking condition occurred, but the settling improved as the calcium and magnesium concentration in the feed was increased. The bulking was due to a low concentration of salts which produced very small, light and weak flocs. The effluent suspended solids were very low during the bulking conditions. As settling improved, the effluent suspended solids initially increased and then decreased when the calcium and magnesium concentration was increased above 2.0 meq/L. As can be seen in Figure 2.4, the SRF and CST decreased as the concentration of calcium and magnesium increased. ANOVA tests
showed that the settling and dewatering properties, measured by SVI, SRF, CST, and cake solids, were all significantly improved by calcium and magnesium additions to the feed at a p-value less than 0.05. Most of the improvement occurred at the first incremental addition of calcium and magnesium of 0.72 meq/L. Therefore, the minimum concentration of calcium and magnesium necessary for a reasonable SVI, SRF, CST is in the range of 0.72 to 2.0 meq/L. These minimum concentrations agree with results reported for monocultures by Endo et al. (1976) and Shimizu and Odawara (1986). Endo et al. (1976) reported that a *Flavobacterium* species required a minimum of 0.60 meq/L Ca\(^{++}\) for flocculation, and Shimizu and Odawara (1986) reported an *Agrobacterium* species required 2.0 meq/L Mg\(^{++}\) for flocculation.

![Graph](image)

**Figure 2.3.** Steady state SVI and effluent suspended solids as a function of calcium and magnesium concentration in the feed. Error bars indicate one standard deviation. For clarity, the standard deviation for the effluent suspended solids is not shown. The average standard deviation for effluent suspended solids was 107% of the value.
Figure 2.4. Steady state floc density, CST, cake solids and SRF as a function of calcium and magnesium concentration added to feed. Concentration is given as meq/L of each cation that was added to the feed. Error bars indicate one standard deviation.
The cake solids and the floc density increased as the calcium and magnesium concentration was increased, and as can be seen in Figure 2.4, the increase was approximately proportional to the calcium and magnesium concentration. Forster and Lewin (1972) reported that calcium ions added to activated sludge decreased the bound water content of the sludge, which would increase floc density and cake solids. Also, divalent cations would increase binding of the exocellular biopolymers. The tighter bound network of exocellular polymers would decrease the amount of bound and or inter-floc water and form a denser floc.

Floc strength measurements indicate flocs were more resistant to shear at higher cation concentrations. The floc strength was analyzed by imparting a constant shear (G=500/s) to a mixed liquor sample and measuring CST over time. Weak flocs are characterized by increasing CST due to floc breakup during mixing, whereas stable flocs show a much smaller increase in CST with mixing. As can be seen in Figure 2.5, the floc strength increases as the concentration of calcium and magnesium increases up to 5.0 meq/L of each and the strongest flocs are those with the highest calcium and magnesium concentrations.
Figure 2.5. Resistance to shear measured by changes in CST over time due to constant shear applied to mixed liquors from reactors with different concentrations of calcium and magnesium added to the feed.

Conditioning and dewatering experiments were performed to determine the optimum polymer dose required for the different feed conditions. These tests were performed on thickened mixed liquor samples from the reactors. In general, as the calcium and magnesium concentration in the feed increased, the optimal polymer dose for conditioning decreased. For example, the optimum dose was five times greater when no salts were added to the feed compared to when 2.5 mM of calcium and magnesium were added to the feed. The decrease in polymer demand is thought to be associated with a change in particle size distribution resulting from cation addition to the feed. Also, the total number of particles was reduced at higher calcium and magnesium concentrations, and most of the reduction was in the particle size range of 5-50 μm as shown in Table 2.3. This reduction in colloidal size particles likely contributed to the decrease in
polymer conditioning dose. Novak et al. (1977) reported that the polymer dose required for conditioning was a function of the concentration of colloidal particles in the supernatant of a biological suspension. Karr and Keinath (1978) reported the "supracolloidal" particles in the size range of 1-100 μm had the greatest affect on the dewaterability of sludges, and as the concentration of the particles in this size range increased, dewaterability decreased. The increase in calcium and magnesium in the feed also decreased the charge of the particles, measured by the zeta potential, which could decrease the polymer dose for conditioning. Novak et al. (1977) also reported that charge neutralization was the major mechanism in polymer conditioning of activated sludge.

Table 2.3. Particle size distributions of reactors with increasing cation concentrations in the feed.

<table>
<thead>
<tr>
<th>Particle Size (μm)</th>
<th>No Salt Added</th>
<th>Ca=Mg=0.72 meq/L</th>
<th>Ca=Mg=2.0 meq/L</th>
<th>Ca=Mg=5.0 meq/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-50</td>
<td>48350</td>
<td>34157</td>
<td>4220</td>
<td>3030</td>
</tr>
<tr>
<td>50-100</td>
<td>2643</td>
<td>8943</td>
<td>57</td>
<td>420</td>
</tr>
<tr>
<td>100-200</td>
<td>1887</td>
<td>1983</td>
<td>13</td>
<td>397</td>
</tr>
<tr>
<td>&gt;300</td>
<td>437</td>
<td>320</td>
<td>123</td>
<td>1177</td>
</tr>
</tbody>
</table>
Effect of Sodium. Excess sodium addition to activated sludge has been shown to result in a deterioration in the settling and dewatering properties of activated sludge (Novak and Randall, 1986 and Bruus et al., 1992). However, the concentration at which sodium begins to effect the sludge properties has not been well established. Therefore, a series of reactors were operated to determine the effect of increasing concentrations of sodium on the settling and dewatering properties of activated sludge. Calcium and magnesium were each added to the feed of all the reactors at 3.0 meq/L, and sodium was then varied from 0.9 to 20.9 meq/L using 5 meq/L incremental additions. The control reactor was not fed additional sodium, although the media contained 0.9 meq/L of sodium.

As shown in Figure 2.6, addition of 5 meq/L (230 mg/L) of sodium to the feed decreased the SVI and effluent suspended solids. Above this sodium concentration, the SVI and effluent suspended solids increased. Floc density, CST, SRF, and cake solids did not change appreciably when up to 10 meq/L of sodium was added to the feed, as shown in Figure 2.7.
Figure 2.6. Effect of sodium concentration in feed on the SVI and Effluent TSS of laboratory activated sludge systems. Each reactor also contained 3.0 meq/L each of Ca++ and of Mg++.
Figure 2.7. Effect of sodium concentration in feed on the floc characteristics of activated sludge reactors. Each reactor also contained 3.0 meq/L of calcium and of magnesium.
Sodium addition above 10 meq/L caused a deterioration in both the settling and dewatering properties of the activated sludge. The SRF, CST, and effluent solids increased while the floc density and the cake solids decreased as shown in Figures 2.6 and 2.7. The SVI at 20 meq/L Na⁺ could not be accurately measured due to deflocculation and flotation problems. The suspension in this reactor contained very fine particles and during the SVI test, many of the particles remained in suspension. In addition, air bubbles adhered to the flocs resulting in floating solids both in the reactor and during the SVI analysis, so the settled volume represented only a fraction of the biomass. The SVI of this system was estimated by adding the biomass volume that had floated to the surface of the graduated cylinder during SVI analysis to the settled volume after 30 minutes.

Adsorption of air bubbles is likely due to the decrease in bound protein and polysaccharide content. As the sodium concentration increased above 10 meq/L, the bound polysaccharide and protein concentrations decreased by approximately 30% and 60%, respectively. The bound biopolymers are generally hydrophilic, which give the flocs an overall hydrophilic nature. Proteins can also have hydrophobic domains, but these domains are typically not exposed to the solution. Therefore, the decrease in hydrophilic biopolymers likely increased the overall hydrophobicity of the flocs, resulting in increased adsorption of air bubbles and flotation. Similar results have been observed in industrial facilities where floating problems occurred and the biomass had a very low exocellular biopolymer content (Higgins, 1995).

It is thought that the poor settling and dewatering that occurred in the reactors with high sodium is a result of ion-exchange processes in which divalent cations were displaced by the sodium. According to the cation-bridging model proposed by others, removal of divalent cations from the floc would decrease the cation bridging in the floc which would lead to a deterioration in the settling and dewatering properties due to a
weakening of the floc structure (Tezuka, 1969, Novak and Haugan, 1978, and Bruus et al., 1992). This was supported by floc strength measurements which showed resistance to shear decreased at the higher sodium concentrations. The CST of the 20 meq/L Na+ suspension increased by 680% during 10 minutes of shear compared to 31% for the 10 meq/L Na+ suspension. The results from the floc strength tests on mixed liquor from these two sodium reactors are shown in Figure 2.8.

![Figure 2.8. Floc Strength measurements of suspensions at different sodium concentrations in the feed.](image)

Poor settling and dewatering of the activated sludge resulted when the sodium to calcium plus magnesium ratio was greater than approximately 2 to 1 on a meq/L basis. If displacement is due to ion-exchange reactions, the settling and dewatering should be improved by a reduction in this ratio. This hypothesis was tested by increasing the
calcium and magnesium concentration to 6.0 meq/L in the feed to the reactor receiving 20 meq/L of sodium. This decreased the ratio of sodium to calcium plus magnesium to 1.7. After several days the CST, SRF and effluent solids decreased and the cake solids and floc density increased. In addition, an increase in the bound biopolymer content was observed and flotation of the flocs ended. This indicates the ratio of sodium to divalent cations is important to the settling and dewatering properties and not simply the absolute concentrations of these cations. A comparison of the steady state values of settling and dewatering properties obtained from both reactor runs with a sodium to calcium plus magnesium ratio of 1.67 are shown in Table 2.4, respectively. For comparison, the data from the reactor with the sodium to divalent ratio of 3.33 is also shown in Table 2.4. The reactors had the same ratio of sodium to calcium plus magnesium, but the total concentration of cations was twice as much in the second data set. Despite the difference in the total cation concentration, the settling and dewatering properties were similar.

The data clearly supports the cation-bridging model proposed by Novak and Haugan (1978) and Tezuka (1969) for bioflocculation in activated sludge and not the double layer compression or DLVO model. The deterioration due to high sodium concentrations cannot be explained by the DLVO model as suggested by Zita and Hermansson (1994), because according to this model, the settling and dewatering should improve at all levels of sodium addition due to double layer compression from increased ionic strength. This did not occur in these experiments.
Table 2.4. Comparison of steady state settling and dewatering properties of two reactors each with a sodium to calcium plus magnesium ratio of 1.67, but with sodium concentrations of 10 and 20 meq/L and a reactor with a ratio of 3.33 and 20 meq/L Na⁺. The sodium to calcium plus magnesium ratio is shown in parentheses.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Na⁺ = 10 meq/L (1.67)</th>
<th>Na⁺ = 20 meq/L (1.67)</th>
<th>Na⁺ = 20 meq/L (3.33)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SVI (mL/g)</td>
<td>100 ± 8.4</td>
<td>124 ± 11</td>
<td>158 ± 37</td>
</tr>
<tr>
<td>Effluent TSS</td>
<td>7.3 ± 3.7</td>
<td>15 ± 7.1</td>
<td>52 ± 35</td>
</tr>
<tr>
<td>CST</td>
<td>10.0 ± 1.0</td>
<td>9.3 ± 0.8</td>
<td>23.9 ± 10.3</td>
</tr>
<tr>
<td>SRF</td>
<td>0.12 ± 0.05</td>
<td>0.48 ± 0.52</td>
<td>6.05 ± 2.10</td>
</tr>
<tr>
<td>Cake Solids</td>
<td>12.3 ± 2.0</td>
<td>9.2 ± 1.3</td>
<td>6.8 ± 1.0</td>
</tr>
</tbody>
</table>

Effect of Potassium. The effect of potassium on the settling and dewatering of the biological system was tested with one reactor that was fed 3.0 meq/L each of calcium and magnesium and also supplemented with 1.5 meq/L of K⁺. The data were compared to a reactor with the same calcium and magnesium concentration but no additional K⁺. The K⁺ concentration in the feed with no additional K⁺ is 0.05 meq/L as shown in Table 2.1. Steady state values of settling and dewatering properties are shown in Table 2.5. The reactor with potassium added to the feed had significantly better settling properties in terms of SVI (p<0.05), however, the dewatering properties deteriorated as shown by a decrease in cake solids and an increase in the SRF. The increase in SRF due to potassium addition to the feed was statistically significant with p<0.05. In addition, a decrease in
the floc density was measured in the reactor with added potassium. The improvement in SVI was likely due to an increase in the particle size of the suspension. Visual inspection of the mixed liquor revealed a number of very large flocs (>1000 μm) that settled rapidly. These type of flocs were not observed in any other system. Potassium addition to the feed had a different impact than addition of sodium. The potassium caused an increase in the SRF at 1.5 meq/L, whereas sodium levels up to 10 meq/L did not cause an increase in the SRF. The effect of potassium on activated sludge settling and dewatering is in need of further study to determine its affects on settling and dewatering.

Table 2.5. Steady state values of reactor with 1.5 meq/L of potassium added to the feed in addition to 3.0 meq/L of Ca++ and Mg++. The control reactor did not have additional potassium added to the feed and also had 3.0 meq/L of Ca++ and Mg++.

<table>
<thead>
<tr>
<th>Reactor</th>
<th>SVI* (mL/g)</th>
<th>CST (s)</th>
<th>SRF* (Tm/kg)</th>
<th>Cake Solids (%)</th>
<th>Floc Density (g/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K+ Added</td>
<td>9.4</td>
<td>9.7</td>
<td>0.21</td>
<td>10.0</td>
<td>1.017</td>
</tr>
<tr>
<td>Control</td>
<td>14.6</td>
<td>10.1</td>
<td>0.10</td>
<td>11.4</td>
<td>1.021</td>
</tr>
</tbody>
</table>

* indicates parameters that are significantly different at p<0.05.

**Batch Tests.** Batch tests were conducted on the bactopeptone fed activated sludge systems with no additional cations added to the feed. Calcium, magnesium and sodium were added to a series of samples and after mixing, the settling and dewatering properties were analyzed. The change in SVI, cake solids and SRF as a function of cation concentration added during the batch test is shown in Figure 2.9. All three salts
improved the settling and cake solids. The greatest improvement in these parameters was due to addition of calcium followed by magnesium and then sodium. No change in floc density occurred during the batch test for any of the cations (data not shown).

Only calcium and magnesium had a positive impact on the SRF, while sodium addition caused a slight increase in the SRF. Bruus et al. (1992) reported similar results for addition of sodium. They attributed the increase in SRF to an ion-exchange mechanism. In these tests, the addition of sodium also caused a release of both calcium and magnesium as shown in Figure 2.10. However, the settling velocity, SVI, and cake solids improved while the SRF deteriorated. The addition of sodium appears to have reduced the strength of the surface binding of the biopolymers. This might produce larger but somewhat weaker flocs which settle well but under the shear created during filtration in the SRF test (Novak and Lynch, 1990), the flocs could disaggregate, thereby causing the SRF to increase.

Addition of calcium and magnesium in batch tests decreased the number of small particles in the suspension, but no change is seen with sodium addition. During particle size measurements, the sample is pumped through a small aperture which imparts shear to the sample. The particle size distribution between 5 and 100 μm for calcium addition during batch tests is shown in Figure 2.11. Similar results were found for addition of magnesium. As can seen in Figure 2.11, calcium (and magnesium) had the greatest impact on the particles in the size range of 5-50 μm. Karr and Keinath (1978) reported the supracolloidal particles within the size range of 1-100 μm had the greatest
Figure 2.9. Changes in SVI, SRF and cake solids with addition of cations in batch tests.
Figure 2.10. Cation release due to addition of sodium during batch tests.

Figure 2.11. Change in particle size distribution due to batch addition of calcium.
impact on sludge dewatering, therefore the change in the particle size distribution due to calcium or magnesium addition could improve the dewatering rate of the sample.

Although the settling and dewatering can be improved by batch addition of calcium and magnesium, a greater improvement occurred when the same amount of the salt was added to the feed and the reactor was allowed to reach steady state. A comparison of SVI and cake solids for batch addition of 5.0 meq/L of calcium or magnesium and for continuous flow steady state values with the feed addition of 5.0 meq/L of calcium or magnesium is shown in Figure 2.12. When cations are present in the feed, they can become incorporated into flocs as they form, resulting in a denser floc that is more resistance to shear. During batch addition the cations may not become completely enmeshed in the biopolymer network. This is supported by the lack of change in floc density during batch tests, while floc density increased over time when the cations were present in the feed.
Figure 2.12. Comparison of improvements in SVI and Cake solids when cations are added to a low salt suspension during batch tests and due to changes in the feed to the activated sludge reactors.
**Relationships of Cations to Biopolymers.** The ion-bridging model states that divalent cations act as a bridge between negatively charged sites on exocellular biopolymer. Therefore, improvements in settling and dewatering due to increased particle sizes should correlate to an increase in the bound content of exocellular biopolymer involved in the aggregation process. The steady state bound polysaccharide concentration varied from 10 to 17 mg/g TSS for all the reactor runs performed, and the bound protein content varied from 40 to 170 mg/g TSS. The increase in divalent cations was associated with an increase in the bound protein content but not the bound polysaccharide content as shown in Figure 2.13. As a result, the improvements in settling and dewatering are also well correlated to the bound protein content.

The role of protein in binding of the flocs from this system was further demonstrated using proteolytic enzymes (Higgins, 1995). Pronase, a non-specific proteolytic enzyme, was added to suspensions from the laboratory reactors. These enzymes have been reported to degrade extracellular protein and not intracellular protein (Kato et al., 1971). During six hours of incubation with the enzyme, the suspension deflocculated as shown by an increase in the CST from 10.2 to 32.2 seconds and the SRF from 0.43 to 4.61 Tm/kg. The particle size distribution also shifted toward smaller particles in suspension. The SRF and CST changed only slightly in the control sample which had no enzymes added to the suspension. Polysaccharide degrading enzymes had a minimal effect on settling and dewatering suggesting that the polysaccharide in the floc matrix is not as important as protein for the aggregation of bacteria into flocs in this laboratory system.

These results suggest that proteins are the most important biopolymer component. However, a number of other researchers have suggested polysaccharides are the most important biopolymer in floc formation (Bruus et al., 1992, Horan and Eccles,
1986, Jorand et al., 1995). For example, Bruus et al. (1992) suggested divalent cations bind with polysaccharides such as alginates in the bioflocculation process. In general, most researchers have overlooked or minimized the role of protein in bioflocculation, although the protein content has frequently been found to exceed the polysaccharide content in activated sludge (Urbain et al., 1993, Jorand et al., 1995, Eriksson and Alm, 1991, and Barber and Veenstra, 1986). The feed to the laboratory reactors was mainly a proteinaceous material which could select for organisms that produce extracellular protein that are involved in the flocculation process, so differences between activated sludges and their biopolymer content can be expected.

![Graph]

**Figure 2.13.** Changes in the bound protein and polysaccharide content with increasing concentrations of calcium and magnesium in the feed to laboratory activated sludge reactors.
SUMMARY AND CONCLUSIONS

The results of this study demonstrate that the cation content in a wastewater can have major impacts on the settling and dewatering characteristics of activated sludge. Two components of the cation balance that have been shown to be important are the concentrations of the cations and the relative ratios of cations.

When present in the feed, cations become incorporated within the microbe-biopolymer network. When the mix of cations was optimum, a dense floc was formed that was more resistant to shear. This results in an improvement in settling and dewatering and reduces conditioning requirements. An equimolar ratio of calcium and magnesium was important to the settling and dewatering of some, but not all, activated sludge systems. The minimum concentration of calcium and magnesium for good settling and dewatering was in the range of 0.72 to 2.0 meq/L of each. A ratio of sodium to divalent cations greater than approximately two resulted in a deterioration in settling and dewatering characteristics. Systems with an imbalance in divalent cations could be improved by batch addition of certain cations to the suspension, however, improvement was greater when the cation was added to the feed. From these results, a recommended cation balance for activated sludge expressed as meq/L is a:

1) monovalent to divalent cation ratio less than two, and ideally near one.
2) calcium to magnesium ratio near one.

These results suggest settling and dewatering in some full-scale activated sludge systems could be improved by adjustment of the cation balance. For example, the use of sodium hydroxide for pH adjustment could lead to poor settling and dewatering similar to
that seen in this study. These problems could be overcome by addition of divalent cations or by the use of an alternative chemical for pH adjustment. The implications are unknown because cations are not routinely measured but results from this study suggest the cation content may be important, especially in industrial systems.

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Authors. Matthew J. Higgins is a Ph.D. Candidate and John T. Novak is a professor in the Charles Edward Via, Jr. Department of Civil Engineering, Virginia Polytechnic Institute and State University. All correspondence should be sent to John T. Novak, 318 Norris Hall, VPI&SU, Blacksburg, VA, 24061.

REFERENCES


III. THE EFFECT OF CATIONS ON THE SETTLING AND DEWATERING OF ACTIVATED SLUDGES-FULL SCALE EXPERIENCE

ABSTRACT

Seven mixed liquor samples from full-scale activated sludge systems were analyzed for settling and dewatering properties and the calcium, magnesium, sodium, and potassium concentrations were measured. The cation concentrations were analyzed in terms of the monovalent to divalent cation ratio and the calcium to magnesium ratio. The monovalent to divalent ratio was positively correlated to activated sludge dewatering properties. In batch tests, the addition of calcium or magnesium to samples with a high monovalent to divalent cation ratio improved the dewatering rate of the suspensions by up to 30%. Conditioning and dewatering experiments were performed on thickened sludges from two of the industrial samples. Calcium was added to each sample, in one case to lower the monovalent to divalent cation ratio, and in the second case to increase the calcium to magnesium ratio. In each case, addition of calcium to the sample reduced the optimum polymer dose for conditioning by 30% compared to control samples with no added calcium. Laboratory reactor studies were performed using mixed liquor and wastewater from three of the activated sludge plants. Two of the systems were deficient in magnesium, and one system was deficient in calcium. In each case, the deficient cation was added to the feed of the reactors, and settling and/or dewatering properties improved. Addition of cations to the feed of two different full-scale activated sludge systems improved the settling dramatically, and in one system the thickened solids content was doubled. These results indicate that cation imbalances are a common cause of sludge settling and dewatering problems in industrial activated sludge plants, and these
imbalances can be corrected by addition of the cation deemed to be deficient by analysis of the monovalent to divalent ratio or the calcium to magnesium ratio.

**Keywords.** activated sludge, bioflocculation, settling, dewatering, cations.

**INTRODUCTION**

Previous research has shown the cation concentrations in the feed of laboratory scale activated sludge reactors had a significant effect on settling and dewatering properties of activated sludge. Higgins and Novak (1995) found that as the concentration of calcium and magnesium in the feed to the reactors was increased from less than 0.5 to 5 meq/L, settling and dewatering properties improved. Sodium added to the feed improved settling until the ratio of sodium to calcium plus magnesium exceeded approximately 1 to 1. At sodium concentrations above this ratio, settling rates decreased and effluent suspended solids increased. Dewatering deteriorated significantly above a 2 to 1 sodium to divalent cation ratio expressed on a meq basis. The poor settling and dewatering that resulted from high sodium concentrations could be reversed by addition of calcium and magnesium to the feed to reduce the sodium to divalent cation ratio. Higgins and Novak (1995) suggested that a cation balance exists for a given system that optimizes settling and dewatering characteristics.

The purpose of this research was to analyze the cation content of several full-scale activated sludge systems and assess the cation content in terms of the monovalent to divalent cation ratio and the calcium to magnesium ratio to determine if the cation content affects the settling and dewatering properties of the activated sludges. In
addition, another objective of this research was to attempt to improve the settling and
dewatering properties of the activated sludge systems by adjusting the cation
concentrations according to the guidelines developed by Higgins and Novak (1995) using
laboratory activated sludge systems.

METHODS AND MATERIALS

Materials. The cations used in the laboratory portion of this study, Ca++, Mg++, Na+, and K+ were laboratory grade chloride salts.

Cation Analysis. Calcium, magnesium, potassium, sodium, and ammonium were
measured on a Dionex Ion Chromatograph using a CS12 column and conductivity detection
with self regenerating suppression of eluent. The eluent was 20 mM methanesulfonic
acid and the flow rate was 1 mL/min.

Settling and Dewatering Properties. Total suspended solids (TSS) and volatile suspended
solids (VSS) were analyzed using Method 2540D and 2540E, respectively, in Standard
Methods (1992). The settling properties of the biological suspension were
characterized by the Sludge Volume Index (SVI) as described by Method 2710D in
Standard Methods (1992). The settling velocity of the mixed liquor was measured by
placing a well mixed sample in a 250 mL graduated cylinder. The height of interface was
then measured as a function of time and plotted to calculate settling velocity. The
dewatering characteristics of the biological suspension were determined by Capillary
Suction Time (CST) using Method 2710G in Standard Methods (1992), and Specific
Resistance to filtration (SRF) according to the method of Christensen and Dick (1985).
Floc density was determined using the isopycnic Percoll method described by Knocke et
al. (1993).
**Conditioning and Dewatering.** Cationic polymer was used for conditioning and dewatering of thickened sludges from the reactors. Polymer solutions were made to a final concentration between 5 and 10% by mixing concentrated polymer with distilled water for 30 minutes. During conditioning and dewatering the polymer was added to a 50 mL sludge sample and mixed for 20 seconds in a square container. The mixing speed was 200 rpm. After mixing, the CST was measured and the optimum dosage was considered as the dose which resulted in the minimum CST.

**Batch Settling and Dewatering Tests with Cation Addition.** Batch tests were performed on 250 mL mixed liquor samples. Cations were added to the sample using the appropriate volume of a concentrated salt solution. Prior to addition of a volume of the salt solution, the suspension was allowed to settle and an equivalent volume of the supernatant was removed. This minimized differences due to volume changes. In general, the volume added was less than 5% of the total sample volume. The samples were then mixed for 15 minutes at 50 rpm on a paddle mixer and then analyzed for settling and dewatering properties.

**Exocellular Protein and Polysaccharide Extraction and Analysis.** Biopolymer extractions were performed to obtain a soluble and bound fraction. A 40 mL sample of the biomass was centrifuged at 8000 x g for 15 minutes. The supernatant was removed and the biopolymer in this supernatant was considered the "soluble" biopolymer fraction. The remaining pellet was then resuspended in 40 mL of 1 mM NaOH (pH=11) by mixing in a Waring blender for three seconds. Next, the sample was mixed at 90 rpm for 15 minutes on a paddle mixer and then centrifuged at 8000 x g for 15 minutes. The resultant supernatant was considered the "bound" biopolymer fraction. Protein was measured using the Hartree (1972) modification of the Lowry et al. (1951) method.
Polysaccharide was measured using the method of Dubois (1956). Bovine serum albumin and glucose were used as protein and polysaccharide standards, respectively.

RESULTS AND DISCUSSION

Sampling Results. Seven full-scale activated sludge plants were sampled and settling and dewatering properties were analyzed along with the soluble cation concentration. A summary of these results are listed in Table 3.1. The type of treatment plant is also listed in Table 3.1. Most of the samples were from industrial activated sludge systems, but two were from municipal systems.

Higgins and Novak (1995) reported that several indices can be used to describe the cation balance and to predict how the cation concentrations may effect the settling and dewatering of an activated sludge. These are the monovalent to divalent cation ratio and the calcium to magnesium ratio, both expressed on a meq/L basis. The relationships between these ratios and the activated sludge settling and dewatering properties were examined for the seven activated sludge plants. The best defined relationship was between the monovalent to divalent ratio and dewatering properties measured as SRF and CST. The relationship between SRF and the monovalent to divalent cation ratio is shown in Figure 3.1. Data from laboratory experiments were also plotted in Figure 3.1 for comparison (Higgins and Novak, 1995). From Figure 3.1 it is evident that an increase in the monovalent to divalent ratio above approximately 2-3 will result in a decrease in the dewatering rate measured by SRF. A similar relationship was found for the CST (Table 3.1). The poor dewatering rate associated with a high monovalent to divalent cation ratio has been suggested to be the result of displacement of divalent cations from
the floc by monovalent cations, resulting in decreased binding between biopolymer and flocs (Higgins and Novak, 1995).

The relationship between the calcium to magnesium ratio and activated sludge characteristics was not as apparent but an examination of the data gives insights into the importance of this parameter. For example, the two lowest SVIs and SRFs were from the

Table 3.1. Sampling results from seven full-scale activated sludge systems.

<table>
<thead>
<tr>
<th>Plant</th>
<th>MLSS</th>
<th>SVI</th>
<th>CST</th>
<th>SPF</th>
<th>Cake</th>
<th>Na⁺</th>
<th>K⁺</th>
<th>Mg++</th>
<th>Ca++</th>
<th>Ca/Mg</th>
<th>Mono/ Dival.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/L</td>
<td>mL/g</td>
<td>s</td>
<td>Tm/kg</td>
<td>%</td>
<td>meq/L</td>
<td>meq/L</td>
<td>meq/L</td>
<td>meq/L</td>
<td>meq/L</td>
<td></td>
</tr>
<tr>
<td>Industry A</td>
<td>3.926</td>
<td>207</td>
<td>22.3</td>
<td>1.99</td>
<td>17.1</td>
<td>17.30</td>
<td>0.22</td>
<td>4.47</td>
<td>6.33</td>
<td>1.42</td>
<td>1.62</td>
</tr>
<tr>
<td>Industry B</td>
<td>1.710</td>
<td>107</td>
<td>47.1</td>
<td>3.40</td>
<td>24.3</td>
<td>24.27</td>
<td>0.82</td>
<td>0.76</td>
<td>9.70</td>
<td>12.73</td>
<td>2.40</td>
</tr>
<tr>
<td>Industry C</td>
<td>3.502</td>
<td>281</td>
<td>31.7</td>
<td>1.33</td>
<td>13.3</td>
<td>3.70</td>
<td>0.19</td>
<td>69.7</td>
<td>1.89</td>
<td>0.03</td>
<td>0.05</td>
</tr>
<tr>
<td>Industry D</td>
<td>2.760</td>
<td>208</td>
<td>64.8</td>
<td>11.40</td>
<td>19.7</td>
<td>10.87</td>
<td>0.25</td>
<td>0.17</td>
<td>0.86</td>
<td>5.13</td>
<td>10.81</td>
</tr>
<tr>
<td>Industry E</td>
<td>2.935</td>
<td>339</td>
<td>12.0</td>
<td>0.81</td>
<td>14.9</td>
<td>3.18</td>
<td>0.25</td>
<td>41.5</td>
<td>1.24</td>
<td>0.03</td>
<td>0.08</td>
</tr>
<tr>
<td>Municip. F</td>
<td>3.640</td>
<td>73</td>
<td>11.5</td>
<td>0.34</td>
<td>13.3</td>
<td>5.85</td>
<td>0.59</td>
<td>0.88</td>
<td>1.81</td>
<td>2.06</td>
<td>2.39</td>
</tr>
<tr>
<td>Municip. G</td>
<td>1.418</td>
<td>85</td>
<td>11.3</td>
<td>0.16</td>
<td>15.6</td>
<td>3.68</td>
<td>0.18</td>
<td>1.06</td>
<td>1.40</td>
<td>1.32</td>
<td>1.58</td>
</tr>
</tbody>
</table>
Municipalities F and G. These systems had calcium and magnesium ratios near one, and they also had relatively low monovalent to divalent ratios. The poorest settling occurred in the systems with a very low calcium to magnesium ratio. The SVI was 281 and 339 for Industry C and D, respectively, and both had a calcium to magnesium ratio equal to 0.03. Therefore, it appears that a combination of factors such as the calcium to magnesium ratio and the monovalent to divalent cation ratio may play a role in determining settling and dewatering characteristics.
Laboratory Tests Using Activated Sludge from Industrial Facilities

**Industry A.** Examination of the cation data for Industry A reveals a monovalent to divalent ratio equal to 1.62. The ratio suggested by Higgins and Novak (1995) for optimum settling and dewatering is one, so it would be expected that addition of calcium or magnesium should result in improved settling and dewatering. Batch tests were conducted within several hours of collecting activated sludge from the aeration basin. The change in CST as a function of calcium or magnesium is shown in Figure 3.2. Addition of either calcium or magnesium produced modest improvement in CST. These improvements in dewatering during batch tests are a good indicator that addition of the cations to the feed of the activated sludge unit would also improve settling and dewatering (Higgins and Novak, 1995).

Sequencing batch reactors (SBRs) were operated to test the effects of cation addition to the wastewater from Industry A. Two, one liter SBRs were operated with a 10 day mean cell residence time and a two day hydraulic retention time. The reactors were seeded with mixed liquor from Industry A's activated sludge plant. Since the calcium to magnesium ratio was greater than one, magnesium was added to the feed to adjust the calcium to magnesium ratio to one. A control reactor, SBR-C, was fed the wastewater obtained from Industry A, while reactor SBR-Mg received the magnesium supplemented feed. The reactors were operated for seven days and settling and dewatering properties were analyzed during this period. The averaged values for settling and dewatering parameters from the last two sampling days are summarized in Table 3.2 for both reactors. As shown in Table 3.2, the settling properties as measured by SVI did not change but dewatering properties measured by SRF and CST improved. Along with improved dewatering properties, the optimum polymer dose of the solids
from the reactors was reduced by 50% in SBR-Mg compared to the control. It is possible that continued feeding for several mean cell residence times would have produced additional improvements.

The addition of magnesium reduced the monovalent to divalent cation ratio and adjusted the calcium to magnesium ratio to one. Both of these changes in the cation balance have been shown to result in improvements in the settling and dewatering of laboratory activated sludge reactors.

Figure 3.2. Effect of calcium and magnesium addition on the dewatering rate measured by CST for Industry A.
Table 3.2. Summary of settling and dewatering results for SBRs operated using wastewater from Industry A. The wastewater feed to SBR-Mg was supplemented with magnesium to achieve an equimolar ratio of calcium and magnesium. Values represent the average of the last two days of operation.

<table>
<thead>
<tr>
<th>Reactor</th>
<th>SVI (mL/g)</th>
<th>CST (s)</th>
<th>SRF (Tm/kg)</th>
<th>Cake Solids (%)</th>
<th>Zeta Pot. (mV)</th>
<th>Optimum Polymer Dose (mg/g TSS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBR-C</td>
<td>98</td>
<td>22.4</td>
<td>1.20</td>
<td>18.8</td>
<td>-21.0</td>
<td>14.3</td>
</tr>
<tr>
<td>SBR-Mg</td>
<td>102</td>
<td>18.1</td>
<td>0.43</td>
<td>18.3</td>
<td>-17.5</td>
<td>7.2</td>
</tr>
</tbody>
</table>

**Industry B.** Industry B also had a monovalent to divalent cation ratio greater than one, as shown in Table 3.1. Therefore, batch addition of divalent cations should result in an improvement in the settling and dewatering properties. The effect of either calcium or magnesium addition to the mixed liquor from Industry B on the CST is shown in Figure 3.3. The CST decreased by approximately 30% with addition of either calcium or magnesium. Since both cations improved the dewatering rate of the mixed liquor, the improvements are likely due to a reduction in the monovalent to divalent cation ratio. If addition of cations to the feed were to be considered for this industry, magnesium would be the recommended choice since it would reduce both the monovalent to divalent ratio and the calcium to magnesium ratio to within recommended ranges.

Previous work with laboratory activated sludge systems also demonstrated that cations could decrease the polymer dose required for activated sludge conditioning and dewatering (Higgins and Novak, 1995). The effect of calcium addition on conditioning and dewatering was examined using a thickened sludge sample from Industry B. One sample was amended with 7.5 meq/L of calcium which was found to be an effective dose
in reducing the CST during batch tests (see Figure 3.3). A sample with no added calcium was used as a control. During the polymer conditioning experiments, the calcium amended sample improved markedly over 10 minutes of mixing, while the control samples changed little during this time. The optimum polymer dose determined after 10 minutes of mixing was 0.50 mg/g TSS for the sample which received calcium and 0.75 mg/g TSS for the unamended sample as shown in Figure 3.4. Thus, the polymer dose was reduced by one third with calcium addition, and the CST at the optimum dose was approximately 10% less than the unamended sample.

![Graph showing the effect of calcium and magnesium addition on CST](image)

Figure 3.3. Effect of calcium and magnesium addition on the dewatering rate measured by CST for Industry B.
Figure 3.4. Effect of the addition of calcium on the polymer requirements for conditioning thickened activated sludge from Industry B.

**Industry C.** Examination of the cation content in Industry C shows the wastewater had a calcium deficiency and as evidenced by an extremely low calcium to magnesium ratio. The wastewater also had a low monovalent to divalent ratio. This sludge dewatered reasonably well but settled very poorly. In this case, the addition of calcium to the mixed liquor would be expected to improve settling. Therefore, the effect of calcium addition on settling was investigated. The settling velocity and SVI of the suspension as a function of the calcium addition is shown in Figure 3.5. Calcium addition is expressed as the resultant calcium to magnesium ratio in Figure 3.5. Settling began to improve at a calcium to magnesium ratio near one, above a ratio of 1.5, the settling properties deteriorated.
A laboratory scale continuous flow reactor study was also performed to determine the effect of calcium addition to the feed on settling and dewatering properties. The reactor setup was the same as described by Higgins and Novak (1995). A two day hydraulic retention time was used, and a 15 day mean cell residence time. The reactor was seeded with mixed liquor from Industry C and wastewater from the treatment plant was used as feed to the reactor. The feed was supplemented with 10 meq/L of Ca$^{++}$ (200 mg/L). The reactor was operated for eight days. As shown in Figure 3.6, the SVI decreased from 676 to 93 mL/g. The decrease in CST and increase in cake solids is also shown in Figure 3.6. Also, the SRF decreased from 9.95 to 0.44 Tm/kg (data not shown). The results from this laboratory activated sludge reactor demonstrate that the addition of calcium to this calcium deficient wastewater dramatically improves the settling and dewatering properties of the activated sludge. The addition of calcium to the reactor feed was ten times less than the concentration of calcium added in the batch settling tests, however the improvement in SVI was greater in the reactor study. This is consistent with laboratory results in that improvements due to cation addition to the feed
Figure 3.5. Effect of calcium addition on the settling velocity and the SVI of Industry C.

Figure 3.6. Settling and dewatering properties as a function of reactor operation time for a laboratory scale reactor treating wastewater from Industry C with 10 meq/L Ca$^{++}$ added to the feed.
were greater than improvements due to addition of the same concentration of cations in batch tests (Higgins and Novak, 1995).

A conditioning and dewatering experiment was also performed using a thickened sludge sample obtained from Industry C. The optimum polymer dose was determined for samples with calcium added to achieve a final concentration of 100 meq/L (calcium/magnesium ratio = 1.5) and a control sample with no additional calcium. After the polymer was added to the sample, the CST was measured at five minute intervals during 10 to 15 minutes of mixing to assess the affect of mixing time on conditioning results. The CST of samples with added calcium decreased over time as slow mixing occurred, but the control samples did not show the same improvement, as indicated in Figure 3.7. The results for conditioning after thirty seconds and ten minutes of mixing are shown in Figure 3.8. After thirty seconds of mixing, the control sample dewatered better than the calcium amended sample, but after 10 minutes of mixing, the optimum dose for the calcium amended sample was approximately one third less than the control sample.

The results from this industry were similar to those of Industry B in that calcium addition reduced the polymer dose by 30%, and the effect of calcium addition on conditioning was dependent on mixing time. The decrease in optimal polymer dose may have been a result of a decrease in the number of colloidal particles. Data from laboratory activated sludge systems had shown divalent cation addition reduced the number of particles in the 5-50 μm range which was associated with better settling and dewatering and decreased polymer dose required for conditioning (Higgins and Novak, 1995). Thus, addition of divalent cations prior to conditioning and dewatering could reduce polymer demand which would result in significant savings since polymers are a significant portion of the overall operation and maintenance costs associated with activated sludge systems. The results also suggest a mixing time of at least ten minutes
after polymer addition and prior to dewatering would maximize benefits due to divalent cation addition.

![Graph](image)

**Figure 3.7.** Changes in CST as a function of mixing time after polymer addition for conditioning. The polymer dose for each sample is 10 mg/g TSS for each sample. One sample was spiked with a calcium dose of 100 meq/L.
Figure 3.8. Effect of calcium addition on polymer requirements for conditioning using mixing times of 30 seconds and ten minutes.
**Industry D.** Examination of the cation content of Industry D in Table 3.1 shows a magnesium deficiency as evidenced by a calcium to magnesium ratio of 5.13. Also, the monovalent to divalent cation ratio is 10.8. As would be expected with a high monovalent to divalent cation ratio, the dewatering is poor. In fact, this system has the highest monovalent to divalent cation ratio of the systems tested, and it also has the highest SRF and CST. The addition of divalent cations should improve the settling and or dewatering due to a reduction in the monovalent divalent cation ratio. Batch tests were performed on the mixed liquor using calcium addition, but no improvement in the CST occurred over a range of calcium concentrations added to the suspension (0-10 meq/L).

Even though the batch tests indicated no improvement with cation addition, reactor studies were conducted to evaluate the effect of cation addition on settling and dewatering properties. Ten liter, continuous flow reactors used by Higgins and Novak (1995) were seeded with the industry’s mixed liquor and fed wastewater from their treatment plant. The feed to one reactor was supplemented with 22 meq/L each of calcium and magnesium, and a second reactor was fed wastewater supplemented with only magnesium at a concentration of 22 meq/L. A third reactor, used as a control, was fed the wastewater with no additional cation. The reactors had a hydraulic retention time of eight days and a mean cell residence time of 20 days. These operational parameters were similar to those at the full-scale plant.

After two weeks of operation, the reactor with calcium and magnesium added to the feed showed a 75% reduction in CST and a 70% reduction in the one hour settled volume. The reactor with only magnesium added to the wastewater also showed a 75% reduction in CST and a 36% reduction in the one hour settled volume. The CST and two hour settled volume after two weeks of operation for the control and the reactors with
added salts are shown in Figure 3.9. The magnesium addition resulted in an equivalent improvement in the dewatering compared to addition of both calcium and magnesium.

![Bar chart showing two hour settled volume and CST for different Reactors](image)

Figure 3.9. The two hour settled volume (in a 250 mL graduated cylinder) and the CST of Industry D's laboratory reactor operated with calcium and magnesium or just magnesium added to the feed. Results from a control reactor with no salts added to the feed are also shown.

**Full-Scale Plant Tests**

The positive results from these laboratory studies using industrial wastes suggested that addition of divalent cations to full-scale activated sludge systems would improve the settling and dewatering properties of the system. The experience with the
addition of cations to full-scale systems is limited, but data were available from two industrial plants, Industry C and Industry D, in which divalent cations were added to the activated sludge system. Both Industry C and Industry D had a history of poor performance as evidenced by SVIs greater than 200. Industry C was calcium deficient as indicated by a calcium to magnesium ratio of 0.03, and Industry D was magnesium deficient with a calcium to magnesium ratio of 5.13. These conditions made the industry good candidates for field testing of cation addition.

**Industry C.** Data from Industry C is from a study by Novak and Randall (1986). Results from full-scale systems support laboratory findings that the cation balance has a significant effect on the settling and dewatering properties of activated sludge. This system had poor settling due to calcium addition which was made worse by addition of sodium. For example, the negative impact on settling due to high sodium concentration at Industry C is shown in Figure 3.10. The high sodium concentration was a result of NaOH addition for pH control. After, several weeks of NaOH addition, a nearly complete deflocculation of the biomass was observed which increased the effluent suspended solids and the height of the sludge blanket, as shown in Figure 3.10 (Novak and Randall, 1986). Input of 2000 kg (=40 mg/L Ca++) of calcium chloride directly into the aeration basin resulted in marked improvement in both the effluent suspended solids and the height of the sludge blanket which is shown in Figure 3.10. The CaCl₂ added to the system was used for road deicing and was dumped directly into the aeration basin. Almost immediately, the operation improved as shown in Figure 3.10.

Settling problems, measured by effluent suspended solids greater than 200 mg/L, recurred 40 days later as NaOH additions continued and no additional calcium was provided as shown in Figure 3.11. The washout of calcium from the system likely
contributed to the recurrence of settling problems. However, a second slug dose of 1900 kg (=38 mg/L) of calcium chloride improved settling and reduced the effluent suspended solids to less than 50 mg/L.
Figure 3.10. Effluent suspended solids and height of the sludge blanket in the clarifier at Industry C during a period of NaOH addition and after addition of CaCl₂. Filled in arrows indicate points when NaOH was added to the system and the open arrow indicates when CaCl₂ was added to the system (adapted from Novak and Randall, 1986).
Figure 3.11. Effluent suspended solids at Industry C during a second period of NaOH addition and after the second addition of CaCl₂. Filled in arrows indicate points when NaOH was added to the system and the open arrow indicates when CaCl₂ was added to the system (adapted from Novak and Randall, 1986).

**Industry D.** Industry D has had chronic settling problems at their treatment plant, although the poor settling was not a result of filamentous organisms. Microscopic examination of the mixed liquor found the filamentous organism concentration to be low using the counting technique of Jenkins et al. (1986). Settling tests performed on mixed liquor from the aeration basins showed the settled volumes were consistently greater than 950 mL after two hours of settling in a one liter graduated cylinder.

From the laboratory reactor data of Industry D, magnesium addition to their wastewater feed was considered the best option for improvement in the settling
properties. This would reduce the monovalent to divalent cation ratio, and also balance the calcium to magnesium ratio. Calcium addition was not considered in this case since the pH in the aeration basins range from 8-9 which could result in precipitation of calcium carbonate. The plant began adding 227 kg/d of MgSO$_4$ to their wastewater which corresponded to 2.0 meq/L of Mg$^{++}$. The two hour settled volume for aeration basin one and four before and after magnesium addition are shown in Figure 3.12. Magnesium addition began on day 22. For clarity, data from aeration basin 2 and 3 were omitted, although the results from these two basins were similar to the values of basin 1 and 4. Prior to magnesium addition, the two hour settled volume had been consistently around 950 mL in all of the aeration basins. As shown in Figure 3.12, the settling began to improve after approximately 10 days of magnesium addition to the system, and after approximately one month the two hour settled volumes ranged from 300-500 mL. Downstream processes were also affected by the addition of magnesium. The thickened solids content from a dissolved air flotation unit doubled after approximately 20 days of magnesium addition to the feed as shown in Figure 3.12.

The improvement in settling and dewatering at Industry D occurred at a relatively low dose compared to those used in batch and reactor studies. The addition of 2.0 meq/L of magnesium results in a calcium to magnesium ratio of two in the system, however, the monovalent to divalent ratio remained greater than five in the system.
Figure 3.12. Thickened solids from a dissolved air flotation unit and the two hour settled volume at Industry D's activated sludge treatment plant before and after addition of magnesium to the wastewater.
IMPLICATIONS TO ACTIVATED SLUDGE TREATMENT

Laboratory studies suggested that the cation content in the feed of activated sludge systems has a major impact on the settling and dewatering properties of these systems. Addition of cations is governed by the monovalent to divalent cation ratio and the calcium to magnesium ratio where ratios of one seem to be optimum. In tests at operating industrial waste treatment plants, dramatic improvements were seen by adjusting the cation balance even though after some of the adjustments, the desired ratios were not attained.

Many of the treatment plants in this study had high sodium concentrations which was shown to be directly associated with poor dewatering properties. These plants, and others with an obvious divalent cation imbalance, are good candidates for addition of divalent cations for the improvement in their settling and dewatering. Divalent cations can be added as simple salts or by modification of plant operation where applicable. For example, the use of magnesium or calcium hydroxide for neutralization in place of sodium hydroxide may be appropriate. This would increase the divalent cation concentration and reduce negative impacts due to high sodium concentrations.

CONCLUSIONS

The settling and dewatering of full-scale activated sludge systems can be improved by adjusting the cation content in the system. Cation addition can also decrease polymer demand for conditioning by 30 to 50%. Therefore, the cation content in an activated sludge system should be included in evaluations where flocculation, settling,
and dewatering problems are evident. Two parameters that have been shown to be useful in evaluating the cation balance are the monovalent to divalent cation ratio and the calcium to magnesium ratio, both expressed on a meq/L basis. The results from this study and laboratory experiments suggest guidelines for these ratios near one.

REFERENCES


IV. THE ROLES OF EXOCELLULAR BIOPOLYMER IN BIOFLOCCULATION WITH AN EMPHASIS ON PROTEIN CHARACTERIZATION

ABSTRACT

Relationships between exocellular biopolymer, cations, and the settling and dewatering properties of laboratory activated sludge samples were examined. An increase in the divalent cation concentration was associated with an increase in the bound protein concentration. The bound polysaccharide was also increased slightly. Addition of high sodium concentrations resulted in a decrease in the bound protein concentration. The changes in bound biopolymer were explained according to the cation bridging model for bioflocculation of activated sludge. Incubation of a laboratory activated sludge with a proteolytic enzyme resulted in deflocculation of the suspension as measured by an increase in the number of particles in the 5-40 μm range. The enzyme addition also increased the CST and SRF of the suspension. Incubation of the laboratory activated sludge sample with a polysaccharide degrading enzyme did not cause an increase in the CST or a change in the particle size distribution. This indicates that the exocellular protein was involved in the aggregation of bacteria into flocs.

The bound exocellular protein from a municipal, industrial and laboratory activated sludge system was isolated and characterized using amino acid analysis, SDS PAGE, gel chromatography, and amino acid sequencing. SDS PAGE results revealed the presence of a single protein in the exocellular biopolymer extract of the three samples. The molecular weight of the protein was approximately 15-17 kD. The amino acid
analysis, gel chromatography and amino acid sequencing results suggested the protein was a lectin-like protein. Binding site inhibition studies showed the protein had lectin-like activity.

**Key Words.** bioflocculation, biopolymers, protein, polysaccharide, lectins, cations.

**INTRODUCTION**

Researchers have shown that interactions between exocellular biopolymers and cations are largely responsible for flocculation in activated sludge systems (Bruus et al., 1992 and Higgins and Novak, 1995). Exocellular biopolymers are produced by bacteria and typically can be attached to the cell as a capsule, or excreted into the surrounding medium as a slime. Extraction of exocellular biopolymer typically yields polysaccharides, proteins, and smaller amounts of DNA and RNA. It has been suggested that divalent cations form bridges between negatively charged sites on the biopolymers which binds the biopolymer to microbial cells and to other biopolymers.

Researchers have suggested polysaccharides are the biopolymer responsible for flocculation, and as a result most research on the characterization of exocellular biopolymer has focused on the exocellular polysaccharides in activated sludge (Forster, 1971; Horan and Eccles, 1986; and Jorand et al., 1995). However, many studies have reported the exocellular protein concentration in activated sludge systems was greater than the exocellular polysaccharide concentration (Tenney and Verhoff, 1973; Brown and Lester; 1980, Barber and Veenstra, 1986; Eriksson and Alm, 1991; Urbain et al., 1993; and Jorand et al., 1995). The presence of protein in the exocellular fraction
suggests it may play a role in flocculation by binding polysaccharides. This is supported by results from gel chromatography that show protein and polysaccharide co-elute in the same fractions (Horan and Eccles, 1986; Goodwin and Forster, 1989; and Yeh, 1988) which indicates the extracted protein and polysaccharide are bound. Researchers have not characterized exocellular protein extracted in activated sludge to elucidate its functions in bioflocculation.

The purpose of this research was to evaluate relationships between the exocellular biopolymer content, cations and the settling and dewatering properties of full scale and laboratory activated sludge systems. In addition, the research focused on the characterization and isolation of exocellular proteins extracted from activated sludge systems in an attempt to understand the role of protein in bioflocculation.

METHODS AND MATERIALS

Laboratory Activated Sludge Sample. Laboratory activated sludge systems with a bactopeptone feed supplemented with different concentrations and ratios of calcium, magnesium, sodium and potassium were sampled for exocellular biopolymer content. The setup and operation of these systems are described by Higgins and Novak (1995).

Settling and Dewatering Properties. Total suspended solids (TSS) and volatile suspended solids (VSS) were analyzed using Method 2540D and 2540E, respectively, in Standard Methods (1992). The settling properties of the biological suspension were characterized by the Sludge Volume Index (SVI) as described by Method 2710D in Standard Methods (1992). The dewatering characteristics of the biological suspension
were determined by capillary suction time (CST) using Method 2710G in Standard Methods (1992) and specific resistance to filtration (SRF) according to the method of Christensen and Dick (1985).

**Exocellular Protein and Polysaccharide Extraction and Analysis.** Biopolymer extractions were performed to obtain a soluble and bound fraction. A 40 mL sample of the biomass was centrifuged at 8000 x g for 15 minutes. The supernatant was removed and the biopolymer in this supernatant was considered the "soluble" biopolymer fraction. The remaining pellet was then resuspended in 40 mL of 1 mM NaOH (pH=11) by mixing in a Waring blender for three seconds. Next, the sample was mixed at 90 rpm for 15 minutes on a paddle mixer and then centrifuged at 8000 x g for 15 minutes. The resultant supernatant was considered the "bound" biopolymer fraction. The protein concentration was measured using the Hartree (1972) modification of the Lowry et al. (1951) method and the polysaccharide concentration was measured using the method of Dubois (1956). Bovine serum albumin and glucose were used as protein and polysaccharide standards, respectively.

**Molecular Weight Determinations.** The molecular weight of the protein was determined by SDS PAGE analysis on a 10% gel according to the method of Laemmli (1970) and by gel chromatography using a G-75 Sephadex column with an eluent consisting of 0.5 mM EDTA, 50 mM NaCl, and a 5 mM phosphate buffer adjusted to pH=7.0.

**Amino Acid Analysis.** Amino acid analysis was performed after hydrolysis with HCl according to the method of Bidlingmeyer et al. (1984) and the amino acids were analyzed on a Water's HPLC PICO-TAG Amino Acid Analysis System.
Amino Acid Sequencing. The amino acid sequencing of the N-terminus of the protein was performed on an automated HPLC amino acid sequencer from Applied Biosystems Inc. with a 477A Sequencer and a 120A Analyzer using the Edman degradation procedure.

Enzymatic Digestions. Enzymes were added to 500 mL activated sludge samples obtained from laboratory activated sludge systems fed bactopeptone. Pronase was used as the proteolytic enzyme and was added at a concentration of 200 activity units to the 500 mL activated sludge sample. Cellulase was used as a polysaccharide degrading enzyme and added at a concentration of 375 activity units. The activated sludge suspensions were maintained at 25°C.

RESULTS AND DISCUSSION

Relationship between cations and bound biopolymer.

According to the cation bridging model for biofloculation in activated sludge, divalent cations bridge between negatively charged functional groups on exocellular biopolymer to bind the biopolymer within the floc. Bruus et al. (1992) theorized that divalent cations bridge negatively charged groups on alginate-like polysaccharides, although they admitted this type of polysaccharides have not been found in activated sludge systems. Exocellular polymer data collected as part of the laboratory study by Higgins and Novak (1995) is used to examine the cation bridging model and the effect of divalent cations on bound biopolymer concentrations.

The normalized mass of exocellular bound protein and polysaccharide was plotted as a function of calcium and magnesium concentration in the feed of laboratory activated
sludge systems, as shown in Figure 4.1. The data shows the increase in divalent cations increases the bound protein concentration and has little effect on the bound polysaccharide concentration. Therefore, the divalent cations in these systems bind exocellular protein within the microbe-biopolymer network. The increase in divalent cations also resulted in an improvement in settling and dewatering characteristics. Results from a study on the effect of sodium addition to the feed of laboratory reactors also support the cation bridging model. As shown in Figure 4.2, the bound biopolymer content is plotted as a function of the sodium concentration added to the

![Graph](image)

**Figure 4.1.** The effect of calcium and magnesium concentration in the feed on the bound biopolymer content.
Figure 4.2. The effect of sodium addition to the feed on the bound biopolymer content.

feed. The calcium and magnesium concentration in the feed was 3.0 meq/L of each. Initially, the bound protein concentration increases due to addition of 5 meq/L Na⁺ to the feed. The bound protein content decreased with sodium addition above 5 meq/L as shown in Figure 4.2. Similar to the addition of divalent cations to the feed, the bound polysaccharide content was not changed by the increase in sodium concentration. The decrease in bound protein content can also be explained by ion-exchange reactions. As the sodium concentration increased, the divalent cations were likely displaced from within the floc. Similar divalent cation displacement by sodium addition was reported by Higgins and Novak (1995) and Bruus et al. (1992). The displacement of divalent cations would reduce binding of biopolymer within the floc leading to a decrease in the
bound biopolymer concentration. The increase in sodium concentration above 10 meq/L also resulted in deterioration of both settling and dewatering properties.

**Enzymatic analysis of Biopolymer Roles in Bioflocculation**

The ion-bridging model helps to explain the relationships between biopolymer, cations and settling and dewatering in activated sludge. Divalent cations bridge negatively charged sites on biopolymers binding them to microbial surfaces and other biopolymers. The binding of biopolymers stabilizes the biopolymer network which improves floc formation and strength, thereby improving settling and dewatering. The results from this study suggest divalent cations bind protein within the floc.

The cation bridging model does not fully explain the possible individual roles of biopolymer in bioflocculation. To better understand the role of protein and polysaccharide in bioflocculation, a more direct examination of the polymers was performed using enzymes that degrade these biopolymers. The enzymes were added to an activated sludge sample, and the dewatering properties and particle size distribution were then measured over time. Pronase was used to hydrolyze extracellular protein. This protease is a nonspecific enzyme that hydrolyzes proteins at a number of cleavage sites. In addition, it has previously been shown to act only on extracellular protein (Endo et al., 1976 and Kato et al., 1971). Cellulase was used to degrade extracellular polysaccharides. Cellulase hydrolyzes β(1-4) bonds between glucose residues. These type of linkages have been shown to be present in activated sludge (Jorand et al., 1995). The experiment was performed on a sample from a laboratory activated sludge system fed bactopeptone.
As shown in Figure 4.3, the particle size distribution shifted to smaller particle sizes due to the proteolytic enzyme but changed much less with addition of the cellulase enzyme. Most of the change in particle size occurred in the 5-40 μm range. As shown in Figure 4.4, the CST increased in the sample incubated with the proteolytic enzyme but not with the cellulase enzyme or the control. In comparison to the control, the SRF also increased and the cake solids decreased due to addition of the proteolytic enzyme as shown in Figure 4.5. Disaggregation or deflocculation resulting from digestion of the exocellular protein suggests the protein plays an important role in the aggregation of floc formation in this system. This supports the previous observed relationships between the bound protein content and settling and dewatering properties.

The addition of the proteolytic enzyme also resulted in a release of polysaccharide. The soluble polysaccharide concentration as a function of incubation time with the pronase is shown in Figure 4.6. The degradation of the exocellular protein released 75% of the bound polysaccharide that was originally in suspension. The release of polysaccharide suggests the protein plays a role in binding polysaccharide within the biopolymer network. This correlates well with gel chromatography data that shows
Figure 4.3. Changes in the particle size distribution during incubation with enzymes.
Figure 4.4. Change in CST during incubation of a biological suspension with the proteolytic enzyme, pronase, the polysaccharide degrading enzyme, cellulase, and a control sample with no added enzyme.
Figure 4.5. The change in SRF and cake solids during incubation of a biological suspension with the proteolytic enzyme pronase and a control sample with no added enzyme.
Figure 4.6. Release of polysaccharide during incubation of a biological suspension with the proteolytic enzyme pronase and a control sample with no added enzyme.

Protein and polysaccharide often co-elute in the same size fractions (Horan and Eccles, 1986; Goodwin and Forster, 1989; and Yeh, 1988).

**Exocellular Protein Characterization and Isolation.**

The previous data suggested the exocellular protein was important to the bioflocculation process. Several classes of proteins exist in the exocellular environment of bacteria. These include extracellular enzymes, proteinaceous S-layers, lectins, intracellular protein from cell lysis or cell wall turnover, or polypeptide capsular
material. The exocellular protein extracted from activated sludge samples could be from a combination of these sources, however, unlike exocellular polysaccharides, the exocellular protein in activated sludge has not been characterized. Therefore, additional studies were performed to better characterize and understand the role of exocellular protein in bioflocculation. Of the possible proteins, lectins are one of the most plausible types of protein that would be involved in bioflocculation. Lectins are non-enzymatic proteins that bind sugar residues on polysaccharide.

Lectins are produced by a variety of bacteria such as Actinomyces, Corynebacterium, E. coli, Enterobacter, K. aerogenes, K pneumoniae, Pseudomonas, Salmonella, and Streptococcus (Mirelman and Ofek, 1986). They are also produced by viruses, plants and other microorganisms. The lectins produced by bacteria are typically located on appendages such as the pili and fimbriae of bacteria, and the terms lectins, fimbriae, and pili have been used interchangeably in the literature (Mirelman and Ofek, 1986; Sharon and Ofek, 1986; and DeGraaf and Mooi, 1986). They have also been referred to as adhesions or agglutinins. The term lectins will generally be used in this text.

Lectins are typically made up of four subunits. These subunits located on bacterial appendages generally have molecular weights in the range of 15,000 to 20,000 Daltons (DeGraaf and Mooi, 1986). Typically, each subunit has a binding site that can bind several sugar residues on a polysaccharide chain, and the binding sites are highly specific for certain residues in a specific sequence. Many lectins produced by bacteria are specific for galactose, mannose, glucose, and fucose or derivatives of these sugars (Mirelman and Ofek, 1986). These sugar residues are also components of bacterial exocellular polysaccharides (Horan and Eccles, 1986 and Forster, 1971).
which suggests lectins could also play a role in binding of bacteria in activated sludge systems.

Also, many lectins have been reported to require calcium and/or magnesium or manganese for binding activity (Goldstein and Poretz, 1986). Lectins play a role in attachment and colonization of bacteria in both animals and plants, and can cause agglutination of cells such as erythrocytes which have polysaccharides extending from the cell in the form of glycolipids and glycoproteins. The multiple binding sites of lectins bind several polysaccharides on different cells resulting in aggregation or agglutination. Agglutination can be inhibited by adding sugar residues specific for the lectin. The residues bind to the lectin functional sites preventing polysaccharides from binding.

**Amino Acid Distribution**

The distribution of hydrophobic and hydrophilic amino acids in the bound exocellular fraction of a laboratory reactor sample and of an industrial sample are shown in Figures 4.7 and 4.8, respectively. As seen in the figures, the distribution of amino acids are very similar for the different activated sludge samples. The protein samples are rich in the carboxyl containing groups such as aspartate (designated asx) and glutamate (designated glx) which may be involved in the binding of divalent cations. Also, a high proportion of amino acids with hydrophobic groups such as glycine (gly) and alanine (ala) are present in the protein. The amino acid distributions also are similar
to lectin-like proteins from E. coli strain K12 located on fimbriae of these bacteria, which are also shown in Figures 4.7 and 4.8 for comparison.

Figure 4.7. Hydrophobic amino acids of exocellular proteins extracted from the biomass of activated sludge samples.
Figure 4.8. Hydrophilic amino acids of exocellular proteins extracted from the biomass of activated sludge samples.

**Molecular Weight of Bound Exocellular Fraction**

SDS PAGE was used to determine the molecular weight of the exocellular bound protein. Samples were obtained from a municipal and an industrial activated sludge system and also a laboratory activated sludge system. Two extractions techniques were
used to obtain the bound biopolymer fraction, sonication and digestion with NaOH, as described in the Methods and Materials section. Therefore, a total of six samples were run on the gel. The results of this analysis showed the presence of a single protein in all six exocellular extracts, and the molecular weight of the protein in all six samples was approximately 15,000-17,000 Daltons, as shown in Figure 4.9.
Figure 4.9. SDS PAGE results for duplicate exocellular biopolymer extracts from three activated sludge samples which are, from left to right, Industry A, Municipality G, and a bactopeptone fed laboratory activated sludge system and molecular weight standards. The duplicates represent two extraction methods, sonication, and NaOH digestion.
Gel chromatography was also used to measure the molecular weight of the sample from the laboratory activated sludge system. This analysis revealed the molecular weight of the protein was approximately 66,000 Daltons. This suggests the protein is a tetramer. The protein is denatured by SDS and broken apart into its subunits. Therefore, the SDS PAGE analysis gives the molecular weight of the protein subunits. During gel chromatography, conditions are such that the protein is not denatured, and it can retain its tertiary structure. Therefore, if the protein is made up of four subunits, each with an approximate molecular weight of 16,000 Daltons, the quaternary structure of the protein would have a molecular weight of approximately 64,000 Daltons which is consistent with the gel chromatography measurements.

The protein isolated from the samples is most likely involved in bioflocculation because the degradation of the exocellular protein by pronase resulted in deflocculation of the suspension. Since the SDS PAGE results found only one protein in the samples, this protein must be the one degraded by the proteolytic enzyme, and therefore, also involved in the bioflocculation process.

The results of the amino acid analysis, SDS PAGE, and gel chromatography all suggest the protein may be a lectin-like protein. This was further confirmed by amino acid sequencing of the protein sample. The first nine amino acids in the protein are listed in Table 4.1. Two methods were used to determine the sequence, the first was generated by automated computer analysis from the HPLC output, and secondly by a graphical method to analyze peaks of the individual amino acids. The largest amino acid peaks were considered the amino acid for the given sequence number. For comparison, amino acid sequences for several fimbrial proteins characteristic of *E. coli* and *Klebsiella pneumoniae* (DeGraaf and Mooi1988) are also shown in Table 4.1. The
sequence was run through a protein sequence data base search and no plausible protein matches were found, however, the data base did not contain the amino acid sequences for the E. coli proteins listed in Table 4.1. The sequences found are consistent in that the first two amino acids are alanine, and the other amino acids, fourth through tenth, also show similarities to those of the E. coli protein.

Table 4.1. N-terminal amino acid sequence of protein extracted from activated sludge samples and several lectin-like proteins located on the pili of E. coli strains.

<table>
<thead>
<tr>
<th>Amino Acid Number</th>
<th>Computer Output</th>
<th>Graphical Analysis</th>
<th>E. coli K12</th>
<th>E. coli J96</th>
<th>Klebsiella pneumoniae</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ala</td>
<td>Ala</td>
<td>Ala</td>
<td>Ala</td>
<td>Ala</td>
</tr>
<tr>
<td>2</td>
<td>Ala</td>
<td>Ala</td>
<td>Ala</td>
<td>Pro</td>
<td>Ala</td>
</tr>
<tr>
<td>3</td>
<td>Ala</td>
<td>Thr</td>
<td>Thr</td>
<td>Thr</td>
<td>Thr</td>
</tr>
<tr>
<td>4</td>
<td>Val</td>
<td>Thr, Val</td>
<td>Thr</td>
<td>Ile</td>
<td>Thr</td>
</tr>
<tr>
<td>5</td>
<td>His</td>
<td>His, Ala</td>
<td>Val</td>
<td>Pro</td>
<td>Val</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td>Asn</td>
<td>Gln</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Gly</td>
<td>Gly, Val</td>
<td>Gly</td>
<td>Gly</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>His</td>
<td>Asn</td>
<td></td>
<td>Gln</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Lys</td>
<td>Gly</td>
<td>Gly</td>
<td>Gly</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Gly</td>
<td>Gly, Val, Thr</td>
<td>Thr</td>
<td>Lys</td>
<td></td>
</tr>
</tbody>
</table>

The protein isolated in this study is likely not from E. coli or Klebsiella pneumoniae since these bacteria typically are not found in large quantities in most activated sludge systems. However, the comparison is made to show the extracted protein is consistent with the basic structure of lectin and may be from other types of
bacteria found in activated sludge systems. Lectins from these bacteria have similar amino acid compositions, and it is likely the amino acid sequence of lectins found in activated sludge would compare well with those listed in Table 4.1.

**Binding Site Inhibition Experiment**

Characterization of the protein by SDS PAGE, gel chromatography, amino acid distribution and sequencing are consistent with the assumption that the exocellular protein is a lectin. Therefore, binding site inhibition experiments were performed to assess a biological suspension for lectin activity. The experiments were similar to those used in agglutination inhibition studies (Leffler and SvanBorg-Eden, 1986). The binding site of lectins are specific for certain sugar residues and binding is noncovalent and reversible. Therefore, if lectins are involved in aggregation, the addition of free sugars to a suspension will inhibit lectin activity due to occupation of the binding site by the free sugars added. This prevents binding of polysaccharides which will cause a release of particles into the suspension as depicted in Figure 4.10. The binding site inhibiting agents used were glucose, mannose, galactose, p-nitrophenyl-α-D-mannose, phenyl-α-D-mannose, mucin and maltotriose. The mannose derivatives were used by Sharon and Ofek (1986) in binding site inhibition studies for mannose sensitive lectins in bacteria such as *E. coli*, *Salmonella*, and *K. pneumoniae*. The derivatized mannose compounds were used because it has been reported that hydrophobic interactions may be involved in binding of sugars by lectins, and hydrophobic units bound to the mannose typically increase binding in mannose sensitive lectins (Goldstein and Poretz, 1986).
Figure 4.10. Depiction of the role of lectins in aggregation of bacteria by cross-linking polysaccharides and the effect of addition of binding site analogues that occupy the binding sites of the lectin.

The inhibiting agents were added to activated sludge samples and the supernatant turbidity after thirty minutes of settling was measured as an indicator of binding site inhibition. The inhibiting agent, the concentration used, and the resultant supernatant turbidity are given in Table 4.2. Since several sets of experiments were performed and
controls were used in each set, the supernatant turbidity is expressed as a percent increase relative to the control which had no compounds added to the suspension. As seen in Table 4.2, the addition of glucose, galactose, and mannose all resulted in an increased of the supernatant turbidity by 75%. Many lectins have binding sites that are inhibited by these sugars (Mirelman and Ofek, 1986). The mannose derivatives with hydrophobic groups linked to the mannose residue only resulted in slight increases in the supernatant turbidity. The dosage of these compounds was two orders of magnitude lower than the monosaccharides used, although other researchers have found them to be more effective inhibitors than the monosaccharides alone. Mannose and galactose together at a concentration of 1.1 mM of each were able to increase the supernatant turbidity by 21%.
Table 4.2. Results of three binding site inhibiting experiments in which the supernatant turbidity of an activated sludge sample was measured after thirty minutes of settling. Results from a control sample with no added inhibiting agent are shown for each experiment.

<table>
<thead>
<tr>
<th>Inhibiting Agent</th>
<th>Final Concentration in Solution</th>
<th>Supernatant Turbidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>-</td>
<td>8</td>
</tr>
<tr>
<td>mannose</td>
<td>20 mM</td>
<td>14</td>
</tr>
<tr>
<td>galactose</td>
<td>20 mM</td>
<td>14</td>
</tr>
<tr>
<td>glucose</td>
<td>20 mM</td>
<td>14</td>
</tr>
<tr>
<td>arabinose</td>
<td>20 mM</td>
<td>12</td>
</tr>
<tr>
<td>control</td>
<td>-</td>
<td>6.2</td>
</tr>
<tr>
<td>mannose and galactose</td>
<td>1.1 mM of each</td>
<td>9.4</td>
</tr>
<tr>
<td>control</td>
<td>-</td>
<td>62</td>
</tr>
<tr>
<td>maltotriose</td>
<td>0.25 mM</td>
<td>73</td>
</tr>
<tr>
<td>mucin</td>
<td>200 mg/L</td>
<td>69</td>
</tr>
<tr>
<td>p-nitrophenyl-α-D-mannose</td>
<td>0.25 mM</td>
<td>63</td>
</tr>
<tr>
<td>phenyl-α-D-mannose</td>
<td>0.25 mM</td>
<td>66</td>
</tr>
</tbody>
</table>

DISCUSSION

The results of this experiment suggest lectins are involved in the bioflocculation process. The supernatant turbidities increased as a result of the addition of binding site
analogues. The occupation of binding sites would prevent binding of polysaccharides that may act as a bridge between adjacent proteins. The lectin activity is affected by mannose, galactose and glucose which have been shown to be present in exocellular polysaccharides of activated sludges (Horan and Eccles, 1986; Forster, 1971; and Steiner et al., 1976). The addition of these monosaccharides may inhibit polysaccharide binding and release small particles into solution that become detached due to loss of polysaccharide bridges.

Therefore, a model of bioflocculation which includes the role of lectins is proposed. Lectins with their multiple binding sites may act to bind polysaccharides within the biopolymer network. The polysaccharides can then bridge between adjacent lectins. These bridges can then stabilize the entire biopolymer network. This is depicted in Figure 4.10. The role of divalent cations may be to bind free lectins to the biopolymer network. This is supported by the relationship between bound protein content and the divalent cation concentrations. In addition, divalent cations such as calcium and magnesium may play a role in stabilizing lectin structure or may be required for binding activity of lectins. For example, some plant lectins have been reported to require calcium, magnesium or manganese in their binding sites for activity as reviewed by Goldstein and Poretz (1986). As a result, addition of EDTA can inhibit agglutination of cells by lectins, although this inhibition is reversible with addition of calcium (Ray et al., 1992). Similar experiments have been performed with activated sludges in which addition of EDTA leads to deterioration of settling or dewatering properties (Eriksson and Alm, 1991; Kakii et al., 1985; and Bruus et al., 1992). Lodeiro et al. (1995) reported the lectin mediated adhesion of *Rhizobium* bacteria to bean roots was greatest and most resistant to shear when both calcium and magnesium
were present. The absence of either calcium or magnesium decreased adsorbance of the bacteria to the roots. Few studies have been reported on the necessity of divalent cations on the activity of bacterial lectins. Sugarman et al. (1982) reported the divalent cation zinc increased the pili (lectin) mediated adhesion of both gram negative and gram positive bacteria to cells. Also, Staley and Wilson (1983) reported that fimbriae mediated binding of *E. coli* to pig intestinal cells was increased in the presence of calcium but not magnesium or manganese. The necessity of calcium or magnesium for enhancement in lectin mediated binding is likely specific for the type of organism.

This may explain results from Higgins and Novak (1995) who reported most activated sludge cultures require both calcium and magnesium for bioflocculation. In general, it appears that the bacteria in activated sludge produce lectin-like proteins that require both calcium and magnesium for activity, and the absence of either of these decreases binding of biopolymer, resulting in poor flocculation.

**CONCLUSIONS**

The presence of a single protein in the exocellular biopolymer fraction which appears to be a lectin suggests the bound protein fraction is involved in the binding of sugar residues on polysaccharides. The polysaccharides act as a bridge between adjacent proteins and these bridges stabilize the biopolymer network. Divalent cations play a role in binding exocellular biopolymers, especially proteins, within the floc matrix, and may also be involved in the structural stability and binding activity of the exocellular protein.
REFERENCES


V. COMPARISON OF THE LOWRY AND BRADFORD METHODS FOR MEASUREMENT OF EXOCYLLULAR PROTEIN IN ACTIVATED SLUDGE

ABSTRACT

The Bradford and Lowry methods were evaluated for their use in measuring the protein concentration in the exocellular extracts from activated sludge. Two activated sludge samples (A and B) were analyzed for exocellular protein content. The exocellular protein concentration of activated sludges from Plant A and B according to the Lowry method were 49.9 and 16.1 mg/L, respectively. The protein concentrations of Plant A and B using the Bradford method were 2.0 and 13.8 mg/L, respectively. The slopes of standard curves determined using bovine serum albumin and ovalbumin differed by a factor of 1.15 for the Lowry method and 1.81 for the Bradford method. The Lowry method is recommended for the analysis of exocellular protein extracted from activated sludge.

INTRODUCTION

Exocellular polymers produced by bacteria play a central role in the flocculation and sedimentation of activated sludge solids. The polymers consist of polysaccharides, proteins, lipids and smaller amounts of DNA and RNA. The analysis of biopolymers in the exocellular matrix requires two main steps. First, the exocellular polymer must be extracted and separated from the bacterial surfaces. A number of different methods have been used for this extraction step. As can be expected, the extraction method used can
affect the amount of polymer that is measured. This was well documented by Brown and Lester (1980). The second step in polymer analysis is to measure the extracted material for polysaccharide or protein concentration by one of several available methods. In the case of protein measurement, the method used can yield different results. These differences have not been evaluated in the measurement of exocellular protein extracted from activated sludge samples.

The two most commonly used colorimetric methods for measurement of exocellular protein in extracts from activated sludge are the method of Lowry et. al. (1951) and Bradford (1976). For example, those using the Lowry method include Tenney and Verhoff (1973), Pavoni et. al. (1972), and Brown and Lester (1980). The Bradford method has been used by Eriksson and Alm (1991), Urbain et. al. (1993) and Eriksson et. al. (1992). The advantages and disadvantages of these methods are given in Table 5.1.

Table 5.1. The advantages and disadvantages of the Lowry and Bradford protein analysis methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lowry</td>
<td>-sensitive</td>
<td>-many interfering compounds¹-²</td>
</tr>
<tr>
<td></td>
<td>-relatively simple</td>
<td></td>
</tr>
<tr>
<td>Bradford</td>
<td>-sensitive³</td>
<td>-different response to different proteins⁴-⁷</td>
</tr>
<tr>
<td></td>
<td>-very simple</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-few interferences</td>
<td></td>
</tr>
</tbody>
</table>

1 Lowry et. al. (1951)  
2 Bensadoun and Weinstein (1976)  
3 Bradford (1976)  
4 Van Kley and Hale (1977)  
5 Read and Northcote (1981)  
6 Pollard et. al. (1978)  
7 Pierce and Suelter (1977)
The purpose of this study was to evaluate the variability in results that can occur between the two protein analysis methods and to determine the suitability of each for analyzing extracellular protein from activated sludge.

METHODS AND MATERIALS

Activated Sludge Samples: Mixed liquor samples from two municipal activated sludge plants were obtained and named Plant A and Plant B.

Exocellular Polymer Extraction: The exocellular polymer fraction of activated sludge mixed liquor samples was extracted with the method used by Eriksson et. al. (1992). A 250 mL mixed liquor sample was mixed in a Waring blender for two minutes followed by centrifugation at 8000 x g for 15 minutes. The supernatant after centrifugation was analyzed for protein content.

Protein analysis. The extracted biopolymer was analyzed for protein by two methods:

1) Lowry et. al. (1951) Method

2) Bradford (1976) Method

These methods will be referred to as the Lowry and Bradford methods for simplicity.

Bovine serum albumin (BSA) and ovalbumin (OVA) were used as protein standards.
RESULTS AND DISCUSSION

The concentrations of exocellular protein measured in the mixed liquor of Plants A and B are summarized in Table 5.2. The results from this test show a significant difference in protein concentration measured by the two methods (p<0.05). Analysis using the Lowry method gave a much greater mean concentration than the Bradford method. In fact, the mean protein concentration found using the Bradford method is 25 times less than the Lowry method. The higher value found using the Lowry method may be due to the presence of interfering material in the extracellular fraction as discussed by Lowry et. al. (1951) and Bensadoun and Weinstein (1976). However, examination of the list of interfering compounds presented by Bensadoun and Weinstein (1976) reveals that, in general, the concentrations of these compounds in activated sludge would not be great enough to cause interference in the analysis of the protein with the Lowry method. Another possible reason for the difference may be due to the different response the Bradford method has for different proteins. In this case, the extracellular proteins found in these activated sludge samples may not be reactive with the dye of the Bradford method.

A second set of tests were performed on a mixed liquor sample from Plant B. In this analysis, both BSA and OVA were used as protein standards. As can be seen from the Figure 5.1, the Lowry method gave a similar response to the different protein standards. Similar results for a number of different proteins were reported by Pierce and Sueltzer
Table 5.2: Exocellular protein concentration measured in Plant A using the Lowry and Bradford methods and BSA standard.

<table>
<thead>
<tr>
<th>Method</th>
<th>Plant A</th>
<th>Plant B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>triplicate sample results (mg/L)</td>
<td>mean concentration (mg/L)</td>
</tr>
<tr>
<td>Lowry</td>
<td>52.3</td>
<td>49.9</td>
</tr>
<tr>
<td></td>
<td>54.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>43.5</td>
<td></td>
</tr>
<tr>
<td>Bradford</td>
<td>1.5</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td></td>
</tr>
</tbody>
</table>

Figure 5.1. Standard protein curves using the Lowry method and two different protein standards: bovine serum albumin (BSA) and ovalbumin (OVA).
Figure 5.2. Standard protein curves using the Bradford method and two different protein standards: bovine serum albumin (BSA) and ovalbumin (OVA).

(1977). Response of the Bradford method to the two proteins differed significantly, especially at higher concentrations, as shown in Figure 5.2. The OVA protein standard gave a lower response than the BSA standard using the Bradford method which corresponds with results reported by Read and Northcote (1981), Van Kley and Hale (1977), Sedmak and Grossberg (1977). The slope of the standard curves for the Lowry method differed by a factor of 1.15, while the standard curves for the Bradford method differed by a factor of 1.81.

It would be expected that a variety of proteins would be found in the extracellular fraction of protein, therefore, the difference in response with the Bradford method makes it inappropriate for use in the measurement of extracellular protein in activated
sludge. The Lowry method yielded more consistent results for different proteins and would therefore be recommended for measuring exocellular protein in activated sludge.

CONCLUSION

The comparison of the two protein analysis methods demonstrated the problems that can be encountered with the methods and the two methods give significantly different results for the same samples. Protein concentrations reported in the literature cannot be appropriately compared unless the same method and protein standard were used, interfering materials were removed or accounted for, and, in the case of the Bradford method, the same type of protein was being measured. The Lowry method is subject to interference by many compounds, although these compounds are not typically found in activated sludge at concentrations great enough to cause interferences. The Bradford method can yield significantly different results for different types of proteins. Therefore, the Lowry method is recommended for measuring exocellular protein in activated sludge samples.

REFERENCES


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VI. SUMMARY AND CONCLUSIONS

The cation content of activated sludge systems has a direct impact on the settling and dewatering properties of the system. Divalent cations bridge negatively charged functional groups on exocellular biopolymer, mainly protein, which binds the biopolymer to the floc. A deficiency of divalent cations or displacement of divalent cations from the floc by high monovalent cation concentrations reduces binding of biopolymer which reduces floc size and density. This leads to a deterioration of settling and dewatering properties. Addition of divalent cations to a system improves settling and dewatering. Laboratory and full-scale results of this research indicate the two most important criteria for the cation balance are the monovalent to divalent cation ratio and the calcium to magnesium ratio, both expressed on a meq/L basis. The data suggest the monovalent to divalent ratio should be less than two and ideally close to one. Similarly, the calcium to magnesium ratio should also be close to one.

The divalent cations bind exocellular protein within the floc. Isolation and characterization of the exocellular protein from two full scale activated sludge treatment plants and a laboratory activated sludge system found a single protein is involved in the bioflocculation process. This protein appears to be a lectin like protein. The protein acts to bind and stabilize the biopolymer network, and also plays a role in binding polysaccharides within the floc. Divalent cations may also play a role in stabilizing the protein structure and in the activity of their binding site.
VITA

Matthew J. Higgins was born on March 13, 1967 in Portland, Maine. He received a Bachelors of Science in civil engineering at the University of Maine in 1990, and his Master of Science in Civil/Environmental Engineering from the University of Maine in 1992. During this time, he worked part time for several engineering consultant firms. He graduated from Virginia Polytechnic Institute and State University in 1995 with a Ph. D. in Environmental Engineering. After completing his dissertation, he accepted an appointment as a visiting professor at Bucknell University in Lewisburg, Pennsylvania.