COMPOSITION, FATE, AND TRANSFORMATION OF EXTRACELLULAR POLYMERS IN WASTEWATER AND SLUDGE TREATMENT PROCESSES

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COMPOSITION, FATE, AND TRANSFORMATION OF EXTRACELLULAR POLYMERS IN WASTEWATER AND SLUDGE TREATMENT PROCESSES

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Extracellular polymers are produced by microorganisms for protection and attachment. They can be loosely-bound (slime) or firmly attached (capsule) to the cell wall. In wastewater and sludge treatment, biopolymers have important roles in flocculation, sludge dewatering, metals removal, and reuse of treated wastewater.

The characterization of biopolymers in treatment processes is incomplete, with most data coming from activated sludge. This research was designed to provide a comprehensive knowledge of the composition and fate of biopolymers in wastewater and sludge treatment processes.

Three sets of samples were collected in nine locations at a full-scale wastewater treatment plant during a three month period. Capsule was extracted using a cation exchange resin, while slime were separated by centrifugation. Biopolymers were analyzed for carbohydrate, protein, RNA, DNA, humic acids, total organic carbon, trace metals, and molecular weight.

Protein, carbohydrate, RNA, DNA, and humic acids were all constituents of biopolymers. Protein was the major compound in both capsule and slime. The inorganic fraction comprised 10 % to 20 % of the biopolymers' weight. Molecules with sizes greater than 2.0×10^6 were present in biopolymers. The capsule content of volatile suspended solids in digested sludge was 65 % of the capsule content of VSS in activated sludge.

Statistical analysis suggested that the quantity of capsular biopolymer changed in different locations, but the composition remained the same. The hypothesis that the

biopolymer contents of volatile suspended solids in digested sludge, primary, and secondary effluents were the same was not rejected. The equality hypothesis for biopolymer contents between activated sludge and secondary effluent VSS was rejected. These results, together with the observation that digested sludge VSS contained less biopolymer than activated sludge VSS, suggest that particles from anaerobic digester were significantly present in effluents, or there was a preferential settling of particles holding more biopolymers in the sedimentation tanks.

Mass balances showed production of capsular biopolymer constituents in the activated sludge process, and destruction during anaerobic digestion. Production and destruction varied in the ranges of 25 % to 40 %, and 60 % to 70 %, respectively.

The contents of trace metals in capsular biopolymer from wastewater were higher than the metal contents at other locations in the plant. Trace metals associated with capsule represented less than 10 % of the metals measured in samples; in samples with low SS concentration, trace metals associated with slime reached 45 % of the metal contents in samples.

BIOGRAPHICAL SKETCH

Antonio Benetti was born on April 18th, 1955, in Porto Alegre, Brazil. The newborn Antonio was the sixth child of Attilio and Neuza Benetti. During his childhood and teenager years, Antonio loved to play and watch soccer games. Antonio also acquired the habit of reading books, which he has maintained throughout his life.

When Antonio was about 12 years old, he went to the airport in the city he lived to give farewell to his sister, who was taking the airplane to travel to the United States. His sister had received a scholarship to attend graduate school in Library Studies. Antonio thought that it should be exciting to study in the United States. Antonio decided that one day he also would go to the United States to study.

Antonio graduated as a civil engineer in 1978 from the Universidade Federal do Rio Grande do Sul. During his college years, Antonio decided that sanitary engineering was the field he liked the most, and where he could do meaningful work. At the same time, Antonio thought that Brazil was in great need for the work of sanitary engineers to control not only the conventional but also the emerging pollution problems.

Upon graduation, Antonio took a job as an environmental engineer for the State Secretariat for Public Health and the Environment. At this job, Antonio had the opportunity to come into direct contact with many different types of environmental problems and with the communities that were affected. As valuable as these experiences were, Antonio did not feel fulfilled in this career. Antonio's mind and heart were in the academic environment. He returned to the university in Brazil and received a M. Sc. degree in Water Resources and Sanitation in 1987. In 1990 Antonio started teaching at the Universidade Federal do Rio Grande do Sul; in 1991, Antonio applied and was granted a scholarship from the Brazilian Government to attend the

Ph.D. program in Environmental Engineering at Cornell University. The wish of the 12 years old Antonio was to be realized. The years at Cornell will be remembered by Antonio as a period of great academic and personal growth.

When Antonio is not working, he likes to stay with his wife, Silvia, and daughters, Lucia and Fernanda.

To Lu and Fe, who have taught me the most important things.

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Chapter 1: Introduction

Extracellular polymers (biopolymers) are natural organic compounds produced by bacteria and other microorganisms for protection and attachment. In wastewater and sludge treatment processes, biopolymers have important roles in flocculation, sludge dewatering, and trace metals removal. In addition, the presence of biopolymers in secondary effluents has implications.regarding disposal and reuse due to toxicity concerns and the potential for soil clogging in aquifer recharge fields.

Several investigators have studied extracellular polymers in activated or digested sludges, with samples collected from laboratory reactors or full-scale plants. However, the physical and chemical characterizations of the polymers extracted from the sludges have been incomplete, especially for anaerobically digested sludge. Knowledge of biopolymer composition in other stages of wastewater and sludge treatment essentially is nil. The present research provides a more complete characterization of biopolymers in wastewater and sludge treatment.

Physical and chemical compositions of biopolymers were measured at eleven locations in a full-scale wastewater and sludge treatment plant during a time period characteristic of summer conditions. Several methods for biopolymer extraction and analytical techniques for chemical analyses were evaluated. An existing biopolymer extraction technique was modified in order to remove non-biopolymer components from the extract.

The chemical analyses included carbohydrate, protein, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), humic acids, total organic carbon (TOC), and the metals copper, lead, zinc, cadmium and chromium. A procedure for humic acid concentration estimation was developed. The physical characterization of biopolymers measured comprised molecular weight and yield.

The information gathered from these analyses, together with data provided by the plant and from existing literature, supported the development of a biopolymer mass balance, which provided insight into the production and destruction of biopolymers within the treatment processes. Statistical tools were used to identify patterns of similarities and differences among biopolymers in different processes. Finally, the presence of trace metals in biopolymers was quantified and compared with trace metals in whole samples.

Chapter 2 reviews prior studies of biopolymers in flocculation, sludge dewatering, and trace metals adsorption. The presence of biopolymers in secondary effluents and the limitations that they sometimes may pose to effluent disposal, reuse, or post-secondary treatment are introduced. Biopolymer extraction methods and their principles are discussed, and a compilation of extraction techniques that have been used is presented. Finally, the current knowledge on the composition of biopolymers in wastewater and sludge treatment is summarized.

Chapter 3 introduces basic information on the wastewater and sludge treatment plant studied, along with a description of sample sites. Methods considered for biopolymer extraction are discussed, and a detailed description of the procedure adopted is presented. A discussion of the methods and procedures for chemical and physical analyses is presented, including an evaluation of alternatives that were considered.

Chapter 4 presents the results of the physical and chemical analyses of the biopolymers. The sources of the compounds found in biopolymers are discussed, and a comparison with results from other studies is presented. Analysis of variance is performed to determine whether biopolymer composition and quantity are the same at each treatment process within the plant. The results of a solids and biopolymers mass balance within the treatment plant are summarized, and an estimation of a biopolymer

yield coefficient for activated sludge is made. The molecular weight distribution of biopolymers is presented, together with chromatograms displayed by biopolymers from selected locations. The results from trace metals analyses in biopolymers and in whole samples are described. The chapter ends with a discussion on the limitations of the study.

Chapter 5 summarizes results, findings and limitations of the research, with recommendations for improvement and further investigations. Appendixes A through H present the limits of detection for the analytical methods, the complete results of analyses, and a demonstration of the calculations used to perform suspended solids mass balances.

This research presented a more complete physical and chemical characterization of biopolymers within wastewater and sludge treatment plant than is currently available. In addition, new knowledge was gained regarding the fate and transformation of biopolymers within the treatment plant. Finally, contributions were made in the areas of biopolymer extraction and the analytical method for humic acids estimation in the presence of protein.

Chapter 2: Literature Review

2.1 Introduction

Most bacteria produce extracellular polymers in the form of slime and capsule (Wilkinson, 1958). Slime is loosely bound while capsule is adherent to the cell wall. Slime can be separated from capsule by centrifugation. Whitefield (1988) suggested that bacteria produce extracellular polymers as a response to selective pressures from the environment. Although not required for viability or growth, biopolymers offer advantages to bacteria that produce them, in many cases increasing the microorganisms chances of survival. Extracellular polymers help to protect bacteria against desiccation, predation and toxic compounds. For example, while the capsulated strain of *Streptococcus pneumoniae* is lethal to mice, the uncapsulated variant is harmless and easily destroyed (Prescott et al., 1996). Bitton (1994) described that capsule complexes with heavy metals, therefore affecting the penetration of metals into the cell. In addition to the function of protection, biopolymers help bacteria to adhere to surfaces and form flocs, keeping the bacteria in favorable environments.

Wilkinson (1958) and Sutherland (1972) observed that maximum polymer production by *Klebsiella aerogenes* and *Escherichia coli* occurred when nitrogen, phosphorus, or sulfur became the limiting nutrient. Polymer accumulation occurred mostly after the end of the logarithmic phase of bacterial growth, although the rate of production was higher during the logarithmic phase. The excess carbon that remained during the stationary phase continued to be used for polymer production. In addition to N, P, and S limitations, capsule and slime synthesis were higher when oxygen supply was not limited (Sutherland, 1972). Capsule and slime from bacteria are generally formed by polysaccharides, either as homopolysaccharides or heteropolysaccharides (Wilkinson, 1958). The former include polymers of glucose (cellulose, dextran) and

fructose (levan). Heteropolysaccharides include polymers of neutral sugars (e.g. glucose, galactose, fucose, mannose, rhamnose) with acidic sugars (e.g. glucuronic acid), which give the bacterial surface an anionic character. For example, *Klebsiella aerogenes* yields polymers composed by L-rhamnose, D-mannose, D-glucose, D-galactose, D-glucuronic acid and D-galacturonic acid; *Echerichia coli* type 27 produces polymers composed by D-glucose, D-galactose, L-fucose and glucuronic acid (Sutherland, 1972, 1985). Due to the nature of their composition, biopolymers are frequently named extracellular polysaccharides or exopolysaccharides.

Extracellular polymers have drawn attention in the field of environmental engineering for their role in biological flocculation, sludge dewatering properties, and heavy metal complexation. In addition, the presence of biopolymers in secondary effluents affects disposal methods and advanced processes of treatment.

2.2 Extracellular Polymers and Biological Flocculation

Part of the energy released during biological oxidation of wastewater is used by microorganisms for the production of new cells. This biomass needs to be efficiently removed in secondary sedimentation tanks in order to achieve the desired level of organic matter and suspended solids removal. The mechanisms of floc formation have been researched for many years.

Butterfield (1935) isolated a bacteria that grew in flocs, *Zoogloea ramigera*, from activated sludge. This organism was described as a strict aerobe, rod-shaped, surrounded by capsule, and with 1.5 μm average diameter. McKinney and Horwood (1952) isolated 11 floc-producing organisms from activated sludge. All formed capsule outside the cell wall. McKinney (1952) fed pure cultures of bacteria with soluble substrates, and observed their growth and flocculation. He noticed that during flocculation bacterial cells were joined together by their capsules. He then postulated

that bacterial surface was the key element in activated sludge flocculation. The carboxyl groups present in capsules give bacteria a negative charge. In aeration tanks, cations present in solution would be attracted to the bacterial surfaces, reducing the surface charge. As the number of bacteria grows due to favorable environmental conditions, the number of collisions would increase, raising the probability of bacteria sticking to each other. Once two bacterium stick, a larger area is available for further collisions, and the floc would grow. Organic wastewater colloids captured by the floc serve as a source of energy, while inorganic colloids add mass to the floc. The growth of flocs is limited by forces of turbulence in the aeration tank. Although the author considered electrical charge reduction as the predominant mechanism in flocculation, he suggested that other reactions such as direct peptide, salt and ester linkages between bacterial surfaces also play a role in flocculation.

Tenney and Stumm (1965), however, argued that reduction in charge density was not a prerequisite to flocculation, as negatively charged polyelectrolytes can flocculate colloids with the same charge. These authors found that flocculation becomes optimal in the endogenous phase of the bacterial growth curve. They hypothesized that, although biopolymers can be produced and excreted in all phases, it was during the endogenous phase that an "optimal" surface area was available for polymers to adsorb. During exponential growth phase, surfaces were being created faster than the availability of polymers. In their experimental work, they found a stoichiometric relationship between the number of microorganisms (total surface area) and the amount of polyelectrolyte required to flocculate the microorganisms such that, because of sedimentation, their concentration in suspension was halved. Flocculation was thus explained as the bridging among hydrophilic biocolloids (bacteria) brought about by large molecules (biopolymers).

Based on their experiments, Crabtree et al. (1966) disagreed with the bridging theory. They postulated that flocculation would occur only if certain carbohydrates and peptides were present in the microorganisms growth medium. These nutrients were precursors of the intracellular polymer poly-β-hydroxybutyric acid (PHB). They noticed that cultures of *Zoogloea ramigera* would flocculate only after this polymer had been accumulated in the cells. They believed that the synthesis of PHB caused cells to divide incompletely, leaving pairs of cells attached. Other cells under the same conditions would attach to each other, first by mechanical means, followed by ester linkage between PHB in adjacent cells.

Busch and Stumm (1968) showed that extracellular polymers extracted from an activated sludge culture grown in laboratory flocculated an inorganic colloid (silica) and *Aerobacter aerogenes* cells. The curves relating residual turbidity with polymer dosage were typical of those that are obtained when synthetic polyelectrolytes are applied to negatively charged colloids. For a given surface area, represented by the colloid or cell concentration, there was an optimal biopolymer concentration that would give maximum flocculation. When too much biopolymer was present relative to the surface area, colloid and cell redispersion occurred. Based on these results, the authors suggested that extracellular polymers may adsorb to available sites on bacteria and inorganic colloids, forming bridges that lead to floc formation. The forces that hold the polymer to the surface must be higher than the electrostatic repulsion that occurs when two particles with the same charge interact. One of the possibilities emphasized by the authors was the interactions with cations in the immediate vicinity of the bacterial surface. In the authors' experiments, flocculation would occur only when a minimum concentration of divalent cations (3×10^{-4} M of Ca^{+2} or Mg^{+2}) was present in the medium. Flocculation would still occur in the absence of polymers, but the cations concentration had to be increased to at least 10^{-1} M. The authors postulated

that the main role of cations in flocculation was to make complex or ion pair formation between the functional groups in the bio or synthetic polymer and counter ions close to the bacterial or inorganic colloid surface. This mechanism would enhance polymer adsorption at the interface. Other observation from their experiments was that positively charged Al_2O_3 particles were imparted a negative electrophoretic mobility when contacted with an aqueous solution of biopolymers extracted from activated sludge, thus proving the negative character of the extracted polymers.

Working with batch systems of bacterial cultures, Pavoni et al.(1972) observed that optimal flocculation and highest concentration of biopolymers occurred during the endogenous phase of the bacterial growth curve. They measured an increase in the negative surface charge of a kaolin suspension upon addition of extracted biopolymers. They concluded that reduction in surface potential was not a requisite for flocculation. In addition, biopolymers should have surface functional groups that were mostly anionic and nonionic at neutral pH. They postulated that flocculation occurs as a result of polymer bonding to cell surfaces and subsequently bridging among cells in the suspension. The authors also found that a lower concentration of biopolymers was required to flocculate inorganic particles if the extracted polymers were kept in the original supernatant, as opposed to drying them. The authors hypothesized that cations present in the liquid enhanced flocculation by coordinating with functional groups in the polymer and on the particle surface.

Harris and Mitchell (1975) investigated the influence of physiological conditions on polymer characterization and flocculative properties. The organism *Leuconostic mesenteroides* grown on sucrose failed to agglomerate at various growth phases and at pH values ranging from 4 to 10. The same organism grown on glucose flocculated at several phases of their growth curve, over a wide range of ionic strength, and at pH above 7. *L. mesenteroides* grown in sucrose is known to produce

the nonionic polymer dextran. Similar results were obtained for other bacteria that synthesize dextran in the presence of sucrose. When dextran was removed by centrifugation, the sucrose-grown bacteria showed aggregation similar to that of glucose-grown bacteria. If dextran was reintroduced, inhibition of aggregation occurred again. If dextran was added to glucose-grown bacteria, they failed to agglomerate. It was concluded that dextran inhibited aggregation of *L. mesenteroides* regardless of the grown medium. A synthetic polyelectrolyte enhanced aggregation of glucose-grown *L. mesenteroides*, but failed to cause flocculation in the sucrose-grown organism in the presence of dextran. The authors postulated that dextran stabilized the suspension by covering the cells, and protecting them from interactions with other cells and polyelectrolytes. Steric repulsion of the adsorbed nonionic species opposes approach from other particles. The same observations were made for dextran produced by *Lactobacillus* grown in sucrose, for levan produced by pseudomonas, and lytic polymers from *L. mesenteroides*. The authors concluded that dextran and levan are detrimental to flocculation. Wastes containing a high amount of sucrose, which induces dextran and levan production by microorganisms, may not be conducive to good flocculation.

Some authors tried to find a correlation between the amount of extracellular polymers and one measure of sludge settleability, the Sludge Volume Index. Beccari et al. (1980) found that when the concentration of biopolymers was below a critical level, increases in their concentration correlated with decreases in SVI, and vice-versa. When the critical level was reached, further increases in biopolymer concentration caused higher values in SVI. Forster (1971) found no strong correlation between the amount of carbohydrates in polymers and SVI. Ryssov-Nielsen (1975), however, postulated that SVI increases with carbohydrate content in extracellular polymers but decreases with higher protein quantities. Steiner et al. (1976) measured higher values

of electrophoretic mobility with higher values in SVI in activated sludge samples taken from three plants in Scotland. At seven wastewater treatment plants in Sweden, Eriksson and Alm (1991) noted a trend towards decreasing SVI and capillary suction time in activated sludges that had lesser amounts of biopolymer. This trend was also observed by Urbain et al. (1993) who measured a positive correlation between SVI and the amount of biopolymers in seven wastewater treatment plants in France. However, comparison of sludge settling properties among different plants based on SVI is of limited utility. The test does not measure physical properties of activated sludge, such as yield strength or plastic viscosity, nor can it be used to predict settling characteristics in full scale plants (Dick and Vesilind, 1969). SVI values are also dependent upon the suspended solids concentration. These issues were not addressed by Eriksson and Alm (1991) and Urbain et al. (1993).

Li and Ganczarczyk (1990) studied the internal structure of activated sludge flocs larger than 100 μm. Qualitatively, the authors described that biopolymers, microorganisms and water were the major components of the flocs. Hansen (1998) described activated sludge flocs as being composed of organic fibers, bacterial colonies, inorganic particles, filamentous bacteria, and divalent cations, all linked together by extracellular polymers. The polymers are compounds excreted from bacterial metabolism or lysis, or they can be compounds that were adsorbed from the wastewater. Vallom and McLoughlin (1984) detected an improvement in flocculation when DNA, originating from cell lysis, was added to bacterial cultures.

Higgins and Novak (1997a) postulated that proteins present in extracellular polymers play an important role in flocculation. They added a protein-degrading enzyme (pronase) to an activated sludge sample and measured a shift in particle size distribution towards smaller particles. Addition of a polysaccharide degrading enzyme (cellulase) caused much less deflocculation. In another experiment, the addition of

divalent cations (Ca^{++} and Mg^{++}) to an activated sludge reactor caused an increase in the quantity of protein in biopolymers, but the amount of polysaccharides remained constant. As the amount of bound protein increased, the reactor effluent total suspended solids concentrations decreased. The authors concluded that protein played a more important role than polysaccharides in bioflocculation.

In addition to flocculation in activated sludge, the role of biopolymers also has been investigated in the formation of granular sludge in upflow anaerobic sludge reactors (UASB). This process depends on the formation of granular sludge that can be retained in the reactor. Washout of granules is detrimental to the process. de Beer et al. (1996) reported that granules that were retained in a UASB reactor contained more extracellular polymers than flocs that were in the washout. Schmidt and Ahring (1994) found that granules grown under mesophilic temperature had more extracellular polymers than granules grown under thermophilic conditions. Both groups of investigators determined that the presence of carbohydrates in the reactors feed increased the retention of granules inside the reactors. The presence of carbohydrates and consequently granule retention was associated with the increase in biopolymers.

2.3 Extracellular Polymers and Sludge Dewatering Properties

Novak et al. (1977) observed that anionic biopolymers influenced sludge dewatering characteristics. The polymers that were found to be detrimental were present in the sludge supernatant, not associated with the flocs. This was concluded upon testing the amount of cationic polymers required to reach a minimum filtration time at several solids to supernatant volume ratios. The optimum amount of polymer was found to be independent of the amount of sludge solids in the sample, but dependent on the amount of the supernatant liquor volume. The minimum filtering

time occurred at the point of zero colloidal charge, indicating the biopolymers in the supernatant were anionic. In another experiment, the simple addition of clay to sludge improved its dewatering properties. The mechanism here would be interparticle bridging by the anionic polymer in the supernatant liquor with the negatively charged clay particles. The conclusion was that "an anionic biopolymer demand" would need to be satisfied in order to improve sludge dewatering properties.

Gulas et al. (1979) found a positive correlation between the ratio of total biopolymer mass per unit of active MLVSS and the percent float solids collected in a dissolved-air flotation apparatus. They also measured an inverse correlation between the ratio of specific resistance to filtration per unit of float solids and the ratio of total polymer mass per unit of active MLVSS. Extracellular polymers in this study included both cell associated and loosely bound polymers. They concluded that the presence of biopolymers enhanced the thickening and filtration properties of activated sludge.

Bowen and Keinath (1984) correlated carbohydrate, protein and lipid contents in primary, activated and digested sludges with a Polymer Index (PI). This index would represent an optimum or near optimum dosage of a synthetic cationic polymer to reach the lowest specific resistance to filtration for the sludge. Polymers performed better if they had lower PI. For several synthetic polymers tested, the authors positively correlated better performance with increasing amounts of carbohydrate and protein in the sludges. The opposite was found for lipids. Although the biochemical analyses were made with whole sludge samples, the authors extrapolated the results to extracellular polymers. In their analysis, the positive correlation that was found between PI with protein and carbohydrate content was due to the presence of these compounds in the extracellular polymers of the sludges.

Kang et al. (1989) measured dewatering characteristics and extracellular polymers in six types of sludges. Dewatering characteristics were given by the specific

resistance to filtration and a consolidation coefficient. The latter measures the ease with which water is removed from the sludge cake during the consolidation period. Higher values of the coefficient indicate more favorable properties for sludge dewatering. The sludges studied were: (1) anaerobically digested sludge from a municipal treatment plant, (2) excess sludge from a pharmaceutical factory, (3) return sludge from a chemical plant, (4) return sludge from a fiber plant, (5) excess sludge from a beer plant, and (6) return sludge from a fiber dyeing plant. All plants used the activated sludge process. Although not specified by the authors, "return" and "excess" sludges were likely to be activated sludges that had been concentrated in secondary clarifiers. With the exception of anaerobic sludge, specific resistance to filtration decreased and consolidation coefficient increased with lower amounts of extracellular polymers in the sludges. Digested sludge had the highest and lowest specific resistance and consolidation coefficient, respectively. In comparison with a sludge that had the same amount of extracellular polymers (beer factory), the specific resistance to filtration and consolidation coefficient of the digested sludge were 18 times higher and 19 times lower, respectively. In one of the authors' experiments, extracellular polymers were extracted from each sludge. They were then added at different concentrations to the digested sludge, and the activated sludges from the beer and fiber dyeing plants. The values of the specific resistance to filtration and the consolidation coefficient consistently increased and decreased, respectively, as the concentration of extracellular polymers added increased. It was concluded that extracellular polymers are harmful to sludge dewatering.

Experiments by Higgins and Novak (1997b) indicated that settling and dewatering of activated sludges required a minimum concentration of the divalent cations calcium and magnesium. In addition, the ratio between mono to divalent cations needed to be kept below a certain level to maintain good sludge properties.

When these conditions were not met, extracellular polymers that were bound to flocs were released into solution, weakening the floc. The function of divalent cations was believed to be the linkage between negatively charged sites within the floc. The action of excess sodium was interpreted as an ion exchange process where divalent cations in the floc were exchanged by sodium in solution. This phenomenon has been detected in treatment plants that receive wastes containing stormwater from snowmelt. For example, Eriksson and Alm (1993) monitored activated sludge properties during periods comprising the beginning and end of snowmelt. During snowmelt, the sludge presented high filtration resistance and low floc strength; effluent turbidity was high and extracellular polymers were more easily extracted from the flocs. When the snowmelt ceased, flocs gained strength, specific resistance to filtration decreased and effluent turbidity dropped. Extracellular polymers extracted from sludge also decreased. The presence of excess sodium in the snowmelt caused the deterioration in sludge and effluent qualities.

The importance of calcium in sludge properties was also verified by Bruus et al. (1992). The removal of calcium ions from thickened sludge through ion exchange or chelation with EDTA caused increases in supernatant turbidity and specific resistance to filtration. The removal of calcium resulted in a release of small particles that were being held in the floc. These authors believed that divalent cations with varying degrees of selectivity hold activated sludge flocs together. They based this belief on the fact that substitution of calcium in the floc by other divalent cations produced sludges with different dewatering properties. For example, substitution of calcium by copper decreased the sludge specific resistance to filtration; when magnesium was substituted for calcium, the resistance to filtration increased.

To investigate how the removal of extracellular polymers affected rheological and dewatering properties of activated sludge, Sanin and Vesilind (1994) used

centrifugation in the range 2,000 to 16,500 times gravity as a method to extract polymers. Little effect on the quantity of polymer extracted was measured with increasing centrifugation speeds. After the polymers had been extracted, the authors observed that (1) sludge filterability coefficient decreased by 50 %, (2) supernatant turbidity increased, and (3) sludge viscosity decreased. The deterioration in dewaterability, as measured by the decrease in the filterability coefficient, was interpreted as being caused by the release of small particles that occurred when polymers were extracted. These small particles were initially being held in the floc by the polymers.

The influence of extracellular polymers in sludge dewaterability was also examined by Poxon and Darby (1997). Instead of extraction methods, they used the adsorption of a cationic dye, ruthenium red, to anionic sites at the sludge surface to estimate the amount of polymers in anaerobically digested sludges. Two sets of data were gathered, representing two different feeds to the digester. The first set of data was measured when the feed to the digester was regular primary and secondary sludges; the second set of data comprised a period when the feed contained waste from a candy plant, thus rich in carbohydrate. The parameter used to measure sludge dewaterability was the same used by Sanin and Vesilind (1994), the filterability coefficient. Although great scatter was present in their results, the authors stated that a increase in the amount of biopolymers caused a deterioration in the dewatering properties of sludge in data set 1, while the opposite occurred for sludges in data set 2. As a conclusion of their study, the authors proposed that biochemical properties were more important than the quantity of extracellular polymers present in sludges.

The results from these investigations are contradictory. An increase in biopolymers was correlated with both better and poorer dewaterability, suggesting that factors other than the amount of biopolymers present also play a role. Among the other

factors that might influence sludge properties are the presence of mono and divalent cations and wastewater composition. For example, Bura et al. (1998) operated bench scale sequencing batch reactors with feed having four different ratios COD:N:P. They measured variations in biopolymer composition, floc bound water, surface charge and hydrophobicity in each of the reactors' flocs. This was also verified in full scale activated sludge plants with feeds from poultry, petroleum, potato processing and municipal sources.

2.4 Extracellular Polymers and Heavy Metal Adsorption

The presence of anionic groups such as carboxyl, phosphoryl and sulfate in extracellular polymers represents potential sites for metal binding (Fleming, 1995). In addition, the possible presence of chelating agents such as humic acids in biopolymers enhance their potential for metal removal. Sterritt and Lester (1986) presented data indicating removal greater than 50 % was achieved for the soluble copper, lead, chromium and zinc entering activated sludge processes. According to these authors, adsorption, complexation, precipitation and active uptake by viable cells were the mechanisms that acted for metal removal. Based on their experiments, the authors suggested that the uptake capacity of activated sludges increases with sludge age, with an optimum at 12-15 days.

Rudd et al. (1984) measured conditional stability constants of metal complexes formed by activated sludge flocs and extracted extracellular polymers. Higher constants indicate that more metal is bound to adsorption sites. Of the four metals studied, copper was the metal most strongly bound to both floc and polymer. The data measured also allowed estimation of the complexation capacity for each metal. This parameter is a measure of the available binding sites for the particular metal. Because the conditional stability constants and the complexation capacities determined for the

activated sludge flocs and extracellular polymers were similar, the authors concluded that polymers are a significant source of metal uptake in activated sludge systems.

Karapanagiotis et al. (1990) measured conditional stability constants and complexation capacity for extracts from activated and digested sludges. For digested sludge, the stability constants indicated affinity for metals in the order Cd > Cu > Ni > Zn; for activated sludge, the order of affinity was Cu > Zn > Ni > Cd. In these experiments, the capacity of activated sludge to complex metals was less than the capacity of digested sludge. This result was interpreted in terms of availability of binding sites. Because cells in anaerobic sludges remain dispersed or in small clusters, more surface area to volume ratio is available than in activated sludge, where cells are enmeshed in large flocs.

The capacity of anaerobic sludges to adsorb metals has found application in reactors especially designed for metals removal. For example, Morper (1986) contacted heavy metal solutions with anaerobic sludge in reactors designed to maximize contact of metals and sludge. Removal efficiencies greater than 99 % for copper, nickel, zinc and mercury, and 75 % for chromium were obtained during the nine month period of reactor operation. The author postulated that active uptake by viable cells, adsorption to cell surfaces, complexation by surface functional groups, hydroxide and sulfide precipitation, particle agglomeration, filtration and sedimentation contributed to metal removal. One criteria that had been suggested to judge the economic feasibility of metal uptake bioreactors was that metal recovery greater than 99 % must be achieved, along with metal loadings greater than 150 mg/g of biomass. Gadd (1990) stated that there are many examples where these criteria have been satisfied.

The capability of extracellular polymers to adsorb and complex heavy metals can be enhanced by providing special nutrients to a microorganism growth culture. For

example, Fukushi (1996) observed that the addition of β-glycerophosphate, peptone, cystine, and cadmium to the growth culture improved metal uptake in comparison to a culture grown in a basal medium without those compounds. The differences in metal uptake were 369 % for copper and 2711 % for cadmium. β-glycerophosphate is an ATP precursor, and was included in consideration to energy demands of biopolymer production. Cystine induces production of proteins with sulfhidryl groups, which have reportedly enhanced metal uptake capability. Peptone was included to increase polymer production, which had been observed in another experiment. The presence of cadmium in the growth medium was related to its capability to induce production of proteins with higher metal binding capacity. The organic compounds were named "biopolymer stimulators", while cadmium was called "biopolymer inducer". Another finding of this research was that viability of microorganisms didn't affect the efficiency to which the metals adsorbed to the polymers. Because metal concentrations in solution were high, viable cell numbers were reduced by at least three orders of magnitude after 24 h. The metal uptake, however, did not decrease. This was interpreted as the capability of intracellular polymers released by lysis to bind metals.

Wang et al. (1999) considered pH and dissolved organic matter as the most important factors affecting metal uptake by sludge particulates. At low pH, hydrogen ions compete with metals for the negatively charged sites at the bacterial surface, consequently, lower metal removal can be achieved. Dissolved organic matter complexes with metals, keeping them partly in solution and unavailable for adsorption to the sludge. These authors reported that carbohydrates and proteins present in extracellular polymers have equal affinity toward heavy metals.

2.5 Extracellular Polymers in Secondary Effluents

Secondary effluents are sometimes used to recharge groundwater through infiltration on sand or soil, or they may undergo further treatment before final disposal or reuse. Both approaches are being applied particularly in regions with limited availability of water. In the United States, a report from the National Research Council stated that indirect potable reuse of reclaimed water is a viable option after consideration given to water conservation, development of new water sources, and nonpotable reuse (Crook et al., 1999). One country that makes extensive use of groundwater recharge is Israel. Each year, 100 million m^3 of groundwater that had been recharged with secondary effluents are transported to the Negev desert area for irrigation of crops (Sitton, 1999). However, one practical limitation exists with the infiltration of treated effluents. When sand or soil is continuously inundated, the infiltration rate decreases with time due to biological clogging of the medium. The clogging is caused by the production and accumulation of polysaccharides on the medium surface (Mitchell and Nevo, 1964). Polysaccharide production occurs as result of nutrient availability to bacteria, while polysaccharide accumulation is triggered by the low oxidation-reduction potential in the inundated sand or soil (Nevo and Mitchell, 1967). The restoration of aerobic conditions in the medium causes partial degradation of the polysaccharide, and limited return to the original infiltration rate. A method that is used to restore aerobic conditions to an infiltration field is periodic resting, but this alternative requires more land area.

The problem of decreasing infiltration rates was also addressed by Vandevivere and Baveye (1992). They measured hydraulic conductivity in sand columns that had been inoculated with four pure cultures of bacteria: slime producing, capsule producing, and the respective non-mucoid strains of the slime and capsule producing bacteria. Reduction in hydraulic conductivity occurred only in the column

that had been inoculated with the slime producing bacteria. The reduction in hydraulic conductivity was two orders of magnitude in relation to the initial condition. Two of the mechanisms that could have been operative in the hydraulic conductivity reduction were increase in the frictional resistance at the solid-liquid interfaces and decrease in effective porosity.

Dean (1969) recognized the difficulty in filtering secondary effluents from biological treatment plants. He attributed the problem to the presence of high molecular weight anionic hydrocolloids, capsule and slime, which accumulate and clog surface filters, cause shorter runs in rapid filters, and support the growth of microorganisms in granular carbon filters. On the other hand, the presence of these macromolecules complicates flocculation of secondary effluents. Before reacting with suspended solids in the effluent, the flocculant reacts preferentially with the dissolved organic matter. The coagulant demand can be very high. For example, Narkis and Rebhun (1997) determined that effective clarification of a secondary effluent could be achieved when 30 to 40 mg/l of a cationic polyelectrolyte or 400 to 500 mg/l of alum had been added to the effluent. They then separated the suspended solids from the effluent, and determined that the optimum flocculant concentrations for turbidity reduction in the effluent solution continued to be the same. When the suspended solids that had been separated from the effluent were resuspended in tap water to the same concentration present in the original effluent, the optimal concentrations of the flocculants were reduced to 2 mg/l for the cationic polyelectrolyte and 25 mg/l for alum.

In recent years membrane bioreactors have been evaluated as a biological treatment alternative when high quality effluent is required. The process is a modification of the conventional activated sludge system. Effluent and mixed liquor separation is accomplished by membrane filters, which substitute for secondary

clarifiers. Membrane bioreactors produce effluent free of suspended solids and microorganisms (Çiçek et al., 1999). The process has found small scale application in Japan since 1979, in buildings with water reuse capability (Nagaoka et al., 1996). In these applications, cross-flow type membrane modules have been used because of their capability to maintain good permeability for long periods. However, the high cross-flow velocity required to remove the sludge that accumulates on the membrane surface implies high energy consumption. The costs associated with this energy consumption have prevented the process to find application in full scale municipal wastewater plants. Attempts have been made to evaluate process alternatives that could reduce the energy requirement. One of these alternatives has been described by Nagaoka et al. (1996). Loop-type hollow membranes are submerged into the aeration tank, with the treated waste permeating the membrane with the aid of a pump. In a set of experiments, however, the authors, identified the accumulation of extracellular polymers on the membrane surface as a constraint, which caused reduction in flux and increase in the membrane filtration resistance with time.

Concerns exist with respect to possible toxic effects of soluble microbial products present in secondary effluents from biologically-treated wastewater (Eckenfelder, 1994; Barker and Stuckey, 1999). Matsui et al. (1998) described the presence of DNA-damaging chemicals in effluents of activated sludge process treating municipal wastewater and nightsoil. Rappaport et al. (1979) used the Ames mutagen bioassay to test the mutagenic responses of organic concentrates prepared from samples of primary, secondary and post-secondary effluents from six wastewater treatment plants from the San Francisco Bay and Los Angeles areas. More mutagenic activity was found in the organic compounds present in secondary and post-secondary effluents than was observed in the organic extracts from the primary effluent. The authors suggested that activated sludge might convert inactive substances into

mutagens. The process by which innocuous compounds are converted to toxicants by microorganisms is known as activation (Alexander, 1999).

2.6 Extracellular Polymers as Recalcitrant Compounds

The biodegradability of extracellular polymers has been a matter of dispute. Wilkinson (1958) stated that microorganisms are generally incapable of degrading their own biopolymers. In the operation of an activated sludge reactor, Tenney and Stumm (1965) observed that the concentration of glucose, the sole carbon source used, was undetectable when the reactor culture reached the endogenous stage. However, even after extensive aeration, a substantial chemical oxygen demand existed in the reactor's effluent, implying that organic compounds other than glucose were present. The authors postulated that part of these compounds was recalcitrant polysaccharides formed by microorganisms that acted as polymers in the floc formation process. Pavoni et al. (1972) also suggested that biopolymers have very low biodegradability due the low BOD:COD ratio they measured, 0.10. Hejzlar and Chudoba (1986) found that only 40 % of biopolymers present in a system operated in the endogenous phase of bacterial growth were biodegradable, and that the lag time before oxidation started was 7 d.

Because liquors from sludge treatment processes are typically returned to wastewater treatment, some of the recalcitrant products formed during sludge digestion will be present in secondary effluents. For example, Warfel (1998) found that particles in the range 1.1 to 28.2 μm present in secondary effluents correlated better in size and morphology with particles from anaerobic digester supernatant and filtrate from belt filter press than with particles from any other location at a treatment plant. Schiener et al. (1998) evaluated the biodegradability of microbial soluble products formed during anaerobic digestion using the biochemical methane potential

assay. Their tests indicated that 62 to 82 % of the products were anaerobically biodegradable, with the refractory fraction being constituted mostly by high molecular weight heteropolysaccharides.

Obayashi and Gaudy (1973) disagreed that extracellular polymers are not biodegradable. They designed an experiment where slimes and capsules were extracted from five types of bacteria (*Aerobacter aerogenes*, *Arthrobacter viscous*, *Azotobacter vinelandii*, *Xanthomonas campestris*, and *Zoogloea ramigera*) and used as feed and the sole carbon source to acclimated microorganisms found in municipal wastewater. Results of batch experiments indicated that 80 to 93 % of the feed COD was oxidized. In addition, yield coefficients had values in the range of those reported from a variety of organic compounds. The authors concluded that extracellular polysaccharides could not be classified as biologically inert material.

The biodegradability of capsule material has implications in other aquatic environments as well. Wilkinson et al. (1997) named biopolymers from microbial origin as aquagenic organic matter. Together with pedogenic (soil derived) organic matter, they constitute a great part of the natural organic matter of lakes. While the low molecular weight pedogenic organic matter (i.e., fulvic acids) helps to stabilize colloids, the high molecular weight biopolymers favor aggregation and sedimentation of the colloids. Because most toxic and trace nutrient compounds are bound to colloids and macromolecules, the destabilization effect of aquagenic organic matter has important effect on the fate of toxic and nutrients in freshwaters. In the oceans, capsule released by bacterioplankton is fairly resistant to microbial degradation. Stoderegger and Herndl (1998) found that carbon present in biopolymers was incorporated by bacterioplankton at a rate three orders of magnitude lower than the rate for carbon present in glucose. In addition, only 0.02 % of the biopolymer carbon was respired per day. Capsule is released from live bacterioplankton as part of their

metabolism or it is set free when bacterioplankton lose viability. Because the number of bacterioplankton is significant in the oceans, maybe even exceeding phytoplankton in biomass, the authors postulated that the organic matter released by bacterioplankton capsules may constitute a substantial fraction of the oceanic recalcitrant organic matter.

2.7 Extracellular Polymers Extraction Methods

Many techniques have been used for extraction of biopolymers. Loosely associated polymers (slime) distinguish from solids-bound polymers (capsule) by centrifugation (Whitefield, 1988). Capsule usually requires an additional step besides centrifugation. After separation, both slime and capsule can be concentrated and/or purified. Concentration is usually achieved by precipitation upon addition of an organic solvent, e.g. acetone, ethanol. Purification may entail several other steps, depending on the objectives. For example, dialysis is used for removal of low molecular weight impurities and inorganic ions, while specific enzymes such as protease are sometimes used to degrade proteins and leave the biopolymer "purified" for carbohydrates. A major concern of extraction is the contamination of extracellular polymers with intracellular cell components. Although many methods for biopolymer extraction have been used, a discussion about the principles by which each method works was not found in the literature. In the following, extraction methods and their principles are discussed, together with a brief description of their potential for cell disruption. A compilation of applications of biopolymer extraction methods is presented in Table 2.1. It can be inferred from Table 2.1 that a standard technique for biopolymer extraction does not exist. Each researcher has chosen his/her own method, in many cases without consideration for the potential of intracellular disruption. In recent years, there seems to be a trend towards the use of the cation exchange resin

Table 2.1 – Extraction Techniques

Author	Sample	Technique
Dudman and Wilkinson (1956)	*Klebsiella* and *Aero. Cloacae* strains	Slime: centrifugation Capsule: washing, (i) boiling; (ii) NaOH
Nishikawa and Kuriyama (1968)	*Escherichia coli* Activated sludge	Slime: undeclared Capsule: EDTA, precipitation
Busch and Stumm (1968)	*Escherichia coli* *Aerobacter aerogenes*	Capsule: high speed centrifugation, precipitation
Wallen and Davis (1972)	Activated sludge;. bacterial strains	Capsule: (i) boiling, (ii) NaOH, precipitation
Pavoni et al. (1972)	Bacterial cultures from activated sludge	Capsule: high speed centrifugation, precipitation
Obayashi and Gaudy (1973)	5 bacterial strains, activated sludge	Slime: centrifugation, precipitation Capsule: NaOH, precipitation
Carr and Ganczarczyk (1974)	Activated sludge	Capsule: boiling, steaming, trichloroacetic acid, sulfuric acid, centrifugation, EDTA, acetone, sonication, NaOH, boiling benzene
Farrah and Unz (1976)	*Zoogloea* strains	Capsule: washing, high speed centrifugation, precipitation
Novak et al. (1977)	Activated sludge	Capsule: high speed centrifugation, precipitation
Tago and Aida (1977)	Bacterial strain culture from activated sludge	Capsule: washing, NaOH, centrifugation, precipitation, dialysis
Mian et al. (1978)	*Pseudomonas aerogenes*	Capsule: EDTA, precipitation
Gulas et al. (1979)	Synthetic activated sludge	Capsule: high speed centrifugation, precipitation
Kiff and Thompson (1979)	Activated sludge	Capsule: (i) high speed centrifugation; (ii) boiling; (iii) sonication; (iv) blending; (v) shear-press; precipitation
Brown and Lester (1980)	Activated sludge; synthetic activated Sludge; *K. aerogenes*	Capsule: (i) high speed centrifugation; (ii) ultrasonication; (iii) combination of (i) and (ii); (iv) steaming; (v) NaOH; (vi) EDTA
Sato and Ose (1980)	Synthetic activated sludge; *E. coli*	Capsule: washing, NaOH, precipitation
Beccari (1980)	Synthetic activated sludge	Capsule: washing, boiling, precipitation
Novak and Haugan (1981)	Activated sludge	Slime: sedimentation, precipitation Capsule: Centrifugation at several speeds
Forster and Clark (1983)	Activated Sludge	Capsule: sedimentation, washing, ethanol
Rudd et al. (1983)	Activated sludge	Capsule: sedimentation, then (i) boiling; (ii) ultrasonication; (iii) homogenization; (iv) sodium tripolyphosphate; (v) NaOH; (vi) steaming; (vii) Dowex; dialysis

Table 2.1 (Continued)

Author	Sample	Technique
Turakhia et al.(1983)	Biofilm from bacteria of Activated sludge	Chelant EGTA
Gehr and Henry (1983)	Activated sludge amended with glucose	Capsule: (i) high speed centrifugation; (ii) boiling; (iii) boiling in acid; (iv) alkaline Boiling (NaOH); (v) homogenization in a blender; (vi) K_2HPO_4, precipitation
Sato and Ose (1984)	Synthetic activated sludge	Capsule: Ammonium hydroxide and EDTA, precipitation
Starkey and Karr (1984)	Synthetic activated sludge	Capsule: NH_4OH, phenol, sulfuric acid
Rudd et al. (1984)	Activated sludge	Capsule: Dowex
Platt et al. (1985)	Sediment freshwater bacterium	Capsule: EDTA, precipitation
Dolfing et al. (1985)	Granular methanogenic sludge	Capsule: washing, (i) EDTA; (ii) steaming; (iii) phenol, dialysis
Horan and Eccles (1986)	Activated sludge	Capsule: washing, heat at 80 °C, precipitation. Purification by dialysis, addition of deoxyribonuclease and protease
Hejzlar and Chudoba (1986)	Synthetic activated sludge	Slime: filtration, concentration under vacuum, filtration, dialysis, lyophilization
Read and Costerton (1987)	*P. putida* *P. fluorescens*	Slime: Centrifugation, dialysis Capsule: NaCl, centrifugation, dialysis
Karapanagiotis et al. (1989)	Digested sludge	Slime: centrifugation Capsule: (i) Dowex; (ii) boiling; (iii)NaOH (iv) phenol; (v) steaming
Domenico et al. (1989)	*Klebsiella pneumoniae*	Capsule: washing, detergent (Zwitergent), centrifugation
Kang et al. (1989)	Activated sludge	Capsule: washing, sodium carbonate, centrifugation, dialysis
Figueroa and Silverstein (1989)	bench-scale sequencing batch reactor	Capsule: ruthenium red adsorption
Hsieh et al. (1990)	*Pseudomonas atlantica*	Slime: centrifugation, dialysis Capsule: EDTA, dialysis
Morgan et al. (1990)	Activated and digested sludges	Capsule: heating at 80 °C, precipitation
Karapanagiotis et al. (1990)	Activated and digested sludges	Capsule: (i) Dowex, (ii) pyrophosphate
Eriksson and Alm (1991)	Activated sludge	Capsule: homogenization with a blender
Urbain et al. (1993)	Activated sludge	Capsule: sonication

Table 2.1 (Continued)

Author	Sample	Technique
Sanin and Vesilind (1994)	Activated sludge	Capsule: sedimentation, centrifugation at several speeds, precipitation
Frølund et al. (1994)	Activated sludge	Capsule: sedimentation, washing, Dowex
Schmidt and Ahring (1994)	Granules UASB reactor: thermophilic and mesophilic, 6 different feeds	Capsule: heating at 70 °C
Jahn and Nielsen (1995)	Biofilms from sewer and from *P. putida*	Capsule: Dowex
Jorand et al. (1995)	Activated sludge	Capsule: Sonication
Chen et al. (1995);	13 Bacterial strains	Slime: centrifugation, dialysis Capsule: EDTA, dialysis
Palmgren and Nielsen (1996)	Activated sludge; *P. putida*	Slime: centrifugation Capsule: Dowex
Jia et al. (1996)	Enriched cultures of hydrogenotrophic methanogens and sulfate reducing bact.	Capsule: washing, ultrasonic homogenization
de Beer et al. (1996)	Synthetic sludge from UASB reactor	Capsule: washing, NaCl
Frølund et al. (1996)	Activated sludge	Capsule: sedimentation, (i) Dowex; (ii) NaOH; (iii) heating at 80 °C
Higgins and Novak (1997)	Synthetic activated sludge	Slime: centrifugation Capsule: Sonication
Schiener et al. (1998)	Synthetic anaerobic sludge	Slime: centrifugation, ultrafiltration, phenol, dialysis
Bura et al. (1998)	Activated sludge (i) municipal; (ii) potato; (iii) poultry (iv) petroleum	Capsule: settling, (i) Dowex; (ii) steaming
Azeredo et al. (1998)	Activated sludge	Capsule: washing, (i) glutaraldehyde; (ii) steaming; (iii) sonication (iv) Dowex
Jahn and Nielsen (1998)	Biofilm from sewer	Capsule: Glass homogenization, Dowex
Nagaoka et al. (1998)	Activated sludge	Capsule: washing, NaOH, dialysis
Dignac et al. (1998)	Activated sludge	Capsule: washing, (i) sonication, (ii) Dowex, (iii) sonication and Dowex

technique to extract biopolymers from activated sludge (Karapanagiotis et al., 1990; Frϕlund et al., 1996; Jahn and Nielsen, 1998; Azeredo et al., 1998; Bura et al., 1998; Dignac et al., 1998).

2.7.1 *Heat*

Heat denatures nucleic acids, enzymes and other proteins by changing the shape of the molecules, and destroying their activity. Heat can also disrupt cell membranes (Prescott et al., 1996) and is likely to contaminate extracellular polymers with intracellular polymers.

Heat has been applied for biopolymer extraction under three variations: (1) boiling water, (2) steaming, and (3) heating at lower temperatures. Cells other than bacterial spores are destroyed when subjected to boiling water. In steaming, besides vegetative cells, spores are also destroyed under the action of saturated air, higher than boiling water temperature and pressure (Prescott et al., 1996). Steaming is done in an autoclave, where the temperature reaches 121 °C at 15 lb/in^2. Goodwin and Forster (1985) proposed the use of heating at temperatures in the range 70 - 80 °C for one hour, as an alternative to boiling water and steaming. They argued that less intracellular contamination occurs when extraction occurs at that temperature range.

2.7.2 *Other Physical Methods – Centrifugation, Sonication, Blending*

High speed centrifugation aims to dislodge biopolymers that are attached to the cell surface by the action of fluid drag forces. Centrifugation with force equivalent to 30,000 times the force of gravity has been applied in biopolymer extraction (Pavoni et al., 1972). In sonication, ultrasound is applied to the suspension through a probe, causing cell disruption by the action of shearing and cavitation. In blending, cells are lyzed, ground, and homogenized by vertical strokes and rotation of a pestle (Robyt and

White, 1987). The efficiency of these methods for biopolymer extraction has been questioned in some instances, either on the grounds of small yields or intensity of cell disruption (Novak and Haugan, 1981; Platt et al., 1985; Azeredo et al., 1998).

2.7.3 *Bases and Acids*

Extreme variation in pH causes the destruction of microorganism's plasma membranes, and inhibition of enzyme activities and membrane transport proteins (Prescott et al., 1996). Chemicals used include sodium hydroxide, sulfuric acid, potassium bi-phosphate, ammonium hydroxide, sodium carbonate and trichloroacetic acid. As in the case for boiling or steaming, cellular disruption is likely to contaminate biopolymers extracted using methods that cause large variations in pH.

2.7.4 *Chelate Compounds*

Divalent cations are considered to be essential elements in flocculation (Busch and Stumm, 1968; Higgins and Novak, 1997). In addition, they take part in polymer-polymer and polymer-cell cross linking (Mian et al., 1978). Chelate compounds such as EDTA form stable complexes with divalent cations (Sawyer et al., 1994). Cell-bound biopolymers extraction is based on the principle that the complexing of Ca^{2+} and Mg^{2+} with EDTA weakens the association of the biopolymer with the cell. Biopolymers can then be more easily separated from the cells by medium speed centrifugation, e.g., 12,000 x g. Platt et al. (1985) concluded that little cell disruption occurred with EDTA extraction, after monitoring the presence of glucose-6-phosphate dehydrogenase (an intracellular enzyme) and 2-keto-3-deoxyoctonate (KDO, a compound from the bacterial cell wall) in the extracted biopolymer. Ten percent of the total cell KDO, and 3.3 % of the whole cell intracellular enzyme were measured in the extracted biopolymer.

Another chelate compound that can be used for polymer extraction is ethylene glycol-bis(β-aminoethyl ether)-N,N-tetraacetic acid (EGTA). This compound is calcium specific (Turakhia et al., 1983).

2.7.5 *Cation Exchange Resin*

Ion exchange is a process by which divalent cations are removed from the floc matrix or cell-biopolymer linkage. Sodium in the cation resin exchanges with Ca^{2+} and Mg^2, resulting in the weakening of the linkage between biopolymer and the solid phase. The biopolymer is then separated by centrifugation from the solid phase. Palmgren and Nielsen (1996) and Frϕlund et al. (1996) monitored the intracellular enzyme glucose-6-phosphate dehydrogenase during extraction of biopolymers with Dowex, and observed negligible activity of this enzyme with extraction times up to 2 h. They concluded that cell lysis was minimum with this method. Another favorable feature of this method is that the resin is easily separated from the suspension by centrifugation.

2.7.6 *Other Miscellaneous Methods*

Ethanol and Phenol

In microorganism control, ethanol and phenol are both used as disinfectants and antiseptics. They denature protein and possibly dissolve membrane lipids (Prescott et al., 1996) causing intracellular contamination. Although Forster and Clark (1983) have used ethanol for extracting extracellular biopolymer, inclusion of intracellular constituents seems likely.

Sodium Chloride

Addition of concentrated salt causes an imbalance in the osmotic equilibrium between the inner and outer cell environments. As a result of osmotic pressure, water leaves the cell, which is dehydrated, retarding partially or completely the metabolic processes (Pelczar et al., 1986). Read and Costerton (1987) used salt addition to extract cell-bound polymers from pure cultures of two bacterial strains. The authors didn't discuss the principle by which polymers are released when this method is applied, but it is possible the major mechanism is the exchange of calcium in the polymer-cell wall linkage by sodium in the salt.

Detergents

Detergents act as wetting agents and emulsifiers due to their property of having polar hydrophilic and nonpolar hydrophobic ends. Detergents are capable of disrupting microbial membranes, and possibly denature proteins (Prescott et al., 1996). Detergents have not found much use in biopolymer extraction. However, Domenico et al. (1989) described a procedure where the detergent Zwittergent was used to extract polymers with minimal cell disruption, as seen with an electron microscope.

Glutaraldehyde

Glutaraldehyde has antimicrobial properties (Pelczar et al., 1986). It inactivates vegetative bacteria, fungi, bacterial and fungal spores, and viruses. The inactivation is likely to occur from the reaction of glutaraldehyde with proteins and nucleic acids (Prescott et al., 1996). Azeredo et al. (1998) reported that glutaraldehyde appears to solubilize biopolymer components without causing much cell lysis.

2.7.7 *In Situ Methods*

In situ methods make use of dyes that bind to surfaces holding an opposite charge. For example, ferritin was used to bind anionic sites in biofilms (Eighmy et al., 1983); congo red and tween 80 dyes were used to stain extracellular polymers in bacteria (Allison and Sutherland, 1984); ruthenium red was used to bind carboxyl groups in biopolymers from activated and anaerobic sludges (Figueroa and Silverstein, 1989; Poxon and Darby, 1997).

2.8 Physical and Chemical Characterization of Extracellular Biopolymers

The chemical composition of biopolymers according to the measurements made by several investigators is presented in Table 2.2. The table is arranged to show the type of sample, the extraction method, the characteristic analyzed, the method of analysis and compound quantification.

By far the major source of biopolymer studies has been activated sludge. From the 54 sources of samples compiled in Table 2.2, 45 were activated sludge (36 from full scale plants, eight from bench scale reactors, and one from pilot plant). Four samples were from anaerobically digested sludge, and five from upflow anaerobic sludge blanket (UASB) reactors.

Of the variety of extraction methods that have been applied, centrifugation, sonication, steaming, EDTA and Dowex exchange resin were the most frequently used. The chemical characterization of biopolymers has been usually incomplete. Biopolymers were analyzed for carbohydrate, protein, DNA and RNA in only five samples – from these, all but one were bench-scale reactors. In the sole full scale plant where those four compounds were assessed (Horan and Eccles, 1986), only the percentage of each compound in biopolymer could be inferred.

Table 2.2 Amount and Chemical Composition of Biopolymers

Author	Sample	Extraction Method	Characteristic	Method of Analysis	Quantification
Nishikawa and Kuriyama (1968)	activated sludge (unrevealed source)	EDTA	DNA RNA	unknown	46.4 mg/g VS 97.8 mg/g VS
Pavoni et al. (1972)	batch bacterial culture grown in glucose	centrifugation [1]	carbohydrate protein DNA RNA mass biop/mass TS	phenol-sulfuric acid Folin-Lowry diphenylanine orcinol	~165 mg/g SS ~ 60 mg/g SS ~ 60 mg/g SS ~ 70 mg/g SS 0.4
	batch bacterial culture grown in nutrient broth	centrifugation [1]	carbohydrate Protein DNA RNA mass biop/mass TS	phenol-sulfuric acid Folin-Lowry diphenylanine orcinol	~75 mg/g SS ~100 mg/g SS ~ 28 mg/g SS ~ 35 mg/g SS 0.25
	batch bacterial culture grown in salicylic acid	centrifugation [1]	carbohydrate Protein DNA RNA mass biop/mass TS	phenol-sulfuric acid Folin-Lowry diphenylanine orcinol	~120 mg/g SS ~ 65 mg/g SS ~ 40 mg/g SS ~ 50 mg/g SS 0.32
	batch bacterial culture grown in acetic acid	centrifugation [1]	mass biop/mass TS		0.06
Carr and Ganczarczyk (1974)	activated sludge Humber plant, Toronto, Canada	boiling	carbohydrate protein	anthrone Folin-Lowry	23.8 mg/g VSS 8.7 mg/g VSS
		steaming	carbohydrate protein	anthrone Folin-Lowry	- 18.0 mg/g VSS

Table 2.2 (Continued)

Author	Sample	Extraction Method	Characteristic	Method of Analysis	Quantification
Carr and Ganczarczyk (1974)	activated sludge Humber plant, Toronto, Canada	centrifugation	carbohydrate protein	anthrone Folin-Lowry	0.7 mg/g VSS 0.1 mg/g VSS
		sonication	carbohydrate protein	anthrone Folin-Lowry	71.0 mg/g VSS 65.8 mg/g VSS
		boiling benzene	carbohydrate protein	anthrone Folin-Lowry	0.3 mg/g VSS 0.0 mg/g VSS
Kiff and Thompson (1979)	activated sludge (unknown source)	centrifugation heat sonication homogenization	ethanol insoluble matter	precipitation and drying	26 to 54 mg/g SS 34 to 36 mg/g SS ~ 35 mg/g SS < < 30 mg/g SS
Brown and Lester (1980) [2]	activated sludge Mogden plant, London	centrifugation	carbohydrate protein DNA	anthrone Folin-Lowry diphenylamine	0.5 mg/g SS 2.9 mg/g SS 0.3 mg/g SS
		sonication	carbohydrate protein DNA	anthrone Folin-Lowry diphenylamine	0.2 mg/g SS 0.1 mg/g SS 0.1 mg/g SS
		steaming	carbohydrate protein DNA	anthrone Folin-Lowry diphenylamine	15.5 mg/g SS 75.1 mg/g SS 3.7 mg/g SS
		sodium hydroxide	carbohydrate protein DNA	anthrone Folin-Lowry diphenylamine	19.9 mg/g SS 75.1 mg/g SS 13.1 mg/g SS

Table 2.2 (Continued)

Author	Sample	Extraction Method	Characteristic	Method of Analysis	Quantification
Brown and Lester (1980) [2]	activated sludge, Mogden plant, London	EDTA	carbohydrate protein DNA	anthrone Folin-Lowry diphenylamine	15.4 mg/g SS 118.2 mg/g SS 4.3 mg/g SS
Sato and Ose (1980)	activated sludge grown in synthetic medium	sodium hydroxide (0.1 N)	carbohydrate protein DNA RNA	anthrone Folin-Lowry u.v. absorbance u.v. absorbance	15.1 % biop. mass 22.1 % biop. mass 23.3 % biop. mass 20.3 % biop. mass
Forster and Clarke (1983)	activated sludge Severn-Trent WA plant, England	ethanol	yield	ethanol precipitation	108 mg/g SS
Gehr and Henry (1983)	activated sludge Humber plant, Toronto	centrifugation K_2HPO_4	yield	ethanol precipitation	12.6 mg/g SS 21.2 mg/g SS
Rudd et al. (1983)	activated sludge Hogsmill Valley Plant, Thames Water Authority	sonication	carbohydrate protein	phenol sulfuric acid Folin-Lowry	7.8 mg/g SS 22.6 mg/g SS
		steaming	carbohydrate protein	phenol sulfuric acid Folin-Lowry	28.5 mg/g SS 86.2 mg/g SS
		boiling	carbohydrate protein	phenol sulfuric acid Folin-Lowry	23.4 mg/g SS 88.2 mg/g SS
		Dowex	carbohydrate protein	phenol sulfuric acid Folin-Lowry	38.9 mg/g SS 147.9 mg/g SS
		sodium hydroxide 2 N	carbohydrate protein	phenol sulfuric acid Folin-Lowry	65.1 mg/g SS 135.5 mg/g SS
Starkey and Karr (1984)	bench-scale reactors fed with dextrose	NH_4OH	carbohydrate	phenol-sulfuric acid	33.0 mg/g VSS

Table 2.2 (Continued)

Author	Sample	Extraction Method	Characteristic	Method of Analysis	Quantification
Sato and Ose (1984)	activated sludge grown in synthetic medium	NH_4OH and EDTA	yield		103.8 mg/g SS
Rudd et al. (1984)	activated sludge pilot plant ϕ_C = 10 d	Dowex	carbohydrate protein	phenol sulfuric acid Folin-Lowry	20.7 mg/g VSS 92.3 mg/g VSS
Dolfing et al. (1985)	upflow anaerobic sludge blanket plant (sugar) Netherlands	steaming, sodium hydroxide, EDTA, phenol	carbohydrate	unknown	10 – 20 mg/g TS
Horan and Eccles (1986)	activated sludge, unrevealed source	heat 80 °C	carbohydrate protein DNA RNA	phenol-sulfuric acid Folin-Lowry thiobarbituric acid modified orcinol	66 % biop 11 % biop 16 % biop 8 % biop
Karapanagiotis et al. (1989)	anaerobic digested sludge, Beckton Sewage Works, Thames Water Authority, UK	sodium hydroxide	carbohydrate protein yield	anthrone Folin-Lowry ethanol precipitation	13.7 mg/g TS 48.1 mg/g TS 34.7 mg/g TS
		Dowex	carbohydrate protein yield	anthrone Folin-Lowry ethanol precipitation	9.7 mg/g TS 31.4 mg/g TS 33.5 mg/g TS
		boiling	carbohydrate protein	anthrone Folin-Lowry ethanol precipitation	11.3 mg/g TS 33.8 mg/g TS 24.1 mg/g TS
		steaming	carbohydrate protein	anthrone Folin-Lowry ethanol precipitation	6.1 mg/g TS 22.4 mg/g TS 14.7 mg/g TS

Table 2.2 (Continued)

Author	Sample	Extraction Method	Characteristic	Method of Analysis	Quantification
Karapanagiotis et al. (1989)	anaerobic digested sludge, Beckton Sewage Works	phenol	carbohydrate protein	anthrone Folin-Lowry ethanol precipitation	10.5 mg/g TS 5.6 mg/g TS 4.1 mg/g TS
Kang et al. (1989)	municipal anaerobic digested sludge	sodium carbonate	carbohydrate protein (6.25·org N)	phenol sulfuric acid Kjeldahl	14.8 mg/g TS 59.4 mg/g TS
	activated sludge pharmaceutical	sodium carbonate	carbohydrate protein (6.25·org N)	phenol sulfuric acid Kjeldahl	24.4 mg/g TS 343.1 mg/g TS
	activated sludge chemical plant	sodium carbonate	carbohydrate protein (6.25·org N)	phenol sulfuric acid Kjeldahl	22.7 mg/g TS 73.1 mg/g TS
	activated sludge fiber plant	sodium carbonate	carbohydrate protein (6.25·org N)	phenol sulfuric acid Kjeldahl	18.0 mg/g TS 67.5 mg/g TS
	activated sludge beer plant	sodium carbonate	carbohydrate protein (6.25·org N)	phenol sulfuric acid Kjeldahl	16.2 mg/g TS 60.0 mg/g TS
	activated sludge fiber dying plant	sodium carbonate	carbohydrate protein (6.25·org N)	phenol sulfuric acid Kjeldahl	14.0 mg/g TS 42.5 mg/g TS
Figueroa and Silverstein (1989)	bench-scale sequencing batch reactor	sodium hydroxide EDTA ruthenium red	carbohydrate	phenol sulfuric acid phenol sulfuric acid absorbance	123 mg/g SS 26 mg/g SS 152 mg/g SS
Morgan et al. (1990)	activated sludge, Kidderminster plant, UK	heat 80 °C	carbohydrate protein yield	phenol sulfuric acid Folin-Lowry ethanol precipitation	23.9 mg/g SS 14.1 mg/g SS 90.2 mg/g SS
	anaerobic digested sludge, Bromsgrove plant, UK	heat 80 °C	carbohydrate protein yield	phenol sulfuric acid Folin-Lowry ethanol precipitation	1.9 mg/g SS 5.4 mg/g SS 13.3 mg/g SS

Table 2.2 (Continued)

Author	Sample	Extraction Method	Characteristic	Method of Analysis	Quantification
Morgan et al. (1990)	upflow anaerobic sludge blanket paperwaste plant, Aberdeen, UK	heat 80 °C	carbohydrate protein yield	phenol sulfuric acid Folin-Lowry ethanol precipitation	2.3 mg/g SS 2.9 mg/g SS 10.4 mg/g SS
	upflow anaerobic sludge blanket dairy waste plant, Gloucester, UK	heat 80 °C	carbohydrate protein yield	phenol sulfuric acid Folin-Lowry ethanol precipitation	8.4 mg/g SS 9.0 mg/g SS 46.8 mg/g SS
Karapanagiotis et al. (1990)	activated sludge, Beckton plant, Thames Water Authority	Dowex	protein	Folin-Lowry	221.7 mg/g SS
		pyrophosphate	carbohydrate protein	phenol sulfuric acid Folin-Lowry	45.8 mg/g SS 171.1 mg/g SS
	anaerobic digested sludge, same source	Dowex	protein	phenol sulfuric acid Folin-Lowry	- 200.9 mg/g SS
		pyrophosphate	carbohydrate protein	phenol sulfuric acid Folin-Lowry	60.8 mg/g SS 215.7 mg/g SS
Eriksson and Alm (1991)	activated sludge (Margretelund plant, Sweden)	homogenization	carbohydrate protein humic acid	anthrone Bradford (dye) Folin-Lowry minus Bradford	11.5 mg/g SS 35.0 mg/g SS 17.0 mg/g SS
	activated sludge (Käpala plant, Sweden)	homogenization	carbohydrate protein humic acid	anthrone Bradford (dye) Folin-Lowry minus Bradford	10.3 mg/g SS 29.4 mg/g SS 18.9 mg/g SS

Table 2.2 (Continued)

Author	Sample	Extraction Method	Characteristic	Method of Analysis	Quantification
Eriksson and Alm (1991)	activated sludge (Rya plant, Sweden)	homogenization	carbohydrate protein humic acid	anthrone Bradford (dye) Folin-Lowry minus Bradford	6.3 mg/g SS 29.3 mg/g SS 16.7 mg/g SS
	activated sludge (Eskilstuna plant, Sweden)	homogenization	carbohydrate protein humic acid	anthrone Bradford (dye) Folin-Lowry minus Bradford	6.0 mg/g SS 16.6 mg/g SS 13.3 mg/g SS
	activated sludge (Henriksdal plant, Sweden)	homogenization	carbohydrate protein humic acid	anthrone Bradford (dye) Folin-Lowry minus Bradford	3.8 mg/g SS 10.4 mg/g SS 6.5 mg/g SS
	activated sludge Himmersfjärden plant, Sweden)	homogenization	carbohydrate protein humic acid	anthrone Bradford (dye) Folin-Lowry minus Bradford	2.7 mg/g SS 8.6 mg/g SS 4.4 mg/g SS
Urbain et al. (1993)	activated sludge extended aeration 0.03 kg BOD/kg VS	sonication	carbohydrate protein DNA	phenol sulfuric acid Bradford (dye) diphenylamine	14.5 mg/g VSS 28.5 mg/g VSS 17.4 mg/g VSS
	activated sludge "low organic load" 0.14 kg BOD/kg VS	sonication	carbohydrate protein DNA	phenol sulfuric acid Bradford (dye) diphenylamine	8.3 mg/g VSS 7.8 mg/g VSS 12.1 mg/g VSS
	activated sludge "intermediate load" 0.29 kg BOD/kg VS	sonication	carbohydrate protein DNA	phenol sulfuric acid Bradford (dye) diphenylamine	14.9 mg/g VSS 30.3 mg/g VSS 21.3 mg/g VSS

Table 2.2 (Continued)

Author	Sample	Extraction Method	Characteristic	Method of Analysis	Quantification
Urbain et al. (1993)	activated sludge "high organic load" 0.61 kg BOD/kg VS	sonication	carbohydrate protein DNA	phenol sulfuric acid Bradford (dye) diphenylamine	24.1 mg/g VSS 71.4 mg/g VSS 24.2 mg/g VSS
Sanin and Vesilind (1994)	activated sludge plant, Durham, NC	centrifugation	yield	ethanol precipitation	20 mg/g TS
Frϕlund et al. (1994)	activated sludge, conventional, ASA plant, Denmark	Dowex	carbohydrate protein	anthrone Folin-Lowry	6.3 mg/g VSS 53.8 mg/g VSS
	activated sludge, advanced, Aalborg plant, Denmark	Dowex	carbohydrate protein	anthrone Folin-Lowry	6.5 mg/g VSS 48.8 mg/g VSS
Schmidt and Ahring (1994)	upflow anaerobic sludge blanket, sugar plant	heat 70 °C	carbohydrate protein lipids	phenol sulfuric acid Kjeldahl Bligh and Dyer	12.6 mg/g VSS 72.6 mg/g VSS 0.2 mg/g VSS
	upflow anaerobic sludge blanket papermill plant	heat 70 °C	carbohydrate protein lipids	phenol sulfuric acid Kjeldahl Bligh and Dyer	11.7 mg/g VSS 68.1 mg/g VSS 0.1 mg/g VSS
Jorand et al. (1995)	activated sludge plant, Nancy, France	sonication	carbohydrate protein DNA	phenol sulfuric acid Folin-Lowry diphenylamine	13 mg/g SS 43 mg/g SS 14 mg/g SS
Palmgren and Nielsen (1996)	activated sludge, Asa and Aalborg plants, Denmark	Dowex	DNA	fluorescence (DAPI)	15 mg/g VS

Table 2.2 (Continued)

Author	Sample	Extraction Method	Characteristic	Method of Analysis	Quantification
Frϕlund et al. (1996)	activated sludge, conventional plant, Asa, Denmark	Dowex [3]	carbohydrate	anthrone	~18 mg/g VSS
			protein	modified Lowry	~ 130 mg/g VSS
			humic acids	modified Lowry	~ 125 mg/g VSS
			DNA	fluorescence DAPI	~ 15 mg/g VSS
	activated sludge, advanced plant, Aalborg, Denmark	Dowex [3]	carbohydrate	anthrone	~20 mg/g VSS
			protein	modified Lowry	~ 120 mg/g VSS
			humic acids	modified Lowry	~ 90 mg/g VSS
			DNA	fluorescence DAPI	~ 12 mg/g VSS
Poxon and Darby (1997)	anaerobic digested sludge, Fairfield-Suisun plant, CA	Adsorption ruthenium red	carbohydrate	absorbance	360 mg/g VS
Bura et al. (1998)	activated sludge plant, Metropolitan Toronto	Dowex	carbohydrate	anthrone	12.7 mg/g VSS
			protein	Folin-Lowry	162.0 mg/g VSS
			DNA	fluorescence DAPI	11.2 mg/g VSS
		steaming	carbohydrate	anthrone	37.5 mg/g VSS
			protein	Folin-Lowry	294.9 mg/g VSS
			DNA	fluorescence DAPI	5.3 mg/g VSS
Dignac et al. (1998)	activated sludge Plaisir, France	sonication	total sugars	gas chromatography	9.5 mg/g VSS
			total aminoacids	HPLC	127 mg/g VSS
		sonication + Dowex	total sugars	gas chromatography	11.4 mg/g VSS
			total aminoacids	HPLC	242.0 mg/g VSS

[1] Chemical quantification was estimated from graphs at 100 h of growth

[2] Samples were filtered through 0.22 μm filters

[3] Chemical quantification was estimated from graphs at 2.0 h extraction time.

Most of the techniques used for chemical analysis were colorimetric. This type of analysis usually involves the hydrolysis of the compound into its basic units, followed by a reaction with a chemical agent, yielding a color with intensity proportional to the amount of the compound present. The chemical agent added usually gives the method its name (e.g., anthrone for carbohydrates, diphenylamine for DNA). However, methods other than colorimetric were also used. For example, the DAPI method for DNA is based on the fluorescence enhancement that occurs when DAPI complexes with DNA. The Kjeldahl method for protein estimation is based on acid-base and oxidation-reduction reactions rather than colorimetric analysis.

The chemical quantification of biopolymers involves the choosing of an extraction technique and methods of biochemical analysis. In addition, samples come from biological processes that may have different features, even if the process is the same. For example, activated sludge might be operating in the endogenous or exponential phases of bacterial growth. Also, influent wastewater may have different strengths or composition. For this reason, a certain degree of variability is expected to occur in the quantity and quality of extracellular polymers. However, a trend in biochemical composition should be detectable.

Data from Table 2.2 indicate that from the five major biochemical compounds, four seem to be significantly present in activated sludge biopolymers – carbohydrate, protein, DNA and RNA. In most of the measurements, protein was the major biochemical component of the solids-associated polymers. The quantity of lipids was very low in the one study in which they were measured (Dignac et al. 1998). In addition to these biochemical compounds, humic acids have also been measured in activated sludge biopolymers (Eriksson and Alm, 1991; Frǿlund et al., 1996). Peter and Wuhrman (1971) had previously postulated that humic acids were well suited flocculant agents due to their high molecular weight and chelant properties. Humic

acids are known to be formed in activated sludge (DeWalle and Chian, 1974) and digested sludge (Riffaldi et al., 1982). Finally, activated sludge biopolymers should contain an inorganic fraction enmeshed in it, coming from the feed wastewater or cell decay.

Fewer data are available regarding the composition of extracellular polymers in anaerobically digested sludge. Protein and carbohydrate have been measured (Karapanagiotis et al., 1989, 1990; Kang et al., 1989; Morgan et al., 1990) but no quantification of DNA, RNA and humic acids in digested sludge biopolymers are known. Lipids were measured by Schmidt and Ahring (1994) in biopolymers of UASB reactors at very low quantities in comparison with carbohydrate and protein.

Molecular weight and yield are two of the physical characteristics of extracellular polymers. The latter normally is represented by the mass of polymer per mass of volatile or total solids. Mass is measured by weighing the polymer after the solution containing it has been evaporated. Another technique makes use of the property in which polymers become insoluble upon addition of an organic solvent to a solution containing the polymer. The precipitate is then either dried or filtered, and weighed.

Table 2.2 presents available information regarding biopolymer yields. In bacterial cultures grown in synthetic medium, Pavoni et al. (1972) measured yields varying from 60 to 400 mg/g SS, while Sato and Ose (1984) measured 104 mg/g SS. In full scale activated sludge plants, yields ranged from 20 mg/g SS (Sanin and Vesilind, 1994) to 108 mg/g SS (Forster and Clark, 1983). In anaerobically digested sludges, Karapanagiotis et al. (1989) measured yields ranging from 4.1 to 34.7 mg/g TS. The variation in this case was due to differences in extraction methods, inasmuch as the sludge was the same.

Information regarding the molecular weight of biopolymers is scarce. In the polymer bridging mechanism of flocculation, the molecular weight of the flocculant is of critical importance. Using synthetic polymers, Walles (1968) has shown that higher settling rates are achieved with higher flocculant molecular weight. Amirtharajah and O'Melia (1990) stated that the molecular weight of an anionic synthetic polymer should be at least 10^6 for effective bridging of negatively charged particles. Novak et al. (1977) also speculated that activated sludge extracellular polymers should have molecular weight above 10^6 because of their flocculative properties. Rudd et al. (1994) stated that biopolymers molecular weight should be in the range $2x10^5$ to $2x10^6$, but didn't actually measure them. Horan and Eccles (1986) used high performance liquid chromatography to measure the molecular weight of extracellular polymers from five activated sludge plants. The polymers had been purified for carbohydrates by using enzymes to degrade nucleic acids (DNase and RNase) and proteins (protease). A fraction corresponding to molecular weights in the range 1.6 to 2.0 x 10^6 was identified in all samples. In addition, one or two more fractions were present in samples from some plants. These fractions corresponded to molecular weights in the ranges 4 to 8 x 10^5, and 1.2 to 4.3 x 10^5.

Chapter 3: Materials and Methods

3.1 Wastewater and Sludge Treatment Plant Information

Samples for biopolymer extraction were all collected at the Ithaca Areawide Wastewater Treatment Plant, located in Ithaca, New York. This plant was designed to treat 10 MGD and has been in operation since 1987 (City of Ithaca, 1994). During the months of June thorough August 1998, when most of the samples were collected, the average flowrate was approximately 6.2 MGD. This was a typical flowrate for the summer period (Denmark, 1998). The plant receives wastewater from the City and Town of Ithaca, part of Town of Dryden, and trucked wastes from septic tanks and restaurants. Trucked wastes are discharged into anaerobic digesters. Plant processes were designed for removal of suspended solids, biodegradable organic matter, phosphorus and pathogens through primary and secondary sedimentation, activated sludge, phosphorus stripper/lime and chlorination/dechlorination. Primary and waste activated sludges are concentrated in thickeners, stabilized in two-stage anaerobic digesters, and dewatered in belt-filter presses. After the plant was built, the biological stripper/lime process was replaced by chemical precipitation for phosphorus removal. Secondary effluent is discharged into Cayuga Lake, and sludge cake is sent to a landfill. The chemicals used in the plant are ferrous sulfate (primary clarifiers), chlorine (secondary effluent), sulfur dioxide (secondary effluent), and synthetic polymer (belt filter press). Overflow from thickeners, supernatant from the second stage anaerobic digester, and filtrate from belt-filter presses are returned to the head of the plant.

3.2 Sample Collection and Characterization

Figure 3.1 presents the sampling sites at the treatment plant. The dates when samples were collected are shown in Table 3.1. A description of each site, methods of collection, and the number of samples collected in each location are presented below.

Location 1: Raw wastewater, grab samples, collected after screening. The collection site was next to an automatic sampling device. A four inch hose was installed in a connector. The valve was opened and the influent was left running for approximately one minute before the sample was collected. The volume gathered was in the range 20 to 30 L. Three samples were collected during the months of June, July and August of 1998.

Location 2: Primary effluent, grab samples, collected at the effluent weir channel. The sampling device, provided by the plant, was a bucket placed at the end of a pole. The bucket was washed with effluent prior to the sample collection. The volume gathered was in the range 20 to 30 L. Three samples were collected during the months of June, July and August of 1998.

Location 3: Activated sludge, grab samples, collected from the aeration tank. Samples were collected approximately one meter from the lateral wall, 1.5 m depth, and 3 to 4 m from the end of the tank. The same sampling device from location 2 was used, with the bucket being washed with activated sludge prior to the sample collection. The volume gathered was approximately four liters. Four samples were collected during the months of June, July, August, and October of 1998.

Location 4: Secondary effluent, grab samples, collected at the effluent weir channel. The sampling device was the same from location 2. The bucket was washed with effluent prior to the sample collection. The volume gathered was approximately 40 L. Three samples were collected during the months of June, July and August of 1998.

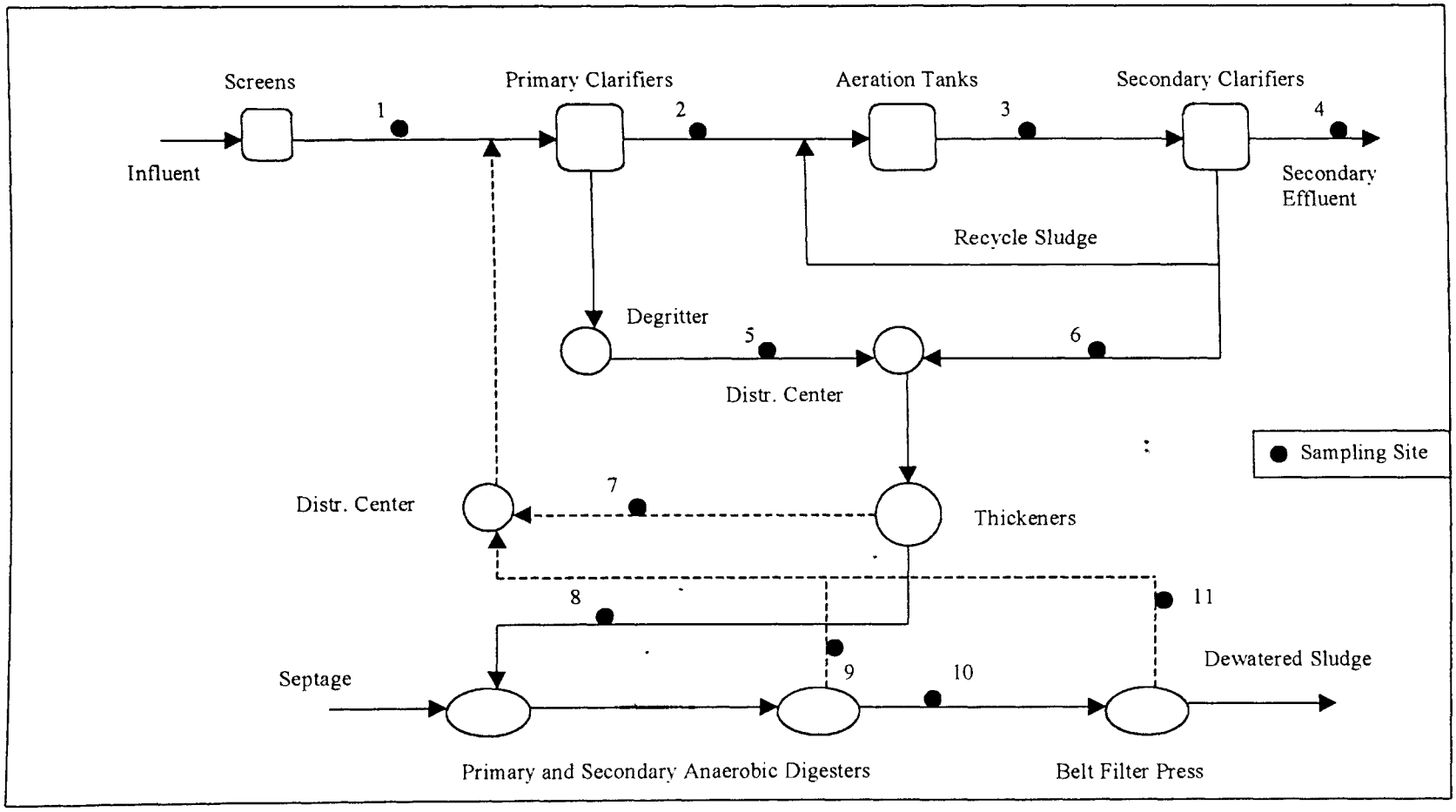

Figure 3.1 Sampling Sites at the Ithaca Wastewater and Sludge Treatment Plant

Table 3.1 – Sampling Dates (Year 1998)

Location	Number	First Set		Second Set		Third Set	
		Date	Weekday	Date	Weekday	Date	Weekday
Influent	1	06/17	Wed.	07/06	Monday	08/10	Monday
Primary Effluent	2	06/12	Friday	07/10	Friday	08/20	Thursday
Primary Sludge	5					08/18	Tuesday
Activated Sludge [1]	3	06/04	Thursday	06/29	Monday	08/05	Wed.
Secondary Effluent	4	06/19	Friday	07/13	Monday	08/06	Thursday
Waste Act. Sludge [2]	6						
Thickened Sludge	8	06/10	Wed.	07/08	Thursday	08/14	Friday
Digested Sludge	10	06/08	Monday	06/30	Thursday	08/12	Wed.
Thickener Overflow	7	06/15	Monday	07/02	Thursday	08/14	Friday
Digester Supernatant	9	06/15	Monday	07/02	Thursday	08/12	Wed.
BFP Filtrate	11	06/17	Wed.	07/08	Wed.	08/17	Monday

[1] A fourth sample was collected on Friday, October 2.
[2] A sample was collected on Friday, October 2.

Location 5: Primary sludge, grab sample, collected at the discharge of a distribution center located before the thickeners. The sampling device was the same as used at location 2, with the bucket being washed with the sludge before collection. One sample was collected in August of 1998.

Location 6: Waste activated sludge, grab sample, collected at the discharge of a distribution center located before the thickeners. The sampling device was the same as used at location 2, with the bucket being washed with the sludge before collection. One sample was collected at the beginning of October of 1998.

Location 7: Thickener overflow, grab samples, collected at the effluent weir channel of thickeners one and two. The sampling device was the same as the one used at location 2, with the bucket being washed with the overflow before collection. Three volumes of sample from thickener one were mixed with two volumes of sample collected from thickener two in order to compose the final sample. The ratio was in accordance with the estimated flowrates to each thickener (Denmark, 1998). Three samples were collected during the months of June, July and August of 1998.

Location 8: Thickened sludge, grab samples, collected from a sampling port in the pipe conducting the sludge to the anaerobic digester. A valve was opened and the thickened sludge was left running for approximately one minute. The sample was collected in a bucket that had been washed with the sludge and transferred to the sample container. Approximately four liters of thickened sludge were collected. Three samples were collected during the months of June, July and August of 1998.

Location 9: Anaerobic digester supernatant, grab samples, collected in a sample port 33 ft above the digester bottom. The supernatant was left running for approximately one minute before the sample was collected by a bucket that had been washed with the supernatant. The sample volume was approximately four liters. Three samples were collected during the months of June, July and August of 1998.

Location 10: Digested sludge, grab samples, collected in a sample port located at the bottom cone of the secondary anaerobic digester. The sludge was left running for approximately one minute before the sample was taken. The sample was collected in a bucket that had been washed with the sludge and transferred to the sample container. The sample volume was approximately four liters. Three samples were collected during the months of June, July and August of 1998.

Location 11: Filtrate mixed with washwater from the belt filter presses, grab samples, collected in a sample port located in the pipe conducting these flows to the primary sedimentation tank. The liquid was left running for approximately one minute before being collected by a bucket that had been washed with the liquid. The sample volume was in the range of five to ten liters. Three samples were collected during the months of June, July and August of 1998.

The samples were collected in three periods during the summer of 1998 with an additional sample collected for activated sludge in the beginning of fall (see Table 3.1). The sampling periods were (1) June 04 to June 19, (2) June 29 to July 13, and (3) August 06 to August 20. The fourth activated sludge sample was collected on October 2. First, second and third sets refer to each group of samples collected within the respective sampling periods. The analytical results from the activated sludge sample collected on October 2 were substituted for the values measured for the activated sludge sample collected in August 5 (see Section 3.8).

The sampling program was designed to collect samples during the daily routine operation of the plant. Samples were collected in weekdays, between 10 and 11 am, while filtrate from belt filter press and thickener overflow were being recycled to the primary clarifier. Supernatant from anaerobic digester was not always being produced because frequently more digested sludge was being withdrawn from the digester than thickened sludge and septage were being fed. During weekends the plant operated

regularly except with fewer personnel, and the belt filter press is not operated. Samples were not collected during weekends because filtrate and washwater from the belt filter press were not produced.

As explained below, the number of samples obtained was restricted by the large amount of time required to prepare samples, extract biopolymers, and perform analyses. At nine sites, the number of samples collected were three. Although limited, three results for the chemical composition of biopolymers in each location provided minimum data that allowed variability to be incorporated into a confidence interval for the population mean. Moreover, all samples but one were collected during the summer months, where strong events such as snowmelt doesn't occur. Collection of samples on rainy days, except for light precipitation, was avoided.

Composite sampling is generally considered to be a better alternative than grab sampling because it can incorporate variations on composition that occur with time. Composite sampling was not used in this investigation because of practical limitations in the time to process the sample. For example, a typical location in wastewater treatment plants where composite sampling is advisable is the wastewater influent. Because the concentration of solids in influent was not enough to provide the amount of biopolymers required for chemical analyses, a volume of approximately 20 l had to be reduced by centrifugation to less than one liter, while most of the original solids were kept in the reduced volume. This process took six to eight hours, after which, four to six more hours were needed for volatile solids analysis, biopolymer extraction, separation, and preparation for dialysis. If a composite sample was to be collected, additional hours would be required; for instance, the total time necessary to process a 24 h composite sample would be in the range of 34 to 38 h. Sample physical, chemical and microbiological characteristics could change in such a period. For this reason, it was considered that the gain in representativeness in using composite sampling would

be offset by possible changes in the sample's characteristics. In samples with lower solids concentrations such as secondary effluent, time limitations were even greater. Also, secondary effluent is not expected to have variations on an hourly basis. In other locations of the treatment plant, such as activated sludge, thickened sludge, and digested sludge, significant variations were not expected to occur in an hourly basis, and little advantage was to be gained in using composite sampling.

The recycling flows at the treatment plant were constituted by thickener overflow, supernatant from the secondary anaerobic digester, and filtrate with washwater from the belt filter press. Overflow was continuously being generated by the thickeners. Supernatant from the anaerobic digester was produced irregularly, and at many times was not being recycled because more digested sludge was being removed from the digester than thickened sludge and septage were being fed. The filtrate and washwater production started with the operation of the belt filter press, which began at 7:00 am and ended at 2:00 pm (Denmark, 1998). Because the detention time in the primary clarifiers was in the range of one to two hours, and sampling occurred between 10 and 11 am, solids from recycling flows that escaped sedimentation would be incorporated into the primary effluent sample. The sampling timing for activated sludge with respect to recycling flows was not considered to be important because the mean cell residence time in the activated sludge system was 8.8 d. Samples collected from digested sludge, digester supernatant, and belt filter press filtrate/washwater could represent conditions that existed in the anaerobic digester prior to the day the collection was made because the mean cell residence time in the digesters was 28 d.

The time elapsed between sample collection and arrival at the laboratory was between 30 min and one hour. Upon arrival at the laboratory, a volume was set aside for the initial measurements of pH, conductivity and solids. In addition, in activated

sludge samples, sludge volume index was measured. The remainder sample volume was kept in the refrigerator at 4 °C. Once the volatile suspended solids content of the sample was known, biopolymer extraction could begin for those samples that didn't need volume reduction for solids concentration. Influent, primary and secondary effluent, and thickener overflow samples required the concentration of solids before extraction could begin. The concentration and extraction procedures are described in Section 3.3.

Procedures described in the Standard Methods for the Examination of Water and Wastewater (APHA et al., 1995) were followed for solids (Methods 2540 B, 2540 D, 2540 E and 2540 G), pH (Method 4500 – H^+ B), conductivity (Method 2510 B) and Sludge Volume Index (Method 2710 D). pH and conductivity were measured with an Accumet® pH/ion/conductivity meter Model 50, Fischer Scientific. Solids analyses were made in duplicate.

3.3 Procedure for Extraction and Partial Purification of Biopolymers

3.3.1 *Loosely-Bound Biopolymers (Slime)*

(a) Nine hundred ml of the sample were centrifuged (Beckman Model J2-21 Centrifuge) at 5,000g, 4°C, for 15 min;

(b) The supernatant was collected and centrifuged twice, at 12,000g and 16,000g, for 30 min each, at 4°C;

(c) The supernatant was collected and placed in dialysis bags (Spectro/Por membrane, molecular weight cutoff 6,000-8,000). The solution was dialyzed against distilled-deionized water (Barnstead MEGA-Pure® System, resistivity 18.9 Ω) for 3 d. Water was changed every 6-12 h;

(d) At the end of the dialysis period, 50 ml of the slime-containing solution was preserved with 0.5 ml of nitric acid for heavy metal analysis. One hundred ml were

used for measurement of ethanol insoluble matter. The remaining volume was used for chemical analysis. It was kept in the refrigerator if not analyzed immediately.

3.3.2 *Solid-Bound Biopolymer (Capsule)*

Two methods discussed in Chapter 2 were considered for extracting capsule biopolymers: (1) Ethylenediaminetetraacetic acid (EDTA) and (2) DOWEX cation exchange resin. These methods were selected because of their property of causing small intracellular contamination during biopolymer extraction (Platt et al., 1985; Frϕlund et al., 1996).

A concern in extraction methods exists with respect to the final separation of the polymer from the extractant after extraction. Residues of the extractant can interfere with chemical analyses. For example, EDTA interferes with the Folin-Lowry method for protein analysis (Peterson, 1979; Brown and Lester, 1980; Davis, 1988). One experiment was designed to investigate the effect of dialysis on EDTA removal. Fifty ml of a 2,000 mg/l Na_4EDTA were combined with 50 ml distilled water. The EDTA concentration was the same as used by Hsieh et al. (1990) for extraction of biopolymers from *Pseudomonas atlantica*. Eighteen replicates were prepared. Three replicates were placed directly into 200 ml bottles and frozen immediately. The other fifteen replicates were placed inside 8,000 molecular weight cutoff dialysis bags. These bags were put into five buckets filled with five liters of distilled-deionized water, over a stirrer. The water volume was replaced every 8 ± 2 h. At the end of each day three replicates were taken from the dialysis bags, frozen (Gibson Frost Clear Commercial Freezer) and lyophilized in a Flexi-Dry™ μP, FTS Systems, Inc. The dried material was removed from the bottle and weighed in an analytical balance (Denver Instrument Model DI-100). Any leftover material in the bottle was

redissolved with as little distilled/deionized water as possible, placed into a pre-weighed aluminum dish, dried for two hours at 105 °C, cooled and weighed. Table 3.2 presents the mean weight of EDTA remaining inside the dialysis bag at the end of each day. Day zero represents the initial condition.

Table 3.2 Weight of EDTA Remaining Inside the Dialysis Bag at the End of Each Day

Day	EDTA Remaining (mg)
Zero	1,059.2
One	145.4
Two	83.3
Three	61.2
Four	33.9
Five	28.3

After three days of dialysis, about six percent of the initial EDTA weight still remained in solution. Based on these results, the EDTA method for biopolymer extraction was abandoned because (1) of possible interference with Folin-Lowry method for protein-humic acids analysis, which was being considered for use, and (2) of interference of EDTA residues in the procedure to weigh biopolymers.

The DOWEX cation exchange resin (CER) method employed was a slight modification of the method used by Frølund et al. (1996). In the final steps of their procedure, three sequential centrifugations were used to separate the extracted biopolymer from the solids/CER suspension. The first centrifugation was at 12,000 g for one minute; the supernatant was collected and centrifuged twice at 12,000 g for 15 min. In the application of this procedure, it was found that some solids still remained in the supernatants after the centrifugation. An alternative to sequential centrifugation

was evaluated. After the one minute centrifugation at 12,000 g, the supernatant was collected and centrifuged at 12,000 g for 30 min. The supernatant, containing the capsule, was then filtered through 1.2 μm filter (Whatman Glass Microfibre Filter, GF/C) and 0.45 μm filter (MSI Micron Separations, Inc.) in order to remove particles that had escaped centrifugation. However, filtration was found to retain part of the biopolymers. The results of one experiment designed to evaluate biopolymer retention in filters are presented in Table 3.3. After the extraction procedure and centrifugation, the supernatant was separated in three portions. The first one was filtered in a 1.2 μm filter, the second, in addition to the first filtration, went through a 0.45 μm filter. Finally, the third portion underwent a third filtration, again through a 0.45 μm filter. Because solids, in addition to biopolymers, could be present in the supernatant, the retention in first and second filtration could include biopolymers and solids. For this reason, only retention in the third filtration was considered to be entirely biopolymers. The results from Table 3.3 indicate that retention occurred, from 7.1 % for carbohydrates to 16.7 % for DNA. Ethanol insoluble matter, a measure of biopolymer yield, dropped by 32 % of the quantity measured before the last filtration.

Table 3.3 Chemical Composition of Biopolymers Measured in Filtrates[1] (Units: mg compound/g VSS)

Filter Pore Size (μm)	Carb.[2]	Prot./HA[3]	RNA	DNA	EIM[4]
1.2	6.1	34.9	12.0	1.8	57.2
1.2/0.45	4.2	24.2	8.4	1.2	37.9
1.2/0.45/0.45	3.9	21.0	7.8	1.0	25.8

[1] Sample from activated sludge;
[2] Carbohydrates;
[3] Protein/Humic acids;
[4] Ethanol Insoluble Matter

Filtration of biopolymers was substituted by sequential centrifugation at 5,000 g for 15 min, 12,000 g and 16,000 g for 30 min each. By visual inspection, solids didn't seem to remain in the supernatant with this procedure. Besides the change in centrifugation, a dialysis step was included with the objective of removing low molecular weight impurities such as salts and inorganic ions that could interfere with chemical analyses. The final procedure used for solid-bound biopolymer extraction was the following:

(a) A sample volume containing a mass of volatile suspended solids between 0.6 to 3.0 g was used for extraction. Samples from influent, primary and secondary effluent, and overflow from thickeners were concentrated by centrifugation at 5,000g, 4°C, for 15 min. Because of limited centrifuge capacity (900 mL), it was necessary to divide dilute samples into many fractions for centrifugation. The low volatile suspended solids concentration in secondary effluent required centrifugation of 20 to 40 L of liquid, which required several hours. The weight of volatile suspended solids collected from secondary effluent varied from 16 and 44 mg. The amount of volatile solids used for capsule extraction at each location and replicate is presented in Appendix A;

(b) The regular or concentrated sample was centrifuged at 5,000g, 4°C, for 15 min. The supernatant was discarded and the pellets were transferred to an extraction beaker. A buffer solution at pH 7.0 (2.0 mM NaH_2PO_4, 1.26 mM Na_2HPO_4, 2.5 mM NaCl and 0.5 mM KCl) was added to give a final volume of 250 or 300 ml. Because less volatile suspended solids were available for biopolymer extraction from secondary effluent, smaller volumes were used (35 to 100 ml). The reduced volume would allow the concentrations of biopolymer constituents extracted from the secondary effluent volatile solids to be above the limit of detection of the analytical methods;

(c) Seventy grams of a cation exchange resin (Dowex 50 x 8, sodium form, Fluka 44445) per gram of volatile suspended solids were added to the extraction beaker. The resin had been previously immersed in water for at least 1 h;

(d) The suspension was stirred (Caframo RZR1 Stirrer) for 2 h at 600 rpm, under a hood. Secondary effluent was stirred at 400 rpm. This reduction in rpm was intended to keep about the same velocity gradient in the suspension, since the suspension volume in secondary effluent was approximately one third of the volumes of other samples;

(e) At the end of the stirring period, the suspension was centrifuged at 5,000g, 4°C, for 15 min. The supernatant was collected and centrifuged twice, at 12,000g and 16,000g, for 30 min each;

(f) The capsule-containing supernatant was placed in dialysis bags (Spectro/Por membrane, molecular weight cutoff 6,000-8,000). The solution was dialyzed against distilled-deionized water (Barnstead MEGA-Pure® System, resistivity 18.9 Ω) for 3 d. Water was changed every 6-12 h;

(g) At the end of the dialysis period, 50 ml of the partially purified biopolymer was preserved with nitric acid for heavy metal analysis. Fifty ml was used for ethanol insoluble matter measurement. The remaining volume was used for chemical analysis. It was kept in the refrigerator if not analyzed immediately.

Figure 3.2 shows the schematic of the extraction/purification procedure used. The extraction container used for extraction was a one liter beaker built with four baffles to improve mixing of the resin with the solids and decrease occurrence of short circuits. Figure 3.3 presents a drawing of the extraction beaker.

An experiment was prepared in order to compare the quantities of capsular biopolymer constituents that were extracted from samples with and without addition of the cation exchange resin. Two identical samples from activated sludge were subjected

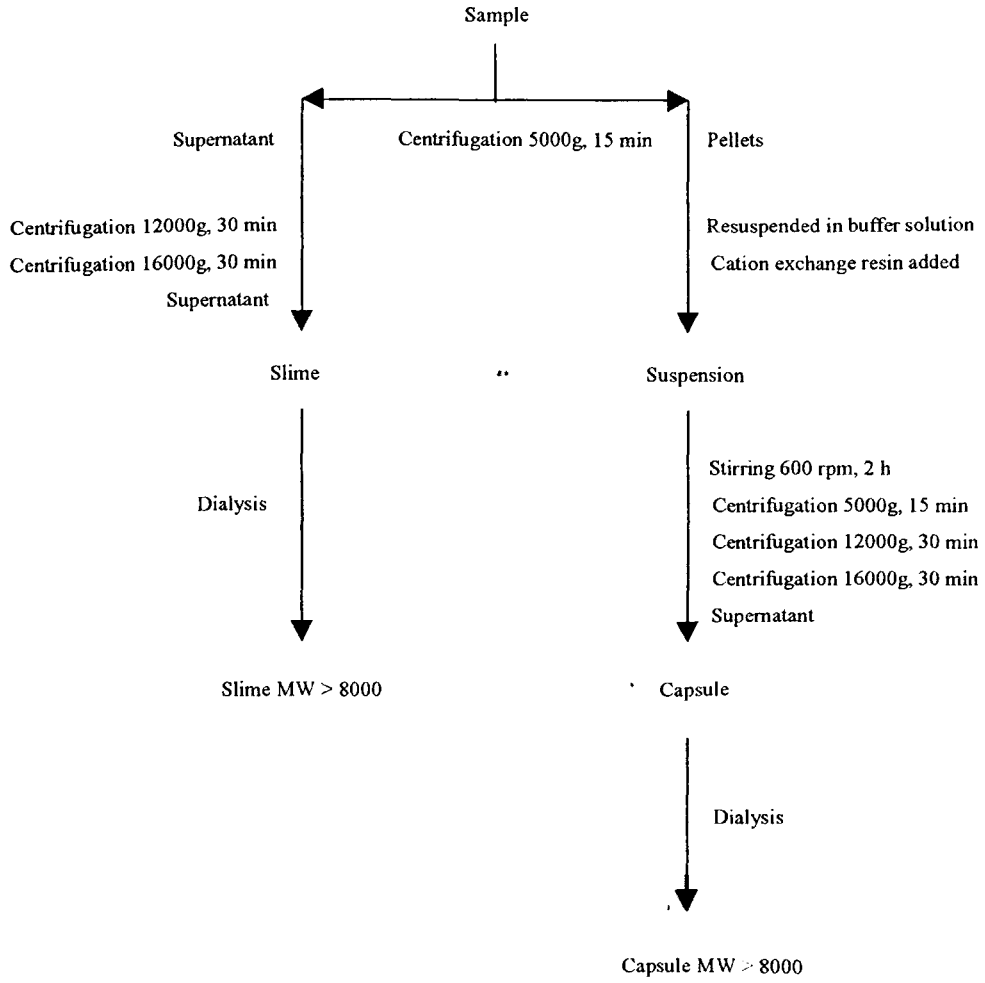

Figure 3.2 Biopolymer Extraction Procedure

to the extraction procedure outlined in Figure 3.2. In one of the samples, however, the cation exchange resin was not added. Table 3.4 presents the weight ratios of capsular biopolymer constituents to volatile suspended solids in both extracts. The data show that more biopolymer was extracted in the sample to which the resin was added.

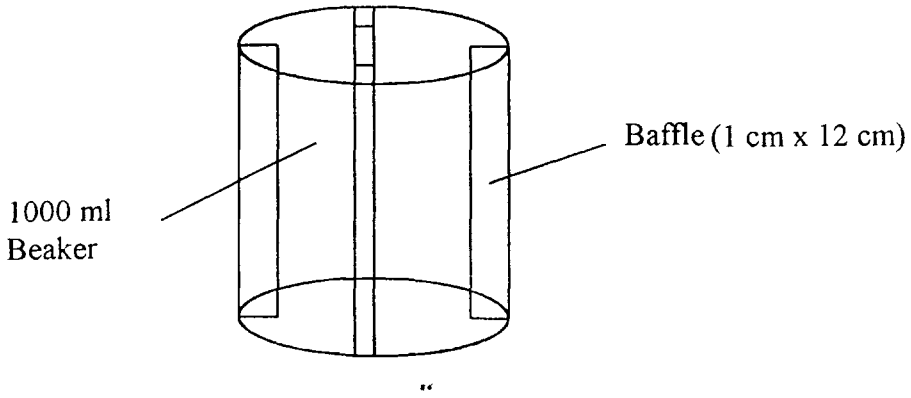

Figure 3.3 Extraction Beaker

Table 3.4 Weight Ratios of Capsular Biopolymer Constituents to Volatile Suspended Solids. Biopolymer Extracted from Activated Sludge with and without Addition of the Dowex Cation Exchange Resin (CER) (Units: mg/g VSS)

Compound	Without CER	With CER	Difference (%)
Carbohydrate	1.2	8.3	592
Protein	3.1	29.7	858
RNA	1.3	9.2	608
DNA	< 1.0	1.5	> 50

3.4 Chemical Analyses

3.4.1 *Carbohydrates*

Anthrone and phenol-sulfuric acid are the most common methods for quantitative determination of carbohydrates (Daniels et al., 1994). Both methods involve addition of sulfuric acid to a sample and heating to hydrolyze polysaccharides and dehydrate monosaccharides to form furfurals. These compounds react with phenol

or anthrone to give a characteristic color with intensity linearly related to the amount of carbohydrates present on the range zero to 100 mg/l.

An experiment was designed to evaluate both methods with respect to their accuracy. Samples with 50 µg of dextran, gum xantham, and alginic acid were prepared according to the anthrone (Gaudy, 1962; Pavlostathis,1985) and phenol-sulfuric acid (Robyt and White, 1987) procedures and were analyzed for carbohydrates. Table 3.5 presents the results. Standards used for calibration curves were prepared using glucose. Dextrans are branched polysaccharides of D-glucose. Upon hydrolysis, the mass of glucose in the dextran sample should have been 50 µg. Gum xantham is produced by fermentation of dextrose by *Xanthomonas campestris*. Xantham is a polymer of glucose, mannose, glucuronic acid, acetate and pyruvate. In commercial xantham, the contents of glucose and mannose should be approximately 60 percent (Sutherland, 1990). Hence, the expected mass of carbohydrates in xantham after hydrolysis was 30 µg. Alginic acid contains D-mannuronic acid units, derived from the oxidation of the carbon bearing the primary hydroxyl group from D-mannose (Lehninger, 1972). The carbohydrate content of alginic acid should have been zero.

Table 3.5 Carbohydrate Contents of 50 µg of Dextran, Gum Xantham and Alginic Acid, as Measured by the Anthrone and Phenol-Sulfuric Acid Methods. Results are the mean of two replicates ± standard deviation. Units: µg

Sample	Anthrone	Phenol Sulfuric Acid	Anticipated Result
Dextran	53.9 ± 0.2	34.7 ± 0.5	50.0
Gum xantham	24.5 ± 1.8	18.4 ± 5.0	30.0
Alginic acid	1.3 ± 0.6	4.0 ± 1.2	0.0

The carbohydrate recoveries by the anthrone method were better than the recoveries by the phenol sulfuric acid method. For dextran and gum xantham, the anthrone measured 108 % and 82 % of the expected carbohydrate amounts, respectively, while the phenol sulfuric acid method measured, for the same compounds, 69 % and 61 %. Also, the anthrone approximated better the expected carbohydrate amount in alginic acid than the phenol sulfuric acid method. In addition, the standard deviations of the means for the anthrone results were smaller than the standard deviations of the means for the phenol sulfuric acid method. For these reasons, the anthrone method was the technique chosen to measure carbohydrates in biopolymers.

The anthrone reagent was prepared by dissolving 200 mg of anthrone in 100 ml of concentrated sulfuric acid. It was prepared freshly, and kept refrigerated for two to six hours prior to use. Standards were prepared from glucose. Two calibration curves were calculated from absorbancies measured in standards, one "low" (0.0 – 10 mg/l), and one "high" (10 – 100 mg/l). Standards were prepared freshly and in duplicates. Stock solutions of 10 mg/l and 100 mg/l were prepared from 1000 mg/l – "low" and "high" standards were prepared from these stocks. Distilled water was used as a reference. The following procedure was used:

1) One milliliter of standard (or appropriate dilution) or sample was delivered to a glass tube and placed into a cool water bath (3-5 °C);
2) Two milliliters of chilled anthrone reagent were added to each tube while still in the cool water bath;
3) Tubes were capped and the solution was immediately mixed in a vortex mixer (Genie, Fischer Scientific). In the instrument's speed scale of 0 to 10, the speed was set at 2;
4) Tubes were placed into a boiling water bath for 15 min;

5) After 15 min, tubes were removed from the boiling water and placed into a cool water bath until room temperature was reached;
6) Absorbance of standards and samples were measured at 625 nm using a Hewlett-Packard 8452 A Diode Array Spectrophotometer.

3.4.2 *Protein*

The Kjeldahl, Biuret, Folin-Lowry and Bradford methods were considered for protein analysis of the biopolymers. As indicated in the review presented in Chapter 2, the Folin-Lowry has been the method more frequently applied for protein analyses in biopolymers. The Bradford and Kjeldahl methods have been used less frequently, while the Biuret method hasn't found application in biopolymer protein analysis.

The Kjeldahl method encompasses the following steps (APHA et al., 1995): (1) distillation of ammonia in the sample at pH 9.5; (2) digestion of organic compounds, with the amino nitrogen being converted to ammonium in the presence of sulfuric acid, potassium sulfate and cupric sulfate; (3) conversion of ammonium to ammonia, after addition of a strong base; (4) distillation of ammonia which is absorbed in boric acid; (5) titration of ammonia in the distillate with 0.02 N sulfuric acid. The outlined procedure measures organic nitrogen. Protein is found by multiplying the organic nitrogen value by the conversion factor 6.25, based on nitrogen comprising 16 % of protein (Sawyer et al., 1994). The method was tested using a protein standard, Bovine Serum Albumin (Sigma Chemical Company). A difference of 2.7 % was observed between the measured value using the Kjeldahl method and the protein content in the standard (4.63 mg vs. 4.76 mg).

The Biuret method is based on the reaction of the peptide bonds of proteins with copper sulfate in alkaline solution. The blue color that develops from this reaction has intensity according to the Beers-Lambert law (Robyt and White, 1987).

The Folin-Lowry method is a modification of the Biuret method to give a more sensitive measurement of protein (Daniels et al., 1994). The copper-treated protein from the Biuret method reduces the phosphomolybdate-phosphotungstic acid of the Folin reagent, giving rise to a blue color of intensity proportional to the concentration of protein present (Lowry et al., 1951). The phenolic functional groups of humic acids also react with the Folin reagent (Box, 1983; Randke and Larson, 1984). For this reason, the Folin-Lowry method gives an overestimation of the protein quantity in samples containing proteins and humic substances.

The Bradford method for protein assay involves the binding of a dye, Coomassie Brilliant Blue G-250, to proteins, causing shifts in the dye color, from red to blue, and in the color absorption wavelength maximum, from 465 to 595 nm (Bradford, 1976). Davis (1988) presented results where several proteins were treated according to the biuret, Lowry and Bradford methods. The color formed was compared with the color developed with the common protein standard used in these methods, Bovine Serum Albumin (BSA). While variation occurred in all three methods, the biuret and the Lowry methods produced results that were closer to the BSA than the Bradford method.

The information just described provided the basis to choose the Kjeldahl nitrogen for protein quantity estimation.

3.4.3 *Humic Acids*

Humic acid concentrations in biopolymers were estimated using a combination of the Kjeldahl and Folin-Lowry methods. The mutual interference of protein and humic acids using the Folin-Lowry method (Section 3.4.2) was evaluated by preparing solutions with pure humic acids (Sigma Chemical Company), pure protein (Bovine Serum Albumin, Sigma Chemical Company), and several combinations of

concentrations of humic acids and protein mixed. The combined absorbancies of humic acids and proteins were, on average, 0.902 times the sum of the individual absorbancies of humic acids and proteins measured in pure solutions. Appendix B shows the complete results.

The procedure for estimating humic acids was as follows:

1) The sample protein concentration was calculated using the Kjeldahl method;
2) The corresponding absorbance was estimated from a calibration curve prepared with pure protein and the Folin-Lowry method;
3) The sample absorbance was measured using the Folin-Lowry method;
4) The absorbance due to humic acids in the sample was calculated using Equation (3.1)

$$A_{HA} = \frac{A_T}{0.902} - A_{Pr} \qquad (3.1)$$

where A_T is the sample total absorbance, A_{Pr} is the sample absorbance due to proteins, and A_{HA} is the sample absorbance due to humic acids.

5) The concentration of humic acids in the sample was calculated from a calibration curve prepared using pure humic acids.

This procedure for humic acids estimation was used for samples collected in all locations, except secondary effluent. The volumes in which solids-bound biopolymers contained in secondary effluents had to be dissolved after extraction (see Appendix A) wouldn't allow the Kjeldahl analysis. The analyses for the sum of protein and humic acid in secondary effluents were made by the Folin-Lowry method. Numerical results were thus an overestimation of protein alone, but an underestimate of the sum of protein and humic acids.

Two calibration curves for both protein and humic acids were made in the ranges 0 – 25 mg/l and 25 – 250 mg/l. Reagents used in the analysis were prepared as follows:

1) Reagent A: 20 g of Na_2CO_3 was dissolved in one liter of 0.1 N NaOH;
2) Reagent B (prepared freshly): 0.5 g of $CuSO_4 \cdot 5H_2O$ dissolved in 100 ml of a 0.1% (wt/vol) aqueous solution of sodium potassium tartrate;
3) Reagent C: just before use, 50 ml of reagent A and 1.0 ml of reagent B were mixed. This reagent was discarded after 1 d;
4) Reagent D: a Folin reagent commercially supplied (Sigma Chemical Company), 2 N, was diluted 1:1.

Protein stock solution was prepared by dissolving 2.0 mg Bovine Serum Albumin (BSA) in 8.0 ml of distilled water; humic acids stock solution was prepared by dissolving 250 mg humic acids in 1.0 L of distilled water. The solution was filtered through 1.2 μm and 0.45 μm filters. The procedure for the analysis was:

1) 0.5 ml of standard (or appropriate dilution) or sample was placed into a glass tube;
2) 2.5 ml of reagent C was added, and the solution immediately was mixed in a vortex mixer (Genie, Fischer Scientific). In the instrument's speed scale of 0 to 10, the speed was set at 2. The mixture was allowed to stand 10 min at room temperature;
3) 0.25 ml of reagent D was added, and the solution was immediately (1 – 2 sec) mixed in the vortex mixer;
4) After a 30 min standing for color development, the absorbance was measured at 750 nm using a Hewlett-Packard 8452 A Diode Array Spectrophotometer.

Standards and samples were prepared in duplicate.

3.4.4 *Deoxyribonucleic acid (DNA)*

Three methods were considered for DNA analyses in biopolymers – the thiobarbituric acid method, a fluorescence method, and the diphenylamine method.

The thiobarbituric acid method was used by Horan and Eccles (1986) to measure DNA in biopolymers from activated sludge. The procedure involves hydrolysis of DNA by sulfuric acid, the oxidation of 2-deoxyribose by periodate, and condensation of the resultant product, malonaldehyde, with thiobarbituric acid (Gold and Shochat, 1980). The intensity of the pink color that develops is proportional to the concentration of DNA in the range 5 – 5,000 mg/l. One experiment with this test was made, but the results didn't reproduce conditions described in the reference, as the development of pink color upon addition of the thiobarbituric acid.. Considering also the hazardous nature of the test's reagents (e.g., arsenite) another procedure was deemed more advantageous.

Frϕlund et al. (1996) used a fluorescence method to measure DNA in biopolymers extracted from activated sludge. The dye, 4',6-diamidino-2-phenylindole (DAPI), complexes with DNA, producing fluorescence that is 20 times higher than the intensity of the dye alone (Brunk et al., 1979). This procedure was evaluated using the Spectrofluorometer Model SLM 8000 C, Spectronic Instruments, at the Cornell Microscopy and Imaging Facility. Salmon testes and calf-thymus, both from Sigma Chemical Company, were used as standards. Measurements were made at excitation and emission wavelengths, respectively, of 360 nm and 450 nm. Figure 3.4 presents the fluorescence as a function of DNA concentration in standards. The relationship between fluorescence and standards was linear for salmon testes in the range 0 to 200 mg/l DNA, and non-linear for calf-thymus above about 20 mg/l DNA. This method for DNA estimation worked well in the biopolymers samples that were tested, but was not selected because the diphenylamine procedure described in the following paragraph more conveniently could be conducted in the Environmental Engineering Laboratories.

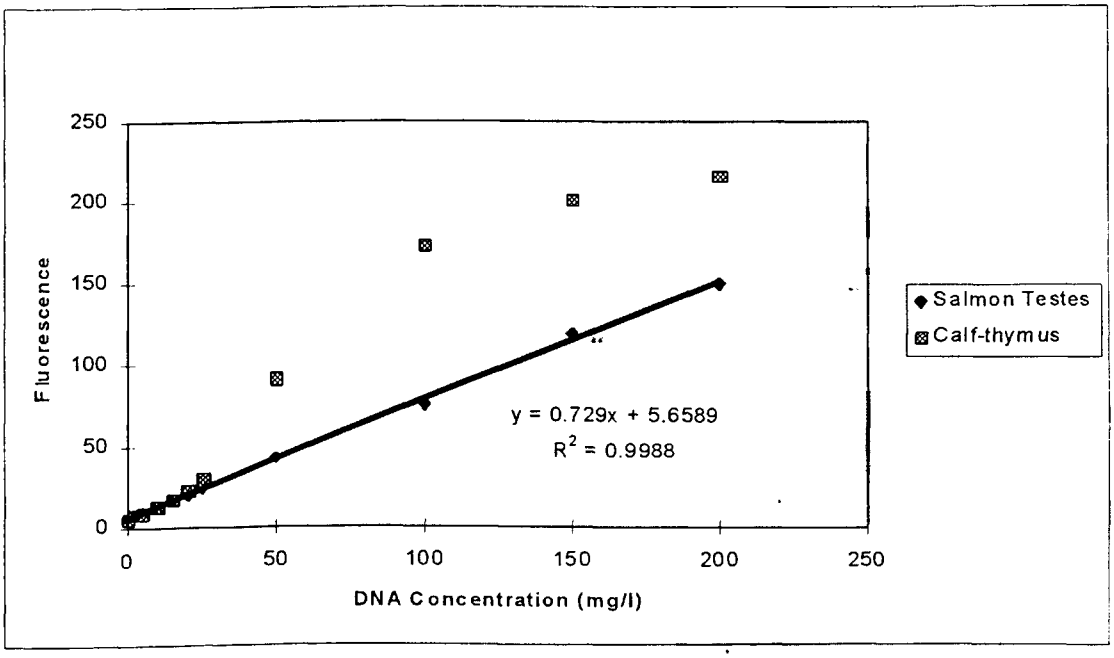

Figure 3.4 Relationship Between Salmon Testes and Calf-Thymus DNA Concentration and Fluorescence

The most common colorimetric procedure for DNA quantification in complex mixtures is the diphenylamine method (Daniels et al., 1994). Under acidic conditions and heat, DNA is broken into purines, pyrimidines, phosphoric acid and 2-deoxy-D-ribose. The acid condition catalyzes the dehydration of 2-deoxy-D-ribose to ω-hydroxylevulinyl aldehyde, which condenses with diphenylamine to produce a characteristic color with maximum intensity at wavelength 595 nm (Clark and Switzer, 1977). The diphenylamine method was the procedure used to estimate DNA in biopolymers. The method is well established, and the required equipment was available in the Environmental Engineering Laboratories. A modification of the diphenylamine procedure, the Burton method, is more sensitive, but involves the use of perchloric acid and takes 16-20 h for completion (Daniels et al., 1994).

The following reagents were used for the diphenylamine procedure:

1) Trichloroacetic acid (TCA) solution: 100 g TCA dissolved in 100 ml distilled water;
2) Diphenylamine reagent: 1 g of diphenylamine dissolved in 100 ml of glacial acetic acid and 2.75 ml of concentrated sulfuric acid.

The procedure for analysis was as follows:

1) 0.1 ml of TCA solution was added to 0.9 ml of sample, standard solution, or appropriate dilution, and mixed in a vortex mixer (Genie, Fischer Scientific). In the instrument's speed scale of 0 to 10, the speed was set at 2.;
2) The resulting solution was heated at 95 °C for 10 min;
3) At the end of the heating period, 2.0 ml of distilled water was added and mixed in the vortex mixer;
4) 5.0 ml of diphenylamine reagent was added and mixed in the vortex mixer;
5) The resulting solution was heated in a boiling water bath for 10 min;
6) The absorbance of the cooled solution was measured at 595 nm using a Hewlett-Packard 8452 A Diode Array Spectrophotometer.

Standards were prepared using 2-deoxy-D-ribose (Sigma Chemical Company) in the ranges 0 – 18 mg/l and 18 – 180 mg/l. The concentration of DNA was calculated by multiplying the 2-deoxy-D-ribose concentration by the conversion factor 2.80. This factor was calculated based on the composition of DNA and the mole fraction of its bases in *E. coli* (Lehninger, 1975).

3.4.5 *Ribonucleic acid (RNA)*

RNA was measured using the Orcinol test (Robyt and White, 1987). Under acidic conditions, D-ribose is dehydrated, forming furfural, which condenses with orcinol in the presence of ferric ion. A blue-green color develops with maximum intensity at wavelength 665 nm.

Orcinol reagent was prepared by dissolving 0.30 g of orcinol in 100 ml of concentrated hydrochloric acid to which 0.2 ml of a 10 % ferric chloride solution had been added. The procedure for the test encompassed the following steps:

1) 2.0 ml of orcinol reagent was added to 0.1 ml of the sample, standard, or appropriate dilution, and immediately mixed in a vortex mixer (Genie, Fischer Scientific). In the instrument's speed scale of 0 to 10, the speed was set at 2.;
2) The solution was heated for 10 min in a boiling water bath;
3) The solution was cooled, and the absorbance was measured at 665 nm using a Hewlett-Packard 8452 A Diode Array Spectrophotometer.

Standards were prepared using D-ribose in the range 0 – 20 mg/l and 20 – 200 mg/l. The concentration of RNA was calculated by multiplying the concentration of D-ribose by 2.52. This conversion factor was calculated based on the composition of RNA and the mole fractions of its bases in *E. coli* (Lehninger, 1975).

3.4.6 *Lipids*

A colorimetric method was tested for lipid estimation in biopolymer. The method was devised for the determination of lipids in serum (Frings and Dunn, 1970). The method is based on the measurement of absorbance of the color that develops when lipids react with a sulfo-phospho-vanillin reagent. The method requires a minimum lipid concentration of 2,000 mg/l. The authors claimed that olive oil, triolein, oleic acid, linoleic acid and cholesterol reacted quantitatively with the reagent. Olive oil is the recommended standard. Goodwin and Forster (1985) used this method to measure lipids in biopolymers extracted from activated sludge. In the test made using this procedure, no color developed in biopolymer extracted from activated sludge, and the absorbance was undistinguishable from the absorbance of distilled water. The method was not considered suitable to lipid analysis in biopolymers

because of its low sensitivity and the use of a standard not meaningful for biopolymers.

Methods presented in the Standard Methods for the Examination of Water and Wastewater (APHA et al., 1995) also were considered for determining lipids in biopolymers. These methods are (1) the partition-gravimetric method, (2) the partition-infrared method, and (3) the Soxhlet method. The first and third methods are gravimetric, and are calculated by the gain in weight of a tared flask that receives extracted oil and grease contained in a sample. Both gravimetric methods require at least 10 mg/l of lipids to ensure accuracy in the result (APHA et al., 1995). For samples that contain less than 10 mg/l of lipids, the partition-infrared procedure is the method of choice, but it is designed for samples that contain volatile hydrocarbons (APHA et al., 1995). In addition to the procedures presented in Standard Methods, two additional methods for lipid determination were evaluated. The first one was designed for the extraction and purification of lipids from biological materials (Bligh and Dyer, 1959). This method requires a minimum of 100 mg of lipids in the sample. The second method is an adaptation of the first one, and it was proposed for the measurement of grease in sewage, sludges or industrial wastes (Loehr and Rohlich, 1962). This procedure requires a minimum of 10 mg of lipids.

None of these techniques were deemed suitable for lipid analysis in biopolymers because they require the presence of 10 mg of lipid for accuracy. A 10 mg/l lipid concentration would require one liter of biopolymer solution, which was not available in all cases. As it will be seen in Chapter 4, the concentrations of capsular biopolymer constituents were below 10 mg/l in most of the locations within the treatment plant. Because no reliable technique was found, lipids in biopolymers were not quantified in this investigation.

3.4.7 *Total Organic Carbon (TOC)*

TOC was measured in a Model 700 TOC Analyzer, O. I. Corporation, College Station, Texas. Standards in the range of 0 – 500 mg/l were prepared using potassium hydrogen phtalate (KHP).

Total organic carbon measured in biopolymers was compared with the sum of the organic carbon content of individual compounds. The ratio of organic carbon molecular weight to chemical compound molecular weight was 0.400 for carbohydrates, 0.450 for proteins, 0.545 for humic acids, 0.360 for DNA, and 0.338 for RNA. Glucose was used as a carbohydrate surrogate. The ratio in proteins was based on the frequency of occurrence of specific amino acids in *E. coli*, as the ratio carbon to molecular weight in each amino acid differs (Lehninger, 1975). The ratio in humic acids was based on the elementary composition of biologically synthesized humic acids (Schnitzer and Khan, 1972). The ratios for DNA and RNA were based on their molecular composition and the frequency of each nitrogen base in *E. coli* (Lehninger, 1975).

3.5 Limit of Detection Determination

For each set of analyses, four or five blanks were prepared using distilled water and appropriate reagents. The minimum distinguishable absorbance was calculated by adding the blank mean absorbance to three times the standard deviation of the mean (Skoog and Leary, 1992). The minimum concentration was calculated using Equation (3.2):

$$C_{min} = \frac{A_{min} - \overline{A}_{blank}}{m} \qquad (3.2)$$

where, C_{min} = minimum distinguishable concentration,

A_{min} = minimum distinguishable absorbance,

$\overline{A}_{blank}$ = blank mean absorbance,

m = slope of the calibration curve of absorbance and standards

A criteria was used to calculate mean and standard deviation when samples from a given location had chemical concentrations below the detection limit. If all samples from the location had chemical concentrations below the detection limits, the result was presented as < LD (less than limit of detection); if one or two from the three samples had chemical concentration below the detection limit, the concentration assigned was one half of the detection limit. Chapman (1992) states that the approach of considering half the concentration for samples below the detection limit is acceptable for mean and variance estimates if the values below detection limit don't predominate.

3.6 Trace Metals

Lead, copper, zinc, cadmium and chromium were analyzed in biopolymers and samples from which they were extracted. Sample preservation and digestion followed methods 3010 B and 3030 E of Standard Methods for the Examination of Water and Wastewater (APHA et al., 1995). The nitric acid used was trace metals grade (Fischer Scientific). Glassware was cleaned with Nochromix®, Godax Laboratories, Inc. Stock solutions for standard preparation were from Fischer Scientific. After digestion, the digestate was centrifuged at 8,000 rpm for 10 min, followed by filtration through a 0.45 μm filter. Distilled and deionized water (Barnstead MEGA-Pure® System) was used for dilution. The same procedure was carried out for blanks.

Zinc and copper were analyzed primarily by flame atomic absorption; however, when the concentration was below 100 μg/l, they were measured using graphite furnace atomic absorption. Lead, cadmium and chromium were measured with graphite furnace atomic absorption. Both methods were applied in a AAnalyst 100 Atomic Absorption Spectrophotometer from Perkin Elmer. The sample

absorptions were background corrected. The wavelengths for absorption were 293.3 nm (lead), 228.8 nm (cadmium), 324.8 nm (copper), 357.9 nm (chromium), and 213.9 nm (zinc).

3.7 Physical Characterization

3.7.1 *Ethanol Insoluble Method*

A separation based on solubility was used to estimate the amount of biopolymer present after extraction and purification. Two volumes of ethanol were added to one volume of biopolymer-containing solution (Gehr and Henry,1983). The volumes used for capsule and slime were 50 ml and 100 ml, respectively. Ethanol and the solution were chilled before being mixed; the solvent was added slowly and with constant stirring (Robyt and White, 1987). The mixture was kept in the refrigerator at 4°C for 24 h. At the end of the standing period, the mixture was filtered in pre-weighed 1.2 μm glass-fiber filter. The filter was dried at 103°C for 1 h, and the amount of ethanol insoluble matter calculated. No difference in weight was measured if the filter was dried at 80°C or 103°C. The ash content of the biopolymers was calculated by placing the filter in a muffle furnace at 550°C for 15 min, and weighing the ignited residue.

3.7.2 *Polymer Freeze-Drying*

An alternative method to estimate polymer weight was used in some samples. The extracted biopolymer was placed in a freezer (Gibson Frost Clear Commercial Freezer), and subsequently freeze-dried in Flexi-Dry™ μP, FTS Systems, Inc. equipment. The dried polymers were then weighed in an analytical balance (Denver Instrument Model DI – 100).

3.7.3 *Molecular Weight*

The molecular weight of the biopolymers was measured in an HP1090 Liquid Chromatograph with a Phenomenex BIOSEP-SEC-S4000 column. The elution fluid was a phosphate buffer at pH 7.0 (30.66 mM NaH_2PO_4 and 19.34 mM Na_2HPO_4). The flow was 1.0 ml/min. Standards used were from Sigma Chemical Company – Blue Dextran (MW 2,000,000), Thyroglobulin (MW 669,000), Apoferritin (MW 443,000), β-amylase (MW 200,000), and Alcohol Dehydrogenase (MW 150,000).

3.8 Statistical Analysis

Analysis of Variance (ANOVA) is a statistical technique that can be used to test the equality of sample means, with the number of samples greater than two (Anderson et al., 1994). ANOVA was used to test if the mean quantities of each chemical compound in biopolymers per gram of volatile suspended solids were the same at the several locations within the wastewater and sludge treatment plant. It was also used to test if the ratios of each compound in biopolymers were the same at those locations. When ANOVA rejected the hypothesis that the means were equal, Fisher's Least Significant Difference was used to make pairwise comparison of sample means. Fisher's procedure arranges means in homogeneous groups, that is, means where the equality hypothesis cannot be rejected (Anderson et al., 1994). Both ANOVA and Fischer's LSD were performed with the statistical software SPSS Release 6.1.3 (SPSS Inc., 1995).

Two-Factor ANOVA (Devore, 1982) was used to test the significance of sample location and sampling period with respect to the values of the ratios of capsular biopolymer constituents to volatile suspended solids. The procedure for the application of the Two-Factor ANOVA to data is described in Section 4.2.4. Two-

Factor ANOVA was performed with the software Minitab for Windows™ (Minitab Inc., 1996).

The means used in ANOVA, Fischer's Least Significance Difference and Two-Factor ANOVA procedures didn't include the values determined for the activated sludge sample collected on August 05 (third set). The values for carbohydrate, protein, RNA, DNA, humic acids, TOC and trace metals were considered to be outliers (see complete results in Appendix E). Chapman (1992) defines an outlier "as a value which does not conform to the general pattern of the data set" (page 481). In the third set activated sludge sample, the values of carbohydrate and TOC in capsular biopolymers were outside the 99 % confidence interval for the population means; the values for protein, RNA, and humic acids were outside the 98 %, 95 %, and 90 % confidence intervals for the population means, respectively; the DNA value was below the limit of detection. All values were located outside the lower end of the confidence interval. In none of the other locations or sampling periods were similar pattern observed.

The reason for such low values in the activated sludge third set sample is not known. It could be contamination of the container used to bring the sample to the laboratory; for example, the presence of residual acid in the container could cause biopolymers hydrolysis and their detachment from the solids. Another hypothesis considered was related to the change that occurred during August in the settling properties of activated sludge. During the months of June and July, the sludge volume index was in the range 70 ml/g, changing to 165 ml/g during August and September. The plant operator reported that the SVI change was being caused by an increase in the amount of filamentous organisms in the activated sludge. It is doubtful, however, that the presence of filamentous organisms would have caused such a reduction in the quantity of biopolymers. The fourth activated sludge sample was collected when the

SVI was still in the same range of the third set, but the values of the chemical constituents were within the range observed for the first and second samples.

Chapter 4: Results and Discussion

4.1 Wastewater, Effluents, and Sample Characteristics

Characterization of plant influent, primary and secondary effluents is given in Table 4.1. Influent wastewater would be characterized as having weak strength if compared with wastewater composition given in Metcalf and Eddy (1991). Primary sedimentation removes approximately 45 % of suspended solids and BOD. The cited reference reports that primary sedimentation tanks should remove 50 % to 70 % of suspended solids, and 25 % to 40 % of BOD_5. The plant's permit contains effluent standards for suspended solids, BOD_5 and total phosphorus of 30 mg/l, 30 mg/l, and 1.0 mg/l, respectively. These standards have been achieved, with removal efficiencies, respectively, of 98 %, 91 %, and 86 %. Biological synthesis and partial nitrification reduce ammonia nitrogen by 50 %.

Table 4.1 Characterization of Wastewater and Primary and Secondary Effluents At the Ithaca Wastewater and Sludge Treatment Plant Numbers represent mean ± SD (n=3) for the period June – August 1998 (Source: Ithaca Area Wastewater Treatment Plant, 1998)

Characteristic (mg/l)	Influent	Primary Effluent	Sec. Effluent
Total Suspended Solids	142 ± 25	79 ± 7	2.5 ± 0.6
BOD_5	140 ± 8	79 ± 9	12 ± 2.5
Total Phosphorus	2.9 ± 1.3	-	0.4 ± 0.2
Ammonia Nitrogen	10.9 ± 1.1	-	5.5 ± 0.7
Total Kjeldahl Nitrogen	16.9 ± 4.8	-	5.2 ± 1.9

Solids, pH, and conductivity were measured every time a sample was collected. In addition, for activated sludge samples, sludge volume index was determined. Tables 4.2 and 4.3 present mean ± standard deviation values for these determinations. With few exceptions, the number of samples was three. There were four samples for activated sludge solids, and one sample for primary and waste activated sludges. Complete results are presented in Tables C1, C2, and C3 in Appendix C. Significant digits for numbers in Table 4.2 are 2, 2, 2, 3, and 3, respectively, for numbers in the range 0 to 10, 10 to 100, 100 to 1,000, 1,000 to 10,000 and 10,000 to 50,000. Chapra and Canale (1998) recommend that significant digits should include the digits known with certainty plus one estimated number. The significant digits were established based on the values obtained in duplicate analyses.

No grit chamber exists in the plant, and grit settles with other solids in the primary sedimentation tank. A cyclone degritter, placed before the sludge thickener, removes grit. The operation of this equipment requires the primary sludge to be very dilute.

The mean cell residence times in the activated sludge process during the months of June, July and August were 8.5, 8.2, and 9.8 days, respectively. The sludge volume index had mean and standard deviation values of 120 ± 55 ml/g. The MCRT in anaerobic digester in the same period was 28 days. The residence time in complete mix activated sludge systems is usually in the range 5 – 15 days; in anaerobic digestion, the residence time varies from 10 to 28 days, according to the operating temperature (Metcalf and Eddy, 1991).

Mixed liquor suspended solids concentration in complete-mix activated sludge system is generally in the range 2,500 – 6,500 mg (Metcalf & Eddy, 1991). It can be seen from Table 4.2 that the MLSS at the plant was well below this range. Approximately 95 % of solids in thickened and digested sludges were in suspended

Table 4.2 Solids Concentrations in Samples at Selected Locations (mg/l)

Location	Total Solids	Volatile Total Solids	Total Susp. Solids	Volatile Susp. Solids	Total Diss. Solids	Volatile Diss. Solids
Influent	700 ± 220	310 ± 150	140 ± 50	120 ± 40	560 ± 170	190 ± 110
Primary Effluent	550 ± 56	150 ± 24	64 ± 13	47 ± 10	490 ± 43	100 ± 14
Primary Sludge	1760	870	1130	720	630	150
Activated Sludge	1,550 ± 150	860 ± 69	1,060 ± 140	750 ± 96	540 ± 15	140 ± 16
Waste Act. Sludge	4,720	3,240	4,350	3,270	370	0
Secondary Effluent	520 ± 23	130 ± 26	2.5 ± 0.5	1.9 ± 0.5	510 ± 23	130 ± 25
Thickened Sludge	37,700 ± 10,900	27,900 ± 8,200	35,400 ± 10,800	26,900 ± 8,100	2,280 ± 280	1,000 ± 100
Digested Sludge	49,300 ± 8,600	27,800 ± 5,900	47,200 ± 9,000	27,200 ± 5,900	2,180 ± 750	670 ± 59
Thickener Overflow	660 ± 40	200 ± 24	85 ± 16	67 ± 13	570 ± 31	140 ± 34
Digester Supernatant	4,450 ± 2,780	2,400 ± 1,800	2,920 ± 2,670	1,970 ± 1,640	1,530 ± 140	440 ± 170
BFP Filtrate	1,870 ± 440	630 ± 140	930 ± 430	490 ± 240	930 ± 20	140 ± 100

form, as it would be expected. Supernatant and filtrate solids concentrations were highly variable, as shown by the large standard deviations.

Table 4.3 pH and Conductivity in Samples at Selected Locations

Location	pH	Conductivity (μS/cm)
Influent	7.3 ± 0.2	840 ± 150
Primary Effluent	7.3 ± 0.1	820 ± 29
Primary Sludge	6.9	820
Activated Sludge	7.0 ± 0.2	730 ± 6
Secondary Effluent	7.1 ± 0.1	770 ± 28
Thickened Sludge	5.9 ± 0.2	2,400 ± 210
Digested Sludge	7.2 ± 0.3	7,900 ± 190
Thickener Overflow	7.5 ± 0.3	940 ± 20
Digester Supernatant	7.7 ± 0.1	7,000 ± 1,100
BFP Filtrate	7.2 ± 0.1	3,200 ± 540

pH was fairly stable, with most values in the range 6.5 – 7.5. The enzyme system of microorganisms present in most biological processes employed in environmental engineering operates at neutral pH (Sawyer et al., 1994). In anaerobic digestion, pH in the range 6.5 – 7.6 is required for good process efficiency (Parkin and Owen, 1986). The pH of thickened sludge was lower than the pH at other locations. It was probably an indication that anaerobic conditions already prevailed, and the acidogenesis stage of anaerobic digestion was taking place. Conductivity, which measures the capacity of a sample to carry electrical current, gives an indication of the

amount of ionized substances in the water. Conductivity remained approximately constant during the wastewater treatment processes, but it was greatly increased during sludge treatment, reaching its maximum in digested sludge. Saywer et al. (1994) report that the dissolved solids content of a sample can be estimated by multiplying the value of conductivity times an empirical factor varying from 0.55 to 0.90. Using values from Tables 4.2 and 4.3, the most extreme factors relating conductivity and total dissolved solids were found to be 0.28 and 0.97, respectively, for digested and thickened sludges.

The sludge volume index values varied appreciably, as shown by their standard deviation. During the months of June and July, SVI was in the range of 70 ml/g, and it changed to 165 mg/g during August and September. Because the mixed liquor suspended solids concentrations did not vary widely (minimum and maximum values were 930 mg/l and 1180 mg/l), the change in SVI was likely to represent alteration in sludge settling conditions. According to the plant operator (Denmark, 1998), an increase in the amount of filamentous organisms occurred during this period. However, effluent suspended solids concentration was not affected.

4.2 Composition and Amount of Biopolymers

4.2.1 *Biopolymers Bound to Solids (Capsule)*

The average chemical concentrations of biopolymers that were bound to solids are shown in Table 4.4. Justification for the use of averages in data presentation and analysis is given in Section 4.2.4. Limits of detection and complete results are presented in Appendixes D and E. The data in Table 4.4 are shown as mean ± standard deviation of three measurements, except primary and waste activated sludges which were sampled only once. Another way to express the chemical compounds of capsule biopolymers has been in terms of mg compound/g VSS (see Table 2.2, Chapter 2).

Table 4.4 Concentration of Solids-Bound Biopolymers Constituents in Wastewater and Sludge Samples (Units: mg/l, except secondary effluent, µg/l)

Location	Carbohydrate	Protein	DNA	RNA	Humic Acids	Total Organic Carbon
Influent	0.8 ± 0.2	2.9 ± 0.4	0.4 ± 0.1	0.8 ± 0.2	1.1 ± 0.6	3.1 ± 1.1
Primary Effluent	0.2 ± 0.0	0.8 ± 0.2	< LD [2]	0.3 ± 0.1	0.2 ± 0.2	0.9 ± 0.2
Primary Sludge	2.9	12	3.7	3.4	4.3	12.0
Activated Sludge	5.9 ± 0.6	21 ± 3	2.4 ± 1.6	8.1 ± 2.8	6.0 ± 2.3	17.8 ± 1.3
Waste Act. Sludge	26	84	7.2	27	11	68.6
Secondary Effluent	10 ± 6	45 ± 11 [1]	< LD	11 ± 5	[1]	28.5 ± 7.2
Thickened Sludge	200 ± 55	800 ± 220	91 ± 32	270 ± 70	160 ± 20	705.8 ± 213.5
Digested Sludge	130 ± 40	480 ± 140	52 ± 37	200 ± 35	150 ± 71	461.1 ± 130.3
Thickener Overflow	0.4 ± 0.1	1.5 ± 0.5	< LD	0.5 ± 0.0	0.2 ± 0.2	1.6 ± 0.3
Digester Supernatant	6.7 ± 6.5	30 ± 28	2.6 ± 2.2	12 ± 14	13 ± 11	28.6 ± 27.2
BFP Filtrate	2.6 ± 1.4	11 ± 6	1.2 ± 1.0	3.2 ± 1.7	3.8 ± 3.0	10.4 ± 6.3

[1] In secondary effluent, protein and humic acids were measured combined, using the Folin-Lowry method (see Section 3.3.3)
[2] Below the limit of detection (see Section 3.4 and Appendix D)

Table 4.5 presents the weight ratios of chemical compounds in capsular biopolymers to volatile suspended solids at the sampled locations at the Ithaca treatment plant. Figures 4.1 to 4.6 present the mean and the 95 % confidence interval of biopolymer compounds at each location sampled. Abbreviations used to describe locations in Figures 4.1 to 4.6 are summarized in Table 4.6. The mean values presented in Tables 4.4 and 4.5 were calculated from measurements in samples collected during the three sampling periods described in Section 3.2. The mean values for activated sludge were calculated using analytical results from the samples collected in June, July, and October. The results for activated sludge sample collected in August were considered to be outliers and were not included in the calculations for the mean and standard deviation (see Section 3.8).

It is apparent that solids-bound biopolymers within the wastewater and sludge treatment plant were composed of carbohydrate, protein, DNA, RNA, and humic acids. Each of these chemical compounds has organic carbon in their molecules. The amount of organic carbon can be estimated if a chemical formula is used to represent the respective compound. An estimation of the total organic carbon of the biopolymer can be made by adding the organic carbon of the compounds forming the polymer. This value can be compared with the total organic carbon actually measured in the biopolymers. As discussed in Section 3.4.7, the weight ratio of organic carbon per unit of compound was estimated to be 0.400 for carbohydrates, 0.450 for proteins, 0.545 for humic acids, 0.360 for DNA and 0.338 for RNA. Table 4.7 presents the estimated and measured TOC for those locations where the analytical results were above the limit of detection. Figure 4.7 presents the data in graphical form.

The estimated and measured TOC values agreed well. The small differences may be due to the approximations used in the chemical formulas, or the presence of

Table 4.5 Weight Ratios of Chemical Compounds in Solids-Bound Biopolymers to Volatile Suspended Solids (Unit: mg/g VSS)

Location	Carbohydrate	Protein	DNA	RNA	Humic Acids	Total Organic Carbon
Influent	6.5 ± 0.9	26.2 ± 4.6	4.1 ± 1.4	7.0 ± 1.0	9.6 ± 5.3	26.9 ± 1.9
Primary Effluent	4.7 ± 0.9	16.6 ± 0.2	< LD [2]	5.7 ± 0.5	4.3 ± 4.3	19.5 ± 4.0
Primary Sludge	4.0	16.6	5.1	4.7	5.9	16.6
Activated Sludge	7.7 ± 0.6	27.8 ± 4.2	3.1 ± 1.9	10.5 ± 3.1	8.2 ± 4.0	23.3 ± 3.1
Waste Act. Sludge	7.9	25.7	2.2	8.3	3.3	21.0
Secondary Effluent	5.3 ± 2.2	24.1 ± 4.1 [1]	< LD	5.6 ± 1.5	[1]	15.3 ± 2.2
Thickened Sludge	7.6 ± 0.8	30.0 ± 3.7	3.4 ± 0.2	10.4 ± 1.9	6.1 ± 1.5	26.5 ± 2.9
Digested Sludge	4.9 ± 0.6	17.6 ± 1.5	1.8 ± 0.9	7.6 ± 2.1	5.3 ± 1.7	16.8 ± 1.1
Thickener Overflow	5.6 ± 1.2	23.1 ± 7.1	< LD	8.1 ± 1.1	3.5 ± 2.9	23.9 ± 4.3
Digester Supernatant	3.2 ± 0.9	14.6 ± 3.4	1.4 ± 1.0	5.1 ± 2.1	6.1 ± 0.6	13.7 ± 2.1
BFP Filtrate	5.2 ± 0.6	21.0 ± 4.1	2.0 ± 1.5	6.3 ± 0.5	8.0 ± 4.0	19.9 ± 4.3

[1] In secondary effluent, protein and humic acids were measured combined, using the Folin-Lowry method (see Section 3.3.3)
[2] Below the limit of detection (see Section 3.4 and Appendix D)

Table 4.6 Abbreviations Used to Describe Locations in Figures 4.1 to 4.6

Abbreviation	Description
Infl	Influent, raw wastewater
Peffl	Primary effluent
ActSl	Activated sludge
Seffl	Secondary effluent
ThSl	Thickened sludge
DigSl	Anaerobic digested sludge
Ovfl	Thickener overflow
Supnt	Anaerobic digester supernatant
Filt	Belt filter press filtrate/washwater

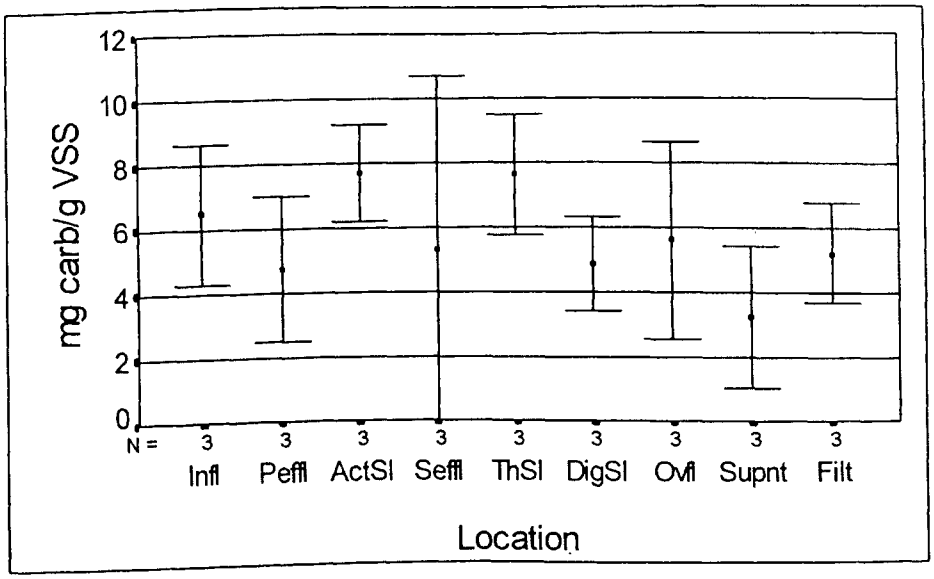

Figure 4.1 Carbohydrate Content of Solids-Bound Biopolymers

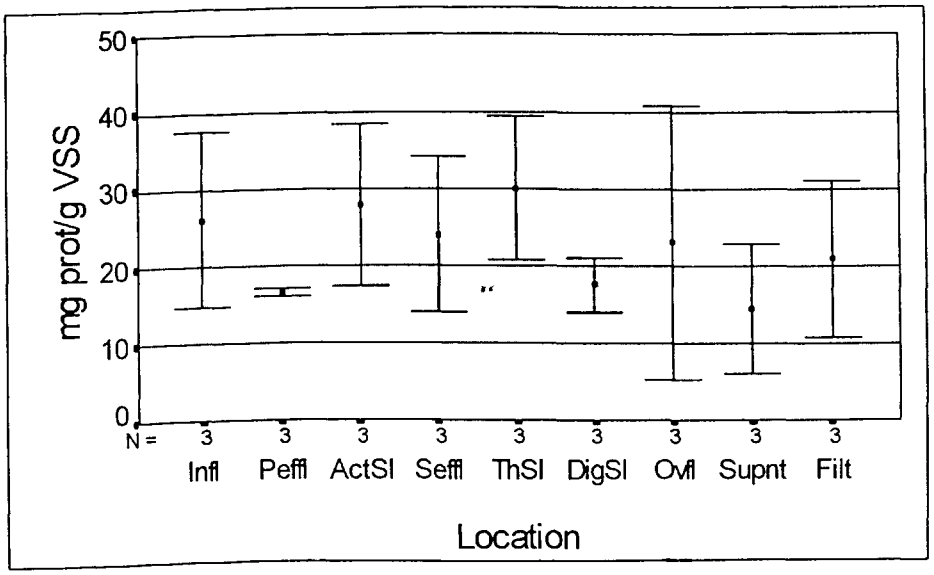

Figure 4.2 Protein Content of Solids-Bound Biopolymers

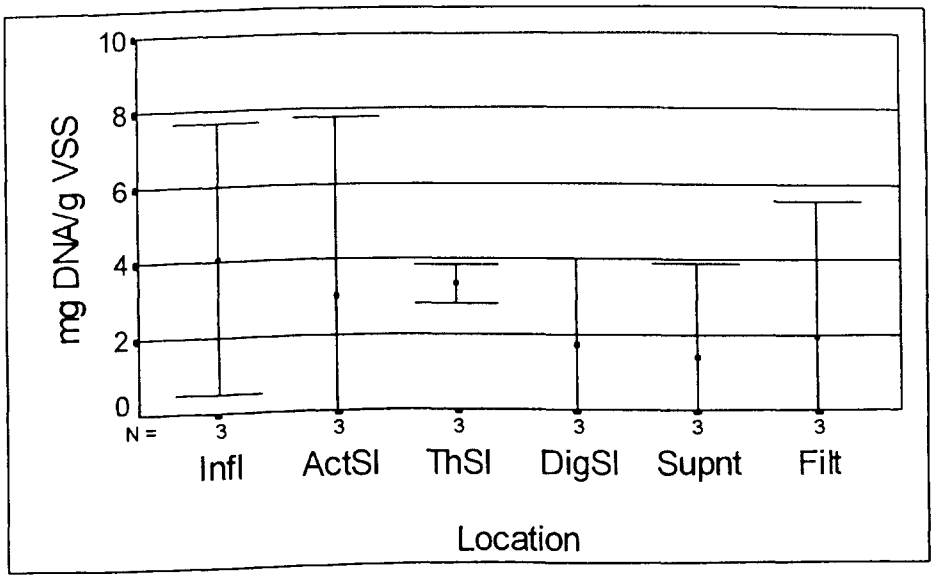

Figure 4.3 DNA Content of Solids-Bound Biopolymers

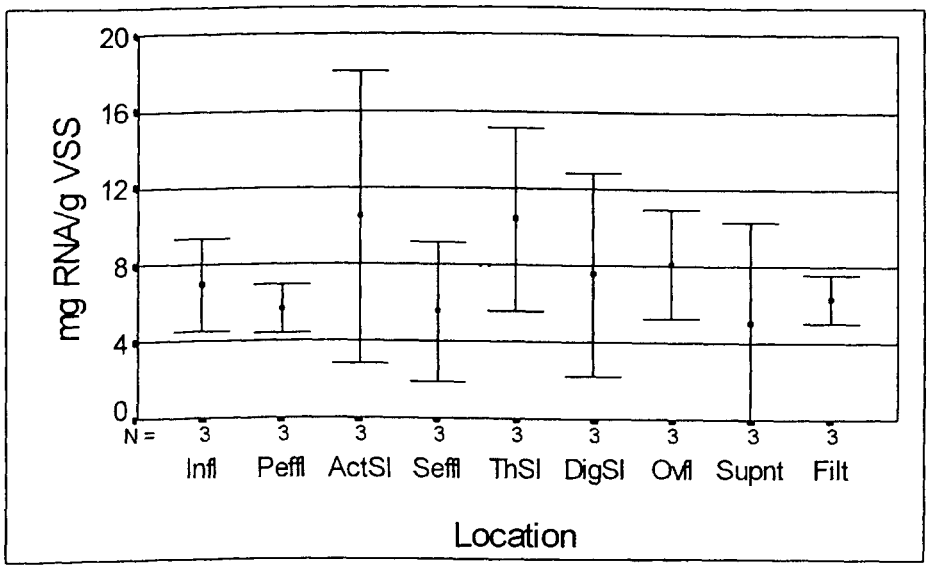

Figure 4.4 RNA Content of Solids-Bound Biopolymers

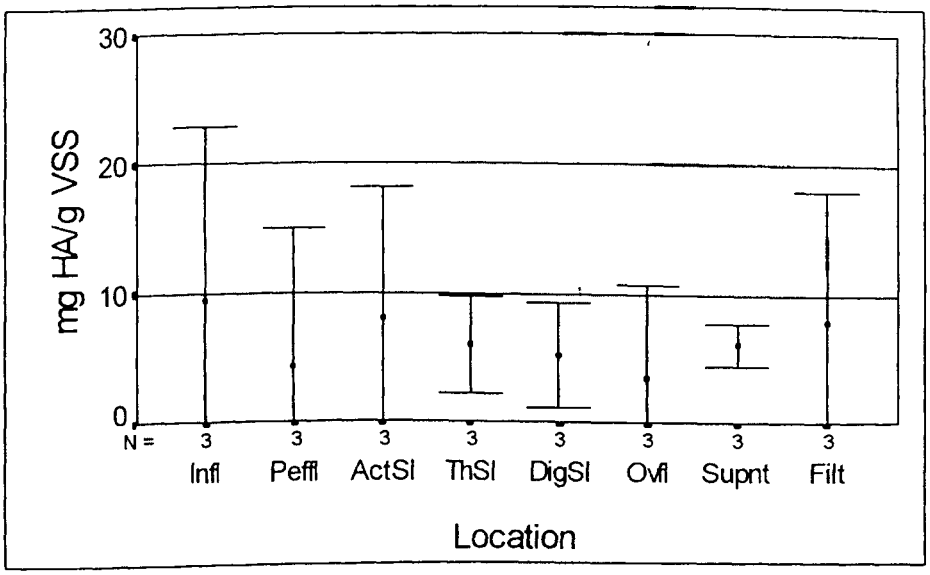

Figure 4.5 Humic Acids Content of Solids-Bound Biopolymers

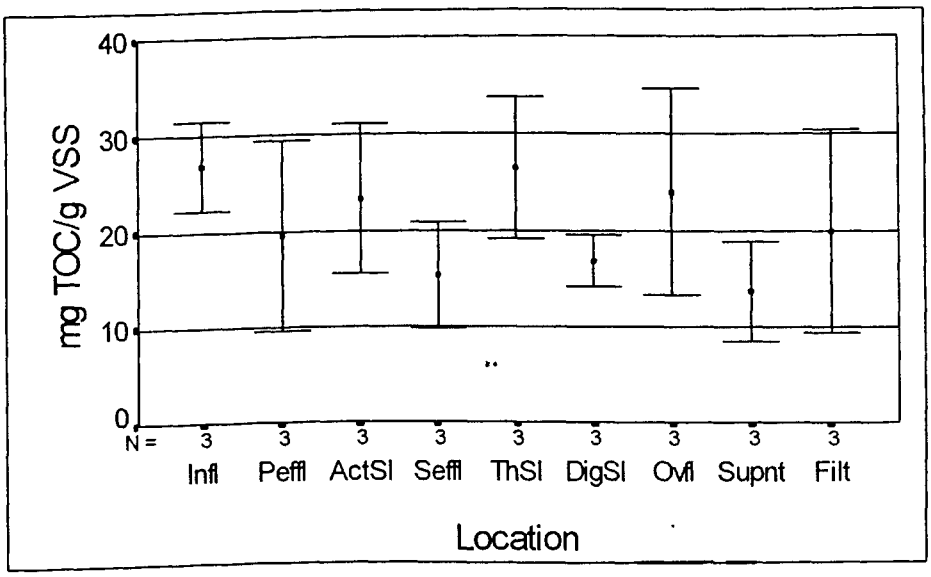

Figure 4.6 Total Organic Carbon Content of Solids-Bound Biopolymers

Table 4.7 Total Organic Carbon of Solids-Bound Biopolymers
Values represent the average ± SD (Unit: mg/g VSS)

Location	TOC Estimated	TOC Measured	Percent Difference
Influent	23.4 ± 5.0	26.9 ± 1.9	- 12.8
Primary Sludge	15.7	16.6	- 5.4
Activated Sludge	24.7 ± 4.8	23.3 ± 3.1	+ 6.0
Waste Act. Sludge	20.1	21.0	- 4.2
Thickened Sludge	24.6 ± 2.6	26.5 ± 2.9	- 7.3
Digested Sludge	15.9 ± 1.4	16.8 ± 1.1	- 5.3
Digester Supernatant	13.4 ± 2.7	13.7 ± 2.1	- 2.1
BFP Filtrate	18.7 ± 2.7	19.9 ± 4.3	- 5.8

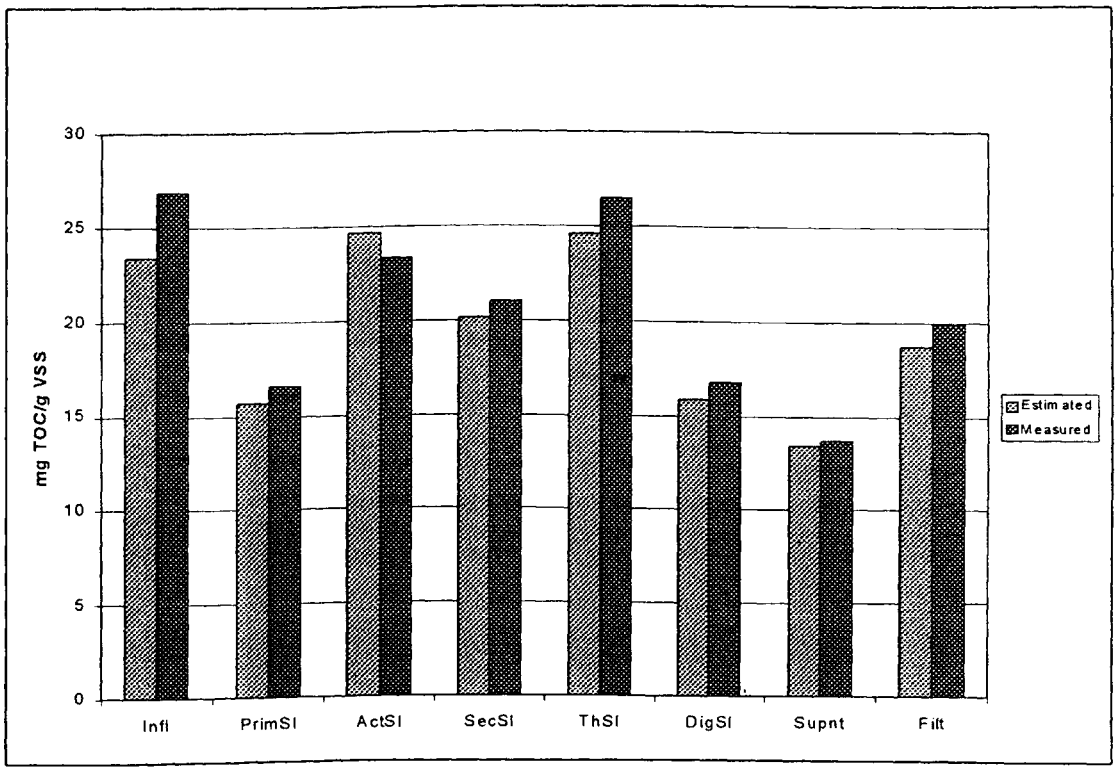

Figure 4.7 Estimated and Measured TOC in Solids-Bound Biopolymers

another component in biopolymer that hasn't been accounted for. For example, Dignac et al. (1998) detected lipids in biopolymers by gas chromatography. The lipids TOC was less than one percent of the biopolymer TOC. Frϕlund et al. (1996) measured a minor amount of uronic acid in biopolymer from activated sludge.

Based on the data presented in Table 4.7 it was assumed that the organic fraction of solids-bound biopolymers was constituted entirely by carbohydrate, protein, DNA, RNA, and humic acids. The chemical composition of biopolymers could then be estimated. Table 4.8 and Figure 4.9 present the fractional composition of solids-bound biopolymers. The fractional compositions in primary and secondary effluents and thickener overflow in Table 4.8 are shown between parentheses to indicate that DNA was not accounted for. DNA values in those samples were below

Table 4.8 Fractional Organic Chemical Composition of Solids-Bound Biopolymers

Location	Total Biopolymer (mg/g VSS)	Fractional Composition				
		Carbohydrate	Protein	DNA	RNA	Humic Acid
Influent	53.4	0.12	0.49	0.08	0.13	0.18
Primary Effluent	31.3 [1]	(0.15)	(0.53)	(< LD) [2]	(0.18)	(0.14)
Activated Sludge	57.3	0.13	0.49	0.05	0.18	0.14
Secondary Effluent	35.0 [1]	(0.15)	(0.69)	(< LD) [2]	(0.16)	(N. M.) [3]
Thickened Sludge	57.5	0.13	0.52	0.06	0.18	0.11
Digested Sludge	37.2	0.13	0.47	0.05	0.20	0.14
Thickener Overflow	40.3 [1]	(0.14)	(0.57)	(< LD) [2]	(0.20)	(0.09)
Digester Supernatant	30.4	0.11	0.48	0.05	0.17	0.20
BFP Filtrate	42.5	0.12	0.49	0.05	0.15	0.19

[1] Does not include DNA, which was below the limit of detection;
[2] < LD = less than limit of detection;
[3] N.M. = not measured

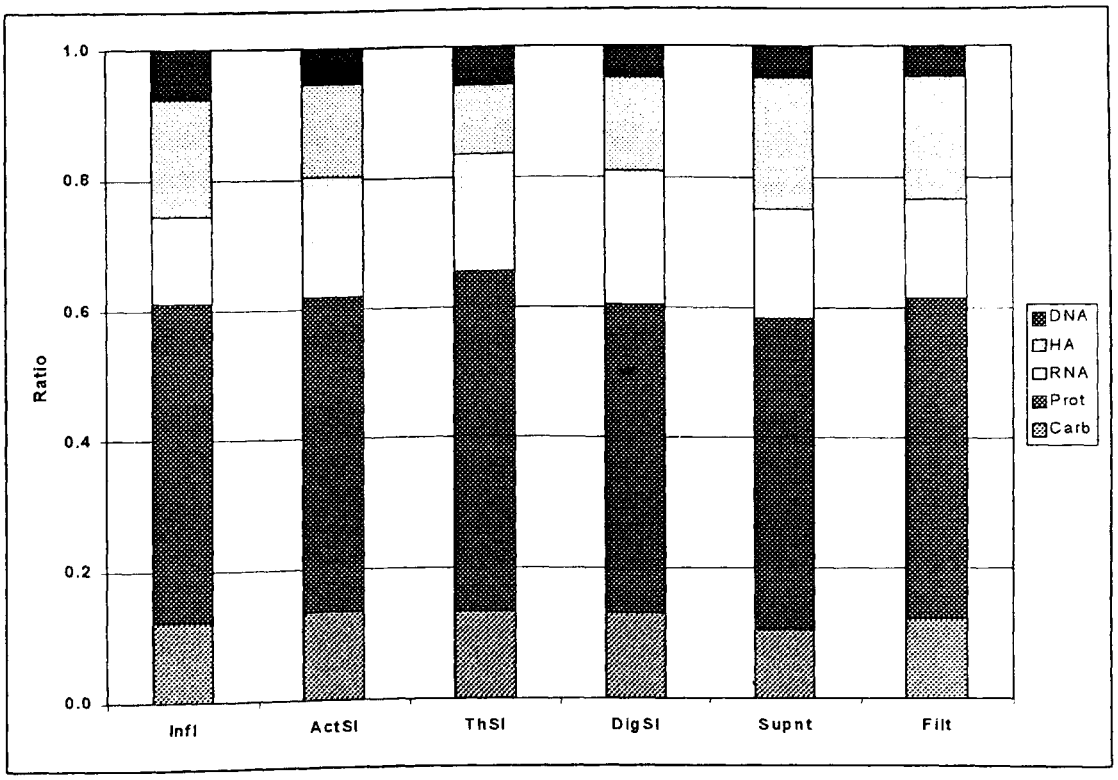

Figure 4.8 Fractional Organic Chemical Composition of Solids-Bound Biopolymers

the limit of detection. DNA could not be ignored in determining the fractional composition of primary and secondary effluent and thickener overflow because DNA's limit of detection was higher than the limits of detection from the other compounds (Table D.1, Appendix D). In addition, as discussed in Section 3.4.3, humic acids were not measured in secondary effluent. The fractional composition of protein in secondary effluent is inflated because the analytical method used (Folin-Lowry) overestimates protein in the presence of humic acids (see Section 3.4.2) .

Protein was by far the major capsular biopolymer compound, constituting approximately 50 % of the organic fraction. Of the compounds measured, DNA consistently was the chemical in smallest amount in biopolymer. DNA was always below the detection limit in primary effluent, secondary effluent, and thickener

overflow. However, this doesn't mean that DNA was not present. The limit of detection for the DNA analytical method was higher than those of the other components (see Appendix D). Also, fewer solids were contained in primary and secondary effluents, and overflow samples. Hence, a smaller quantity of biopolymer (and DNA) could be extracted from those samples. RNA, humic acids and carbohydrate were present in fractions intermediate between protein and DNA.

Data presented in Table 4.8 indicate that there was a trend towards uniform composition in capsular biopolymers at the locations sampled. In particular, the composition of biopolymers in activated and digested sludges was very similar. On the other hand, the total quantities of capsular biopolymer per unit of VSS in digested sludge and associated supernatant and filtrate were smaller than the quantities in activated sludge, influent and thickened sludge.

The capsular biopolymer composition measured in samples from wastewater and sludges was quite different from the reported capsule and slime composition in pure cultures of bacteria. In these, polymers are composed mostly by homo or heteropolysaccharides. What is the origin, then, of compounds other than carbohydrates in solids bound polymers in wastewater and sludges? In addition to other compounds, carbohydrate, protein, RNA and DNA are also cellular components. In biological treatment systems, cells at several ages are present, with many of them being lysed, that is, the cells are being broken down, and their intracellular components exposed to the outer environment. Because these cell components have large molecular weight, they may attach to surfaces already present in the medium, such as the activated sludge flocs. However, cell lysis is not the only source of protein and nucleic acids in biopolymers. Cells produce extracellular protein in the form of exoenzymes, for example. Exoenzymes have been measured in extracellular polymers extracted from activated sludge flocs (Frϕlund et al., 1995). It has also been observed

that a strain of *Pseudomonas aeruginosa* produces extracellular DNA in quantities many times more than intracellular DNA, suggesting that the release of extracellular DNA could be a normal physiologic function of the cell (Paul and David, 1989).

In addition to cellular biochemical components, humic acids were also found to be present in capsular biopolymers. Humic acids result from organic matter decomposition, and can be isolated in raw wastewater, activated sludge and digested sludge (Peter and Wuhrmann, 1971; Riffaldi et al., 1982). A mechanism by which humic acids can be formed in biological treatment of wastewater is chemical polymerization. Using food sources, microorganisms synthesize, intracellularly, products such as phenols and amino acids, which are excreted into the surrounding environment where chemical oxidation and polymerization to humic acids occur (Schnitzer and Khan, 1972). Humic acids have high molecular weight and are quite refractory to biodegradation (Shevchenko and Bailey, 1996). Another characteristic of humic acids is their property to chelate metals (Windholz, 1983). The combination of these properties may explain the presence of humic acids in extracellular polymers. High molecular weight compounds act in interparticle bridging; also, humic acids may form complexes with divalent cations, which are found to be an important element in flocculation.

Carbohydrate, protein, RNA and DNA in extracellular polymers can be used as substrate for microorganisms. It is possible, however, that not all of these compounds will be present in a form available for utilization. In the discussion about the presence of biopolymers in secondary effluents (Section 2.5), and biopolymers as recalcitrant products (Section 2.6), emphasis was given to the fact that of some of the polysaccharides might be difficult to degrade. Tanoue et al. (1996) suggested that a cell membrane- derived protein, porin, is refractory to biological degradation. Nishikawa and Kuriyama (1968) postulated that nucleic acids released by cell

autolysis are enmeshed in activated sludge flocs and protected by metal ions from enzymatic degradation. This hypothesis seemed to be confirmed by Palmgren and Nielsen (1996), who measured accumulation of DNA in extracellular polymers with fermentation time, even though cells were not being lysed. The adsorption of DNA to organic compounds or inorganic materials retards its enzymatic degradation (Aardema et al., 1983). Also, the presence of humic acids was found to inhibit the activity of some enzymes (Tebbe and Vahjen, 1993). All these factors might combine to render some components of the biopolymers not readily available for biodegradation.

It is possible that the composition of solids-bound biopolymers reflects a dynamic equilibrium from a combination of several factors – the contribution from wastewater components, cell lysis, cell excretion of macromolecules, substrate degradation, and the accumulation of refractory or difficult-to-degrade compounds.

As an illustration, Table 4.9 presents the composition of bacterial cell, as given by two sources, together with the composition of extracellular polymers in activated sludge measured in this research. Polysaccharide in data presented by Neidhardt et al. (1990) was composed of glycogen, and parts of the murein and lipopolysaccharide molecules. Glycogen is a storage polysaccharide while murein is a cell wall structure composed of polysaccharide chains covalently cross-linked by peptide chains (Lehninger, 1975). Lipopolysaccharide is another component of cell wall formed by polysaccharide and a fatty acid molecule.

Data from Table 4.9 indicate that the major molecules of bacterial cells are protein and RNA, comprising about 75 % of the cell dry weight. The percentage of each compound in capsular biopolymers and the bacterial cell cannot be directly compared because lipid was not measured in biopolymer and humic acids are not a component of bacterial cell. Table 4.10 shows data comparing the common compounds presented in Brock and Madigan (1991) and capsular biopolymer from

Table 4.9 Bacterial Cell and Extracellular Polymer Composition (Dry Weight, %)

Molecule	Bacterial Cell		Activated Sludge Capsular Biopolymer
	Brock & Madigan (1991)	Neidhardt et al. (1990)	
Protein	55	55	48.5
Polysaccharide	5	-	13.4
Lipid	9	9.1	-
DNA	3	3.1	5.4
RNA	20	20.5	18.3
Humic acids	-	-	14.3
Lipopolysaccharide	-	3.4	
Murein	-	2.5	
Glycogen	-	2.5	
Amino acids and precursors	0.5	-	
Sugars and precursors	2.0	-	
Nucleotides and precursors	0.5	-	
Building blocks molecules, vitamins	-	2.9	
Inorganic ions	1.0	1.0	

activated sludge. Protein and RNA are the major molecules in cells and in biopolymers.

Lipids were not analyzed in this study, but it is not unlikely that lipids are present in biopolymers, since other cell components were found to be biopolymers constituents. Current available information, however, shows insignificant presence

Table 4.10 Comparable Compounds in Bacterial Cells and Extracellular Polymer (%)

Molecule	Bacterial Cell (Brock & Madigan, 1991)	Activated Sludge Capsular Biopolymer
Protein	66	57
Polysaccharide	6	16
DNA	4	6
RNA	24	21

of lipids in activated sludge capsular biopolymers (Dignac et al., 1998). The low molecular weight cell components, amino acids, sugars, nucleotides, vitamins, and precursors shown in Table 4.9 were not expected to be present in biopolymers because these compounds should have been removed during the dialysis step of the extraction.

Table 4.11 presents the weight ratios of chemical compounds in capsular biopolymers to total or volatile suspended solids in this and other studies where the Dowex exchange resin was used for biopolymer extraction. The total amount of biopolymer per unit of volatile suspended solids was not determined in the studies of Rudd et al. (1983), Karapanagiotis et al. (1989), Frǿlund et al. (1994, 1996) and Bura et al. (1998). Also, the chemical characterization is incomplete. For this reason, the complete composition of capsular biopolymers in the cited studies are not known.

In all studies presented in Table 4.11, protein was the compound in solids-bound biopolymers with highest mass per unit of volatile suspended solids. Using the information presented in Table 4.11, the ratios between the mass of protein and the mass of other chemical compounds in solids-bound biopolymers could be calculated. These ratios are shown in Table 4.12. The ratio between protein and carbohydrate in

Table 4.11 Weight Ratios of Chemical Compounds in Solids-Bound Biopolymers to Total or Volatile Suspended Solids. In All of the Studies, Biopolymers Were Extracted with Dowex Cation Exchange Resin

Author	Sample	Compound	Method of Analysis	Quantification
Rudd et al. (1983)	Activated sludge Full scale	Carbohydrate	Phenol sulfuric acid	38.9 mg/g SS
		Protein	Folin-Lowry	147.9 mg/g SS
Karapanagiotis et al. (1989)	Anaerobic sludge Full scale	Carbohydrate	Anthrone	9.7 mg/g TS
		Protein	Folin-Lowry	31.4 mg/g TS
Frϕlund et al. (1994)	Activated sludge conventional	Carbohydrate	Anthrone	6.3 mg/g VSS
		Protein	Folin-Lowry	53.8 mg/g VSS
	Activated sludge advanced	Carbohydrate	Anthrone	6.5 mg/g VSS
		Protein	Folin-Lowry	48.8 mg/g VSS
Frϕlund et al. (1996) [1]	Activated sludge conventional	Carbohydrate	Anthrone	~18 mg/g VSS
		Protein	Modified Lowry	~130 mg/g VSS
		DNA	DAPI	~15 mg/g VSS
		Humic acids	Modified Lowry	~125 mg/g VSS
Bura et al. (1998)	Activated sludge	Carbohydrate	Anthrone	12.7 mg/g VSS
		Protein	Folin-Lowry	162.0 mg/g VSS
		DNA	DAPI	11.2 mg/g VSS
This Study	Activated sludge Conventional	Carbohydrate	Anthrone	7.7 mg/g VSS
		Protein	Kjeldahl	27.8 mg/g VSS
		DNA	Diphenylamine	3.1 mg/g VSS
		Humic acids	Modified Lowry	8.2 mg/g VSS
	Anaerobic digested sludge	Carbohydrate	Anthrone	4.9 mg/g VSS
		Protein	Kjeldahl	17.6 mg/g VSS
		DNA	Diphenylamine	1.8 mg/g VSS
		Humic acids	Modified Lowry	5.3 mg/g VSS

[1] The quantities were estimated from graphs, at 2 h extraction time.

Table 4.12 Ratios Between the Mass of Protein and Other Chemical Compounds in Activated Sludge Solids-Bound Biopolymers Presented in Table 4.11

Author	Prot:Carb	Prot:HA	Prot:DNA
Rudd et al. (1983)	3.8	-	-
Frϕlund et al. (1994)	8.5	-	-
Frϕlund et al. (1996)	7.2	1.0	8.7
Bura et al. (1998)	12.8	-	14.5
This Study	3.6	3.4	9.0

the solids-bound biopolymers at the Ithaca plant was smaller than the ratios found by the other authors. On the other hand, the ratio between protein and humic acids at the Ithaca plant was higher than the ratio measured by Frϕlund et al. (1996). The ratios between protein and DNA were about the same in this study and in the study by Frϕlund et al. (1996), but they were smaller than the ratio in Bura et al. (1998). The ratios between protein and carbohydrate in capsular biopolymers of anaerobic sludge were 3.2 in the Karapanagiotis et al. (1989) and 3.6 at the Ithaca plant.

Data presented in Table 4.11 show that, in activated sludge, the weight ratio of protein in capsular biopolymer to volatile suspended solids was smaller at the Ithaca plant than in the other studies. Also, the weight ratio of carbohydrate in capsular biopolymer to volatile suspended solids at the Ithaca plant was smaller than the ratios of carbohydrate to VSS at other plants, except in Frϕlund et al. (1994). The weight ratios of humic acids and DNA in capsular biopolymer to VSS at the Ithaca plant were about 15 times and 5 times, respectively, less than the ratios measured by Frϕlund et al. (1996). In anaerobic sludge, the weight ratios of protein and carbohydrate to volatile suspended solids were smaller at the Ithaca plant than the ratios measured by

Karapanagiotis et al. (1989). These findings might indicate that fewer quantities of capsular biopolymer are attached to solids at the Ithaca plant than are attached to solids at the treatment plants studied by the other authors cited in Table 4.11. The wastewater feed at the Ithaca plant is characterized as having weak strength, while the mixed liquor suspended solids concentration is less than half the lower limit of concentration found in similar activated sludge systems (Section 4.1). Maybe influent strength and MLSS concentration are factors that contribute to the production and binding of capsular biopolymers to solids in the treatment systems. Information regarding wastewater strength and MLSS concentration are only partly provided for the studies cited in Table 4.11. Frϕlund et al. (1994, 1996) and Bura et al. (1998) did not present information regarding influent or the mixed liquor suspended solids concentrations. In the plant investigated by Rudd et al. (1983), the MLSS was 1,900 mg/l, which can be considered low for activated sludge (Metcalf & Eddy, 1991). The anaerobic sludge studied by Karapanagiotis et al. (1989) had dry solids content of 1.9 %, which was lower than the dry solids content of digested sludge at the Ithaca plant (4.0 %).

One reason that might have contributed to smaller weight ratios of chemical compounds to volatile suspended solids at the Ithaca plant in relation to other plants was the extraction method. As discussed in Section 3.3.2, the extraction procedure used in this research introduced modifications in the technique used by Frϕlund et al (1996) to extract solids-bound biopolymers. The modifications reduced the presence of solids remaining in the supernatant after extraction, and removed compounds with molecular weights smaller than 8,000 daltons. It is possible that these steps removed compounds that otherwise would be counted as biopolymer by the procedures used by the other authors. Finally, the protein values measured by Rudd et al. (1983), Karapanagiotis et al. (1989), and Frϕlund (1994) are likely to have been overestimated

because they didn't take into account the presence of humic acids, which interfere with the Folin-Lowry method (Box, 1983).

It is interesting to note in Table 4.11 that capsular biopolymers extracted with the same technique, at the same plant, but with a two year separation, had different weight ratios of protein and carbohydrate in biopolymer to volatile suspended solids (Frϕlund et al., 1994, 1996). The 1996 data were 2.4 and 2.9 times greater than the 1994 data, respectively, for protein and carbohydrate. The authors didn't address this topic, but changes in biopolymer composition or quantity could explain the differences observed.

Eriksson and Alm (1991) used homogenization to extract capsular biopolymers from activated sludge in six plants in Sweden. In all plants, protein was the biopolymer compound with greatest mass per unit of suspended solids (see Table 2.2). The protein content varied from 8.6 mg/g SS to 35.0 mg/g SS. Carbohydrate content varied from 2.7 mg/g SS to 11.5 mg/g SS. The values measured at the Ithaca plant were intermediate between the values determined by Eriksson and Alm (1991). Urbain et al. (1993) used sonication to extract capsular biopolymers from activated sludge in four plants in France. The protein mass in biopolymer per unit of volatile suspended solids varied from 7.8 mg/g VSS to 71.4 mg/g VSS, while the carbohydrate values varied from 8.3 mg/g VSS to 24.1 mg/g VSS. The mass of DNA in capsular biopolymer per unit of volatile suspended solids varied from 12.1 mg/g VSS to 24.2 mg/g VSS. The values of chemical compounds in biopolymers per unit of volatile suspended solids at the Ithaca plant were intermediate between those measured by Urbain et al. (1993) for protein, but lower for carbohydrate and DNA. Also using sonication to extract capsular biopolymers from activated sludge in a plant in London, Brown and Lester (1980) measured the following ratios of chemical compounds in biopolymers per unit of suspended solids: protein, 0.1 mg/g SS; carbohydrate,

0.2 mg/g SS; DNA, 0.1 mg/g SS. When steaming was used to extract capsule from the same sludge, the values for protein, carbohydrate, and DNA increased, respectively, to 75.1 mg/g SS, 15.5 mg/g SS, and 3.7 mg/g SS. The values measured at the Ithaca plant were higher than the values observed when sonication was used to extract biopolymers at the London plant; on the other hand, when steaming was used, the weight ratios of protein, carbohydrate and DNA in capsular biopolymers to suspended solids at the London plant were higher than the ratios measured at the Ithaca plant using ion exchange to separate biopolymers.

In some instances, investigators have found that the mass of carbohydrate in capsular biopolymer per unit of volatile suspended solids in activated sludge was greater than the mass of protein in capsule per unit of VSS. For example, Carr and Ganczarczyk (1974) found 71.0 mg carbohydrate/g VSS and 65.8 mg protein/g VSS in capsular biopolymers from an activated sludge plant in Toronto. The polymers were extracted by sonication. Morgan et al. (1990) measured 23.9 mg carbohydrate/g SS and 14.1 mg protein/g SS in capsular biopolymers extracted using heat from activated sludge in a plant in England (Table 2.2).

The data presented in Tables 4.5 and 4.8 allow observation of some trends. The quantity of capsular biopolymer per unit of volatile solids in digested sludge was lower than the quantities of capsular biopolymer per unit of volatile suspended solids in influent, activated sludge and thickened sludge. For example, the quantity of biopolymer per unit of volatile suspended solids in digested sludge was 65 % of the quantity in activated sludge (Table 4.8). Another observation relates to the reduction that occurred in the quantity of solids-bound biopolymers per unit of volatile suspended solids in primary effluent in comparison to influent and activated sludge. Total capsular biopolymers per unit of VSS in influent and activated sludge were, respectively, 53.4 mg/g VSS and 57.3 mg/g VSS. The value for primary effluent was

31.3 mg/g VSS. Reduction occurred also in secondary effluent, which had total capsular biopolymer per unit of VSS of 35.0 mg/g VSS. However, in both primary and secondary effluents, the contribution of DNA to total polymer could not be quantified. The reduction in capsular biopolymer per unit of volatile solids in primary and secondary effluent also can be deduced from the TOC data in Table 4.5. The weight ratios of TOC in capsular biopolymers to VSS in primary and secondary effluents were 19.5 and 15.3 mg TOC/g VSS, respectively, while the ratios in influent and activated sludge were 26.9 and 23.3 mg TOC/g VSS, respectively. There are three possible ways by which the observed reductions could occur. First is preferential settling of particles holding more biopolymers than particles with less biopolymers in primary and secondary sedimentation tanks. Second is a significant contribution from particles coming from digester supernatant and belt filter press filtrate to primary and secondary effluent solids (the weight ratios of TOC in biopolymer to VSS were lower in supernatant and filtrate than in influent and activated sludge). The third possibility is a combination of the first two phenomena.

The conclusion by Warfel (1998) that particles in the range 1.1 to 28.2 μm present in the secondary effluent of the Ithaca plant correlated better in size and morphology with particles from anaerobic digester supernatant and belt filter press filtrate than particles from other locations gives strength to the second hypothesis. The limitation of the second hypothesis has to do with the load of solids that were recycled through the digester supernatant and BFP filtrate to the primary sedimentation tank. According to the mass balance for solids calculated for the Ithaca plant (Section 4.4.1), the load of solids from supernatant and filtrate comprised only 13 % of the load of solids coming from the influent to the primary sedimentation tank. In the next section, analytical techniques will be used to test hypothesis about equality in the composition of capsular biopolymers among locations within the treatment plant, as

well as the identification of locations where the chemical composition of the biopolymers were similar.

4.2.2 *Testing the Equality of Sample Means*

Analysis of Variance (ANOVA) is a statistical tool that can be used to test the equality of sample means (Anderson et al., 1994). ANOVA was used to test if the average values presented in Tables 4.4, 4.5, and 4.8 were the same at each location sampled within the Ithaca wastewater and sludge treatment plant. Tables 4.4, 4.5, and 4.8 present, respectively, the average concentrations of chemical compounds bound to capsular biopolymers in wastewater and sludge, weight ratios of chemical compounds in capsular biopolymers to volatile suspended solids, and fractional compositions of capsular biopolymers.

The ANOVA test is formulated as null (H_0) and alternative hypotheses (H_a). The former states that the means are equal, while the latter specifies that not all the means are the same. Mirer (1988) suggests the following five steps when developing a hypothesis testing:

1. State the hypothesis clearly;
2. Choose the level of significance, α, for the test;
3. Construct the decision rule;
4. Determine the value of the test statistic;
5. State the conclusion of the test.

In order to determine the test statistic for ANOVA, two estimates of the population variance are required. These estimates are called "*mean square between*", MSB, and "*mean square within*", MSW. The sampling distribution (i.e., a probability distribution showing all possible values of a sample statistic) of the ratio MSB/MSW has a F distribution with $k - 1$ degrees of freedom for the numerator and $n_T - k$

degrees of freedom for the denominator (Anderson et al., 1994). k is the number of populations being tested, and n_T is the total number of observations. The value of MSB is given by Equation (4.1),

$$MSB = \frac{\sum_{j=1}^{k} n_j \cdot (\bar{x}_j - \bar{\bar{x}})^2}{k-1} \qquad (4.1)$$

where n_j = the number of observations within each group;

$\bar{x}_j$ = mean value within each group;

$\bar{\bar{x}}$ = overall mean from all observations.

Using the compound carbohydrate in Table 4.4 as an example, there will be nine groups being tested – influent, primary effluent, activated sludge, secondary effluent, thickened sludge, digested sludge, thickener overflow, digester supernatant, and belt filter press filtrate. There are three observations for each group. Hence, k = 9, $n_j = 3$, $n_T = 27$, $\bar{\bar{x}}$ = mean concentration of carbohydrate in the 27 observations, and $\bar{x}_j$ = mean concentration of carbohydrate in influent, in primary effluent, and so on. The value of MSW is given by Equation (4.2),

$$MSW = \frac{\sum_{j=1}^{k} (n_j - 1) \cdot s_j^2}{n_T - k} \qquad (4.2)$$

where s_j^2 = the variance within each group.

The test statistic for ANOVA is $F = \frac{MSB}{MSW}$. The decision rule of the test corresponds to the value of the test statistic that will cause the null hypothesis to be rejected. The decision rule is, then,

$$\text{Reject } H_0 \text{ if } F = \frac{MSB}{MSW} \geq \text{critical value}$$

The critical value depends on the choice of α, the *level of significance*. α is the maximum probability of making a Type I error, that is, the error of rejecting H_0 when

H_0 is true. Mirer (1988) defines α as the probability amount that corresponds to occurrences of the test statistic that will cause the null hypothesis to be rejected. The critical values for a F distribution are found in statistical tables. Besides α, the number of degrees of freedom in numerator and denominator are required. For the carbohydrate example in Table 4.4, the number of degrees of freedom in the numerator is k - 1 = 9 - 1 = 8; the number of degrees of freedom in the denominator is $n_T - k = 27 - 9 = 18$. For $\alpha = 0.05$, the critical value is $F_{0.05;\,8;\,18} = 2.51$.

Another way to state the decision rule is through the use of the *p-value*. This is a probability, defined as

$$\text{p-value} = \Pr(t \geq t^*)$$

under the assumption that the null hypothesis is true. t^* is the value of the test statistic, which is $F = \frac{MSB}{MSW}$ for the F distribution. t is the value corresponding to $F_{p-value;\,\upsilon_1;\,\upsilon_2}$, where υ_1 and υ_2 are the degrees of freedom in the numerator and denominator of the F distribution. The decision rule can be formulated as

$$\text{Reject } H_0 \text{ if p-value} \leq \alpha$$

The five steps procedure outlined by Mirer (1988) are set as follows for the ANOVA test of data from Table 4.4:

1. Hypothesis statement:

$H_0: \mu_1 = \mu_2 = \mu_3 = \mu_4 = \mu_5 = \mu_6 = \mu_7 = \mu_8 = \mu_9$

H_a: not all μ_i are equal

where μ_1 = mean concentration of capsular biopolymer constituent *x* in influent;

μ_2 = mean concentration of capsular biopolymer constituent *x* in primary effluent;

μ_3 = mean concentration of capsular biopolymer constituent *x* in activated sludge;

.....

μ_9 = mean concentration of capsular biopolymer constituent x in belt filter press filtrate.

The capsular biopolymer constituents are carbohydrate, protein, humic acids, RNA, DNA, and TOC. The units of concentration are mg/l.

2. Choice of the level of significance, α:

The most common choice for α is 0.05 (Mirer, 1988).

3. Decision rule:

Reject H_0 if $F = \frac{MSB}{MSW} > F_{0.05;\upsilon_1;\upsilon_2}$, where υ_1, υ_2 are the degrees of freedom of the numerator and denominator of the F distribution, respectively.

4. Determine the value of the test statistic:

The value of the test statistic is $F = \frac{MSB}{MSW}$.

5. State the conclusion of the test:

The null hypothesis will be rejected if $F = \frac{MSB}{MSW} > F_{0.05;\upsilon_1;\upsilon_2}$; otherwise, the hypothesis that the mean concentrations of each chemical compound present in capsular biopolymers in all locations are the same can not be rejected.

Table 4.13 presents, for each chemical compound, the degrees of freedom for the numerator and denominator of the F distribution, the value of the test statistic $F = \frac{MSB}{MSW}$, the critical value $F_{0.05;\upsilon_1;\upsilon_2}$, the p-value, and the test conclusion. The values displayed in Table 4.13 result from the application of ANOVA to the data from Table 4.4. The null hypothesis was that the mean concentrations of capsular biopolymers constituents were the same for the locations tested. There were nine locations for carbohydrate, protein, RNA, and TOC, eight locations for humic acids, and six locations for DNA. Humic acids were not measured in secondary effluent, while the concentrations of DNA in primary and secondary effluents, and thickener overflow were below the limit of detection for the method. These were the reasons for the smaller number of locations tested for humic acids and DNA. The results from the

ANOVA test displayed in Table 4.13 indicate that the null hypothesis is rejected for all chemical compounds. Hence, the mean concentrations of capsular biopolymer constituents – carbohydrate, protein, RNA, DNA, humic acids and TOC – were not the same in the locations tested.

Table 4.13 Information Resulting from the Application of Analysis of Variance to the Mean Concentrations of Capsular Biopolymers Constituents in Samples Collected at the Ithaca Wastewater and Sludge Treatment Plant (Data from Table 4.4)

Compound	Degrees of Freedom				p-Value	Decision
Carbohydrate	8	18	32.4	2.5	0.0000	Reject H_0
Protein	8	18	30.5	2.5	0.0000	Reject H_0
RNA	8	18	46.3	2.5	0.0000	Reject H_0
DNA	5	12	11.1	3.1	0.0004	Reject H_0
Humic Acids	7	16	20.5	2.7	0.0000	Reject H_0
TOC	8	18	29.0	2.5	0.0000	Reject H_0

Table 4.14 presents the information that results from the application of ANOVA to the data from Table 4.5. The null hypothesis in this case was that the mean weight ratios of the chemical compounds in capsular biopolymers to volatile suspended solids were the same at the locations tested. The locations were the same as those tested from Table 4.4. The information presented in Table 4.14 indicates that the null hypothesis was rejected for carbohydrate, protein, RNA and TOC, but not for humic acids and DNA.

Table 4.14 Information Resulting from the Application of Analysis of Variance to the Mean Weight Ratios of Chemical Compounds in Capsular Biopolymers to Volatile Suspended Solids (Data from Table 4.5)

Compound	Degrees of Freedom		$F = \frac{MSB}{MSW}$	$F_{0.05;\upsilon_1;\upsilon_2}$	p-Value	Decision
	υ_1	υ_2				
Carbohydrate	8	18	5.4	2.5	0.0014	Reject H_0
Protein	8	18	5.0	2.5	0.0021	Reject H_0
RNA	8	18	4.0	2.5	0.0070	Reject H_0
DNA	5	12	2.0	3.1	0.1495	Accept H_0
Humic Acids	7	16	1.1	2.7	0.4115	Accept H_0
TOC	8	18	7.2	2.5	0.0003	Reject H_0

Table 4.15 presents the information resulting from the application of ANOVA to the data from Table 4.8. The null hypothesis was that the mean fractional composition of the chemical compounds in capsular biopolymers were the same at the locations tested. For this particular case the locations tested were influent, activated sludge, thickened sludge, digested sludge, digester supernatant, and belt filter press filtrate. These were the locations where the fractional composition could be determined. The fractional composition could not be determined in primary and secondary effluents, and thickener overflow because DNA was below the limit of detection. In addition humic acids were not analyzed in secondary effluents. The information displayed in Table 4.15 indicates that the hypotheses that the fractional compositions of the chemical compounds in capsular biopolymers were the same for the six locations tested could not be rejected.

Table 4.15 Information Resulting from the Application of Analysis of Variance to the Mean Fractional Composition of Chemical Compounds in Capsular Biopolymers (Data from Table 4.8)

Compound	Degrees of Freedom		$F = \frac{MSB}{MSW}$	$F_{0.05;\upsilon_1;\upsilon_2}$	p-Value	Decision
	υ_1	υ_2				
Carbohydrate	5	12	1.2	3.1	0.3767	Accept H_0
Protein	5	12	0.5	3.1	0.7658	Accept H_0
RNA	5	12	0.9	3.1	0.5215	Accept H_0
DNA	5	12	1.4	3.1	0.2894	Accept H_0
Humic Acids	5	12	1.3	3.1	0.3396	Accept H_0

ANOVA was also applied to test if the mean weight ratios of chemical compounds in capsular biopolymer to volatile suspended solids were the same at the six locations where the test for fractional composition was applied. The same conclusion reached when the test was applied for nine locations resulted, i.e., the null hypothesis was rejected for carbohydrate, protein, RNA and TOC, but not for humic acids and DNA (see Table 4.14).

The information displayed in Tables 4.13, 4.14, and 4.15, which resulted from the application of ANOVA to the data from Tables 4.4, 4.5 and 4.8 allows the following conclusions:

(1) The hypotheses that the mean concentrations of capsular biopolymers constituents (carbohydrate, protein, RNA, DNA, humic acids and TOC) were the same at all locations within the treatment plant were rejected;

(2) The hypotheses that the mean weight ratios of carbohydrate, protein, RNA and TOC in capsular biopolymer to VSS were the same in the locations tested were

rejected. The hypotheses for humic acids and DNA were not rejected, but not all locations were tested for those compounds;

(3) The hypotheses that the fractional composition of carbohydrate, protein, RNA, DNA, and humic acids were the same in six locations within the treatment plant were not rejected.

Because the hypothesis that the fractional compositions of capsular biopolymers of activated sludge and digested sludge were the same was not rejected, one question that arises is why doesn't digested sludge flocculate the same way as activated sludge does. The difference between capsular biopolymers from activated sludge and digested sludge occurs in the quantity per unit of volatile suspended solids, not in composition. It can be shown from the data presented in Table 4.8 that the quantity of capsular biopolymer per unit of volatile suspended solids in digested sludge was 65 % of the quantity of capsular biopolymer per unit of VSS in activated sludge. Perhaps, in digested sludge, the minimum number of surfaces to be bridged before flocculation can occur greatly surpasses the quantity of polymers available for bridging. On the other hand, the capacity of capsular biopolymers to flocculate solids was observed in other locations of the plant. For example, in samples that had to be concentrated in order to gather enough solids for polymer extraction – influent, primary effluent, secondary effluent and thickener overflow samples – flocculation occurred as the concentration process proceeded.

4.2.3 *Multiple Comparison Between Means with Fischer's Least Significant Difference*

The rejection of the null hypothesis in ANOVA allows the conclusion that not all means are equal, but it does not indicate in which samples the means differ. A statistical procedure called *Fischer's Least Significant Difference* (LSD) makes

pairwise comparison between the means, showing where the differences detected in ANOVA occurred. The test statistic, t, for Fischer's LSD is (Anderson et al., 1994)

$$t = \frac{(\bar{x}_1 - \bar{x}_2) - (\mu_1 - \mu_2)}{\sqrt{MSW\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}} \qquad (4.3)$$

where $\bar{x}_1, \bar{x}_2$ = mean values of samples 1 and 2;

μ_1, μ_2 = mean values of populations 1 and 2;

n_1, n_2 = number of observations in samples 1 and 2;

MSW = mean square within, as defined in Equation 4.2

The sampling distribution of Equation 4.3 is a *t distribution* with $n_1 + n_2 - 2$ degrees of freedom. The critical values for the t distribution are found in statistical tables (Anderson et al., 1994), given the level of significance, α, and the number of degrees of freedom. The five steps for hypothesis testing outlined by Mirer (1988) are:

1. Hypothesis statement

   H_0: $\mu_1 = \mu_2$

   H_a: $\mu \neq \mu_2$

where μ_1, μ_2 are, for example, the mean concentrations of capsular biopolymer carbohydrate in influent and primary effluent, respectively.

2. Choice of the level of significance, α:

   $\alpha = 0.05$

3. Decision rule:

   Reject H_0 if $t < -t_{\alpha/2}$, or $t > t_{\alpha/2}$. The number of degrees of freedom is $3 + 3 - 2 = 4$. From a statistical table (Anderson et al., 1994), $t_{0.025;4} = 2.776$.

3. Determine the value of the test statistic:

   The value of the test statistic is $t = \frac{(\bar{x}_1 - \bar{x}_2)}{\sqrt{MSW\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$. μ_1, μ_2 don't appear in the

test statistic because, under the null hypothesis, $\mu_1 = \mu_2$. For the carbohydrate example, $\bar{x}_1 = 0.8$, $\bar{x}_2 = 0.2$, MSW = 521.8, and t = 0.0322.

5. State the conclusion of the test:

The null hypothesis will be rejected if $t < -t_{\alpha/2}$ or $t > t_{\alpha/2}$; otherwise, the hypothesis that the mean concentrations of capsular biopolymer carbohydrate in influent and primary effluent are the same can not be rejected. Because t = 0.0322 < $t_{0.025;4} = 2.776$, the null hypothesis can not be rejected.

A different conclusion would be reached if the mean concentrations of capsular biopolymer carbohydrate in influent and thickened sludge were being compared. In this case, $\bar{x}_1 = 0.8$, $\bar{x}_2 = 152.3$, MSW = 521.8, and t = -8.1. Because -8.1 < -2.776, the null hypothesis is rejected.

The test was applied to each pair of locations, and for each chemical compound. For each chemical compound, the locations where the null hypotheses were not rejected were grouped together, as homogenous groups. The application of Fischer's LSD to the results presented in Table 4.4 (mean concentrations of capsular biopolymers constituents in wastewater and sludge samples) resulted in the following homogenous groups, for each compound:

Carbohydrate:

Group 1: influent, primary effluent, activated sludge, secondary effluent, thickener overflow, digester supernatant, belt filter press filtrate

Group 2: thickened sludge

Group 3: digested sludge

Protein: same groups as in carbohydrate

RNA: same groups as in carbohydrate and protein

TOC: same groups as in carbohydrate, protein, and RNA

DNA: (tested in six locations)

Group 1: influent, activated sludge, digester supernatant, belt filter press filtrate

Group 2: thickened sludge

Group 3: digested sludge

Humic acids: (tested in eight locations)

Group 1: influent, primary effluent, activated sludge, thickener overflow, digester supernatant, belt filter press filtrate

Group 2: thickened sludge and digested sludge

The Fischer's LSD results indicate that the mean concentrations of capsular biopolymers carbohydrate, protein, RNA, DNA, humic acids, and TOC in thickened sludge and digested sludge were significantly different from the other locations. Also, the same compounds, with the exception of humic acids, have concentrations in thickened and digested sludges that were significantly different. The concentrations of capsular biopolymer humic acids in thickened and digested sludge were not significantly different.

Fischer's LSD also was used to make pairwise comparisons between the mean weight ratios of chemical compounds in capsular biopolymers to volatile suspended solids, as presented in Table 4.5. The test was performed for carbohydrate, protein, RNA and TOC, as ANOVA had not rejected the null hypotheses for DNA and humic acids (see Table 4.14). The application of the Fischer's LSD resulted in the following homogeneous groups:

Carbohydrate:

Group 1: primary effluent, digested sludge, digester supernatant

Group 2: influent, primary effluent, secondary effluent, thickener overflow, digested sludge, BFP filtrate

Group 3: influent, activated sludge, thickened sludge

Protein:

Group 1: primary effluent, digested sludge, digester supernatant, BFP filtrate

Group 2: primary effluent, digested sludge, thickener overflow, BFP filtrate

Group 3: secondary effluent, digested sludge, thickener overflow, BFP filtrate

Group 4: influent, activated sludge, secondary effluent, thickener overflow, BFP filtrate

Group 5: influent, activated sludge, secondary effluent, thickened sludge, thickener overflow

RNA:

Group 1: influent, primary effluent, secondary effluent, digested sludge, digester supernatant, BFP filtrate

Group 2: influent, primary effluent, secondary effluent, digested sludge, thickener overflow

Group 3: activated sludge, thickened sludge, digested sludge, thickener overflow

TOC:

Group 1: secondary effluent, digested sludge, digester supernatant;

Group 2: primary effluent, secondary effluent, digested sludge, BFP filtrate;

Group 3: primary effluent, activated sludge, thickener overflow, BFP filtrate;

Group 4: influent, activated sludge, thickened sludge, thickener overflow.

It is significant that activated sludge and secondary effluent were placed in different groups for carbohydrate, RNA and TOC. Because TOC is an overall measure of the quantity of capsular biopolymer per unit of VSS, it is possible to conclude that the total quantity of capsular biopolymer per unit of VSS in the secondary effluent is significantly different than the quantity of capsular biopolymer per unit of VSS in activated sludge. The TOC data in Table 4.5 show that less biopolymer per unit of

VSS is associated with solids in secondary effluent than in activated sludge. One way this could happen is through preferential settling of solids holding more polymers than solids with less polymers. Another possibility would be that the main sources of solids in secondary effluent were digester supernatant and belt filter press filtrate. The hypotheses that the weight ratios of TOC in capsular biopolymer to VSS in secondary effluent, digester supernatant, and BFP filtrate were equal were not rejected (see TOC group above). In the same treatment plant, Warfel (1998) found that particles in the range 1.1 to 28.2 μm present in secondary effluents correlated better in size and morphology with particles from anaerobic digester supernatant and BFP filtrate.

It was not possible to determine if the quantities of DNA and humic acids in capsular biopolymers per unit of VSS in activated sludge and secondary effluent were significantly different, as well. In secondary effluent biopolymer, the DNA values were below the limit of detection, while humic acids was not measured.

The weight ratios of TOC in capsular biopolymer to VSS in influent and primary effluent were significantly different. The weight ratio for TOC in influent was 26.9 mg biopolymer TOC/g VSS while the weight ratio in primary effluent was 19.5 mg biopolymer TOC/g VSS. This fact could have been caused by the preferential settling of particles holding more polymers, or by the contribution from anaerobically digested liquors. Solids-bound biopolymers in primary effluent, digested sludge, and BFP filtrate were considered to be homogeneous as a result of the Fischer's LSD test.

Capsular biopolymers in influent, activated sludge, and thickened sludge were considered to be homogeneous for carbohydrate, protein, and TOC. Activated sludge and secondary effluent capsular biopolymers were grouped together for protein. Influent and secondary effluent biopolymers were considered homogeneous for carbohydrate, protein, and RNA.

The major findings regarding the chemical characterization of solids-bound biopolymers were: (1) carbohydrate, protein, RNA, DNA and humic acids were components of the biopolymers; (2) protein was the major compound, comprising approximately 50 % of the biopolymer weight; (3) the concentrations of capsular biopolymers constituents were not the same in the locations sampled; (4) the weight ratios of capsular biopolymer carbohydrate, protein, RNA and TOC to VSS were significantly different among locations; (5) the weight ratios of capsular biopolymer TOC to VSS were significantly different for activated sludge and secondary effluent; (6) the hypotheses that the weight ratio of capsular biopolymer TOC to VSS in secondary effluent was the same as the weight ratios of TOC to VSS in primary effluent, digested sludge, digester supernatant, and BFP filtrate were not rejected; (7) the hypotheses that the fractional compositions of carbohydrate, protein, RNA, DNA, and humic acids in capsular biopolymer were the same at the various locations sampled within the wastewater and sludge treatment plant were not rejected.

4.2.4 *Justification for Summarizing and Analyzing Data as Averages*

In Sections 4.2.1 to 4.2.3, data were presented and analyzed as the average of three samples. Samples from all locations were collected during the months of June, July and August, with an additional sample collected in one location in October. The use of average for the data collected during this period allowed the application of ANOVA and Fischer's LSD to test hypotheses regarding differences and similarities between biopolymers present in different locations within the treatment plant.

The mean and corresponding standard deviation are the most commonly used descriptive statistics (Chapman, 1992). The arithmetic mean provides a measure of central location for the data set. The mean is sensitive to the presence of extreme values, either low and high. In this circumstance, the mean can give a distorted view

of the data (Chapman, 1992). Figures 4.9 and 4.10 present the weight ratios of capsular biopolymer constituents to VSS for the three sampling periods, and their respective averages in activated and digested sludges. While variations among months occurred, they were not extreme. As will be shown in the following application of a *Two Factor Analysis of Variance*, the mean values did not distort the data.

Figure 4.11 presents the overall average weight ratios of capsular biopolymers constituents to volatile suspended solids for the months of June, July and August. For example, the carbohydrate value in June was the average of carbohydrate for all locations (influent, primary effluent, etc.). It can be seen that the variation was small between the months. The column "average" represents the mean for the three months.

A statistical procedure known as *Two-Factor Analysis of Variance* was used to test how location and sampling period affected the values measured for capsular biopolymer constituents. The procedure is described in Devore (1982). Table 4.16 presents the weight ratios of carbohydrate in capsular biopolymer to volatile suspended solids for each location and sampling period. These data, presented in Table E.1 of Appendix E, will be used to illustrate the procedure for the Two-Factor ANOVA.

In Table 4.16, factor A is location and factor B is sampling period. Factor A has 9 levels, corresponding to each location (I = 9); factor B has three levels, corresponding to each sampling period (J = 3). Treatment refers to the possible combinations consisting of one level of factor A and one of factor B; the number of treatments is given by the product I x J. K_{IJ} denotes the number of observations made on treatment (i, j). In Table 4.16, the number of treatments is 27, and the number of observations made on each treatment (i, j) is 1 ($K_{IJ} = 1$).

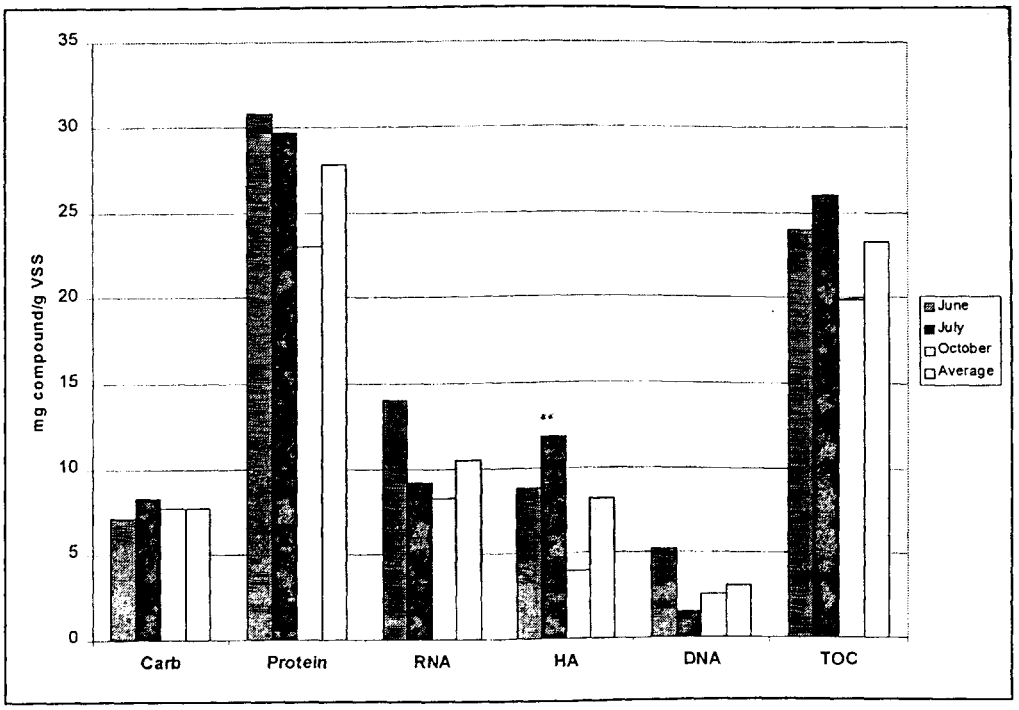

Figure 4.9 Weight Ratios of Capsular Biopolymers Constituents to Volatile Suspended Solids - Activated Sludge

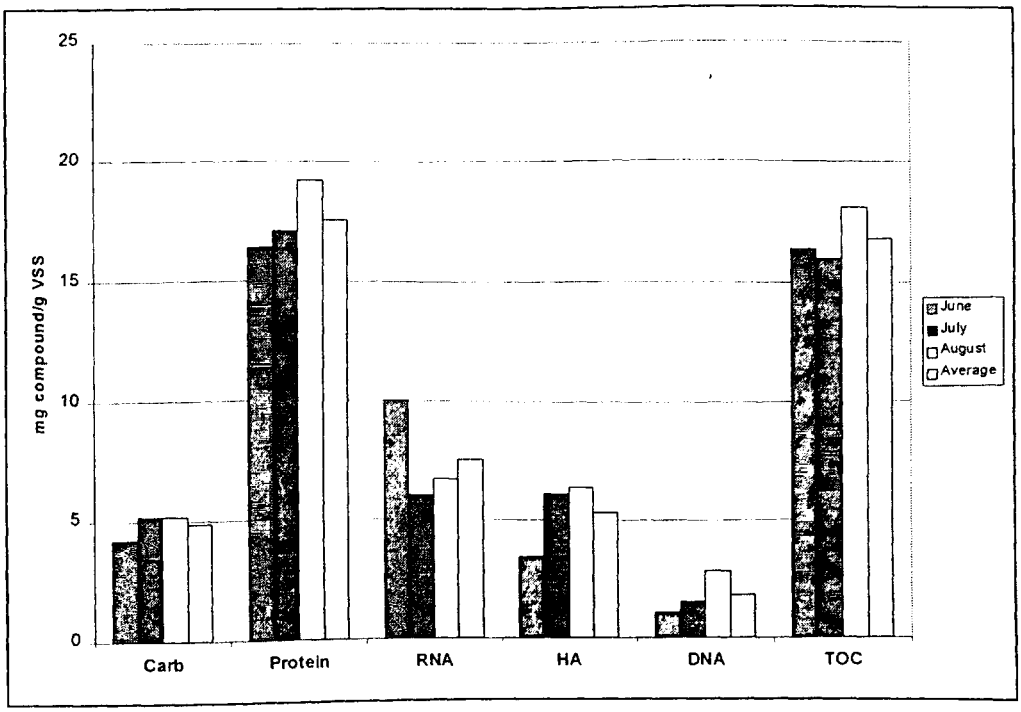

Figure 4.10 Weight Ratios of Capsular Biopolymers Constituents to Volatile Suspended Solids – Digested Sludge

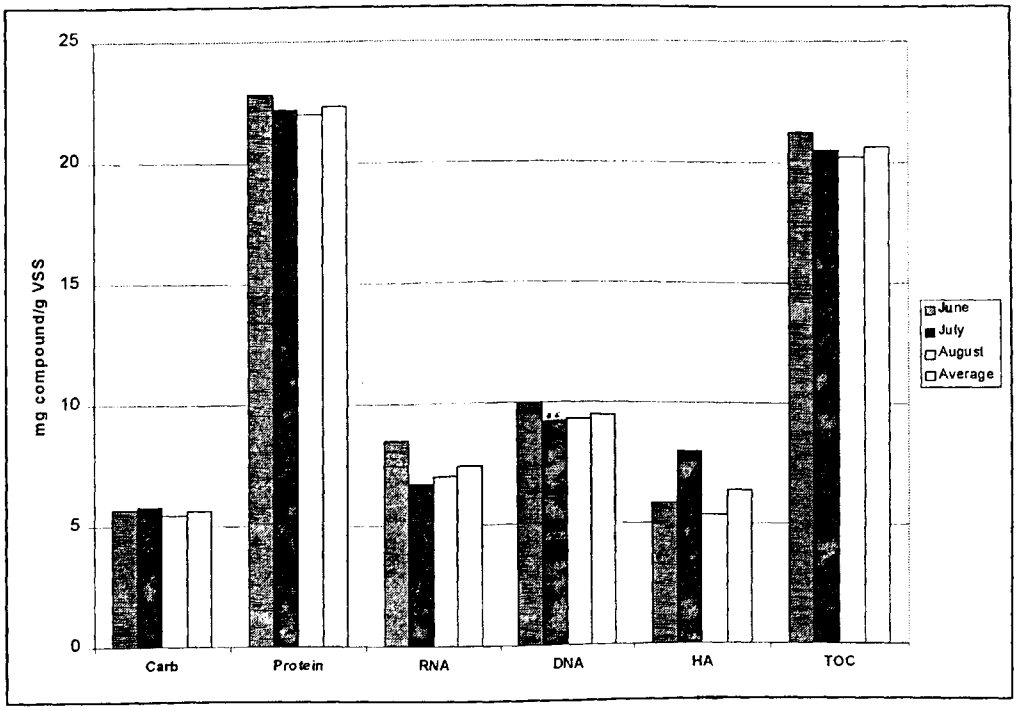

Figure 4.11 Average Weight Ratios of Capsular Biopolymers Constituents to Volatile Suspended Solids, in All Locations

Table 4.16 Weight Ratios of Carbohydrate in Capsular Biopolymer to Volatile Suspended Solids (Unit: mg/g VSS)

		Factor B: Sampling Period		
		June	July	August
Factor A: Location	Influent	5.9	7.5	6.1
	Primary Effluent	5.4	5.1	3.7
	Activated Sludge	7.1	8.3	7.7
	Secondary Effluent	7.8	4.5	3.7
	Thickened Sludge	6.9	7.6	8.4
	Digested Sludge	4.2	5.2	5.2
	Thickener Overflow	4.2	6.5	6.1
	Digester Supernatant	3.7	2.2	3.8
	BFP Filtrate	5.9	5.0	4.7

A model for Two-Factor ANOVA with $K_{IJ} = 1$ is given by Equation (4.4) (Devore, 1982)

$$X_{ij} = \mu_{ij} + \varepsilon_{ij} \tag{4.4}$$

where X_{ij} = the random variable which denotes the measurement when factor A is held at level i and factor B is held at level j; x_{ij} is the observed value of X_{ij};

μ_{ij} = the true average response when factor A is at level i and factor B at level j;

ε_{ij} = the random amount by which the observed value differs from its expectation.

Assuming the existence of I parameters $\alpha_1, \alpha_2, \ldots\ldots, \alpha_I$ and J parameters $\beta_1, \beta_2, \ldots\ldots, \beta_J$ such that

$$\mu_{ij} = \alpha_i + \beta_j \tag{4.5}$$

In Equation (4.5), each mean response μ_{ij} is the sum of an effect due to factor A at level i (α_i) and an effect due to factor B at level j (β_j). Substituting Equation (4.5) into Equation (4.4),

$$X_{ij} = \alpha_i + \beta_j + \varepsilon_{ij} \quad i = 1, 2, \ldots\ldots, I; \quad j = 1, 2, \ldots\ldots, J \tag{4.6}$$

In Equation (4.5), α_i's and β_j's are not uniquely determined (Devore, 1982). The non-uniqueness is eliminated by use of Equation (4.7)

$$X_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij} \quad \text{with } \sum_{i=1}^{I} \alpha_i = 0, \text{ and } \sum_{j=1}^{J} \beta_j = 0 \tag{4.7}$$

where μ = true grand mean (mean response averaged over all levels of factors A and B)

α_i = the effect of factor A at level i, measured as a deviation from μ;

β_j = the effect of factor B at level j, measured as a deviation from μ.

The unbiased estimators of μ, α_i, and β_j are:

$$\hat{\mu} = \overline{\overline{X}}$$
$$\hat{\alpha}_i = \overline{X}_{i\bullet} - \overline{\overline{X}} \qquad (4.8)$$
$$\hat{\beta}_j = \overline{X}_{\bullet j} - \overline{\overline{X}}$$

where $\overline{\overline{X}} = \dfrac{\sum_{i=1}^{I}\sum_{j=1}^{J} X_{ij}}{I \cdot J}$ = the overall mean

$\overline{X}_{i\bullet} = \dfrac{\sum_{j=1}^{J} X_{ij}}{J}$ = the average of measurements obtained when factor A is held at level i

$\overline{X}_{\bullet j} = \dfrac{\sum_{i=1}^{I} X_{ij}}{I}$ = the average of measurements obtained when factor B is held at level j

The sums of squares relevant to Two-Factor ANOVA are:

Total sum of squares = $SST = \sum_{i=1}^{I}\sum_{j=1}^{J}\left(X_{ij} - \overline{\overline{X}}\right)^2$ (4.9)

Sum of squares factor A = $SSA = \sum_{i=1}^{I}\sum_{j=1}^{J}\left(\overline{X}_{i\bullet} - \overline{\overline{X}}\right)^2$ (4.10)

Sum of squares factor B = $SSB = \sum_{i=1}^{I}\sum_{j=1}^{J}\left(\overline{X}_{\bullet j} - \overline{\overline{X}}\right)^2$ (4.11)

Error sum of squares = $SSE = \sum_{i=1}^{I}\sum_{j=1}^{J}\left(X_{ij} - \overline{X}_{i\bullet} - \overline{X}_{\bullet j} + \overline{\overline{X}}\right)^2$ (4.12)

The number of degrees of freedom of SSA, SSB, and SSE are, respectively, (I – 1), (J – 1), and (I – 1)(J – 1). The mean squares of errors and factors A and B, are, respectively:

$$MSA = \frac{SSA}{I-1};\ MSB = \frac{SSB}{J-1};\ MSE = \frac{SSE}{(I-1)\cdot(J-1)} \qquad (4.13)$$

There are two different hypotheses in a two-factor ANOVA. The first hypothesis, denoted H_{0A}, states that the different levels of factor A have no effect on

X_{ij}, while the alternative hypothesis, denoted H_{aA}, states that at least one level of factor A affects X_{ij}.

H_{0A}: $\alpha_1 = \alpha_2 = \ldots\ldots = \alpha_i = 0$

H_{aA}: *at least one* $\alpha_i \neq 0$

The second hypothesis, denoted H_{0B}, states that the different levels of factor B have no effect on X_{ij}, while the alternative hypothesis, denoted H_{aB}, states that at least one level of factor B affects X_{ij}.

H_{0B}: $\beta_1 = \beta_2 = \ldots\ldots = \beta_j = 0$

H_{aB}: *at least one* $\beta_i \neq 0$

The test procedure for the hypotheses are (Devore, 1982):

Hypotheses: H_{0A} versus H_{aA}

Test statistic: $F_A = \dfrac{MSA}{MSE}$

Rejection region: $F_A \geq F_{\alpha, I-1, (I-1)(J-1)}$

Hypotheses: H_{0B} versus H_{aB}

Test statistic: $F_B = \dfrac{MSB}{MSE}$

Rejection region: $F_B \geq F_{\alpha, J-1, (I-1)(J-1)}$

where $F_{\alpha, I-1, (I-1)(J-1)}$ = value from a F distribution associated with a level of significance α, I – 1 degrees of freedom in the numerator and (I – 1)(J – 1) degrees of freedom in the denominator. For $\alpha = 0.05$, I = 9, J = 3, $F_{0.5, 8, 16} = 2.59$ (from the F distribution statistical table).

$F_{\alpha, J-1, (I-1)(J-1)}$ = value from a F distribution associated with a level of significance α, J – 1 degrees of freedom in the numerator and (I – 1)(J – 1) degrees of freedom in the denominator. For $\alpha = 0.05$, I = 9, J = 3, $F_{0.5, 2, 16} = 3.63$ (from the F distribution statistical table)

The sum of squares, calculated using data from Table 4.16, are:

SST = 69.91

SSA = 49.37

SSB = 0.36

SSE = 20.17

The mean squares of factors A and B and error are:

$$MSA = \frac{SSA}{I-1} = \frac{49.37}{9-1} = 6.17$$

$$MSB = \frac{SSB}{J-1} = \frac{0.36}{3-1} = 0.18$$

$$MSE = \frac{SSE}{(I-1)\cdot(J-1)} = \frac{20.17}{(9-1)\cdot(3-1)} = 1.26$$

The tests statistic F_A and F_B are:

$$F_A = \frac{MSA}{MSE} = \frac{6.17}{1.26} = 4.90$$

$$F_B = \frac{MSB}{MSE} = \frac{0.18}{1.26} = 0.14$$

Because $F_A = 4.90 \geq F_{0.5,8,16} = 2.59$, $H_{0A}: \alpha_1 = \alpha_2 = \alpha_3 = \ldots\ldots = \alpha_9 = 0$ is rejected; hence factor A (location) affects the value of X_{ij}.

Because $F_B = 0.14 < F_{0.5,2,16} = 3.63$, $H_{0B}: \beta_1 = \beta_2 = \beta_3 = 0$ is not rejected; therefore, factor B (sampling period) does not affect the value of X_{ij}.

The conclusion of the Two-Factor ANOVA for the data presented in Table 4.16 is that location affects the value of the weight ratio of carbohydrate in capsular biopolymer to volatile suspended solids, while sampling period has no effect in that value. The test was repeated for the weight ratios of protein, RNA, DNA, humic acids and TOC in capsular biopolymer to volatile suspended solids. Table 4.17 summarizes information resulting from these applications of Two-Factor ANOVA.

The results presented in Table 4.17 indicate that the sampling periods in which the samples were collected did not affect the values of the weight ratios of the

chemical compounds in capsular biopolymer to volatile suspended solids. This conclusion supports averaging the data collected in three different months for analysis. These results also indicate that location affected the values of the weight ratios of carbohydrate, protein, RNA and TOC in capsular biopolymer to volatile suspended solids; however, location did not affect the weight ratios for humic acids and DNA. This conclusion was equivalent to the conclusion reached when ANOVA was applied to test the equality of weight ratios in capsular biopolymers to VSS (Table 4.14). It was found that the weight ratios were different among locations for carbohydrate, protein, RNA and TOC, but not for humic acids and DNA.

Table 4.17 Information Resulting from the Application of Two-Factor ANOVA to Data from Tables E.1, E.2, E.3, E.4, E.5, and E.6 from Appendix E

Compound	Factor	D. F.		$F_{0.05;\upsilon_1;\upsilon_2}$	F_{Data}	p-value	Decision
		υ_1	υ_2				
Carbohydrate	Location	8	16	2.59	4.90	0.003	Reject H_{0A}
	Time	2	16	3.63	0.14	0.867	Accept H_{0B}
Protein	Location	8	16	2.59	4.53	0.005	Reject H_{0A}
	Time	2	16	3.63	0.09	0.915	Accept H_{0B}
RNA	Location	8	16	2.59	4.48	0.005	Reject H_{0A}
	Time	2	16	3.63	3.34	0.061	Accept H_{0B}
TOC	Location	8	16	2.59	6.60	0.001	Reject H_{0A}
	Time	2	16	3.63	0.21	0.810	Accept H_{0B}
Humic Acids	Location	7	14	2.76	1.15	0.389	Accept H_{0A}
	Time	2	14	3.74	1.39	0.282	Accept H_{0B}
DNA	Location	5	10	3.33	1.84	0.193	Accept H_{0A}
	Time	2	10	4.10	0.49	0.628	Accept H_{0B}

4.2.5 *Loosely-Bound Biopolymers (Slime)*

The concentration of loosely-bound biopolymers constituents in wastewater and sludge samples are shown in Table 4.18. Limits of detection and complete results are presented in appendixes D and E. The data in Table 4.18 are given as mean ± standard deviation of three measurements, except primary sludge, which was sampled only once.

Table 4.18 Concentration of Chemical Compounds in Loosely-Bound Biopolymers in Wastewater and Sludge Samples (Units: mg/l)

Location	Carbohydrate	Protein	DNA	RNA	Humic Acids	Total Organic Carbon
Influent	3.6 ± 2.2	6.9 ± 2.6	< LD [2]	2.4 ± 2.4	3.3 ± 3.0	9.3 ± 5.5
Primary Effluent	2.2 ± 1.3	2.4 ± 0.9	< LD	< LD	< LD	3.4 ± 0.4
Primary Sludge	2.0	4.0	-	-	< LD	4.0
Activated Sludge	2.1 ± 1.0	2.1 ± 0.6	< LD	< LD	< LD	3.0 ± 0.8
Secondary Sludge	-	-	-	-	-	-
Secondary Effluent	1.4 ± 1.2	2.3 ± 0.5 [1]	< LD	< LD	-	4.2 ± 3.1
Thickened Sludge	40 ± 12	57 ± 9.0	18 ± 4.1	48 ± 21	7.1 ± 10.3	83.7 ± 11.0
Digested Sludge	19 ± 11	66 ± 46	< LD	25 ± 10	5.4 ± 5.4	68.3 ± 44.8
Thickener Overflow	2.0 ± 0.9	4.6 ± 2.0	< LD	1.5 ± 0.9	< LD	5.5 ± 1.4
Digester Supernatant	12 ± 1.5	66 ± 30	< LD	19 ± 5.6	< LD	60.2 ± 17.2
BFP Filtrate	2.9 ± 0.5	6.2 ± 1.7	< LD	6.6 ± 3.8	< LD	8.2 ± 2.4

[1] In secondary effluent, protein was measured using the Folin-Lowry method (see Section 3.3.3)
[2] Below the limit of detection (see Section 3.4 and Appendix D)

In many samples, the concentrations of humic acids, DNA, and RNA were below the limits of detection for the respective methods. The only sample where all the chemicals measured were above the limits of detection was thickened sludge. Protein was found to be the chemical compound with the highest concentration in most of the locations sampled. Protein also had been found to be the major capsular biopolymer constituent (Table 4.4). However, the ratios between protein and carbohydrate in slime and capsular biopolymers differed. Table 4.19 presents the ratios between protein and carbohydrate in capsular and slime biopolymers at four locations. The ratios protein to carbohydrate were appreciably higher in capsular than in slime polymers, except in digested sludge, where the ratio was about the same. This fact probably reflects the greater contribution of protein from the cells to capsular polymers. Another possibility is that protein associated with slime is more labile than protein associated with the capsule. Compounds that are sorbed to surfaces can be less available to biodegradation than compounds that are in solution (Alexander, 1999). Also, as discussed in Sections 2.5 and 2.6, some of the carbohydrates present in biological systems might be recalcitrant.

Table 4.19 Ratios Between Protein and Carbohydrate in Capsular and Slime Biopolymers

Location	Ratio	
	Capsule	Slime
Influent	3.6	1.9
Activated Sludge	3.6	1.0
Thickened Sludge	3.9	1.4
Digested Sludge	3.6	3.5

Humic acids could be detected in slime polymers only in influent, thickened and digested sludges. On the other hand, humic acids were measured above the limit of detection in all capsular biopolymers. This might be an indication that humic acids were associated mostly with surfaces, rather than being in solution.

Table 4.20 presents a comparison of the concentration of capsule and slime biopolymers constituents at selected locations of the treatment plant (data from Tables 4.4 and 4.18). These data indicate that loosely-bound biopolymers were the major polymers present in locations with low suspended solids concentration (influent, secondary effluent), while capsular biopolymers were the major polymers in samples with high suspended solids concentrations (activated sludge, thickened and digested sludges). The availability of surfaces in locations with high suspended solids concentration favors the attachment of molecules with high molecular weight, which are present in biopolymers (see Section 4.3.2). This could be the principal reason why more capsular than slime biopolymers are present in locations with high suspended solids concentrations.

Table 4.20 Concentration of Capsular and Slime Biopolymers Constituents (Units: mg/l)

Location	Carbohydrate		Protein		Humic Acids		TOC	
	capsule	slime	capsule	slime	capsule	slime	capsule	slime
Influent	0.8	3.6	2.9	6.9	1.1	3.3	3.1	9.3
Act. Sludge	5.9	2.1	21.3	2.1	6.0	< LD	17.8	3.0
Sec. Effl.	0.0	1.4	0.0	2.3	-	-	0.0	4.2
Thick. Sludge	202.3	39.6	795.2	57.1	155.3	7.1	705.8	83.7
Dig. Sludge	134.0	18.5	483.0	65.5	149.5	5.4	461.1	68.3

4.2.6 *Elemental Composition of Capsule and Slime Biopolymers*

The inorganic content of extracellular polymers were determined by igniting polymers that had been dried for one hour in an oven at 103 °C in a muffle furnace at 550 °C for 15 min (Section 4.3.1). The inorganic fraction of the biopolymers extracted at the Ithaca treatment plant varied in the range of 10 to 20 % of the biopolymer weight.

Little information exists with regard to the nature of the inorganic fraction of biopolymers. Sato and Ose (1980) measured approximately 15 % ash in activated sludge capsular biopolymers. Morgan et al. (1990) found that ash comprised 18 % of capsular biopolymers from an activated sludge plant. In another facility, ash formed 35 % of the capsular biopolymers extracted from anaerobically digested sludge. Using electron dispersive X-ray analysis, these authors found that calcium and phosphorus were the predominant elements in the inorganic fraction of capsular biopolymers from activated and digested sludges.

X-ray analysis is a technique that can be used to identify the elemental composition in a sample (Postek et al., 1980). In this technique, a concentrated beam of electrons strikes the sample and dislodges electrons in the atoms. In an atom, electrons occupy different energy levels called shells. Electrons located in inner shells (in relation to the atom nucleus) carry less energy than electrons located in outer shells. Because electrons seek the lowest possible energy level, an electron from an outer shell will immediately replace an electron in an inner shell that had been dislodged by the electron beam. The high energy electron loses energy in this "jump". The energy is released as an x-ray, with energy equal to the difference in energy between the shells. The energy of the x-ray that is emitted is a characteristic of each chemical element. This property allows the identification of the elements in a sample. In a x-ray spectrometer, the x-rays that are emitted strike a semi-conductor crystal.

Electrons in the crystal absorb the energy, which is converted to electrical signals and amplified. The electrical signals are introduced into a multichannel analyzer, which sorts the signals and counts the number of x-rays at each energy level. The information is plotted to form a representative spectrum of the elements present in the area traversed by the electron beam. This is called a *characteristic x-ray spectrum.*

Table 4.21 presents the major elements detected in x-ray analyses performed in capsular and slime biopolymers extracted from samples collected at the Ithaca wastewater treatment plant. The samples differed from those considered elsewhere in this thesis; they were collected in April 1996, and the extraction procedure for capsular biopolymers used EDTA in a sodium form (Na_4EDTA) instead of the cation exchange resin. Slime was extracted by centrifugation at 8,000 g for 45 min.

Table 4.21 Elemental Composition of Capsular and Slime Biopolymers

Sample	Capsule	Slime
Influent	C, N, O, Na, Mg, Si, P, S, Cl, K, Ca	C, N, O, Na, Mg, Si, P, S, Cl, K, Ca
Activated Sludge	C, N, O, Na, Mg, Si, P, S, Cl, K, Ca	C, N, O, Na, Mg, P, S, Cl, Ca
Secondary Effluent	C, N, O, Na, Mg, Si, P, S, Cl, K, Ca	C, N, O, Na, Mg, Si, P, S, Ca
Digested Sludge	C, N, O, Na, Mg, Si, P, S, Cl, K, Ca	C, N, O, Fe, Mg, P, S, Ca
BFP Filtrate	C, N, O, Na, Mg, Si, P, S, Cl, K, Ca	C, N, O, Na, Mg, Si, P, S, Cl, K, Ca

The same elements were present in the capsular biopolymer at the five locations. The elemental composition of slime biopolymers varied. For example, silicon was absent in activated sludge and digested sludge slime, while iron was detected only in digested sludge slime. Figures 4.12 and 4.13 present the x-ray spectra of capsule and slime biopolymers extracted from digested and activated sludges

samples from the Ithaca plant. In each spectrum, keV is the x-ray energy, which has units of electron volts (eV). For example, phosphorus has a x-ray energy of 2.0 keV. In Figures 4.12 and 4.13, the characteristic spectra are superimposed on a continuous spectra. The latter is formed by x-rays produced by electrons from the beam that are decelerated after striking the sample. The detection of lead, in Figure 4.13 was likely due to an inability of the instrument to distinguish x-rays that have approximately the same energy (Daugherty, 1996). The peak of sodium in Figure 4.13 might be due to its presence as a component of the EDTA used to extract the polymers. While phosphorus was the element with higher peak intensity in capsular biopolymers, the peak for sulfur was higher than the peaks of other elements in slime biopolymers. The presence of phosphorus in capsular biopolymer might come from its presence in nucleic acids. Phosphorus is a component of ribonucleotides and deoxyribonucleotides, the building blocks of RNA and DNA, respectively (Lehninger, 1972). Nucleic acids, especially RNA, were found to be capsule biopolymer components (Table 4.5). Sulfur is an essential element in the formation of some proteins (Sawyer et al., 1994), which were components of both capsule and slime. Based on composition of capsule and slime, it would be expected that the elements carbon and nitrogen would have the major peaks in the x-rays spectra. However, peak intensity is not a direct quantitative measurement of the element (Postek et al., 1980). Several factors affect peak intensity; for example, x-ray production varies with atomic number, and heavy elements produce more x-rays than light elements.

4.3 Physical Characterization of Biopolymers

4.3.1 *Biopolymer Yield*

A method that is commonly used to estimate the quantity of extracellular polymers extracted from a sample is the addition of an organic solvent, such as ethanol

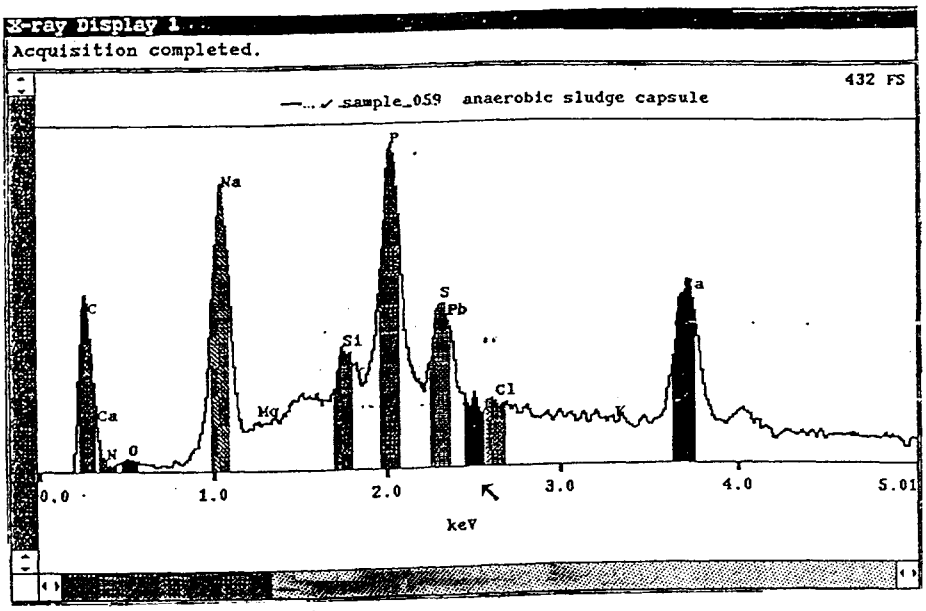

Figure 4.12 X-Ray Spectrum from Digested Sludge Capsular Biopolymer

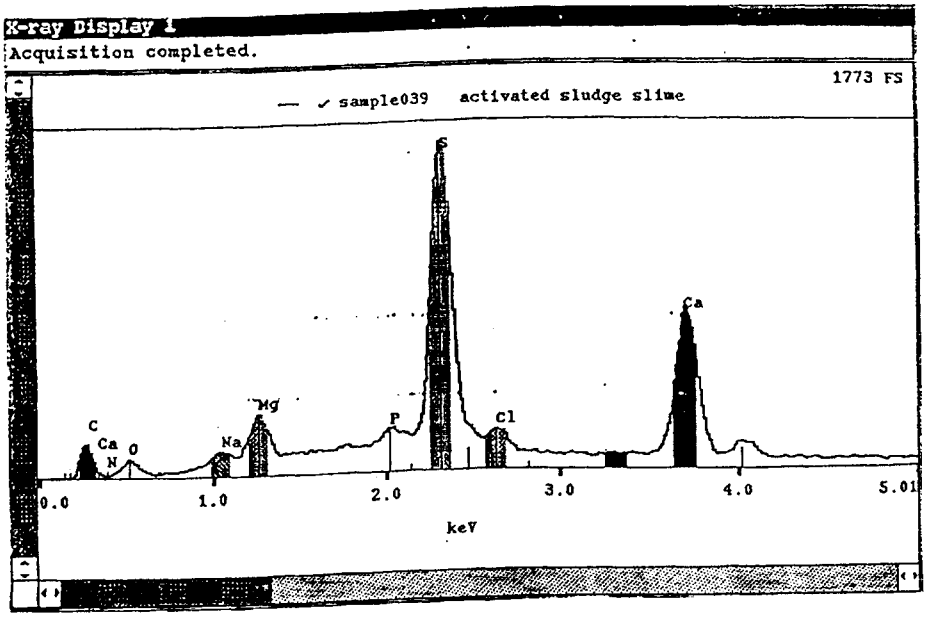

Figure 4.13 X-Ray Spectrum from Activated Sludge Slime Biopolymer

or acetone, to the biopolymer containing solution, at a ratio of 2:1, to precipitate the polymers (Gehr and Henry, 1983). The precipitated polymer is then separated by centrifugation or filtration, dried for one hour in an oven at 103 °C, and weighed. This procedure to measure the quantity of polymers results in ethanol insoluble matter (EIM). The inorganic content of the polymer can be determined by weighing the ignited residue after the dried biopolymer is placed in a muffle furnace at 550 °C for 15 min. Table 4.22 presents the mean ethanol insoluble matter measured at the sampled locations in the treatment plant. Also presented in Table 4.22 are the sums of the organic chemical compounds measured in the solids-bound biopolymers (from Table 4.8).

Table 4.22 Ethanol Insoluble Matter and Organic Compounds in Solids-Bound Biopolymer (mg/g VSS)

Location	Ethanol Insoluble Matter (mg/g VSS)			Sum of Compounds [1]
	Organic	Inorganic	Total	
Influent	33.2	3.7	36.9 ± 3.9	53.4
Prim. Effluent	23.9	3.7	27.6 ± 5.4	31.3 [2]
Prim. Sludge	24.6	5.8	30.4	36.3
Act. Sludge	32.0	4.6	36.6 ± 5.1	57.3
Waste Act. Sl.	42.4	4.5	46.9	47.4
Thick. Sludge	24.0	5.0	29.0 ± 0.8	57.5
Digested Sludge	26.6	5.9	32.5 ± 9.7	37.2
Thick. Overflow	33.5	6.0	39.5 ± 10.1	40.3 [2]
Dig. Supernatant	17.9	3.3	21.2 ± 6.0	30.4
BFP Filtrate	24.1	2.0	26.1 ± 6.0	42.5

[1] The sum of carbohydrate, protein, RNA, DNA, and humic acids values, as presented in the column "Total Biopolymer" in Table 4.8. They are means of three results. Primary and waste activated sludge values are from only one sample, and were obtained from Table 4.5.

[2] Does not include DNA values, which were below the limit of detection.

Ethanol insoluble matter underestimated the quantity of biopolymer present in a sample. This can be concluded by comparing the values in the *organic* column in Table 4.22 with the values in the column *sum of compounds*. EIM values were in the range 10 to 60 % lower than the sum of organic chemical compounds.

Another method that can be used to quantify biopolymer weight is by freeze-drying the solution containing the polymer. The dried material can then be weighed. This technique was used in some samples in addition to the ethanol insoluble matter procedure. Table 4.23 presents the yields obtained by the two methods, as well as the sum of the organic chemical compounds measured. In four of the six samples, the freeze drying technique approximated better the weight calculated by adding the chemical compounds of the biopolymers.

Table 4.23 Total Biopolymer Yields at Selected Locations (mg/g VSS)

Location	Set [1]	EIM [2]	Freeze-Dried [3]	Sum of Compounds [4]
Prim Effl.	2	30.3	38.5	30.5 [5]
Prim. Sludge	3	30.4	34.5	36.3
Act. Sludge	2	34.4	59.7	60.5
Act. Sludge	4	33.0	49.6	45.4
Sec. Sludge	4	46.9	55.0	47.4
Thick. Sludge	2	29.6	58.3	55.2

[1] Corresponds to sampling period;
[2] Refers to the polymer weight that was obtained by ethanol precipitation;
[3] Refers to the polymer weight that was obtained by freeze drying;
[4] Sum of carbohydrate, protein, RNA, DNA and humic acids;
[5] Does not include DNA because it was below the limit of detection.

In activated sludge, ethanol insoluble matter has been measured in the range of 12.6 mg EIM/g SS (Gehr and Henry, 1983) to 108 mg EIM/g SS (Forster and Clark, 1983). In anaerobically digested sludge, Morgan et al. (1990) measured 13.3 mg EIM/g SS while Karapanagiotis et al. (1989) measured 33.5 mg EIM/g TS. Table 4.24 presents a compilation of the values reported in other studies for ethanol insoluble matter in capsular biopolymers. The values of EIM measured at the Ithaca plant were higher in some cases, but lower in others.

4.3.2 *Molecular Weight of Biopolymers*

Molecular weight is the sum of atomic weights of the atoms in a molecule. Molecular weight is a measure of the size of a molecule (Skoog and Leary, 1992). One technique that can be used to determine the molecular weight of a compound is size-exclusion chromatography. In this technique, a column is packed with silica or polymer particles containing a network of pores. The solute, containing the compound of interest, is applied with a solvent to the column. The molecules in the solute that are larger than the pores of the packing material are not retained in the pores, and are the first ones to be eluted from the column. They have the highest molecular weight. The exclusion limit is the molecular weight beyond which no retention occurs. On the other extreme are the solute molecules that are smaller than the packing material pores. These molecules will penetrate into the pores and will suffer the greatest retention – they will be the last ones to be eluted, and have the lowest molecular weight. The permeation limit is the molecular weight below which the molecule can penetrate into all the pores completely. Between the exclusion and permeation limits, there are molecules that can penetrate some pores but are too big for other pores. These molecules will suffer partial retention, and their elution time will be between

Table 4.24 Weight Ratios of Ethanol Insoluble Matter to Suspended Solids in Reported Studies

Author	Sample	Extraction Method	Quantity
Kiff and Thompson (1979)	Activated sludge (Unknown source)	Centrifugation Heat Sonication Homogenization	26 to 54 mg/g SS 34 to 36 mg/g SS 34 to 36 mg/g SS << 30 mg/g SS
Forster and Clark (1983)	Activated sludge Severn-Trent, U.K.	Ethanol	108 mg/g SS
Gehr and Henry (1983)	Activated sludge, Toronto, Canada	Centrifugation K_2HPO_4	12.6 mg/g SS 21.2 mg/g SS
Sato and Ose (1984)	Activated sludge, Synthetic medium	NH_4OH and EDTA	103..8 mg/g SS
Karapanagiotis et al. (1989)	Anaerobic digested sludge, Beckton Sewage Works Thames Water Authority, U.K.	Sodium hydroxide Dowex CER Boiling Steaming Phenol	34.7 mg/g TS 33.5 mg/g TS 24.1 mg/g TS 14.7 mg/g TS 4.1 mg/g TS
Morgan et al. (1990)	Activated sludge, Kidderminster plant, U.K.	Heat 80 °C	90.2 mg/g SS
	Anaerobic digested sludge, Bromsgrove plant, U.K.	Heat 80 °C	13.3 mg/g SS
	UASB reactor, dairy plant, Gloucester, U.K.	Heat 80 °C	46.8 mg/g SS
	UASB reactor, paperwaste plant, Aberdeen, U.K.	Heat 80 °C	10.4 mg/g SS
Sanin and Vesilind (1994)	Activated sludge, Durham, NC	Centrifugation	20 mg/g TSS
This Study	Activated Sludge, Ithaca, N.Y.	Dowex CER	36.6 mg/g VSS
	Anaerobic Digested Sludge, Ithaca, N.Y.	Dowex CER	32.5 mg/g VSS

the elution times for molecules at the exclusion and permeation limits (Skoog and Leary, 1992). In order to determine the molecular weight of a compound, standards with known molecular weights are injected into the column, and their elution times are

monitored with an ultra-violet detector. A plot of elution time versus the logarithm of the molecular weight should give a straight line (Sigma, 1987; Skoog and Leary, 1992).

Table 4.25 presents the elution times for selected standards, while Figure 4.14 displays the graph of the logarithm of molecular weight versus elution time. Typical chromatograms for solids-bound biopolymers are presented in Figures 4.15 and 4.16. Figures 4.17 presents chromatograms from solids-bound biopolymers at four locations within the treatment plant. The numbers that appear on the right-side of Figures 4.15 through 4.18 correspond to the elution times of molecules having sizes greater than the exclusion limit and smaller than the permeation limit, respectively. In Figures 4.15 and 4.16, A and C mean that two signals were being detected by the ultra-violet detector, respectively, at wavelengths of 254 and 195 nm. The numbers next to the wavelength in Figures 4.15 and 4.16 correspond to bandwidths. For example, at wavelength 254 nm and bandwidth 20 nm: absorbance is measured between 244 and 264 nm. The data contained within the bandwidth are bunched by the data acquisition system to give one data point for the center wavelength. Wavelength bunching minimizes the signal to noise ratio (Hewlett Packard, Inc., 1985). In Figures 4.15 and 4.16, range and zero % are functions that control the monitor display in the computer. Although not shown, the same conditions of signals, wavelength, bandwidth, range and zero % were set for the chromatograms presented in Figure 4.17.

The chromatograms show that molecules with molecular sizes greater than the exclusion limit and smaller than the permeation limit were present in capsular biopolymers. The exclusion limit corresponds to the first peak in the chromatograms, while the permeation limit corresponds to the second peak. According to the manufacturer of the column used in this experiment (Phenomenex, Inc., 1996), the

Table 4.25 Elution Times for Standards

Standard	Molecular Weight (Dalton)	Elution Time (min)
Blue Dextran	2,000,000	6.16
Tyroglobulin	669,000	7.86
Apoferritin	443,000	8.53
β-amylase	200,000	8.84
Alcohol dehydrogenase	150,000	10.83

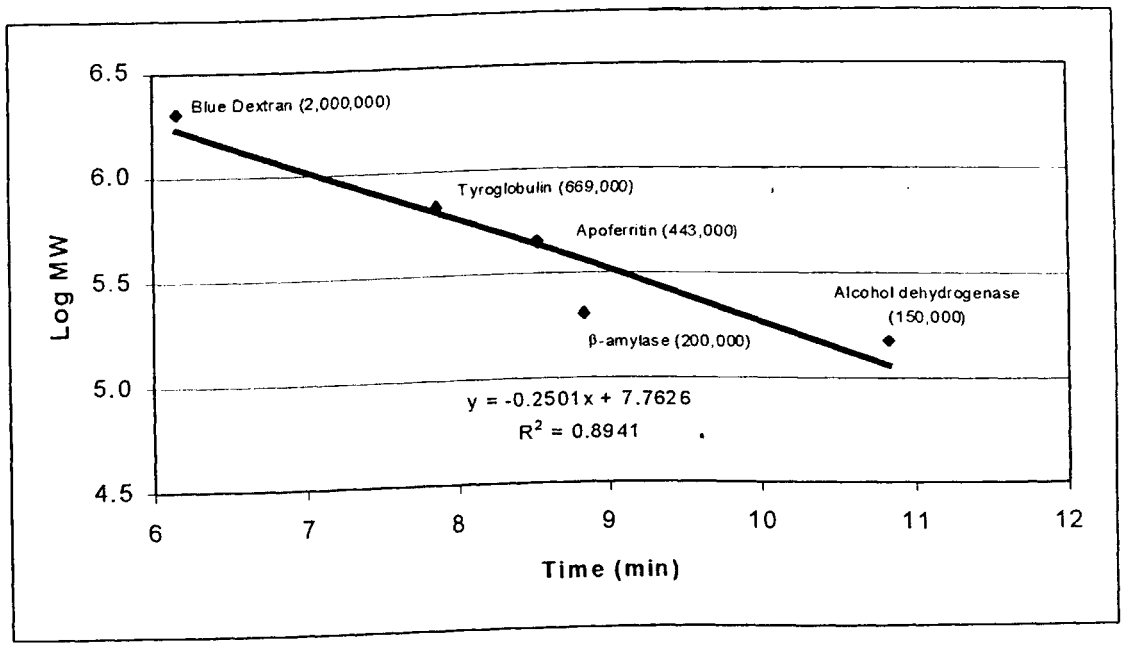

Figure 4.14 Molecular Weights Versus Elution Times for Standards

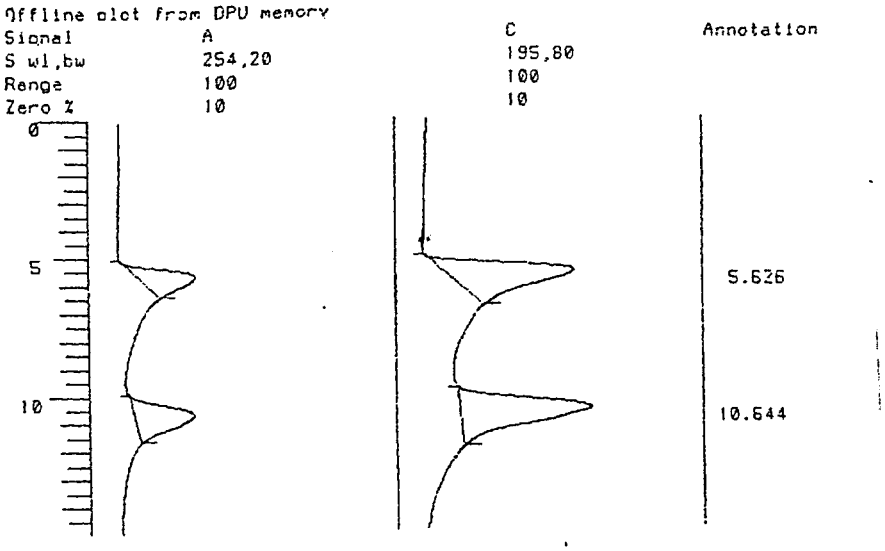

Figure 4.15 Activated Sludge Chromatogram - Capsule

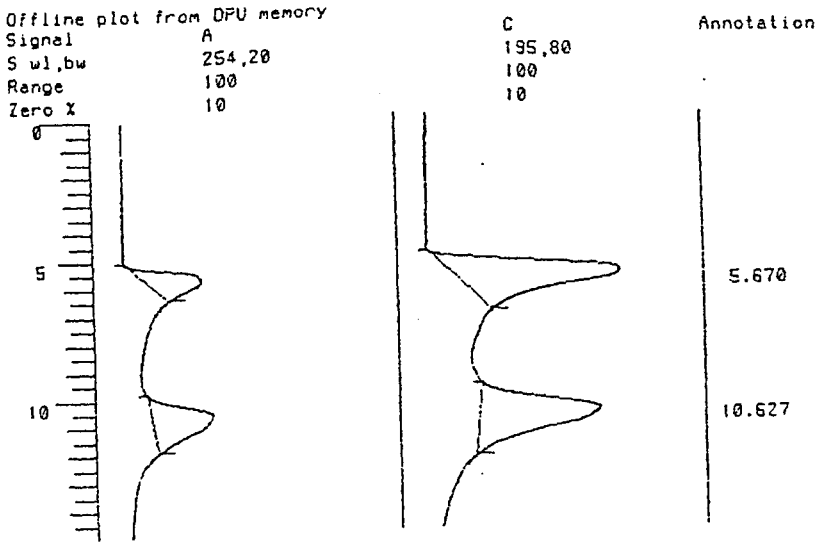

Figure 4.16 Digested Sludge Chromatogram – Capsule

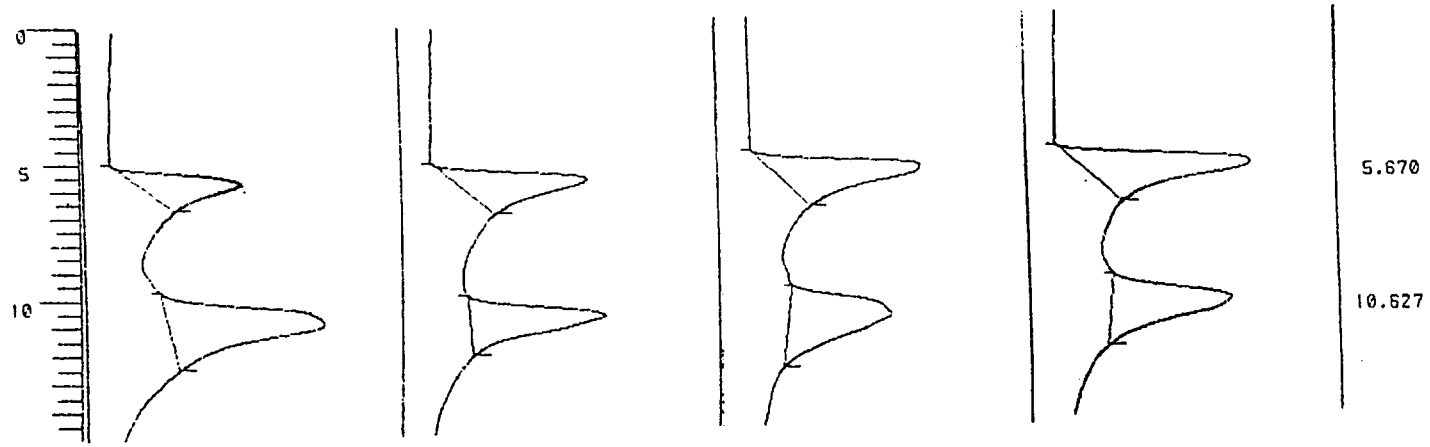

Figure 4.17 Capsule Chromatograms – (a) Influent, (b) Activated Sludge, (c) Thickened Sludge, (d) Digested Sludge

permeation and exclusion limits for the column were, respectively, 15,000 and 2,000,000 daltons. Separation between the exclusion and permeation limits was not accomplished in the column inasmuch as no peak was discernible between the limits. It can be seen that the elution times corresponding to the permeation limit in Figures 4.15 to 4.17 were close to the elution time corresponding to the standard with molecular weight 150,000 daltons (Table 4.25). Thus, the best interpretation of the chromatograms in Figures 4.15 to 4.17 is that molecules with sizes greater than 2,000,000 daltons and smaller than 150,000 daltons were present in capsular biopolymers, in addition to molecules with molecular size between the two limits.

The high molecular weight fraction is in accordance with the flocculative property of the biopolymers. The effective bridging of negatively charged particles requires molecular weight above 1 x 10^6 daltons (Amirtharajah and O'Melia, 1990). Sutherland and Elwood (1979) showed examples of exopolysaccharides produced by bacteria having molecular weights in the range 2 x 10^5 to 2 x 10^6. Horan and Eccles (1986) identified a fraction with molecular weight 1.6 – 2.0 x 10^6 in purified biopolymers extracted from five activated sludge plants. Extracellular DNA excreted by the bacterium *Pseudomonas aeruginosa* KYU-1 has molecular weight of 9.6 x 10^6 (Paul et al., 1987). Cellular components have a wide range of molecular weights: for example, DNA molecules can exceed 2 x 10^9 daltons, proteins vary in the range 5 x 10^3 to 4 x 10^7, storage polysaccharides from 5 x 10^5 to 1 x 10^8, and RNA in the range 2 x 10^3 to 1 x 10^6 (Lehninger, 1975). Molecules with size less than 150,000 daltons include proteins, polysaccharides, RNA (Lehninger, 1975), humic acids (DeWalle and Chian, 1974), residues of larger molecules, and molecules resulting from polymerization of low molecular weight compounds.

Figure 4.18 presents the chromatograms of loosely-bound biopolymers (slime) at the same locations from Figure 4.17. In influent and activated sludge, only

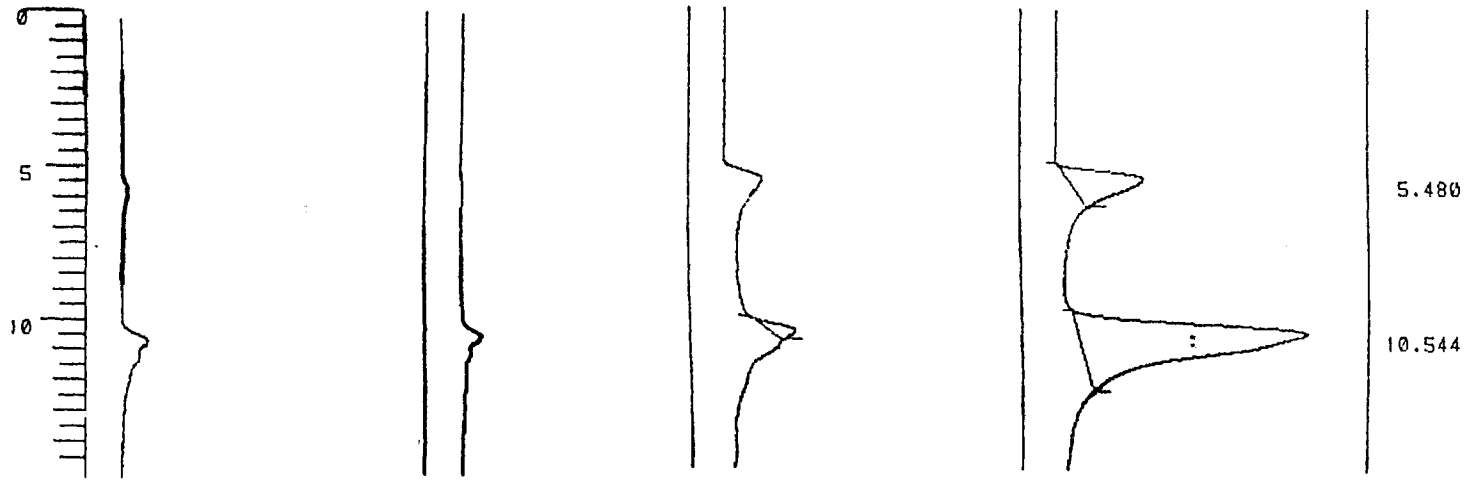

Figure 4.18 Slime Chromatograms – (a) Influent, (b) Activated Sludge, (c) Thickened Sludge, (d) Digested Sludge

molecules with size below the permeation limit were detected. In thickened sludge, molecules with molecular weight greater than 2,000,000 daltons and lower than 150,000 daltons were discernible. These two peaks increased substantially in digested sludge, with the fraction corresponding to the lower size predominating. All peaks in the chromatograms of slime biopolymers were substantially smaller than the peaks in the chromatograms of capsule biopolymers, except the peak corresponding to the permeation limit in digested sludge.

4.4 Mass Balances

Previous sections described the composition of capsular and slime biopolymers either in terms of concentration (i.e., mass of biopolymer constituent per unit volume of wastewater or sludge) or as mass ratio (i.e., mass of biopolymer constituent to mass of volatile suspended solids). Mass balances within the wastewater and sludge treatment processes were another approach that was used to investigate biopolymers. A mass balance is an accounting procedure that keeps track of the fate of a compound within a well defined control volume. Equation (4.14), adapted from Schnoor (1996), describes the mass balance for any compound within a control volume.

Accumulation or depletion of the compound within the control volume = mass input rate of the compound – mass output rate of the compound ± reactions of the compound within the control volume (4.14)

Each term of Equation (4.14) has units of mass per time. If the process is at steady-state, the term in the left-hand side of Equation (4.14) is zero; if no reaction is taking place, the term for reaction in the right-hand side of Equation (4.14) is zero.

In the mass balances for biopolymers, the control volume was a treatment process. Thus, mass balances were prepared for primary sedimentation tanks, degritter, activated sludge process, thickeners, anaerobic digesters, and belt filter

press. The input and output loads of slime biopolymers constituents to any treatment process were calculated by multiplying the constituents concentrations in slime (Table 4.18) by the flowrates. Capsule biopolymer loads were calculated by multiplying the weight ratios of capsule constituents to volatile suspended solids (Table 4.5) by the loads of VSS. Flowrates and suspended solids loads were estimated from mass balances within the treatment plant.

4.4.1 Flowrates and Total and Volatile Suspended Solids Mass Balances

Flowrates and suspended solids mass balances were prepared for each of the following treatment systems: primary sedimentation tanks, degritter, activated sludge process, thickeners, anaerobic digesters, and belt filter press. Figure 4.19 presents the general layout of the Ithaca Wastewater and Sludge Treatment Plant. The treatment processes function as a system, with the output of one process being the input of the following process. In addition, there is feedback between wastewater and sludge treatment processes; the former generate sludges, which are treated by the sludge treatment processes, while the latter generate liquors which are recycled to the wastewater treatment processes. Figure 4.20 depicts this feature of wastewater and sludge treatment processes. The feedback system that characterized the treatment plant required that the mass balances calculations be made iteratively until a steady-state condition was reached. This is described in the next sections. Appendix F contains the detailed calculations performed in the first iteration of the mass balance for each treatment system.

4.4.1.1 Flowrates and Mass Balances on "No Reaction" Processes

Mass balances on the primary sedimentation tanks, degritter, thickeners, and belt filter press were developed assuming that (1) no reaction was taking place within

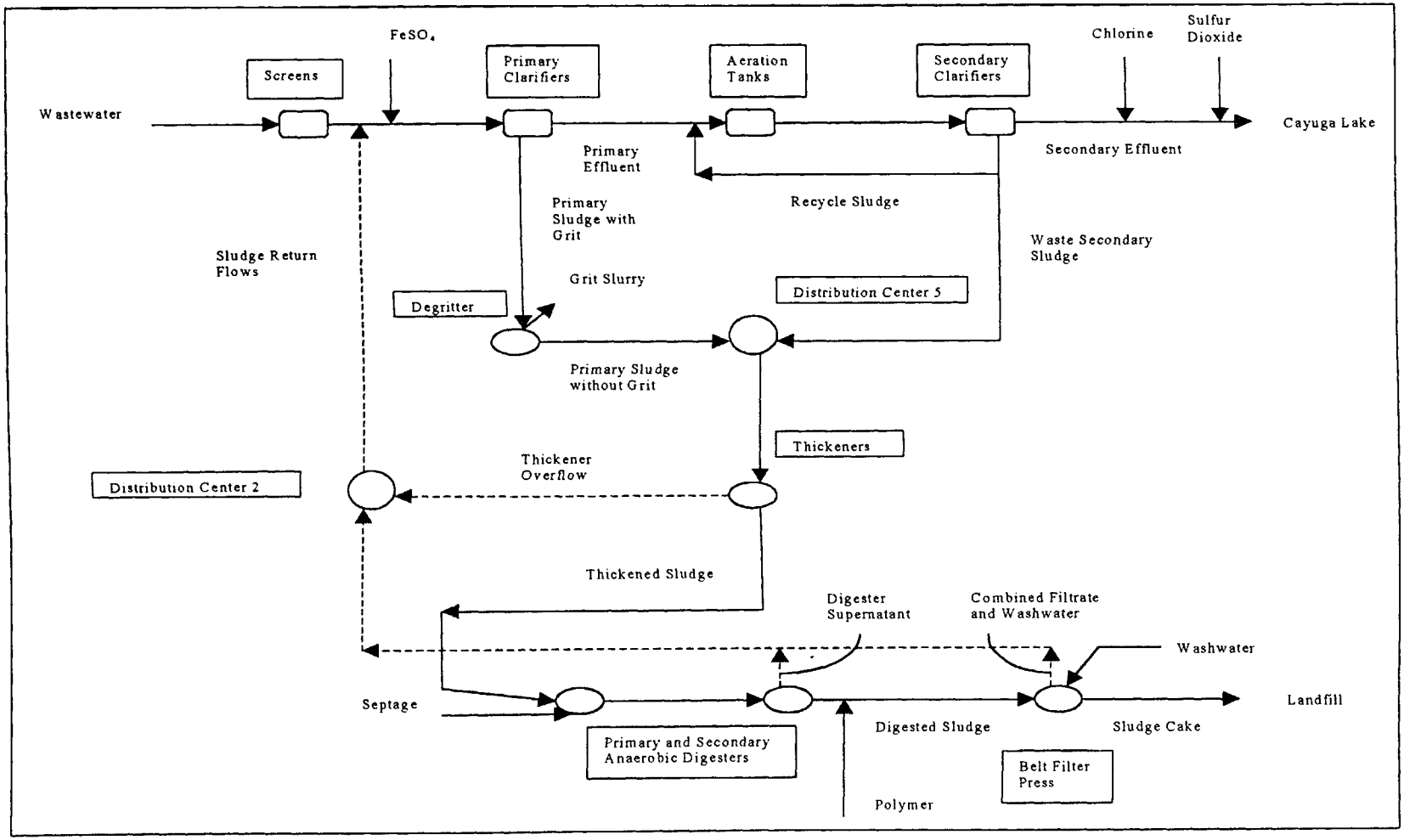

Figure 4.19 General Layout of the Ithaca Wastewater and Sludge Treatment

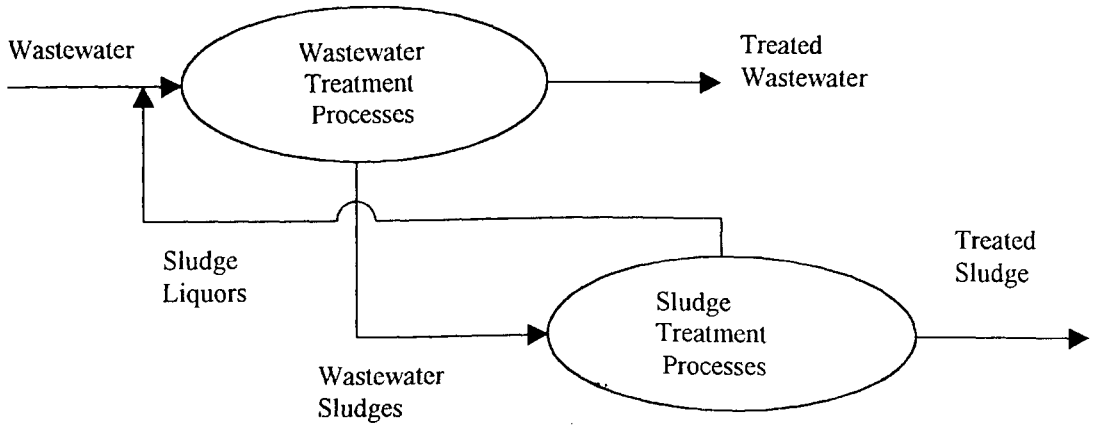

Figure 4.20 Wastewater and Sludge Treatment as a Feedback System

the process, and (2) accumulation or depletion was zero. Equations for conservation of flow and mass on any of the "no reaction" process were given by Equations (4.15) and (4.16).

$$\text{Conservation of flow: } \sum_{i=1}^{m} Q_i = \sum_{j=1}^{n} Q_j \tag{4.15}$$

$$\text{Conservation of mass: } \sum_{i=1}^{m} Q_i \cdot X_i = \sum_{j=1}^{n} Q_j \cdot X_j \tag{4.16}$$

where Q and X = flowrate and total (or volatile) suspended solids concentration, respectively;

m and n = number of contributions in and out of a given process;

i and j = input i, and output j of a given process

In the system of Equations (4.15) and (4.16), the suspended solids concentrations were usually known; they were either taken from the plant's records or measured in samples collected for this study. Thus, Equations (4.15) and (4.16) were solved for two unknown flowrates.

The input to the primary sedimentation tanks were wastewater, return flows recycled from sludge treatment processes and ferrous sulfate solution. The output was primary effluent and primary sludge with grit (Figure 4.19). Wastewater flowrate is not directly measured at the Ithaca plant. For this reason, it had to be estimated with an equation incorporating secondary effluent flowrate (which is measured), inflows and outflows throughout the plant. Inflows were septage, ferrous sulfate and synthetic polymer solutions, and washwater from the belt filter press; outflows were dewatered sludge cake and grit slurry (Figure 4.19).

Return flows were comprised of the overflow from thickeners, secondary anaerobic digester supernatant, and filtrate with washwater from the belt filter press. Initial values had to be established for the flowrates of these contributions, prior to the first iteration of calculations, in order for the system of Equations (4.15) and (4.16) to be solvable (see Appendix F).

Ferrous sulfate solution is applied in the primary sedimentation tank with the objective of providing ferrous ions that are oxidized to ferric ions in the aeration tank of the activated sludge process. Ferric ions react with phosphate ions in solution, precipitate and form chemical sludge, which is removed together with waste activated sludge in the secondary sedimentation tanks.

The system of Equation (4.15) and (4.16) for the primary sedimentation tanks were solved for primary effluent and primary sludge with grit flowrates. The loads of volatile and total suspended solids were calculating by multiplying the flowrates by their respective concentrations.

Equations (4.15) and (4.16) were also applied for the degritter, thickeners, and belt filter press. Primary sludge with grit was the input to the degritter, while grit and primary sludge without grit were the output. Primary sludge without grit, chemical sludge, and waste activated sludge were the input to the thickeners, while thickener

overflow and thickened sludge were the output. The input to the belt filter press was thickened digested sludge, synthetic polymer solution, and washwater, while dewatered sludge cake, and combined filtrate/washwater constituted the output.

4.4.1.2 Flowrates and Mass Balances on Processes with "Reaction"

In activated sludge and anaerobic digestion processes, the conservation of mass equation took the form of Equation (4.17), assuming accumulation or depletion of suspended solids within the process was zero.

$$\text{Conservation of mass: } \sum_{i=1}^{m} Q_i \cdot X_i - \sum_{j=1}^{n} Q_j \cdot X_j \pm \text{Reaction} = 0 \qquad (4.17)$$

The term for "reaction" in Equation (4.17) accounts for (1) the destruction of biodegradable volatile suspended solids present in primary effluent and anaerobic digester feed; (2) the production of new biomass from cell synthesis in activated sludge and anaerobic digester, and (3) the reduction of a portion of the new biomass by the mechanism of endogenous decay.

The fractions of biodegradable volatile suspended solids in primary effluent and digester feed were estimated by the ratios of BOD_L to COD in VSS of primary effluent and digester feed. Production of new biomass in activated sludge and anaerobic digestion were calculated using yield coefficients, which relate mass of cells formed (VSS) with mass of organic matter used (BOD_L). Yields coefficients for activated sludge and anaerobic digestion were calculated using thermodynamic principles presented in McCarty (1971, 1975). Details of the procedure used to calculate the yield coefficients is presented in Appendix F. The reduction of biomass by endogenous decay was calculated assuming that a fraction of the microorganisms in the system (activated sludge, anaerobic digestion) loses viability every day; these

non-living organisms are used as substrate by the living organisms in the system to the extent of their biodegradability (Metcalf and Eddy, 1991).

4.4.1.3 Summary of Flowrates and Suspended Solids Mass Balances

Table 4.26 presents the flowrates and total suspended solids loads to each treatment process after the end of the first, second, third and fourth iterations. The system quickly achieved a steady-state condition; the differences in the values of the third and fourth iterations were less than 0.7 %. The numbers in Table 4.26 were not rounded to significant digits. The mass balance calculations resulted in numbers that varied from 50 to 6,300,000, approximately. Rounding numbers in the range of 6,300,000 would mean to consider numbers in the range of 50 to be insignificant. However, this was not the case. For example, although the estimated flowrate of the grit slurry was only 53 gal/d, the load of total suspended solids it carried was 500 lb/d; on the other hand, secondary effluent, with a flowrate of approximately 6,200,000 gal/d, carried only 130 lb/d of total suspended solids. In addition, consideration of all input to, and output from, the treatment processes, regardless of their numerical values, helped to have a better understanding of the plant's functioning.

Table 4.27 presents data taken from the plant's records and those calculated through the mass balance. There was good agreement in the waste secondary sludge flowrate and volume of methane produced; on the other hand, the hydraulic detention time in the activated sludge process used in the mass balance was twice the time measured at the plant. The weight of total suspended solids in the sludge cake was approximately 75 % of the measured value at the plant. The differences observed might originate from failure of the model to account for all variables acting on the processes. In addition, the model requires the estimation of many coefficients, as well as composition of volatile suspended solids. Reported coefficients and the composition

Table 4.26 Flowrates and Total Suspended Solids Loads Resulted from the Mass Balances Calculations

Location	Flowrate (gal/d)				Total Suspended Solids (lb/d)			
	Iteration				Iteration			
	First	Second	Third	Fourth	First	Second	Third	Fourth
Wastewater	6,172,558	6,179,435	6,178,333	6,178,223	7,315	7,323	7,322	7,321
Return Flows [1]	41,567	496,130	532,051	535,621	324	895	947	952
Primary Effluent	5,888,474	6,324,424	6,356,863	6,360,121	3,882	4,170	4,191	4,193
Primary Sludge	326,133	351,287	354,003	354,204	3,257	3,546	3,578	3,580
Secondary Effluent	5,741,480	6,167,641	6,199,334	6,202,517	120	129	129	129
Waste Sec. Sludge	146,993	156,783	157,529	157,604	3,496	3,729	3,747	3,749
Thickened Sludge	20,179	21,738	21,884	21,896	6,432	6,930	6,977	6,981
Thickener Overflow	452,947	486,332	489,648	489,912	321	345	347	348
Thick. Dig. Sludge	13,364	14,296	14,391	14,397	4,501	4,818	4,850	4,853
Dig. Supernatant	14,317	14,810	14,859	14,865	349	361	362	363
Sludge Cake	2,318	2,481	2,497	2,498	4,276	4,578	4,608	4,610
Filtrate/Washwater	28,866	30,909	31,114	31,127	225	241	243	243

[1] The values are for the antecedent iteration. For example, the value in the second iteration is the sum of the values of thickener overflow, digester supernatant and filtrate/washwater from the first iteration.

Table 4.27 Measured and Calculated Values from Mass Balance for Variables at the Ithaca Wastewater and Sludge Treatment Plant

Location	Variable	Measured	Calculated
Activated Sludge	Hydraulic Det. Time	0.24	0.48
	VSS Concentration	850	850
	Ratio VSS:TSS	0.71	0.63
Secondary Effluent	Flowrate (gal/d)	6,203,333	6,202,517
Waste Sec. Sludge	Flowrate (gal/d)	148,500	157,604
Thickened Sludge	Ratio VSS:TSS	0.74	0.77
Anaerobic Digester	Methane Volume	32,401	32,635
Sludge Cake	TSS Load	6,150	4,610
	Ratio VSS:TSS	0.54	0.60

Units: hydraulic detention time, d; VSS concentration, mg/l; flowrate, gal/d; methane volume, ft^3/d; TSS load, lb/d.

of VSS vary and could contribute to the differences observed between the predicted and actual values.

Table 4.28 presents the flowrates, total and volatile suspended solids loads and concentrations in each of the return flows from the sludge treatment processes. The flowrate and total suspended solids load in the return flows corresponded to 8.7 % and 13.0 % of the flowrate and TSS load in wastewater, respectively. These values were within the range estimated by a model used by Grulois et al. (1993) to simulate the contribution of return flows from sludge processes to wastewater treatment. The authors found values in the range 6 % to 10 % for flowrates, and 2 % to 17 % for total suspended solids. The variations were due to differences in the types of thickening and dewatering processes.

Figure 4.21 presents the average flowrates and total and volatile suspended solids loads at each process of the Ithaca wastewater and sludge treatment plant for the months of June, July and August 1998, after the fourth iteration of the mass balance calculations. The values in the dashed box on the arrow coming from the sludge

processing facilities represent the values of flowrate, TSS and VSS loads in the return flows at the end of the third iteration. The values in the solid box are products of the fourth iteration.

Table 4.28 Flowrates, Total and Volatile Suspended Solids Concentrations and Loads in Return Flows from Sludge Treatment Processes (Average Values for June, July and August 1998)

Source	Flowrate (gal/d)	Total Suspended Solids Conc. (mg/l)	Total Suspended Solids Load (lb/d)	Total Volatile Solids Conc. (mg/l)	Total Volatile Solids Load (lb/d)
Thickener Overflow	489,912	85	348	67	274
Digester Supernatant	14,865	2,927	363	1,757	218
BFP Filtrate/ Washwater	31,127	935	243	562	146
Total	535,904	213	953	143	637

4.4.2 Mass Balance for Biopolymers

4.4.2.1 Solids-Bound Biopolymers

A measurement of the quantity of biopolymer present in each process is given by the sum of biopolymer constituents, i.e., carbohydrate, protein, RNA, DNA, and humic acids, as shown in Table 4.8. Unfortunately, DNA was below the detection limit in three locations, and a mass balance using the total quantity of biopolymer constituents was not possible to perform. As discussed in Section 4.2, the limit of detection of the analytical method for DNA was higher than the limits of detection from the other compounds. For this reason, it was not deemed suitable to ignore the DNA contribution. Total organic carbon was used as a surrogate for the total quantity of biopolymer. The next section describes the mass balance for capsular TOC within the treatment plant.

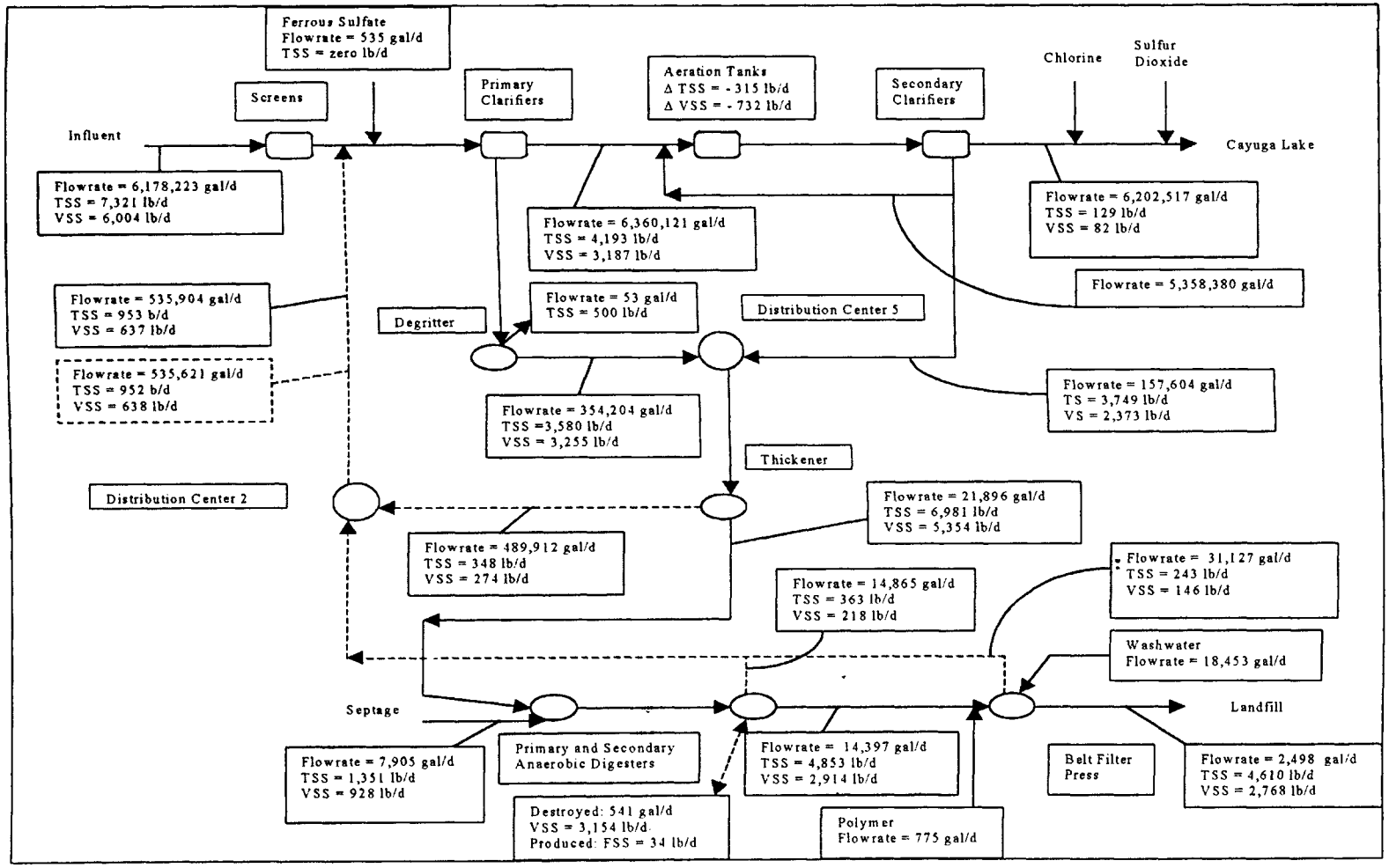

Figure 4.21 Flowrates, Total and Volatile Suspended Solids Mass Balance at the Ithaca Wastewater and Sludge Treatment Plant – Average Values for June, July and August 1998 (End of Fourth Iteration)

4.4.2.1.1 Mass Balance for Capsular Biopolymer Total Organic Carbon

The loads of capsular biopolymer total organic carbon were calculated by multiplying the loads of volatile suspended solids presented in Figure 4.21 by the weight ratios of capsular biopolymer TOC to volatile suspended solids presented in Table 4.5. Table 4.29 presents the volatile suspended solids loads, weight ratio of TOC in capsular biopolymer to volatile suspended solids, and biopolymer TOC loads, in selected locations at the treatment plant. Figure 4.22 presents the capsular biopolymers TOC loads to the plant's treatment processes in graphical form. Mass balances for TOC on individual processes are discussed next.

a) Mass balance on the primary sedimentation tanks

The input loads of capsular biopolymer TOC to the primary sedimentation tanks were wastewater and return flows, while the output loads from the tanks were primary effluent and primary sludge with grit. The load of capsular TOC in primary sludge was calculated by subtracting the load in primary effluent from the loads in wastewater and in return flows.

In order for a mass balance to be achieved in the primary sedimentation tanks, the weight ratio of capsular TOC to volatile suspended solids in primary sludge should be 32.4 mg biopolymer TOC/g VSS. This value was calculated by dividing the load of biopolymer TOC (111.8 lb/d TOC) by the load of volatile suspended solids (3,455 lb/d) in primary sludge. The value calculated for the weight ratio of capsular TOC to VSS in primary sludge, 32.4 mg biopolymer TOC/g VSS, was higher than the weight ratio in primary effluent and wastewater, which were 19.5 and 26.9 mg biopolymer TOC/g VSS, respectively. This is in agreement with the hypothesis formulated in Sections 4.2.1 and 4.2.3 that there might be a preferential settling of particles holding more polymers in relation to particles having less capsular biopolymers. However, the hypothesis could not be proved based on one measurement made on a sample

Table 4.29 Loads of Capsular Biopolymer TOC in Locations at the Ithaca Wastewater and Sludge Treatment Plant – Average Values, June, July and August 1998

Location	VSS Load (lb/d)	Biopolymer TOC (mg/g VSS)	Biopolymer TOC (lb/d)
Influent	6,004	26.9	161.5
Return Flows	638	19.4 [1]	12.4 [4]
Primary Effluent	3,187	19.5	62.1
Prim. Sludge w/ Grit	3,455	32.4 [1]	111.8 [5]
Secondary Effluent	82	15.3	1.3
Waste Sec. Sludge	2,373	23.3	55.3
Thickened Sludge	5,354	26.5	141.9
Septage	928	26.9 [2]	25.0
Anaer. Digester Feed	6,282	26.6 [1]	166.9 [6]
Thick. Dig. Sludge	2,914	16.8	49.0
Sludge Cake	2,768	16.8 [3]	46.5
Thickener Overflow	274	23.9	6.5
Digester Supernatant	218	13.7	3.0
BFP Filt./Washwater	146	19.9	2.9

[1] Value = biopolymer TOC ÷ VSS load;
[2] Value assumed to be the same value as influent;
[3] Value assumed to be the same value as thickened digested sludge;
[4] Value corresponds to the sum of the loads of thickener overflow, digester supernatant, and belt filter press filtrate;
[5] Value = load influent + load return flows – load primary effluent;
[6] Value corresponds to the sum of the loads of thickened sludge and septage

collected from primary sludge. The weight ratio of capsular TOC to VSS measured in this sample was 16.6 mg biopolymer TOC/g VSS, about one-half of the value predicted by mass balance.

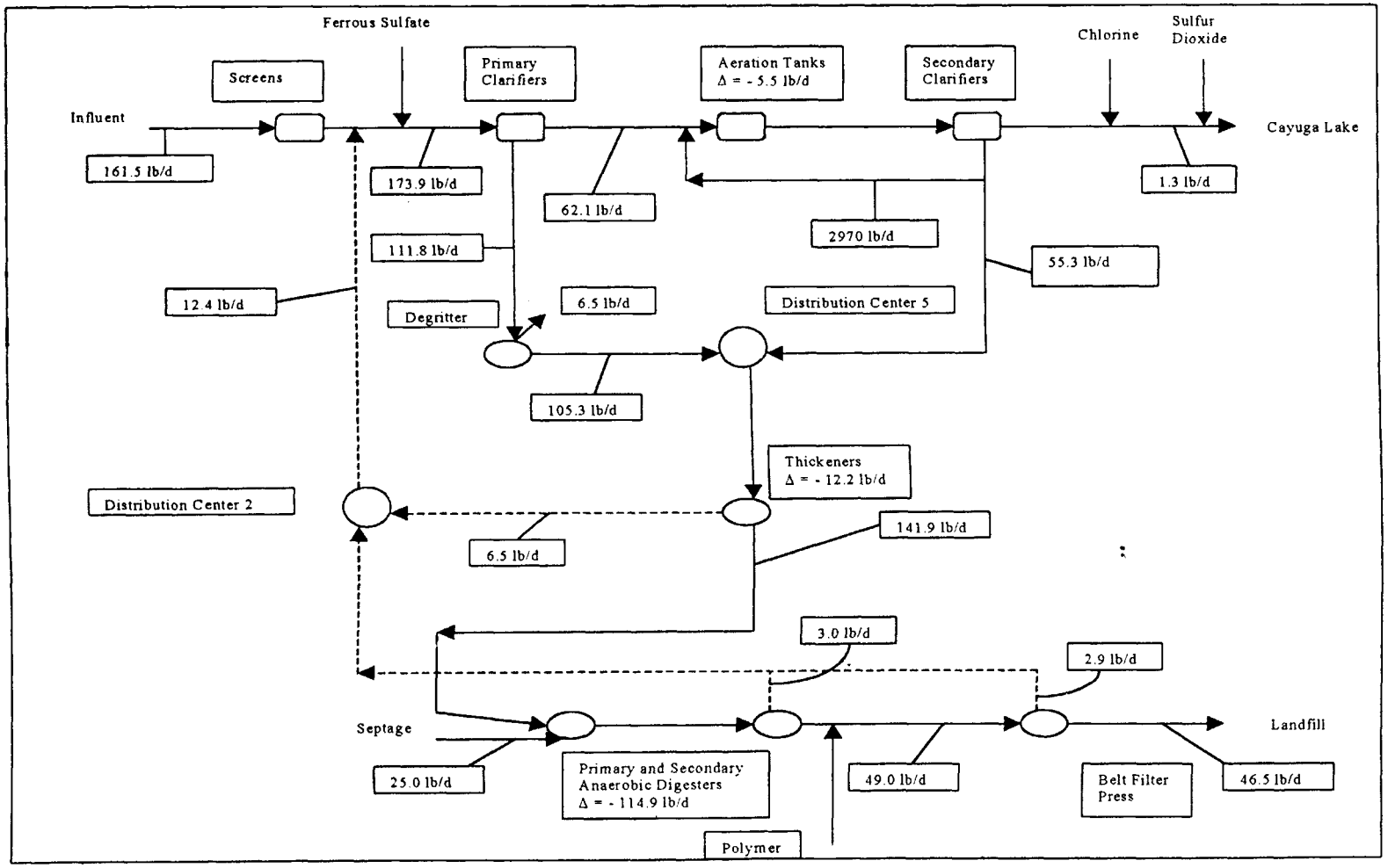

Figure 4.22 Mass Balance for Solids-Bound Biopolymer TOC at the Ithaca Wastewater and Sludge Treatment Plant - Average Values for June, July and August 1998.

b) *Mass Balance on the activated sludge process*

Biodegradable volatile suspended solids in primary effluent are used as substrate by microorganisms in the activated sludge process. A portion of the substrate is used for energy, while another portion is used for cell synthesis. This feature of substrate destruction/biomass formation is shown in the equation that models the concentration of volatile suspended solids in the aeration tank (Equation (4.18) and Appendix F).

$$X_V = \frac{\theta_C}{\theta} \cdot \left[X_V^{PE} \cdot \left(1 - f_d^{PE}\right) + \frac{Y \cdot (S_{PE} - S)}{1 + b \cdot \theta_C} \cdot \left[1 + (1 - f_d) \cdot b \cdot \theta_C\right] \right] \qquad (4.18)$$

where X_V = mixed liquor volatile suspended solids concentration, mg/l;

θ_C = mean cell residence time, 8.8 d;

θ = hydraulic residence time, 0.48 d;

X_V^{PE} = primary effluent volatile suspended solids concentration, 60 mg/l;

f_d^{PE} = biodegradable fraction of primary effluent volatile suspended solids, 0.65;

f_d = biodegradable fraction of the biomass produced in the aeration tank, 0.80;

Y = biomass yield coefficient, 0.45 mg VSS produced/mg BOD used;

S_{PE} = primary effluent BOD_L, 102 mg/l;

S = activated sludge effluent BOD_L, 12 mg/l;

b = endogenous decay coefficient, 0.1 d^{-1}.

The values shown for the variables of Equation (4.18) were the values used to perform the mass balance calculations (Appendix F). Equation (4.18) shows that the concentration of volatile suspended solids in the aeration tank is formed by three components:

(1) concentration of volatile suspended solids originating from primary effluent. This fraction is given by the term $\frac{\theta_C}{\theta}\left[X_V^{PE}\cdot\left(1-f_d^{PE}\right)\right]$, and it represents volatile suspended solids from primary effluent in activated sludge that are not degraded in the aeration tank;

(2) concentration of active biomass produced in the activated sludge process. This fraction is given by the term $\frac{\theta_C}{\theta}\cdot\left[\frac{Y\cdot(S_{PE}-S)}{1+b\cdot\theta_C}\right]$;

(3) concentration of volatile suspended solids remaining from decay of biomass that was produced in the activated sludge. This fraction is given by the term $\frac{\theta_C}{\theta}\cdot\left[\frac{Y\cdot(S_{PE}-S)}{1+b\cdot\theta_C}\cdot\left[(1-f_d)\cdot b\cdot\theta_C\right]\right]$.

Equation (4.18) assumes that all biodegradable volatile suspended solids that are not viable biomass are rapidly converted to soluble form by hydrolysis.

It is possible that similar mechanisms occur with capsular biopolymers entering the activated sludge process. The biodegradable portion of the capsule in primary effluent might be used as a substrate (degradation), while the new biomass formed in the activated sludge process produces new capsular material. Some of the new capsular material might be consumed as a result of microorganisms decay. According to the mass balance shown in Figure 4.22, there was a net destruction of 5.5 lb/d of capsular TOC in the activated sludge process.

The measured weight ratio of capsular biopolymer TOC to volatile suspended solids in the activated sludge process was 23.3 mg biopolymer TOC/g VSS (Table 4.5). This value represents an overall ratio within the activated sludge process, reflecting both destruction and production of capsule material. If the assumption that capsular biopolymer in primary effluent had the same biodegradability as primary effluent volatile suspended solids is made, then it is possible to calculate the weight ratio of capsular biopolymer TOC to VSS in new biomass formed in the activated

sludge process. This is made through a mass balance for capsular biopolymer TOC within the aeration tank (Equation (4.19)).

$$\frac{\theta_C}{\theta}\left[X_V^{PE}\cdot\left(1-f_d^{PE}\right)\right]\cdot Q_{PE}\cdot\left(19.5\frac{mg\ TOC}{gVSS}\right)+\frac{\theta_C}{\theta}\left[\frac{Y\cdot\left(S_{PE}-S\right)}{1+b\cdot\theta_C}\right]\cdot Q_{PE}\cdot(x)$$
$$+\frac{\theta_C}{\theta}\left[\frac{Y\cdot\left(S_{PE}-S\right)}{1+b\cdot\theta_C}\right]\cdot\left[\left(1-f_d\right)\cdot b\cdot\theta_C\right]\cdot Q_{PE}\cdot(x) \qquad (4.19)$$
$$=\frac{\theta_C}{\theta}\left[X_V^{PE}\cdot\left(1-f_d\right)+\frac{Y\cdot\left(S_{PE}-S\right)}{1+b\cdot\theta_C}\right]\cdot\left[1+\left(1-f_d\right)\cdot b\cdot\theta_C\right]\cdot Q_{PE}\cdot\left(23.3\frac{mg\ TOC}{gVSS}\right)$$

The first term in the left hand side of Equation (4.19) represents the contribution of capsular biopolymers from primary effluent, with 19.5 mg TOC/g VSS being the weight ratio of capsular biopolymer TOC to VSS in primary effluent. The second term in the LHS of Equation (4.19) represents the contribution of capsular biopolymers from the viable biomass in the aeration tank, with x being the unknown weight ratio of capsular TOC to VSS. The third term in the LHS of Equation (4.19) represents the contribution of capsular biopolymers from microorganism decay; it was assumed that capsular biopolymer from new cells had the same biodegradability than new cells (f_d). The term in the right hand side of Equation (4.19) represents the total load of capsule biopolymer in the aeration tank, with 23.3 mg TOC/g VSS being the measured weight ratio of capsular TOC to VSS in activated sludge.

Substituting the corresponding values into Equation (4.19) (the values are presented together with Equation (4.18)), and canceling θ_C/θ and Q_{PE} in each side of the equation, a value of 26.5 mg biopolymer TOC/g VSS was obtained. Thus, the ratio of capsular TOC to VSS associated with new biomass in activated sludge was 26.5 mg biopolymer TOC/g VSS. This value is higher than the value measured in samples from activated sludge (23.3 mg biopolymer TOC/g VSS), is about the same as measured in

wastewater (26.9 mg biopolymer TOC/g VSS) and is the same as in thickened sludge (26.5 mg biopolymer TOC/g VSS) (Table 4.29).

The biopolymer yield coefficient for the activated sludge process was estimated as the product between 26.5 mg biopolymer TOC/g VSS and the cell yield coefficient Y.

$$Y_{Biop} = 26.5 \frac{mg\ biop\ TOC}{g\ new\ VSS} \cdot 0.45 \frac{mg\ new\ VSS}{mg\ BOD_L\ used} \cdot 10^{-3} \frac{g}{mg} = 0.012 \frac{mg\ biop\ TOC}{mg\ BOD_L\ used}$$

In order to compare biopolymer yield coefficient with cell yield coefficient, it was necessary to convert both yields to homogeneous units. If the composition of cells formed in the activated sludge process is assumed to be $C_5H_7O_2N$ (Sawyer et al., 1994), then the organic carbon fraction in the cells corresponds to $\frac{5 \cdot 12}{(5 \cdot 12 + 1 \cdot 7 + 2 \cdot 16 + 1 \cdot 14)} = 0.53$, where 12 g, 7 g, 16 g, and 14 g are the atomic weights of carbon, hydrogen, oxygen, and nitrogen, respectively. The cell yield coefficient, based on organic carbon, would be

$$0.45 \frac{mg\ new\ VSS}{mg\ BOD_L\ used} \cdot 0.53 \frac{mg\ TOC}{mg\ new\ VSS} = 0.24 \frac{mg\ TOC}{mg\ BOD_L\ used}$$

Therefore, the biopolymer yield coefficient, $0.012 \frac{mg\ biop\ TOC}{mg\ BOD_L\ used}$, represented 5 % of the cell yield coefficient in the activated sludge process.

c) *Mass balance on the thickeners*

The mass balance for capsular biopolymer TOC on the thickeners showed a net destruction of 12.2 lb/d, which corresponded to 7.6 % of the load of the thickeners' capsular biopolymer feed. Eastman and Fergusson (1981) postulated that hydrolysis of particulate organic carbon to soluble substrates occurs in primary clarifiers and sludge thickeners. Hydrolysis and acidogenesis seemed to take place in the thickeners at the

Ithaca plant; the measured pH was 5.9, while the pH at all other locations in the treatment plant was in the range 7.0 to 7.7 (Table 4.3).

d) Mass balance on the anaerobic digesters

The mass balance for capsular biopolymer TOC on the anaerobic digesters showed a net destruction of 115 lb/d, representing 69 % of the capsular TOC in the digester feed. This value was obtained by assuming that septage had the same ratio of capsular biopolymer TOC to VSS as wastewater. Even if the contribution of septage was zero, there would still be a net destruction of 90 lb/d, representing 63 % of the capsular TOC in the feed. From the suspended solids mass balance (Figure 4.21), the destruction of volatile suspended solids in anaerobic digestion was 50 %. These values suggest that capsular biopolymers in the digester feed had greater biodegradability that volatile suspended solids in the feed.

4.4.2.1.2 Mass Balances for Capsular Biopolymer Constituents

This section describes mass balances within the wastewater and sludge treatment plant for the capsular biopolymers constituents protein, carbohydrate, RNA and humic acids. Mass balance for DNA was only performed on the anaerobic digester; in other processes, at least one input or output capsular DNA concentration was below the limit of detection. The objective in preparing these mass balances was to investigate the patterns of biopolymer production and destruction in the treatment processes.

The loads of capsular biopolymers protein, carbohydrate, RNA, humic acids, and DNA were calculated by multiplying the loads of volatile suspended solids presented in Figure 4.21 by the weight ratios of capsular biopolymer constituents to volatile suspended solids presented in Table 4.5. Tables 4.30 through 4.33 presents the capsular biopolymers protein, carbohydrate, RNA, and humic acids loads in locations

at the treatment plant, together with the weight ratios of capsular biopolymer constituent to volatile suspended solids. Figures 4.23 through 4.26 present the loads in graphical form. The following paragraphs contain discussion of the mass balances on the primary sedimentation tanks, activated sludge process, thickeners, and anaerobic digesters.

a) *Mass Balance on the primary sedimentation tanks*

As described in the discussion of TOC (Section 4.4.2.1.1), the load of capsular biopolymer constituents in primary sludge was calculating by subtracting the load in primary effluent from the loads in wastewater and sludge return flows.

In order to achieve mass balance on the primary sedimentation tanks, the weight ratio of capsular protein to VSS in primary sludge would need to be 33.9 mg capsular protein/g VSS (Table 4.30). A value of only 16.6 mg protein/g VSS was measured in one sample of primary sludge, about one-half the value predicted from mass balance. The same was true for the other compounds. According to the mass balance estimations, the weight ratios of capsular carbohydrate, RNA, and humic acids to volatile suspended solids in primary sludge should be, respectively, 7.8 mg/g VSS, 8.1 mg/g VSS, and 13.7 mg/g VSS. However, the weight ratios of carbohydrate, RNA, and humic acids measured in one sample of primary sludge were, respectively, 4.0 mg/g VSS, 4.7 mg/g VSS, and 5.9 mg/g VSS (Table 4.5). More data are needed to confirm the values measured in this sample. If they represent the true values for capsular biopolymers in primary sludge, it means that degradation of biopolymers occurs in the primary sedimentation tanks.

b) *Mass Balance on the activated sludge process*

Mass balances on the activated sludge process showed net production of 15.1 lb/d of capsular protein, 3.7 lb/d of capsular carbohydrate, 7.2 lb/d of capsular RNA, and 5.8 lb/d of capsular humic acids. These results are in contradiction with the

Table 4.30 Loads of Capsular Biopolymer Protein in Locations at the Ithaca Wastewater and Sludge Treatment Plant – Average Values, June, July and August 1998

Location	VSS Load (lb/d)	Biop. Protein (mg/g VSS)	Biop. Protein (lb/d)
Influent	6,004	26.2	157.3
Return Flows	638	19.7 [1]	12.6 [4]
Primary Effluent	3,187	16.6	52.9
Prim. Sludge w/ Grit	3,455	33.9 [1]	117.0 [5]
Secondary Effluent	82	24.1	2.0
Waste Sec. Sludge	2,373	27.8	66.0
Thickened Sludge	5,354	30.0	160.6
Septage	928	26.2 [2]	24.3
Anaer. Digester Feed	6,282	29.4 [1]	184.9 [6]
Thick. Dig. Sludge	2,914	17.6	51.3
Sludge Cake	2,768	17.6 [3]	48.7
Thickener Overflow	274	23.1	6.3
Digester Supernatant	218	14.6	3.2
BFP Filt./Washwater	146	21.0	3.1

[1] Value = biopolymer protein load ÷ VSS load
[2] Value assumed to be the same value as influent;
[3] Value assumed to be the same value as thickened digested sludge;
[4] Value corresponds to the sum of the loads of thickener overflow, digester supernatant, and belt filter press filtrate;
[5] Value = load influent + load return flows – load primary effluent;
[6] Value corresponds to the sum of the loads of thickened sludge and septage

mass balance for TOC, which showed net destruction of 5.5 lb/d. This topic will be discussed later.

c) *Mass Balances on the thickeners*

Good mass balance was achieved on the thickeners for protein, carbohydrate and RNA. The loads of capsular protein in the thickened sludge and thickener

Table 4.31 Loads of Capsular Biopolymer Carbohydrate in Locations at the Ithaca Wastewater and Sludge Treatment Plant – Average Values, June, July and August 1998

Location	VSS Load (lb/d)	Biop. Carb. (mg/g VSS)	Biop. Carb. (lb/d)
Influent	6,004	6.5	39.0
Return Flows	638	4.7 [1]	3.0 [4]
Primary Effluent	3,187	4.7	15.0
Prim. Sludge w/ Grit	3,455	7.8 [1]	27.0 [5]
Secondary Effluent	82	5.3	0.4
Waste Sec. Sludge	2,373	7.7	18.3
Thickened Sludge	5,354	7.6	40.7
Septage	928	6.5 [2]	6.0
Anaer. Digester Feed	6,282	7.4 [1]	46.7 [6]
Thick. Dig. Sludge	2,914	4.9	14.3
Sludge Cake	2,768	4.9 [3]	13.6
Thickener Overflow	274	5.6	1.5
Digester Supernatant	218	3.2	0.7
BFP Filt./Washwater	146	5.2	0.8

[1] Value = biopolymer carbohydrate load ÷ VSS load
[2] Value assumed to be the same value as influent;
[3] Value assumed to be the same value as thickened digested sludge;
[4] Value corresponds to the sum of the loads of thickener overflow, digester supernatant, and belt filter press filtrate;
[5] Value = load influent + load return flows – load primary effluent;
[6] Value corresponds to the sum of the loads of thickened sludge and septage

overflow represented 95 % of the capsular protein loads from primary sludge and waste secondary sludge; the loads of capsular carbohydrate and RNA in the outflows from the thickeners were, respectively, 97 % and 113 % of the loads in the inflows, primary sludge and waste secondary sludge. Capsular humic acids showed higher difference: the outflow load represented only 52 % of the load brought by primary

Table 4.32 Loads of Capsular Biopolymer RNA in Locations at the Ithaca Wastewater and Sludge Treatment Plant – Average Values, June, July and August 1998

Location	VSS Load (lb/d)	Biop. RNA (mg/g VSS)	Biop. RNA (lb/d)
Influent	6,004	7.0	42.0
Return Flows	638	6.5 [1]	4.2 [4]
Primary Effluent	3,187	5.7	18.2
Prim. Sludge w/ Grit	3,455	8.1 [1]	28.0 [5]
Secondary Effluent	82	5.6	0.5
Waste Sec. Sludge	2,373	10.5	24.9
Thickened Sludge	5,354	10.4	55.7
Septage	928	7.0 [2]	6.5
Anaer. Digester Feed	6,282	9.9 [1]	62.2 [6]
Thick. Dig. Sludge	2,914	7.6	22.1
Sludge Cake	2,768	7.6 [3]	21.0
Thickener Overflow	274	8.1	2.2
Digester Supernatant	218	5.1	1.1
BFP Filt./Washwater	146	6.3	0.9

[1] Value = biopolymer RNA load ÷ VSS load
[2] Value assumed to be the same value as influent;
[3] Value assumed to be the same value as thickened digested sludge;
[4] Value corresponds to the sum of the loads of thickener overflow, digester supernatant, and belt filter press filtrate;
[5] Value = load influent + load return flows – load primary effluent;
[6] Value corresponds to the sum of the loads of thickened sludge and septage.

sludge and waste secondary sludge.

d) *Mass balance on the anaerobic digesters*

During anaerobic digestion, 70 %, 66 %, 63 %, and 60 % of capsular biopolymers protein, carbohydrate, RNA, and humic acids, respectively, were destroyed. These values were calculated assuming that septage had the same weight

Table 4.33 Loads of Capsular Biopolymer Humic Acids in Locations at the Ithaca Wastewater and Sludge Treatment Plant – Average Values, June, July and August 1998

Location	VSS Load (lb/d)	Biop. HA (mg/g VSS)	Biop. HA (lb/d)
Influent	6,004	9.6	57.6
Return Flows	638	5.4 [1]	3.5 [5]
Primary Effluent	3,187	4.3	13.7
Prim. Sludge w/ Grit	3,455	13.7 [1]	47.4 [6]
Secondary Effluent	82	N.M.[2]	-
Waste Sec. Sludge	2,373	8.2	19.5
Thickened Sludge	5,354	6.1	32.7
Septage	928	9.6 [3]	8.9
Anaer. Digester Feed	6,282	6.6 [1]	41.6 [7]
Thick. Dig. Sludge	2,914	5.3	15.4
Sludge Cake	2,768	5.3 [4]	14.7
Thickener Overflow	274	3.5	1.0
Digester Supernatant	218	6.1	1.3
BFP Filt./Washwater	146	8.0	1.2

[1] Value = biopolymer humic acids load ÷ VSS load
[2] Not measured;
[3] Value assumed to be the same value as influent;
[4] Value assumed to be the same value as thickened digested sludge;
[5] Value corresponds to the sum of the loads of thickener overflow, digester supernatant, and belt filter press filtrate;
[6] Value = load influent + load return flows – load primary effluent;
[7] Value corresponds to the sum of the loads of thickened sludge and septage.

ratios of capsular constituents as wastewater. If the contributions of septage to the loads of capsular constituents in the digester feed were zero, the reductions of capsular biopolymer during digestion would be 66 % (protein), 61 % (carbohydrate), 58 % (RNA), and 49 % (humic acids). A mass balance for DNA on the anaerobic digester

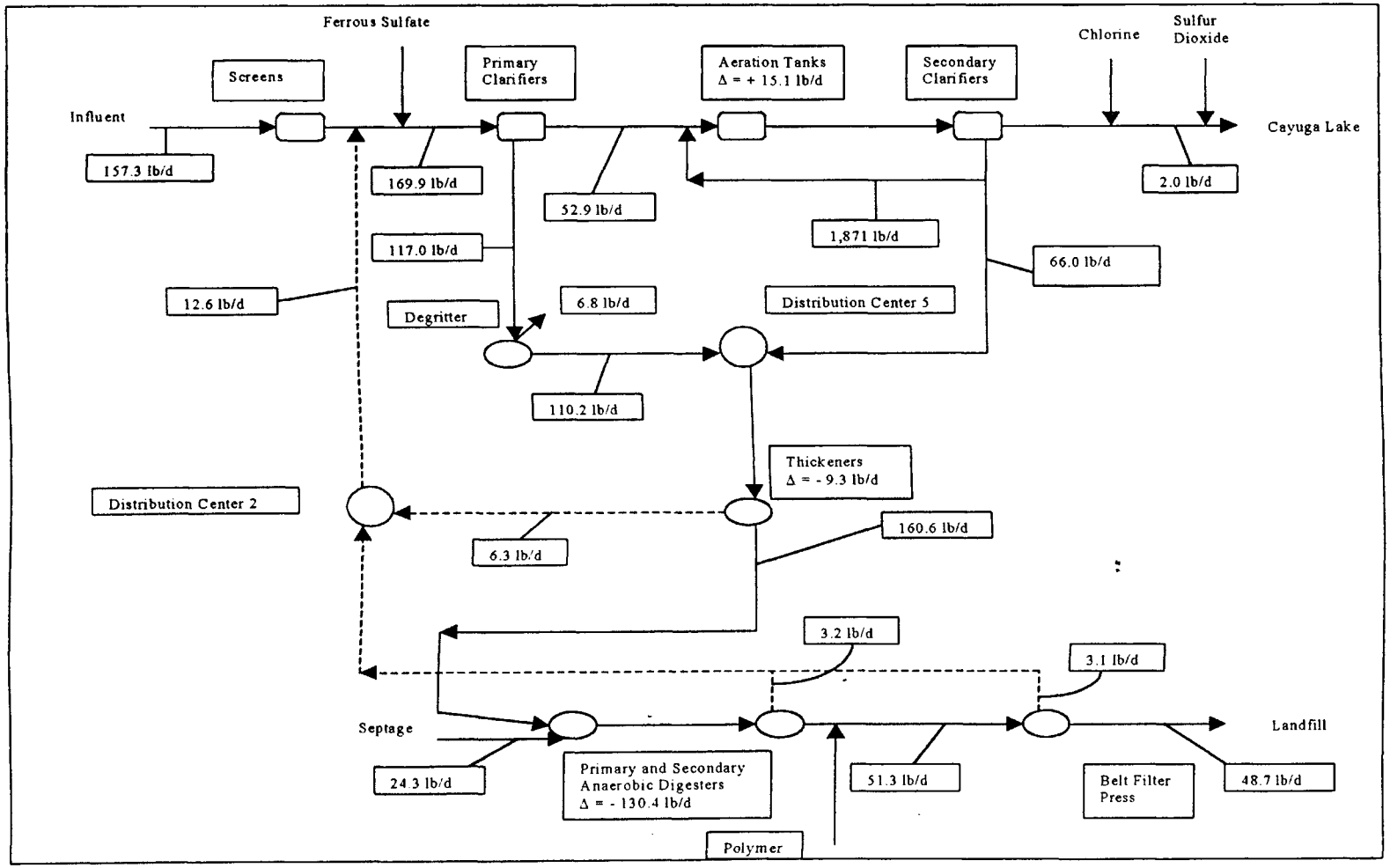

Figure 4.23 Mass Balance for Solids-Bound Biopolymer Protein at the Ithaca Wastewater and Sludge Treatment Plant Average - Values for June, July and August 1998.

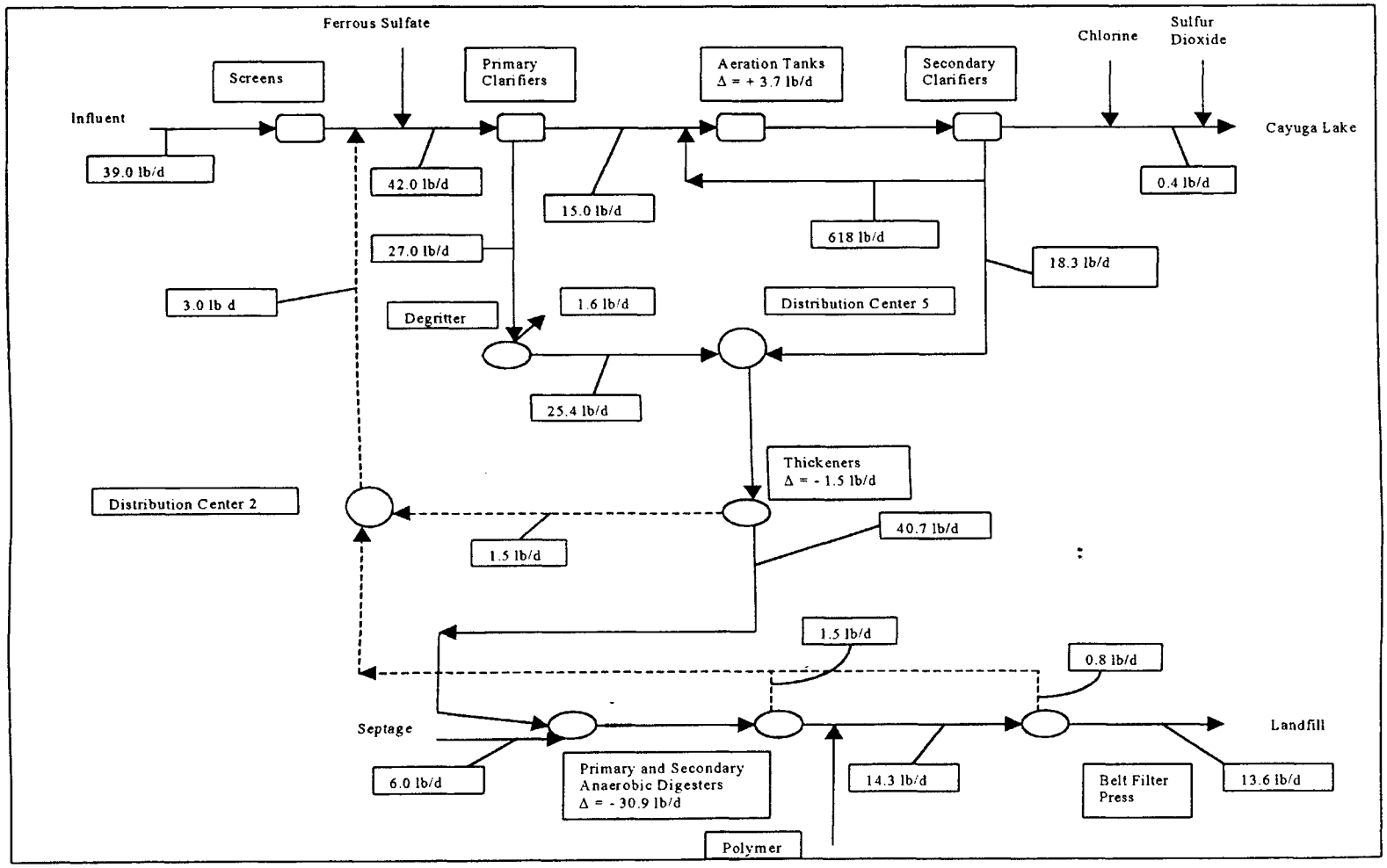

Figure 4.24 Mass Balance for Solids-Bound Biopolymer Carbohydrates at the Ithaca Wastewater and Sludge Treatment Plant - Average Values for June, July and August 1998.

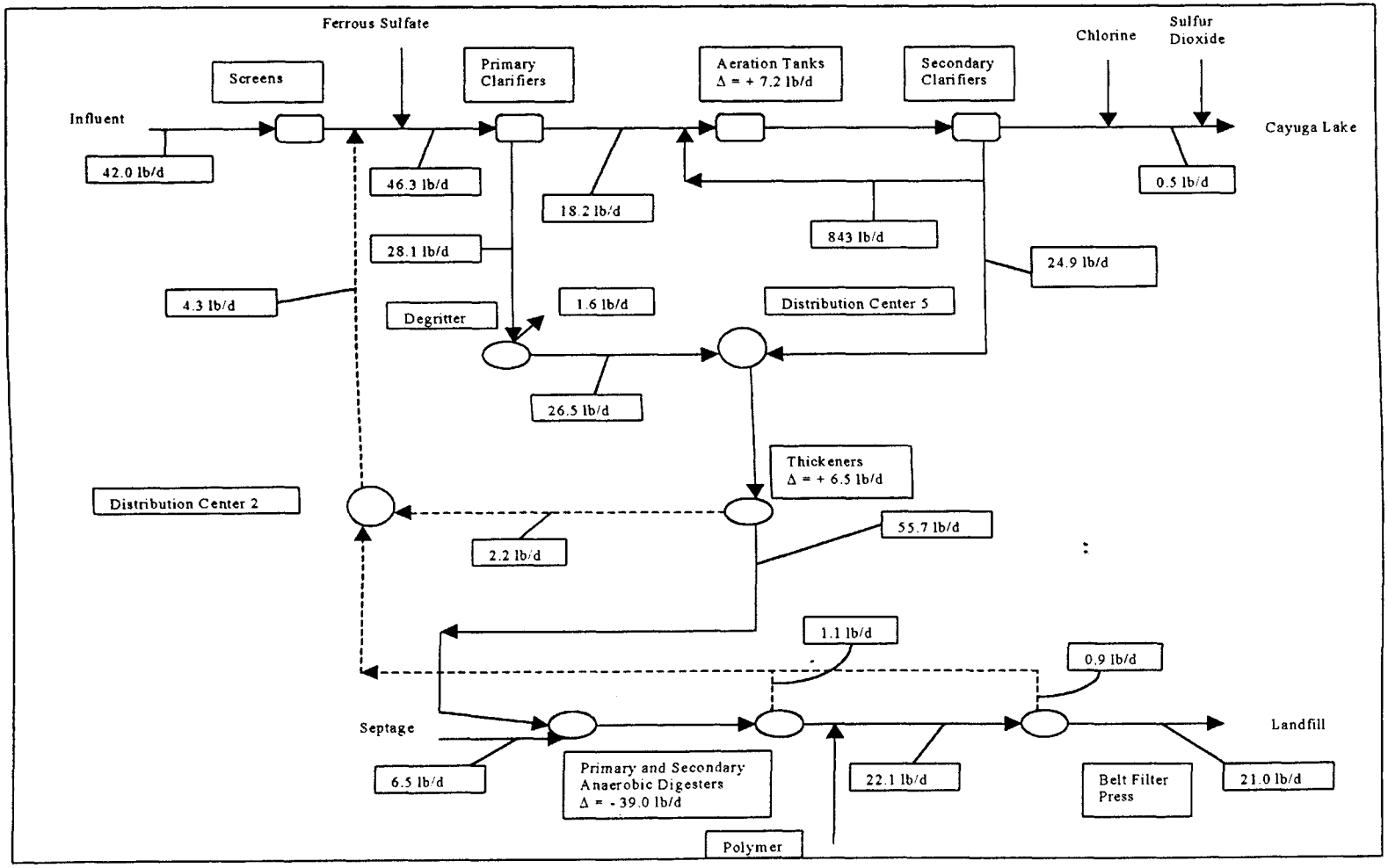

Figure 4.25 Mass Balance for Solids-Bound Biopolymer RNA at the Ithaca Wastewater and Sludge Treatment Plant - Average Values for June, July and August 1998.

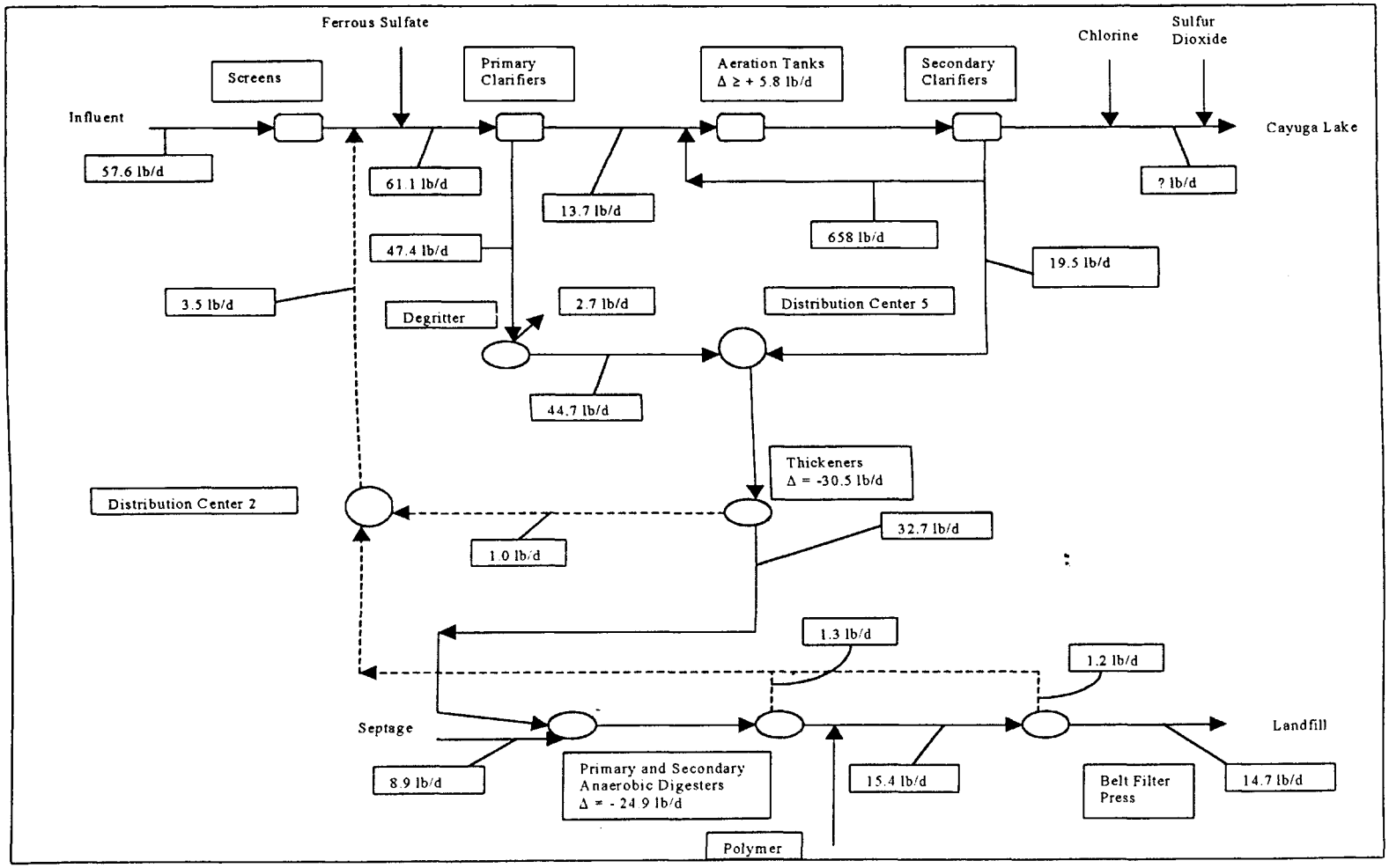

Figure 4.26 Mass Balance for Solids-Bound Biopolymer Humic Acids at the Ithaca Wastewater and Sludge Treatment Plant - Average Values for June, July and August 1998.

showed degradation of 70 % of capsular DNA (with septage contribution), and 64 % (without septage contribution). These results are compatible with the observed reduction in TOC, which was 69 %. These results show that fractions of capsular protein, carbohydrate, RNA, DNA, and humic acids were refractory, or were not available to biodegradation, under the conditions that existed in the anaerobic digesters. Ryssov-Nielsen (1976) postulated that biopolymers in anaerobic digesters are rapidly converted to carbon dioxide and methane, but no quantification was presented to support the hypothesis.

Discrepancy between the mass balance results of capsular TOC and chemical constituents in the activated sludge process

As noted before, the mass balances for capsular biopolymers protein, carbohydrate, RNA and humic acids showed net productions in the activated sludge process of 15.1, 3.7, 7.2, and 5.8 lb/d, respectively (Figures 4.23 through 4.26). These quantities were converted to total organic carbon quantities by multiplying them by the conversion factors 0.450 (protein), 0.400 (carbohydrate), 0.338 (RNA), and 0.545 (humic acids) (see Section 3.3.7). The net capsular TOC production in the aeration tank would be (15.1 lb/d x 0.450) + (3.7 lb/d x 0.400) + (7.2 lb/d x 0.338) + (5.8 lb/d x 0.545) = 13.9 lb/d capsular biopolymer TOC. However, the mass balance for capsular TOC in the activated sludge process showed negative accumulation of 5.5 lb/d, which represented about 9 % of the incoming load from primary effluent (Figure 4.22).

One way this difference could happen was if the TOC of capsular biopolymer in primary effluent was higher than the value predicted from the composition of the capsule. Results presented in Table 4.7 (Section 4.2.1) showed that, in general, there was good agreement between the measured TOC and the predicted TOC based on

composition, with the percent differences ranging from - 12.8 % to + 6.0 %. Table 4.7 did not present, however, the measured and predicted TOC of primary effluent capsular biopolymer because one of the components of the capsule, DNA, was below the limit of detection. The comparison between measured and predicted TOC of capsular biopolymers in primary effluent was made below to see if the difference could explain why the mass balance in the activated sludge process showed production of biopolymers constituents, but destruction of TOC.

The weight ratios of capsular biopolymer protein, carbohydrate, RNA, DNA, and humic acids to volatile suspended solids in primary effluent were, respectively, 16.6 mg/g, 4.7 mg/g, 5.7 mg/g, < LD, and 4.3 mg/g (Table 4.5). Multiplying these ratios by the respective conversion factors 0.450, 0.400, 0.338, 0.360, and 0.545 gives a predicted TOC of 13.6 mg biopolymer TOC/g VSS. An additional contribution would be from DNA, but this was not possible to estimate from the available data. The actual TOC measured in capsular biopolymers of primary effluent was 19.5 mg biopolymer TOC/g VSS (Table 4.5). A difference of 30 % between the measured and the predicted TOC of primary effluent occurred. If, in the mass balance on the activated sludge process, the predicted value of TOC in primary effluent was used instead of the measured TOC, the mass balance in the activated sludge process would be:

Capsular TOC in (from primary effluent) = 13.6 mg/g VSS x 3,187 lb/d VSS = 43.3 lb/d capsular TOC, where 3,187 was the load of VSS in primary effluent (Figure 4.25);

Capsular TOC out (from secondary effluent and waste secondary sludge) = 1.3 + 55.3 = 56.6 lb/d capsular TOC;

Net TOC = 56.6 - 43.3 =13.3 lb/d, which is close to 13.9 lb/d, the value calculated using the net production values of carbohydrate, protein, RNA and humic

acids in the activated sludge process. The predicted TOC value in capsular biopolymer of primary effluent provided a mass balance that was compatible with the estimated net productions of protein, carbohydrate, RNA, and humic acids in the activated sludge process. The reason for the 30 % difference between measured and predicted TOC based on composition is not known. A fraction of the 30 % difference could come from DNA contribution. In other locations at the treatment plant, DNA contribution to TOC ranged from 4 % to 6 %. DNA was below the limit of detection in primary effluent, but had DNA been measured exactly at the limit of detection, it would have contributed 6 % of the capsular TOC. Other factors that might have contributed to the difference between measured and predicted capsular TOC of primary effluent are (1) presence of a compound not measured in this study, and (2) conversion factors of protein, carbohydrate, RNA and humic acids for TOC different from the values used to calculate predicted TOC based on composition.

4.4.2.2 Loosely-Bound Biopolymers (Slime)

Mass balances for slime biopolymers were limited to TOC, carbohydrate and protein because many values measured for RNA, DNA and humic acid concentrations were below the limits of detection. Table 4.34 presents the loads of TOC, carbohydrate and protein at selected locations in the treatment plant. The loads were calculated by multiplying flowrates from the mass balance (Figure 4.21) by the respective concentration of the compound in slime (Table 4.18). Figures 4.27 through 4.29 present the loads of slime constituents in graphical form.

There were great uncertainties associated with the mass balances for slime TOC, protein and carbohydrate. In the activated sludge process, for example, the mass balance depended on the concentrations of each compound in slime biopolymers of primary effluent, secondary effluent, and activated sludge. As can be seen in

Table 4.34 Estimated Loads of Slime Biopolymer Constituents in Selected Locations at the Ithaca Wastewater and Sludge Treatment Plant

Location	Protein (lb/d)	Carbohydrate (lb/d)	TOC (lb/d)
Influent	355.8	185.6	479.5
Return Flows [1]	28.6	10.4	32.1
Primary Effluent	127.4	116.8	180.5
Primary Sludge [2]	257.0	79.3	331.1
Secondary Effluent	119.1	72.5	217.4
Waste Secondary Sludge	2.8	2.8	3.9
Thickened Sludge	10.4	7.2	15.3
Septage [3]	N.K.	N.K.	N.K.
Feed Anaerobic Digester [4]	> 10.4	> 7.2	> 15.3
Digested Sludge	7.9	2.2	8.2
Dewatered Sludge	6.3	1.5	6.1
Thickener Overflow	18.8	8.2	22.5
Digester Supernatant	8.2	1.5	7.5
BFP Filtrate	1.6	0.8	2.1

[1] Values correspond to the sum of values of thickener overflow, digester supernatant and BFP filtrate
[2] Value = (influent load + return load) - primary effluent load
[3] Not known
[4] Values correspond to the sum of thickened sludge and septage

Table 4.18, the concentrations of carbohydrate, protein, and TOC in slime biopolymers of primary and secondary effluents, and activated sludge were not significantly different from each other. In addition, the concentrations were close to their limits of detection (Appendix D, Table D.1), where more uncertainty in the values measured occurs (Skoog and Leary, 1992). In the activated sludge process, the mass balances showed net accumulation of slime TOC of 40.8 lb/d, while slime

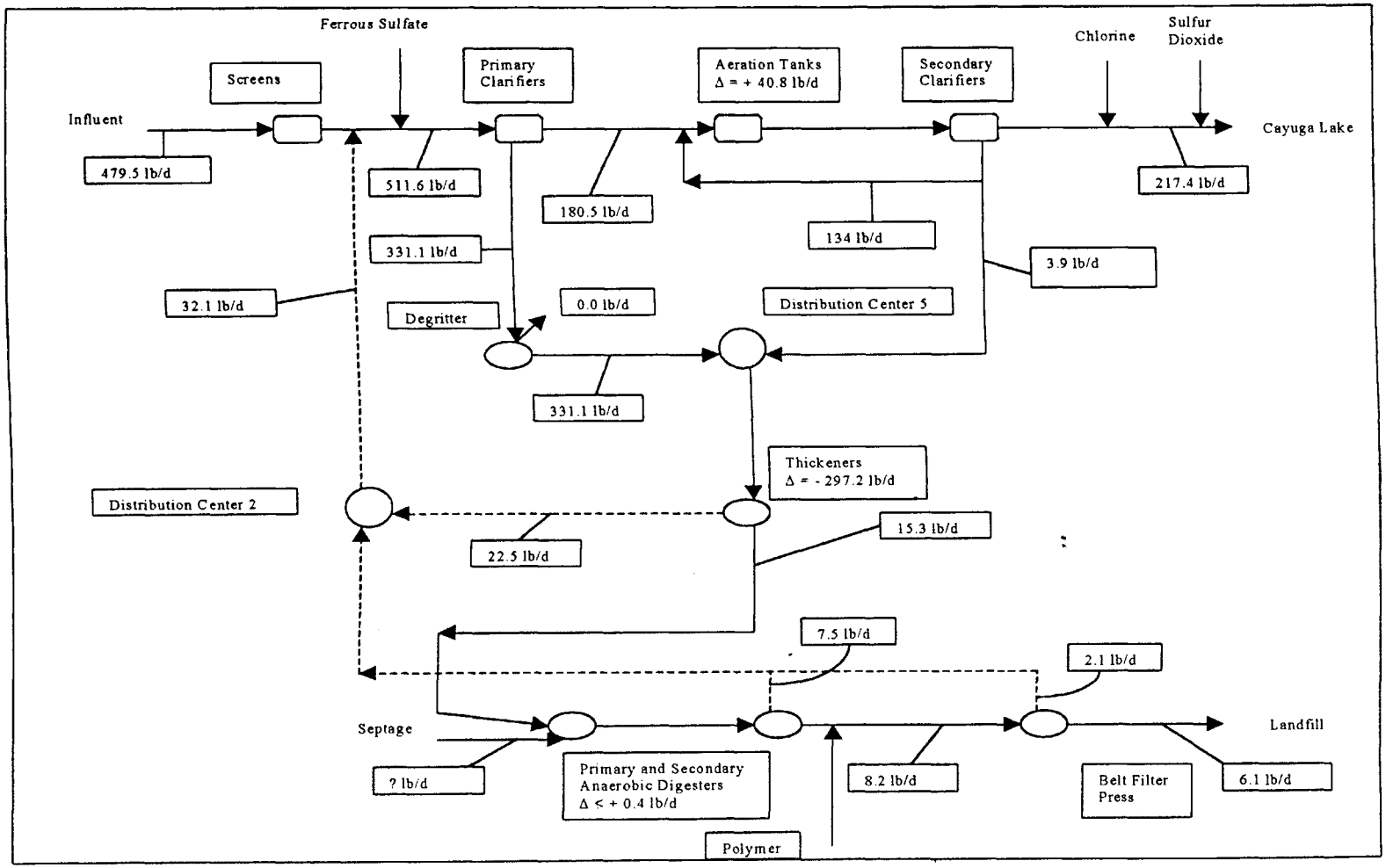

Figure 4.27 Mass Balance for Loosely-Bound Biopolymer TOC at the Ithaca Wastewater and Sludge Treatment Plant – Average Values for June, July and August 1998.

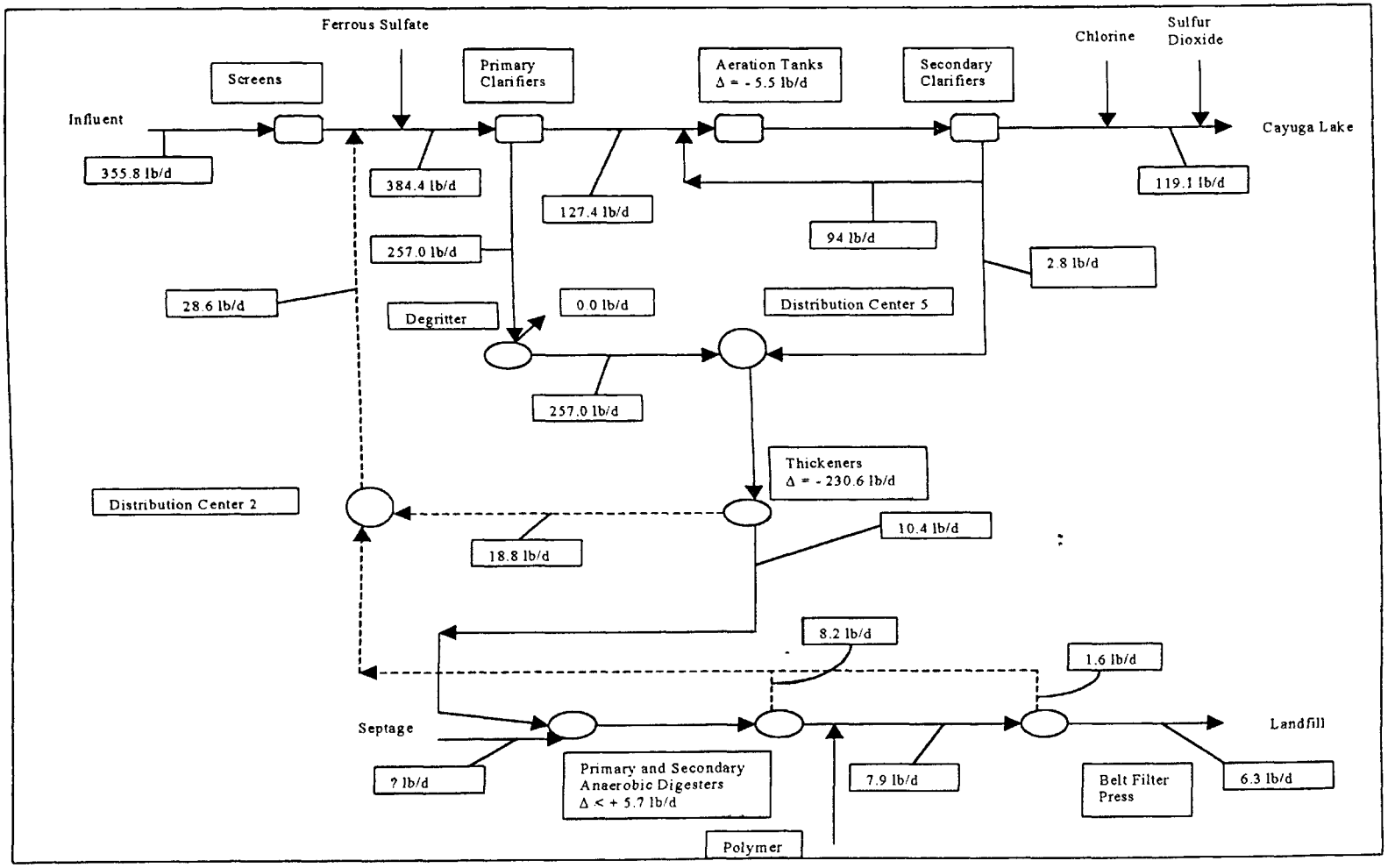

Figure 4.28 Mass Balance for Loosely-Bound Biopolymer Protein at the Ithaca Wastewater and Sludge Treatment Plant – Average Values for June, July and August 1998.

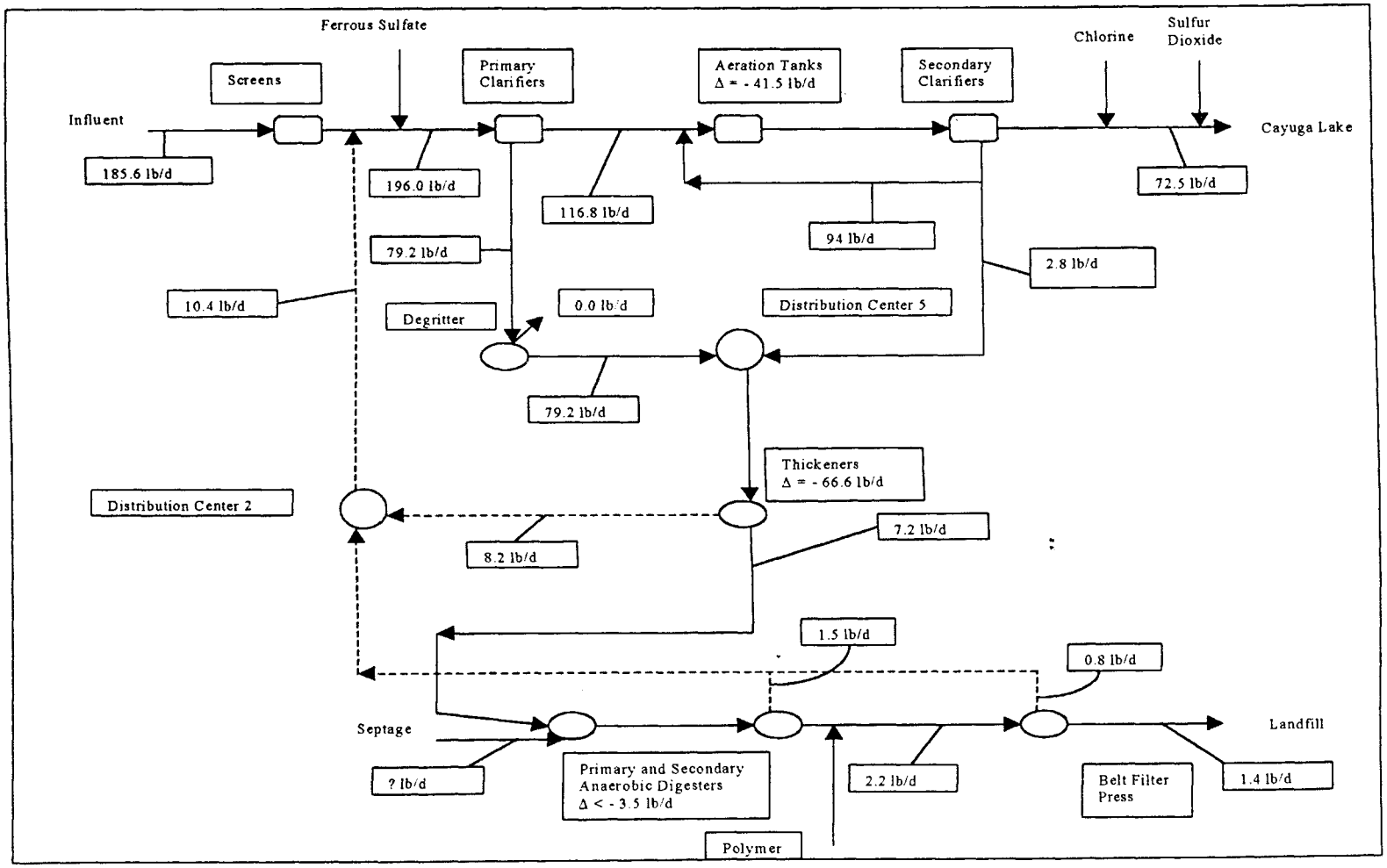

Figure 4.29 Mass Balance for Loosely-Bound Biopolymers Carbohydrate at the Ithaca Wastewater and Sludge Treatment Plant – Average Values for June, July and August 1998.

carbohydrate and protein had destruction of 41.5 lb/d and 5.5 lb/d, respectively.

Mass balances for slime biopolymer on the thickeners showed net destruction of 89 % of TOC, 89 % of protein, and 81 % of carbohydrate. Slime load to the thickeners came almost entirely from primary sludge (Figures 4.27 through 4.29). According to the mass balance on the primary sedimentation tanks, the loads of carbohydrate, protein and TOC in primary sludge were, respectively, 79.2 lb/d, 257.0 lb/d and 331.1 lb/d. By dividing these loads by the primary sludge flowrate, the concentrations of carbohydrate, protein, and TOC in slime biopolymers from primary sludge should be, respectively, 27 mg/l, 87 mg/l, and 112 mg/l, for mass balance to occur. However, in one measurement made in slime biopolymers of primary sludge, the concentrations were only 2.0 mg/l carbohydrate, 4.0 mg/l protein, and 4.0 mg/l TOC (Table 4.18). These data suggested that slime biopolymers were degraded in the primary sedimentation tanks.

The estimated loads of slime biopolymer TOC, protein, and carbohydrate in the anaerobic digester feed were, respectively, 9 %, 6 %, and 15 % of the capsular biopolymers load. The TOC load of slime biopolymers in the digester feed was almost identical of the load of slime TOC leaving the digester in the supernatant and in the thickened digested sludge. There was an increase in slime protein, and a decrease in slime carbohydrate in digested sludge with respect to the digester feed. Slime carbohydrate also showed a larger destruction than slime protein in the activated sludge process.

4.4.3 Summary of Findings from the Biopolymer Mass Balances

Some conclusions that were drawn based on the mass balances for capsular and slime biopolymers are discussed below.

1) Capsular biopolymer protein, carbohydrate, RNA, and humic acids had net production in the activated sludge process. The production represented 29 % (protein), 25 % (carbohydrate), 40 % (RNA), and 42 % (humic acids) of the respective incoming loads to the activated sludge process (primary effluent).

2) Capsular biopolymer TOC in activated sludge showed a net degradation representing 9 % of the incoming load from primary effluent. This finding was inconsistent with the net production of capsular biopolymer constituents. It was estimated that the measured TOC of capsular biopolymer in the feed of the activated sludge process (i.e., the primary effluent) was about 30 % above the TOC predicted based on composition. The reason for the difference is not known. A fraction of the 30 % difference could be contributed by DNA; in other locations at the plant, DNA comprised about 5 % of the capsular TOC.

3) The estimated yield coefficient for capsular biopolymers in activated sludge, $0.012 \frac{mg\ biop.\ TOC}{mg\ BOD_L\ used}$, represented 5 % of the estimated cell yield coefficient, based on organic carbon, in the activated sludge process, $0.24 \frac{mg\ cell\ TOC}{mg\ BOD_L\ used}$.

4) Mass balances on the primary sedimentation tanks supported the hypothesis that there was a preferential settling of particles holding more capsular biopolymers than particles with less capsule. In one sample collected for primary sludge, however, this hypothesis was not confirmed. The results obtained in this sample showed that particles in primary sludge had about the same quantity of capsular biopolymers as particles in primary effluent. If these results are confirmed, then it will be possible to conclude that there is destruction of capsular biopolymers in primary sedimentation tanks.

5) Fair mass balances were achieved in the thickeners for capsular TOC, protein, carbohydrate, and RNA. The output loads of these constituents represented, respectively, 92 %, 95 %, 97 %, and 113 % of the input loads of TOC, protein, carbohydrate, and RNA. On the other hand, the output loads of capsular humic acids represented 53 % of the input loads.

6) Net destruction of all capsule constituents occurred in the anaerobic digestion process. The destruction represented 69 % (TOC), 70 % (protein), 70 % (DNA), 66 % (carbohydrate), 63 % (RNA), and 60 % (humic acids) of the loads in the digester feed. These results indicate that capsular biopolymers had a greater degree of biodegradability than volatile suspended solids in the digester feed, which were reduced by 50 % during digestion. These results indicate also that fractions of capsular biopolymer protein, carbohydrate, RNA, DNA, and humic acids are recalcitrant to biodegradation under the conditions prevailing in the anaerobic digester, an hypothesis that was discussed in the literature review chapter (Section 2.6).

7) Great uncertainties were associated with mass balances for slime biopolymers. Mass balances were not performed for RNA, DNA, and humic acids because their concentrations were below the limits of detection in many flows. In addition, the concentrations of slime protein, carbohydrate, and TOC were close to their detection limits in many locations.

4.5 Trace Metals

4.5.1 *Trace Metals in Samples*

Copper, zinc, lead, cadmium and chromium were analyzed in samples collected for biopolymer characterization. Trace metals were not analyzed in secondary effluent samples and biopolymers because small amounts of capsular biopolymers extracted from secondary effluent didn't allow trace metal analyses.

Table 4.35 presents the mean concentration of trace metals with their respective standard deviations. For all trace metals, there was a substantial decrease in concentration from influent to primary effluent. Another observation from Table 4.35 is the large variability in trace metals concentrations in the thickener overflow, supernatant from anaerobic digester and belt filter press filtrate. The metals, concentrated in primary and waste secondary sludges, were further concentrated in the anaerobic digester, where the highest concentrations occurred. Even so, the digested sludge from the Ithaca plant can be classified as having exceptional quality by EPA standards (U.S. EPA, 1993). Figure 4.30 presents trace metals in digested sludge solids at the Ithaca plant and the EPA standards for metals in sludges classified as exceptional quality.

Figures 4.31 and 4.32 present the loads of copper and zinc in selected locations within the treatment plant. The loads were computed as the product of flowrates (Figure 4.21) and copper and zinc concentrations (Table 4.35). Differences between calculated input and output of copper and zinc to each process in the plant were in the range of zero to 70 %. The measured loads of copper and zinc leaving the primary sedimentation tanks were, respectively, 60 % and 40 % of the inflow load. The measured load of copper leaving the activated sludge process was 70 % higher than the load entering the process; for zinc, the inflow and outflow loads in activated sludge were about the same. The outflows of the thickeners carried 70 % (copper) and 83 % (zinc) of the combined loads of primary sludge and waste secondary sludge. The digester outflows, supernatant and thickened digested sludge, had loads of copper and zinc that were about 30 % higher than the thickened sludge load. These results were considered to be satisfactory, taking into consideration variations that can occur, for example, with the concentrations of trace metals in influent wastewater. Mass balances

Table 4.35 Concentration of Trace Metals in Wastewater and Sludge Samples. Numbers represent mean ± SD (n = 3), except primary sludge, waste activated sludge and chromium (n = 1). Unit: µg/l

Location	Copper	Zinc	Lead	Cadmium	Chromium
Influent	120 ± 46	240 ± 60	6.0 ± 0.6	< LD	17
Primary Effluent	39 ± 12	61 ± 13	< LD	< LD	< LD
Primary Sludge	750	700	61	0.8	25
Activated Sludge	570 ± 80	570 ± 49	27 ± 5.0	1.3 ± 0.6	19
Waste Sec. Sludge	2760	2,350	53	3.4	62
Thickened Sludge	19,700 ± 1,600	21,500 ± 1,790	910 ± 220	33 ± 6	570
Digested Sludge	36,200 ± 8,100	41,000 ± 6,270	1,500 ± 330	62 ± 10	2,700
Thickener Overflow	110 ± 79	100 ± 86	6.1 ± 6.2	< LD	< LD
Digester Supernatant	2,240 ± 2,330	2,700 ± 2,500	100 ± 100	4.3 ± 5.2	32
BFP Filtrate	1,180 ± 840	870 ± 490	27 ± 15	1.3 ± 0.7	17

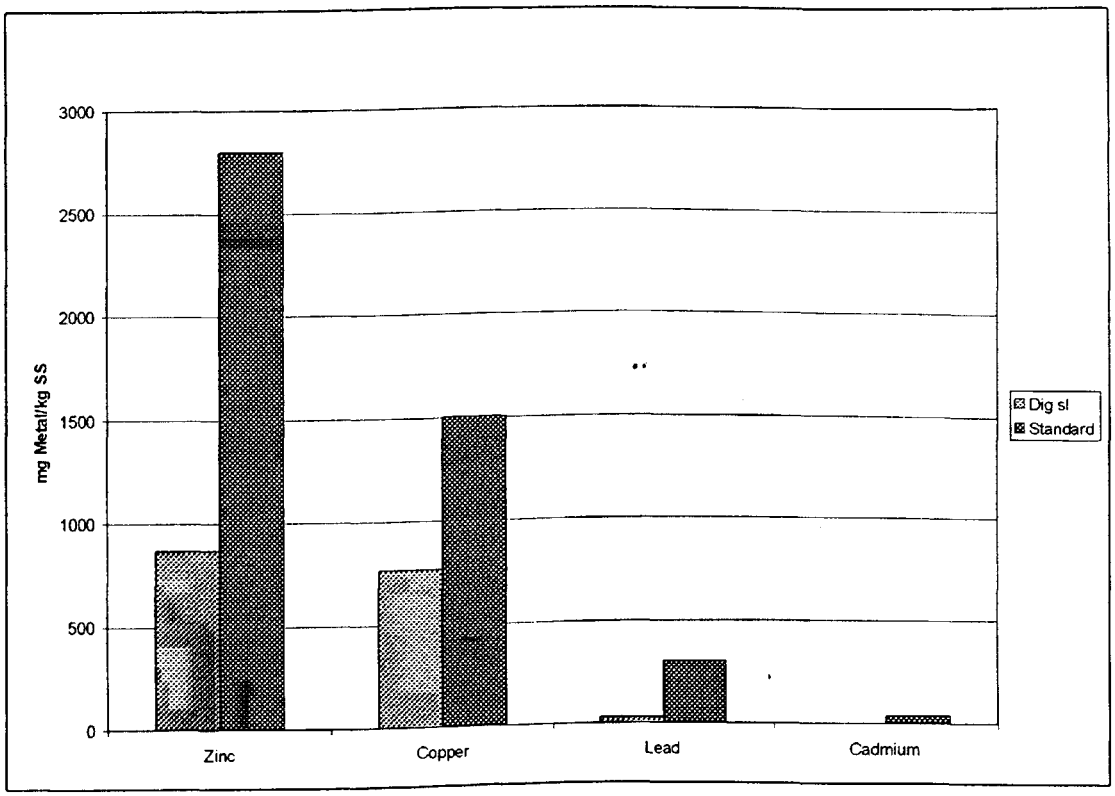

Figure 4.30 Trace Metals in Digested Sludge Solids and Standards for Exceptional Quality Sludges

for lead, cadmium and chromium were not prepared due to measurements below the limits of detection.

4.5.2 *Trace Metals in Solids-Bound Biopolymers*

Table 4.36 presents the weight ratios of metals in solids-bound biopolymers to volatile suspended solids. Figures 4.33 to 4.36 present the mean trace metals content in capsular biopolymers to VSS with their respective 95 % confidence intervals. Abbreviations used to describe locations in Figures 4.33 to 4.36 are summarized in Table 4.6. The weight ratios of trace metals in capsular biopolymers to VSS are also depicted in Figure 4.37. The information presented in Table 4.36 and Figures 4.33 through 4.37 indicate that the ratios of trace metals in capsular biopolymers to volatile

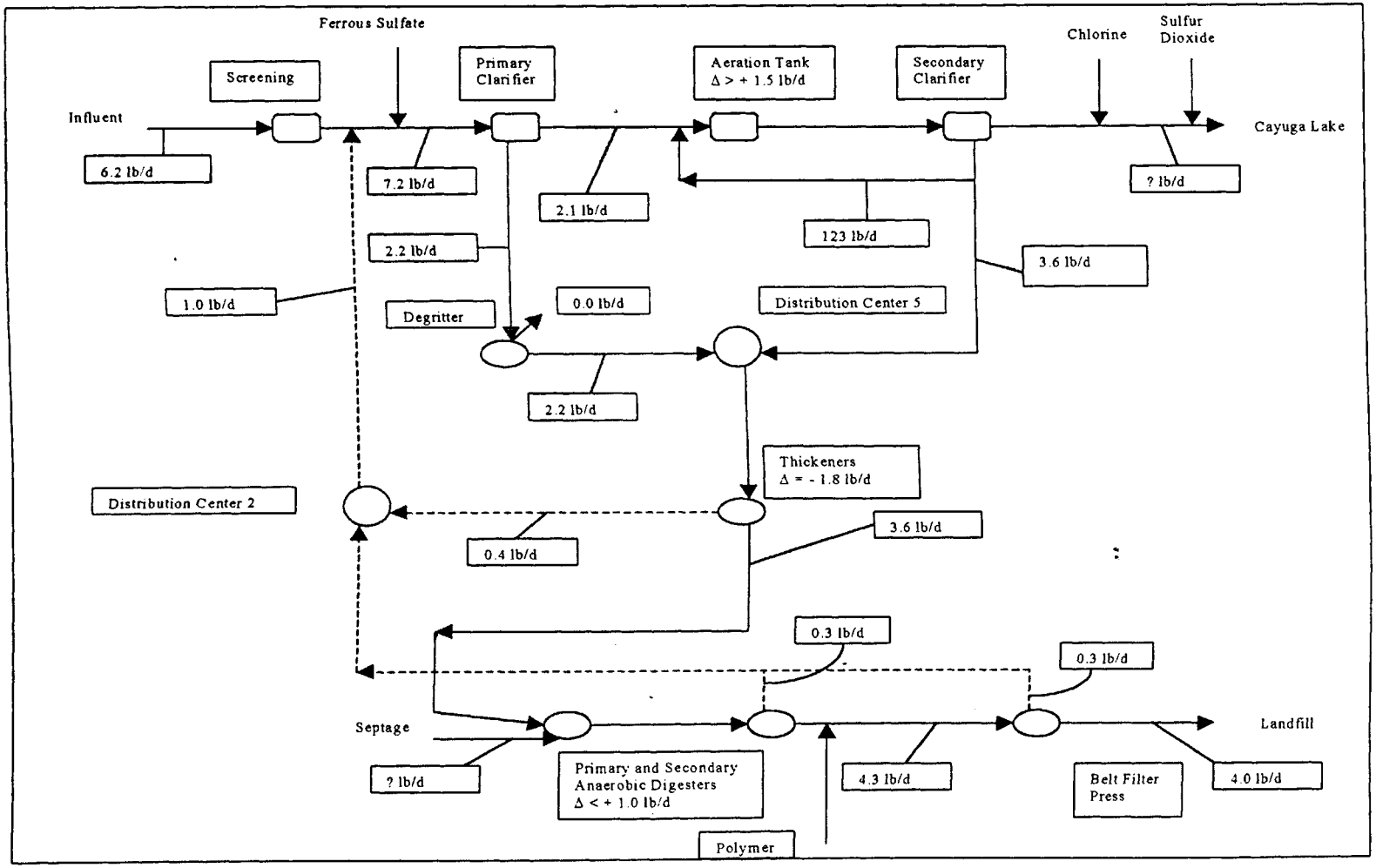

Figure 4.31 Mass Balance for Copper at the Ithaca Wastewater and Sludge Treatment Plant Average Values for June, July and August 1998.

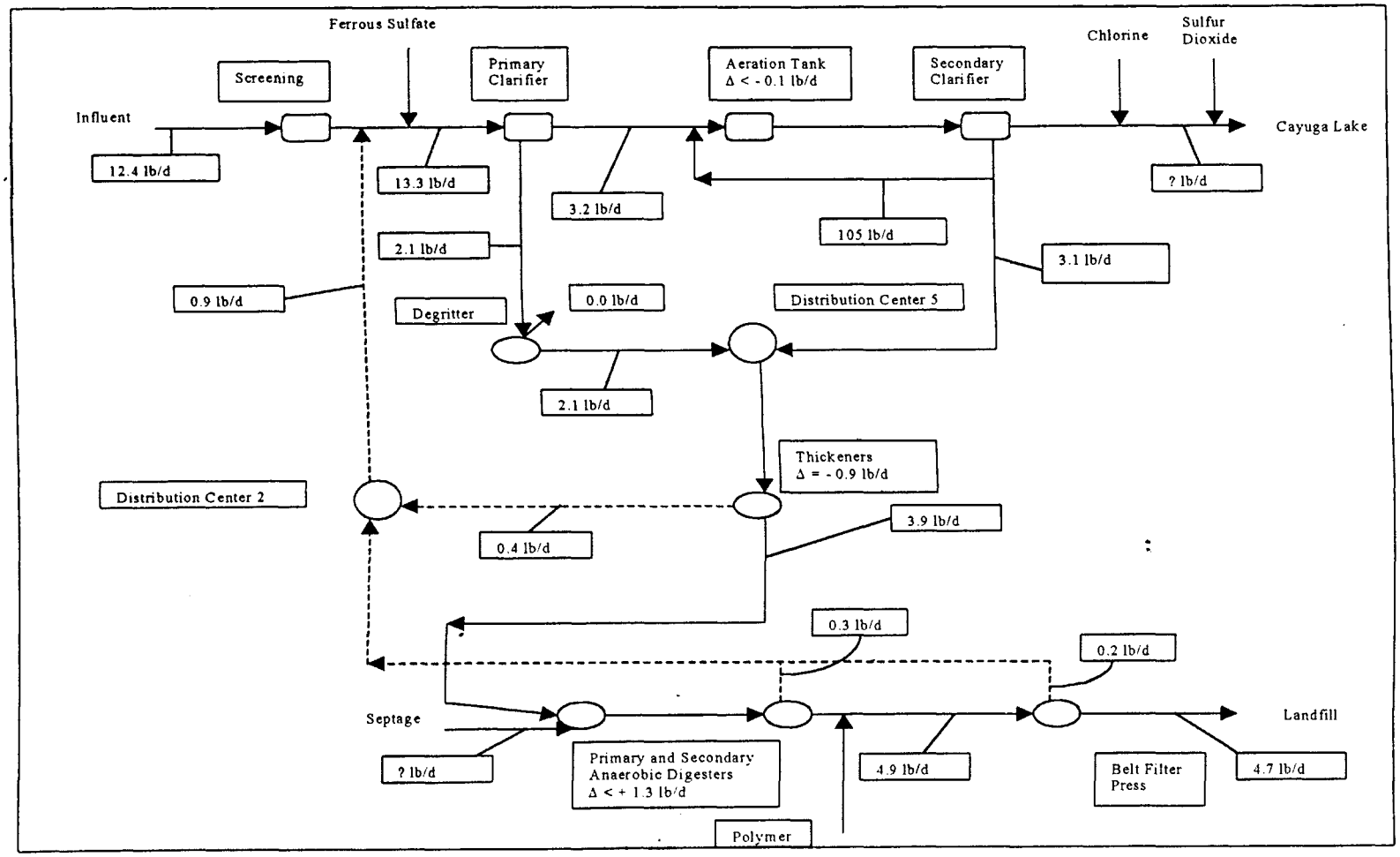

Figure 4.32 Mass Balance for Zinc at the Ithaca Wastewater and Sludge Treatment Plant Average Values for June, July and August 1998.

Table 4.36 Weight Ratios of Trace Metals in Solids-Bound Biopolymers to Volatile Suspended Solids
Numbers represent mean ± SD (n = 3), except primary, waste activated sludge and chromium (n = 1). Unit: μg metal/g VSS

Location	Copper	Zinc	Lead	Cadmium	Chromium
Influent	91 ± 21	110 ± 25	5.2 ± 1.8	0.24 ± 0.12	2.2
Primary Effluent	24 ± 6	50 ± 22	2.3 ± 0.9	< LD	< LD
Primary Sludge	40	35	3.4	< LD	N.M.
Activated Sludge	51 ± 34	32 ± 4	1.8 ± 0.2	0.20 ± 0.06	< LD
Waste Act. Sludge	27	27	1.3	0.11	N.M.
Thickened Sludge	45 ± 11	37 ± 12	2.3 ± 0.3	0.06 ± 0.01	1.2
Digested Sludge	33 ± 17	31 ± 9	1.3 ± 0.4	0.05 ± 0.02	1.1
Thickener Overflow	31 ± 9	27 ± 2	2.8 ± 0.6	0.18 ± 0.03	< LD
Digester Supernatant	16 ± 7	16 ± 5	1.9 ± 0.5	0.10 ± 0.01	< LD
BFP Filtrate	22 ± 3	26 ± 5	1.5 ± 0.2	0.12 ± 0.02	1.7

N.M.: not measured

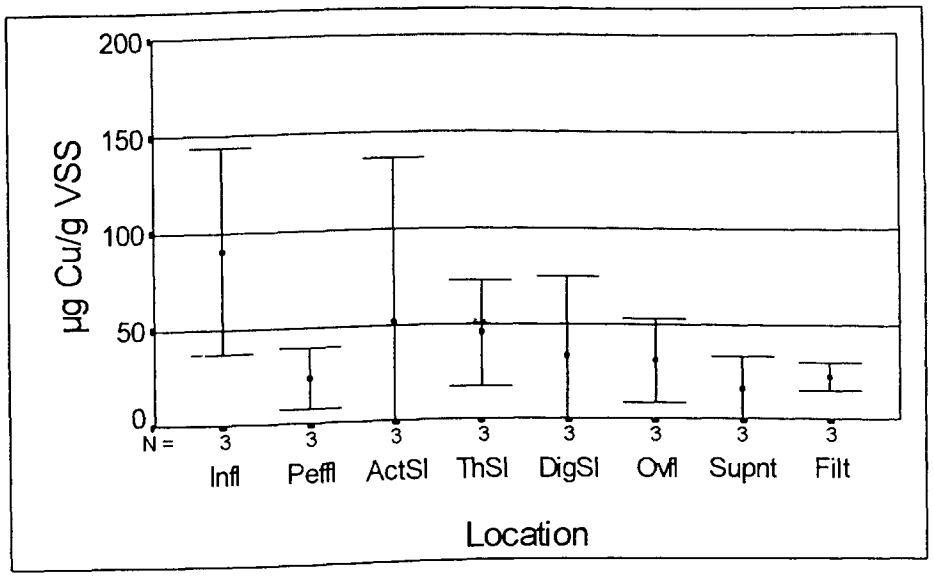

Figure 4.33 Ratio of Copper in Solids-Bound Biopolymers to Volatile Suspended Solids

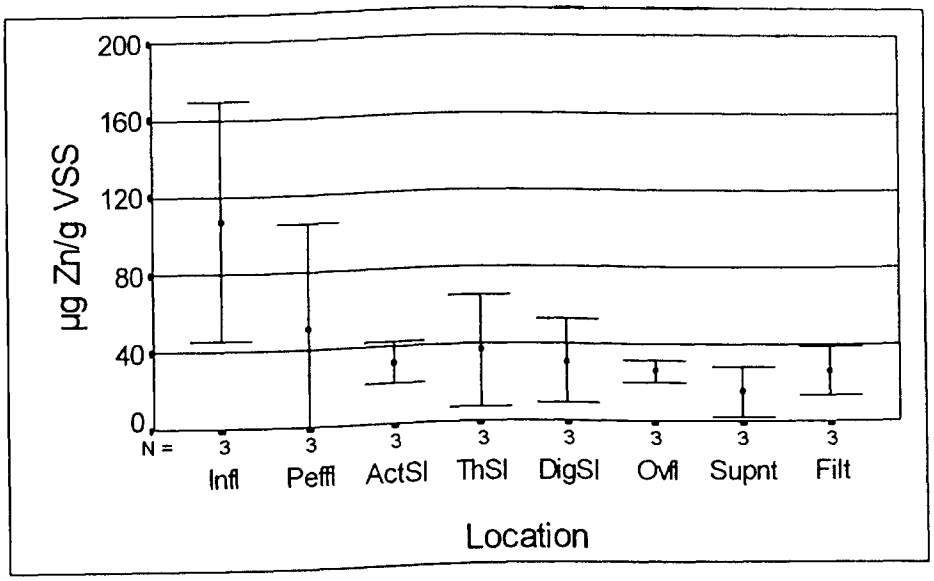

Figure 4.34 Ratio of Zinc in Solids-Bound Biopolymers to Volatile Suspended Solids

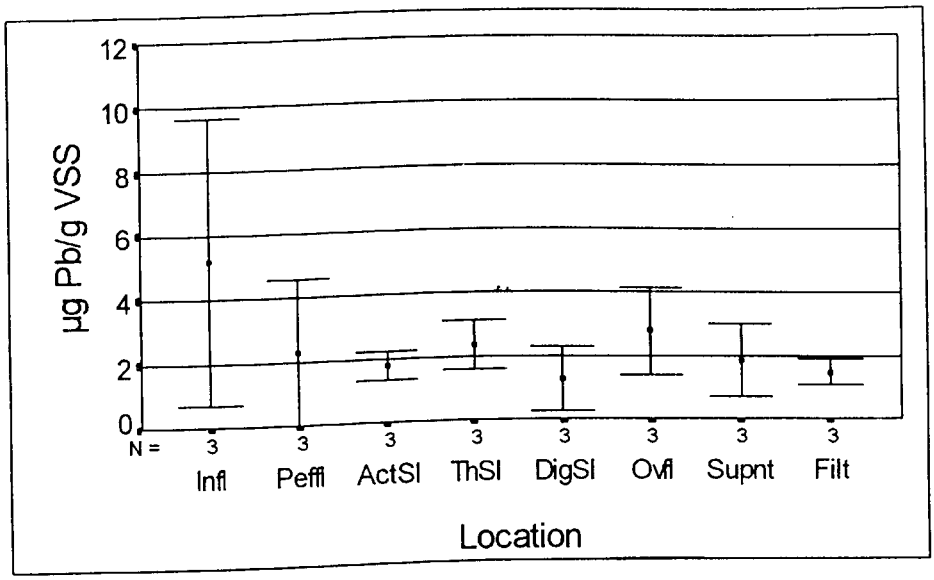

Figure 4.35 Ratio of Lead in Solids-Bound Biopolymers to Volatile Suspended Solids

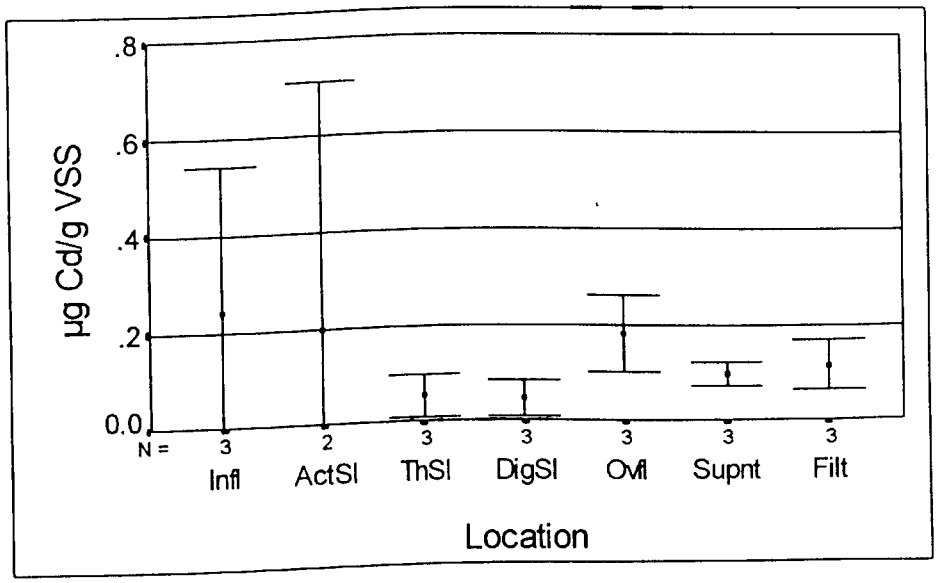

Figure 4.36 Ratio of Cadmium in Solids-Bound Biopolymers to Volatile Suspended Solids

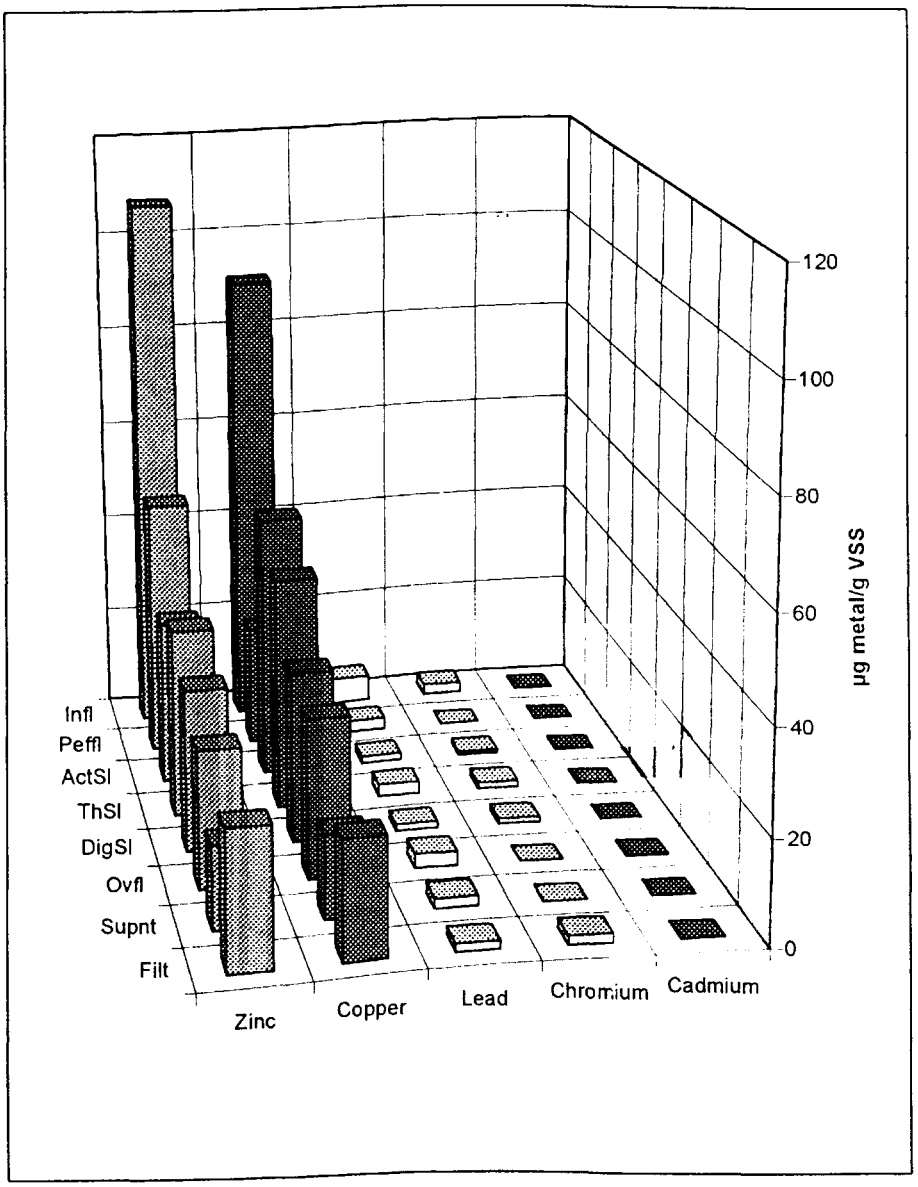

Figure 4.37 Weight Ratios of Trace Metals Bound to Capsular Biopolymer to Volatile Suspended Solids

suspended solids were greater in the influent than the ratios at other locations of the plant.

Table 4.37 presents the weight ratios of metals in capsular biopolymer to capsular biopolymer, while Table 4.38 displays the concentration of capsular biopolymer trace metals in wastewater and sludge samples. A comparison between the results presented in Tables 4.35 and 4.38 indicates that only a fraction of the trace metals present in wastewater and sludge treatment processes was retained in solids-bound biopolymers.

Figure 4.38 presents the mean weight ratios of trace metals in capsular biopolymer to capsular biopolymer. Figures 4.39 through 4.42 present the same information with the 95 % confidence intervals. Adsorption, complexation, precipitation and uptake by cells are the mechanisms by which trace metals can be removed during biological treatment of wastewater (Sterritt and Lester, 1986). Adsorption and complexation are the two major processes of interaction between trace metals and extracellular polymers (Lion et al., 1988). Biopolymers contain anionic and cationic groups, which represent potential sites for metal adsorption (Fleming, 1995); for example, metallic cations can be electrostatically bound to negatively charged functional groups present in biopolymers (Alexander, 1999). The presence of a known chelating agent such as humic acid (Windholz, 1983) in biopolymers enhances the potential for trace metal interaction through the process of complexation.

Fukushi (1996) measured metal uptake of copper and cadmium by biopolymers from microorganisms grown in a chemostat at the specific growth rate of one day^{-1}. Removal of trace metals when microorganisms were grown at this rate was observed to be higher than metals removal when microorganisms were grown at higher growth rates. Copper and cadmium uptakes were 1.21 mmol/g protein (76.9 mg Cu/g protein) and 0.09 mmol/g protein (10.1 mg Cd/g protein), for initial metal concentrations of 40 mg/l. The corresponding Cu and Cd ratios measured in biopolymers at four locations at the Ithaca plant are shown in Table 4.39. The

Table 4.37 Weight of Trace Metals Bound to Solids-Bound Biopolymers per Unit of Solids-Bound Biopolymers. Solids-bound biopolymers are given as TOC. Numbers represent mean ± SD (n = 3), except primary, waste activated sludge and chromium (n = 1). Units: copper and zinc, mg metal/g TOC; lead, cadmium and chromium, µg metal/g TOC.

Location	Copper	Zinc	Lead	Cadmium	Chromium
Influent	3.4 ± 0.8	4.0 ± 0.7	195 ± 69	9.0 ± 5.0	75
Primary Effluent	1.2 ± 0.2	2.6 ± 1.1	115 ± 40	< LD	< LD
Primary Sludge	2.3	2.1	205	< LD	N.M.
Activated Sludge	2.2 ± 1.4	1.4 ± 0.0	78 ± 4	8.6 ± 0.8	< LD
Waste Act. Sludge	1.3	1.3	62	5.2	N.M.
Thickened Sludge	1.7 ± 0.3	1.4 ± 0.3	88 ± 6	2.4 ± 0.2	42
Digested Sludge	1.9 ± 0.9	1.8 ± 0.4	77 ± 20	2.8 ± 0.8	69
Thickener Overflow	1.3 ± 0.3	1.1 ± 0.2	115 ± 7	7.8 ± 2.0	< LD
Digester Supernatant	1.1 ± 0.3	1.2 ± 0.3	135 ± 23	7.5 ± 2.0	< LD
BFP Filtrate	1.1 ± 0.2	1.3 ± 0.0	79 ± 24	6.0 ± 1.3	109

N.M.: not measured

Table 4.38 Concentration of Solids-Bound Biopolymers Trace Metals in Wastewater and Sludge Samples
Numbers represent mean ± SD (n = 3), except primary, waste activated sludge and chromium (n = 1).
Units: μg metal/l solution

Location	Copper	Zinc	Lead	Cadmium	Chromium
Influent	10 ± 2	12 ± 4	0.6 ± 0.1	0.0 ± 0.0	0.2
Primary Effluent	1.1 ± 0.2	2.2 ± 0.6	0.10 ± 0.0	0.0 ± 0.0	0.0
Primary Sludge	29	25	2.5	0.0 ± 0.0	N.M.
Activated Sludge	40 ± 33	25 ± 1	1.4 ± 0.1	0.1 ± 0.0	0.5
Waste Act. Sludge	87	88	4.2	0.4	N.M.
Thickened Sludge	1,160 ± 190	950 ± 260	63 ± 24	1.6 ± 0.4	40.0
Digested Sludge	960 ± 670	890 ± 440	37 ± 19	1.3 ± 0.7	29.0
Thickener Overflow	2.0 ± 0.3	1.8 ± 0.5	0.2 ± 0.0	0.0 ± 0.0	0.0
Digester Supernatant	36 ± 41	31 ± 26	3.6 ± 3.1	0.2 ± 0.1	0.9
BFP Filtrate	11 ± 6	14 ± 8	0.7 ± 0.4	0.1 ± 0.0	0.4

N.M.: not measured

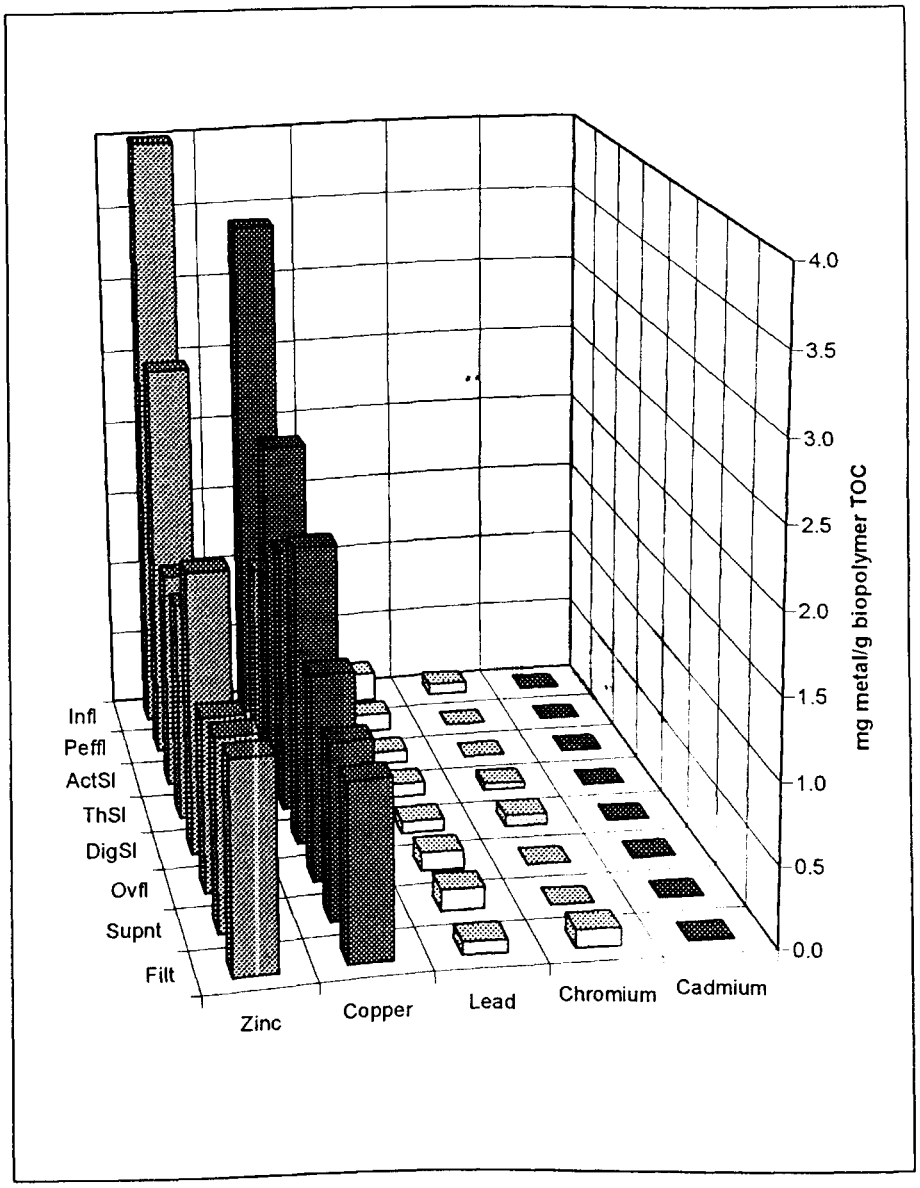

Figure 4.38 Trace Metals Contents of Solids-Bound Biopolymer

Table 4.39 Metal Content per Unit Mass of Biopolymer (mg/g Protein) (Ithaca Plant)

Location	Copper	Cadmium
Influent	3.5	0.009
Activated Sludge	1.9	0.006
Thickened Sludge	1.5	0.003
Digested Sludge	1.9	0.003

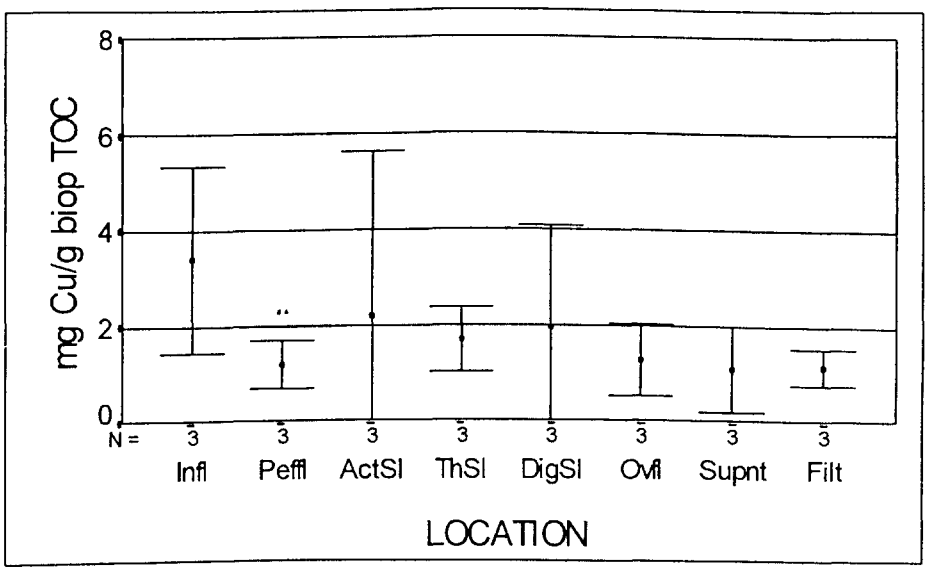

Figure 4.39 Ratio of Copper in Solids-Bound Biopolymers to Solids-Bound Biopolymers

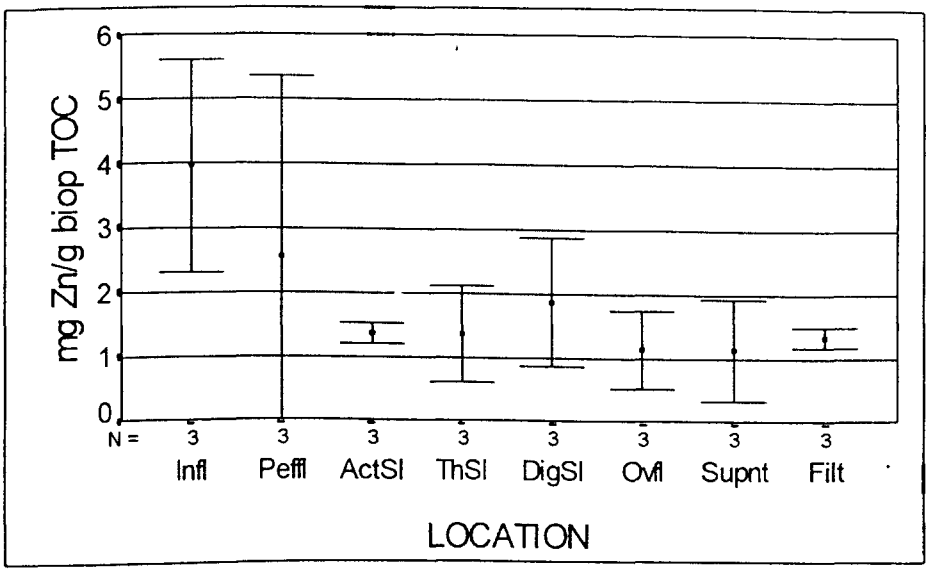

Figure 4.40 Ratio of Zinc in Solids-Bound Biopolymers to Solids-Bound Biopolymers

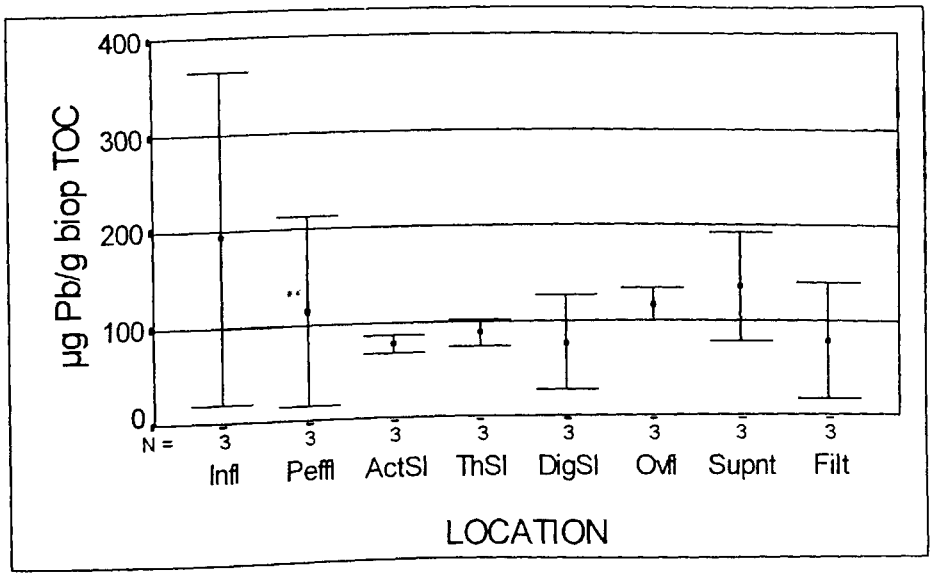

Figure 4.41 Ratio of Lead in Solids-Bound Biopolymers to Solids-Bound Biopolymers

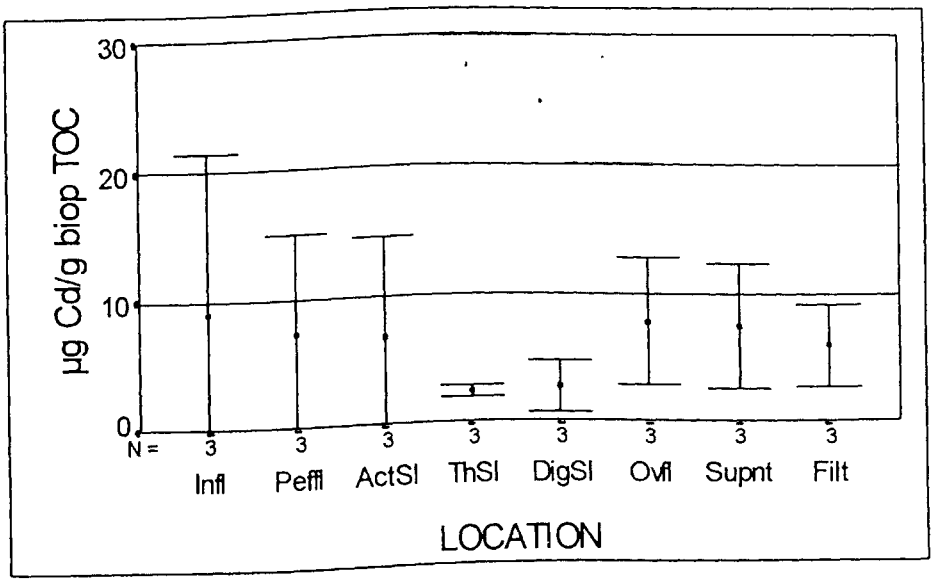

Figure 4.42 Ratio of Cadmium in Solids-Bound Biopolymers to Solids-Bound Biopolymers

contents measured at the Ithaca plant were lower than those observed by Fukushi (1996). This would be expected because the initial concentration of the metal in solution affects uptake and equilibrium. While Fukushi (1996) added copper and cadmium to obtain initial concentrations of 40 mg/l in the biopolymers solution, the concentrations of copper and cadmium in activated sludge of the Ithaca plant were 0.57 mg/l Cu and 1.3 µg/l Cd (Table 4.35). In addition, biopolymers extracted from samples from the Ithaca plant were dialyzed, with metals associated with low molecular weight biopolymers transported across the dialyzing membrane. Also, microorganisms from Fukushi experiment were optimized for metal uptake.

Rudd et al. (1984) measured maximum binding capacities for extracellular polymers extracted from activated sludges in the range 0.019 mmol/g biopolymer (1.2 mg/g) to 0.175 mmol/g biopolymer (11.1 mg/g) for copper, and 0.011 mmol/g biopolymer (1.2 mg/g) to 0.530 mmol/g biopolymer (59.6 mg/g) for cadmium. At the Ithaca plant, the ratios for activated sludge were 0.9 mg Cu/g biopolymer, and 0.003 mg Cd/g biopolymer. The initial concentrations for copper and cadmium in the experiment of Rudd et al. were 9.2×10^{-5} M Cd (10.3 mg/l) and 7.4×10^{-5} M Cu (4.7 mg/l), which were much higher than the concentrations measured in activated sludge at the Ithaca's plant (0.57 mg/l Cu and 1.3 µg/l Cd). As it was the case in the experiment of Fukushi (1996), the initial conditions established by Rudd et al. probably determined the higher contents of copper and cadmium in their capsular biopolymers in relation to the biopolymers from the plant in Ithaca.

The content of trace metals in capsular biopolymer at the Ithaca Wastewater and Sludge Treatment Plant didn't vary widely among locations. From Table 4.37, trace metals varied within the following ranges: copper, 1.1 to 3.4 mg Cu/g biopolymer TOC; zinc, 1.1 to 4.0 mg Zn/g biopolymer TOC; lead, 62 to 193 µg Pb/g biopolymer TOC; cadmium, 2.4 to 9.0 µg Cd/g biopolymer TOC.

The results presented in Table 4.37 suggest that the amounts of trace metals bound to capsular biopolymers per unit of capsular biopolymer in the influent were higher than the amounts of trace metals per unit of capsule at other stages of the treatment plant. For example, the weight ratios of capsular copper to capsular biopolymer of the influent were 1.5 and 1.8 times the weight ratios of capsular copper to capsular biopolymer of activated sludge and digested sludge, respectively. For zinc, these values were 2.9 and 2.2, respectively. Similar trend was observed for lead. This observation was confirmed by performing analysis of variance and Fischer's Least Significant tests. ANOVA rejected the hypotheses that the mean weight ratios of copper, zinc, lead, and cadmium to capsular biopolymers presented in Table 4.37 were the same at nine locations within the treatment plant. ANOVA was not performed for chromium because only one sample was measured. Fischer's Least Significant Difference test placed influent as a separate category from all other locations for copper, zinc and lead. The following homogeneous groups were found from Fischer's LSD:

Copper:

Group 1: influent

Group 2: primary effluent, activated sludge, thickened sludge, digested sludge, thickener overflow, digester supernatant, belt filter press

Zinc:

Group 1: influent

Group 2: activated sludge, thickened sludge, digested sludge, thickener overflow, digester supernatant, belt filter press filtrate

Group 3: primary effluent, digested sludge

Lead:

Group 1: influent

Group 2: primary effluent, activated sludge, thickened sludge, digested sludge, thickener overflow, belt filter press filtrate

Group 3: primary effluent, thickened sludge, thickener overflow, digester supernatant

Cadmium:

Group 1: influent, activated sludge, thickener overflow, digester supernatant, BFP filtrate

Group 2: thickened sludge, digested sludge, BFP filtrate

These results indicate that the hypotheses that the weight ratios of copper, zinc, and lead to capsular biopolymer were the same for activated sludge, thickened sludge, digested sludge, thickener overflow, and belt filter press filtrate, were not rejected.

Biopolymer chemical composition at the level analyzed in this research cannot explain why more metals were bound to capsular biopolymers in wastewater than in other locations, since it was found that the composition of capsular biopolymers was similar within the treatment plant. A pattern that could explain the higher metal binding to capsular biopolymer in the influent was explored. Perhaps more biopolymer per unit of metal could explain the differences. For example, Table 4.40 shows the ratios between mass of metals in samples to the mass of biopolymer, as TOC, at several locations within the treatment plant. The results presented in Table 4.40 are the quotients between concentrations of trace metals in samples (Table 4.35) and concentrations of capsular biopolymers TOC (Table 4.4). The influent had higher ratios of metals to biopolymers than activated and thickened sludges, but had lower ratios than digested sludge and, in most cases, than the liquors from sludge treatment. This result means that higher total metal concentrations in samples per unit of biopolymer do not alone explain higher metal binding to biopolymers. Future research is necessary to determine what causes the possible desorption of trace metals as wastewater enters the treatment plant.

Table 4.40 Ratio Between the Total Mass of Individual Trace Metals in Sample to Mass of Capsular Biopolymer (Unit: mg metal/g biopolymer TOC)

Location	Copper	Zinc	Lead	Cadmium	Chromium
Influent	38.7	77.3	1.9	< LD	5.4
Prim. Effluent	43.0	67.8	< LD	< LD	< LD
Act. Sludge	32.3	32.3	1.5	0.1	1.1
Thick. Sludge	27.8	30.5	1.3	< LD	0.8
Dig. Sludge	78.5	88.8	3.3	0.1	5.8
Thick. Overfl.	67.8	62.0	3.8	< LD	< LD
Dig. Supern.	78.2	94.4	3.4	0.1	1.1
BFP Filtrate	113.2	84.0	2.6	0.1	1.7

4.5.3 *Trace Metals in Loosely-Bound Biopolymers*

Table 4.41 presents the concentration of trace metals associated with loosely-bound biopolymers in wastewater and sludge samples at selected locations within the treatment plant. Of the trace metals analyzed, zinc had the highest concentrations in all locations, except in thickened sludge. Lead, cadmium and chromium concentrations were below their detection limits.

A comparison between concentrations of trace metals associated with capsular and slime biopolymers is presented in Table 4.42. In influent, primary effluent and thickener overflow, the concentrations of slime copper and zinc were higher than the concentrations of capsular copper and zinc. In samples with higher solids concentration, thickened sludge, digested sludge, primary sludge, waste secondary sludge, the concentrations of copper and zinc associated with capsular biopolymers were higher than the concentrations associated with slime. In activated sludge, the concentration in capsule was higher than slime for copper, but about the same for zinc.

Table 4.41 Concentration of Trace Metals Associated with Loosely-Bound Biopolymers in Wastewater and Sludge Samples. Numbers represent mean ± SD (n = 3), except primary and waste activated sludge, and chromium (n = 1). Units: (μg metal/l solution)

Location	Copper	Zinc	Lead	Cadmium	Chromium
Influent	19 ± 7	44 ± 11	< LD	< LD	< LD
Primary Effluent	7.6 ± 1.7	27 ± 8	< LD	< LD	< LD
Primary Sludge	3.5	16	< LD	< LD	-
Activated Sludge	4.7 ± 0.8	25 ± 11	< LD	< LD	< LD
Secondary Sludge	4.1	-	-	-	-
Thickened Sludge	21 ± 17	21 ± 2	< LD	< LD	< LD
Digested Sludge	34 ± 27	43 ± 29	< LD	< LD	< LD
Thickener Overflow	7.7 ± 2.6	24 ± 2	< LD	< LD	< LD
Digester Supernatant	21 ± 17	48 ± 8	< LD	< LD	< LD
BFP Filtrate	8.1 ± 1.6	24 ± 9	< LD	< LD	< LD

Table 4.42 Concentration of Trace Metals Associated with Capsular and Slime Biopolymers in Wastewater and Sludge Samples. Numbers represent mean ± SD (n = 3), except primary and waste activated sludge, and chromium (n = 1). Units: (µg metal/l solution)

Location	Copper		Zinc		Lead		Cadmium		Chromium	
	Capsule	Slime	Capsule	Slime	Capsule	Slime	Capsule	Slime	Capsule	Slime
Influent	10	19	12	44	0.6	< LD	0.0	< LD	0.2	< LD
Prim Effl	1.1	7.6	2.2	27	0.1	< LD	< LD	< LD	< LD	< LD
Prim Sludge	29	3.5	25	16	2.5	< LD	< LD	< LD	NM	NM
Act Sludge	40	4.7	25	25	1.4	< LD	0.1	< LD	< LD	< LD
Sec Sludge	87	4.1	88	NM	4.2	NM	0.4	NM	NM	NM
Thick Sludge	1,160	21	950	21	63	< LD	1.6	< LD	40	< LD
Dig Sludge	960	34	890	43	37	< LD	1.3	< LD	29	< LD
Thick Overfl	2.0	7.7	1.8	24	0.2	< LD	0.0	< LD	< LD	< LD
Supernatant	36	21	31	48	3.6	< LD	0.2	< LD	< LD	< LD
Filtrate	11	8.1	14	24	0.7	< LD	0.1	< LD	0.4	< LD

NM = Not measured;
< LD = below detection limit

Table 4.43 presents the percentage represented by each trace metal bound in capsule and slime biopolymers in relation to the total concentrations of trace metals in the samples. The results indicate that solids bound biopolymers contained 10 % or less of the metal contents in samples. The presence of metals in loosely bound biopolymers represented a very small fraction of the metals for those samples with high solids concentrations. For example, in thickened and digested sludges, trace metals bound to slime represented 0.1 % of the metals in the samples. In diluted samples, however, such as primary effluent and thickener overflow, the presence of metals in loosely bound biopolymers represented as much as 45 % of the total metals in samples.

The trace metals in samples that were not bound to biopolymers could have been (1) associated with low molecular weight biopolymers, or were in solution, and left the dialysis bag during the biopolymer purification process; (2) associated directly with the solids fraction of the samples, and (3) exchanged with sodium on the cation exchange resin during the biopolymer extraction process.

4.5.4 *Mass Balance Evaluation for Trace Metals in Biopolymers*

Figures 4.43 and 4.44 present the loads of copper and zinc associated with capsular biopolymer in locations within the treatment plant. The loads were calculated by multiplying the values in Table 4.36 by the volatile suspended solids loads calculated from mass balance (Figure 4.21). The calculated loads of copper and zinc in the primary sedimentation tanks outflows (primary effluent and primary sludge) were about 40 % of the calculated loads in the inflow. In the activated sludge process, the calculated loads of copper and zinc in the waste secondary sludge were, respectively, 50 % more and 50 % less than the calculated loads coming from primary effluent. Calculated loads of copper and zinc in the thickener's feed were almost the same as the calculated loads leaving the thickener, in thickened sludge and supernatant. In the

Table 4.43 Percentage of Metals in Capsule and Slime Biopolymers in Relation to Total Metals in Samples

Location	Copper		Zinc		Lead		Cadmium		Chromium	
	Capsule	Slime	Capsule	Slime	Capsule	Slime	Capsule	Slime	Capsule	Slime
Influent	8.4	16	5.1	18	10	< LD	< LD	< LD	1.2	< LD
Prim Effl	2.8	20	3.7	45	< LD	< LD	< LD	< LD	< LD	< LD
Prim Sludge	3.9	0.5	3.6	2.3	4.0	< LD	< LD	< LD	NM	NM
Act Sludge	6.9	0.8	4.3	4.4	5.2	< LD	7.7	< LD	< LD	< LD
WAS	3.2	0.1	3.8	NM	7.9	NM	11.7	NM	NM	NM
Thick Sludge	5.9	0.1	4.4	0.1	6.9	< LD	4.8	< LD	7.0	< LD
Dig Sludge	2.7	0.1	2.2	0.1	2.5	< LD	2.1	< LD	1.1	< LD
Overflow	1.8	7.1	1.8	24	3.3	< LD	< LD	< LD	< LD	< LD
Supernatant	1.6	1.0	1.1	1.8	3.7	< LD	4.6	< LD	< LD	< LD
Filtrate	1.0	0.7	1.6	2.7	2.6	< LD	7.7	< LD	2.4	< LD

NM = Not measured;
< LD = below detection limit

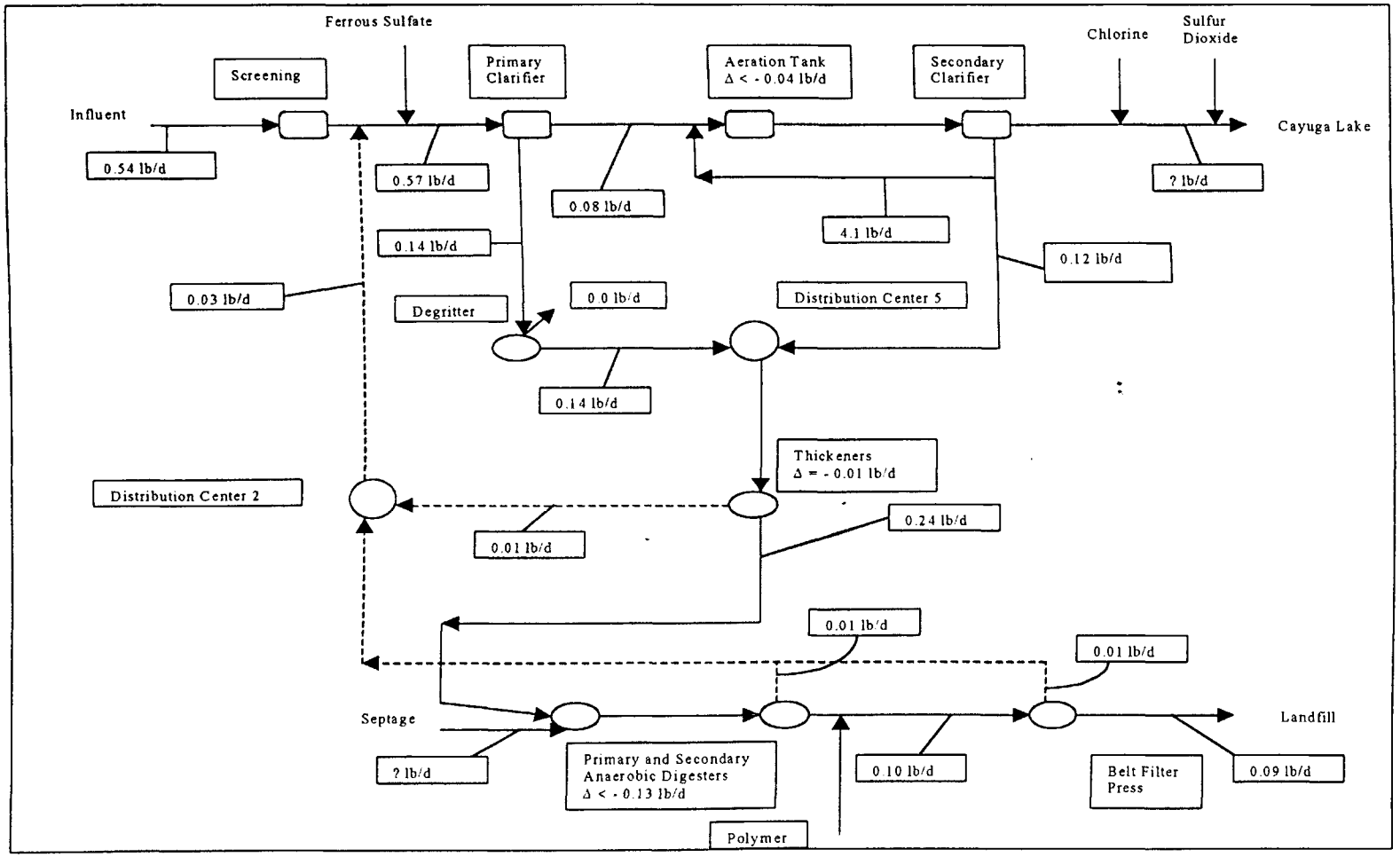

Figure 4.43 Mass Balance for Copper Associated with Solids-Bound Biopolymer at the Ithaca Wastewater and Sludge Treatment Plant – Average Values for June, July and August 1998.

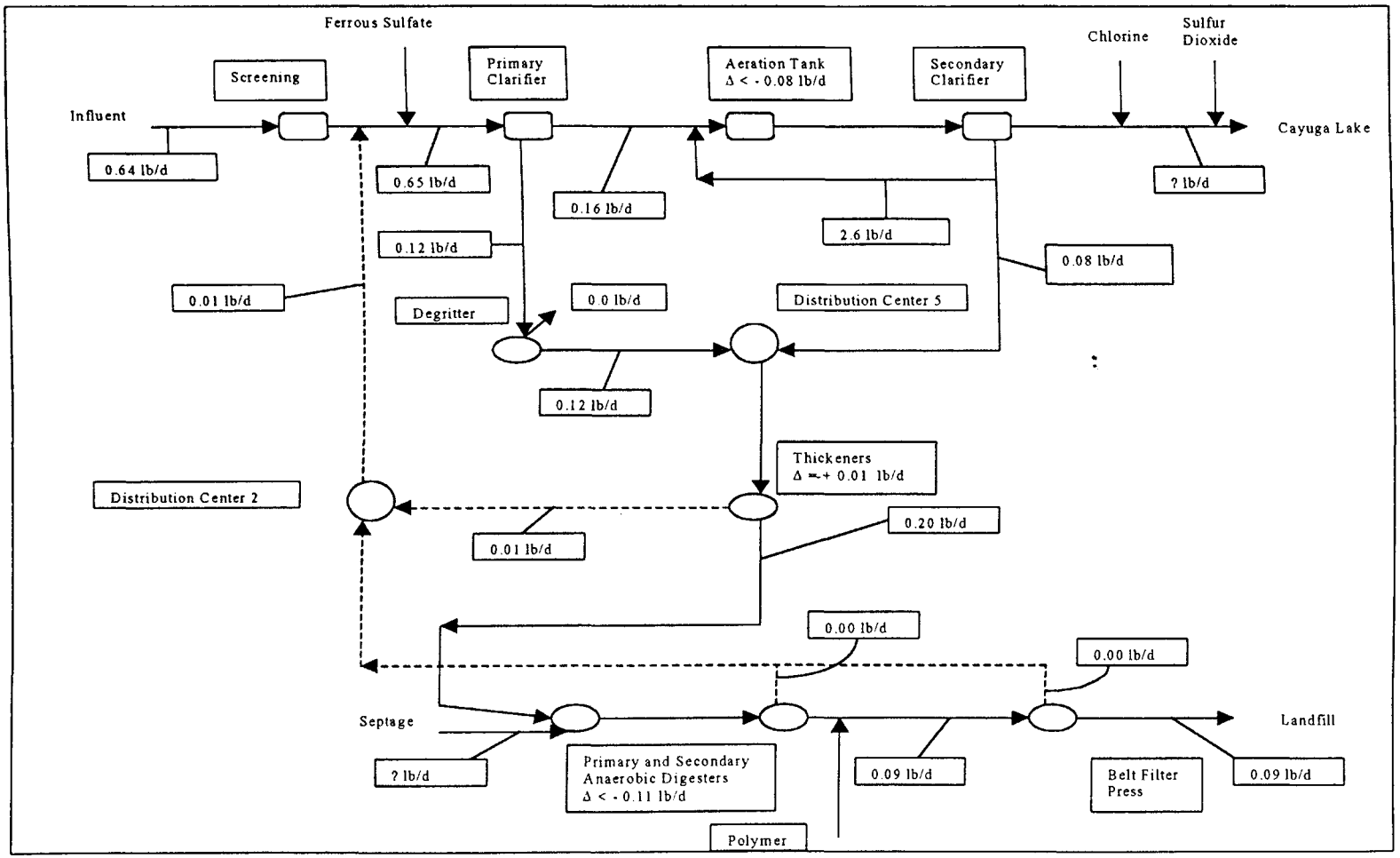

Figure 4.44 Mass Balance for Zinc Associated with Solids-Bound Biopolymer at the Ithaca Wastewater and Sludge Treatment Plant – Average Values for June, July and August 1998.

anaerobic digesters, the calculated loads of copper and zinc leaving the secondary digester through thickened digested sludge and supernatant were approximately 45 % lower than the calculated loads in thickened sludge. Although mass balances were not achieved, the differences between the loads in input and output to any process were in the range of zero to 50 %, which was considered reasonable. Mass balances for total metals (Section 4.5.1) showed differences in the range of zero to 70 %.

4.5.5 *Summary of Findings for Trace Metals*

The study of trace metals in samples and in biopolymers extracted from samples collected at 11 locations at the Ithaca Wastewater and Sludge Treatment Plant allowed the following conclusions:

1) The digested sludge can be classified as having exceptional quality, by EPA standards, with respect to trace metals contents;

2) Input and output loads of trace metals to each process differed in the range of zero to 70 %;

3) The trace metal content of capsular biopolymer was greater in wastewater than in any other location within the plant;

4) Biopolymer composition and ratio between total mass of individual metals in samples to mass of capsular biopolymer were not the reasons for the observed differences described in 3);

5) The hypotheses that the copper, zinc, and lead contents of capsular biopolymers in activated, thickened, and digested sludges were the same were not rejected by the Fischer's Least Significance Difference test;

6) Trace metals associated with capsular biopolymers represented 10 % or less of the metals content of samples;

7) Trace metals associated with slime represented less than 0.1 % of the metals content in samples of thickened and digested sludge; on the other hand, in samples with low suspended solids concentrations, the percentage of metals in slime represented up to 45 % of the metals in the samples;

8) Trace metals measured in samples, but not associated with capsular or slime biopolymers, could have left the dialysis bags either in association with low molecular weight biopolymers or in solution during the purification step of the biopolymer extraction. Trace metals could also be associated directly with the solids fraction of the samples, or they could have exchanged with sodium during the extraction procedure;

9) Input and output loads of trace metals associated with capsular biopolymers to each process differed in the range of zero to 50 %.

4.6 Study Limitations

4.6.1 *Limitations on Sampling*

The characterization of biopolymers relied on the collection of grab samples at selected locations at the Ithaca Wastewater and Sludge Treatment Plant. Nine main locations within the plant were sampled once in each of the months of June, July and August, with an additional sample taken at the beginning of the month of October. Two additional locations, primary and secondary sludges, were collected once. Samples were collected during daytime, between 10 to 11 am. Overflow from thickeners and filtrate from belt filter press were being produced during sampling, but the anaerobic digester was not always releasing supernatant.

No restraint on variations in the composition of wastewater coming into the plant could be made. Also, no control over any of the plant's operational procedures could be exercised. In order to capture how these variations could have caused

changes in biopolymer composition, a much more detailed sampling program would be required. This was beyond the possibilities of this investigation.

Samples were not collected simultaneously and consideration of hydraulic residence time was given to primary effluent only. In this case, the collection of the effluent sample awaited the time required for primary feed to flow through the primary sedimentation tanks. Because the mean cell residence times of activated sludge and digested sludge were approximately 8.8 and 28 d, respectively, the suspended solids in samples collected in those locations represented long-term conditions. On the other hand, the dissolved solids in samples represented conditions prevailing the day the sample was collected.

It is recognized that composite sampling is a better alternative than grab sampling, if variations in composition are expected to occur with time. A grab sample is composed by a volume withdrawn from the flow in a very short, almost instantaneous, period of time. On the other hand, a composite sample is formed by the addition of individual volumes of samples withdrawn from the flow over an extended period of time (Chapman, 1992). However, the gain obtained in representativeness by using composite sampling could have been offset by changes in biopolymer characteristics during the holding period. Influent was a typical location where composite sample would have been preferable. However, the process of concentration of solids and polymer extraction for the influent required 8 to 14 h, which would be added to the hours taken to collect the composite sample.

The small number of samples collected couldn't capture all variations that might have occurred in the treatment plant. However, by gathering three samples from the same locations in a three month period, some of the variation could be incorporated into a confidence interval for the mean. Also, the application of the Two-Factor Analysis of Variance to the data showed that the sampling periods in which the

samples were collected were not significantly related to the analytical values measured.

4.6.2 *Limitations of the Chemical Analysis*

4.6.2.1 Limitations of the colorimetric methods

Chemical analyses such as the anthrone and the Folin-Lowry methods require the use of standards to prepare a linear regression equation relating absorbancies with concentrations. A compound needs to be chosen as the method standard. For example, the carbohydrate glucose and the protein bovine serum albumin are suitable standards for the anthrone and the Folin-Lowry methods, respectively (Daniels, 1994).

When a sample is treated for carbohydrate analysis, for example, the homopolysaccharides and polysaccharides present in the sample are first broken down into their monomers constituents, e.g., glucose, mannose, fructose and galactose. These monomers react with the anthrone reagent to produce colored compounds. The color intensities that monomers other than glucose develop after reacting with anthrone are different than the intensity of the color resulting when glucose (the standard) reacts with anthone. If the color intensity is smaller than that from glucose, the test will underestimate carbohydrate content, while the opposite occurs if the color is more intense than the one developed by glucose.

4.6.2.2 Limitations of the humic acids method

The method used to estimate humic acids in biopolymers was an approximation only, since it used the protein quantity calculated in one method (Kjeldahl) to estimate the equivalent absorbance that that quantity would have in another method for protein analysis (Folin-Lowry). With this procedure, it is possible to decompose a sample absorbance into protein and humic acid absorbancies. The

humic acids can, then, be estimated from a regression equation relating absorbance and concentration of humic acids.

4.6.2.3 Limitations of the protein method

Protein was estimated as the product between the organic nitrogen concentration measured by the Kjeldahl method and the conversion factor 6.25. This conversion factor was used because organic nitrogen is, on average, 16 % of the protein content (Sawyer et al., 1994). However, other conversion factors may apply depending on the main source of organic nitrogen. For example, Skoog et al. (1994) suggest the conversion factors 5.70, 6.25 and 6.38 for cereals, meat and dairy products respectively.

4.6.2.4 Limitations on trace metal estimation

The metals copper, zinc, lead and cadmium that were originally bound to biopolymers could have been exchanged with sodium from the cation exchange resin during the extraction procedure. This could explain, in part, the relatively small quantity of metals measured in the biopolymers, relative to the whole sample. However, the extent of the exchange is unknown. Chromium was present in capsule in about the same amount as the other metals, i.e. less than 10 % (Table 4.43). One of the oxidation states of chromium, Cr (VI), occurs as cromate, CrO_4^{2-}, and dicromate, $Cr_2O_7^{2-}$, in the neutral pH range (Patterson, 1972). These forms of chromium can be removed only by an anion exchange resin (Patterson, 1972). On the other hand, at neutral pH, Cr (III), the other oxidation state of chromium that can occur in aqueous systems, hydrolyzes to form soluble cationic complexes such as $Cr_3(OH)_4^{5+}$ (Clifford, 1990). In this case, chromium can be removed by a cation exchange resin (Patterson, 1972). The form of chromium present in samples, capsular and slime biopolymers is

not known. If the main oxidation state of chromium was Cr (VI), it would mean that exchange with the resin was probably not important in causing the small percentage of metals associated with capsular biopolymers. On the contrary, if Cr (III) was the major form of chromium, then the results suggest that exchange was important.

4.6.3 *Limitations in the Statistical Analysis*

Analysis of Variance was used in this study to test the equality of variable means at nine locations in the Ithaca Wastewater and Treatment Plant (e.g. Section 4.2.2). When ANOVA rejected the hypothesis that the means in the nine locations were equal, Fischer's Least Significant Difference was used to make pairwise comparison between means. The locations where the equality hypothesis could not be rejected were grouped together as homogeneous groups (e.g. Section 4.2.3).

The Fischer's LSD test is one in a group of tests that could be used with the same purpose. For example, Bonferroni Adjustment and Tukey's Least Significant Difference are two other tests that perform pairwise comparison between means. In practice, Fischer's LSD, Bonferroni Adjustment, and Tukey's LSD tests differ in how large the difference between the means must be before the hypothesis that the means are equal is rejected. The difference between the means before the null hypothesis (equal means) is rejected increases from Fischer to Tukey and to Bonferroni tests. As an illustration, the homogeneous groups for the weight ratios of capsular biopolymer carbohydrate to volatile suspended solids were, according to Fischer's LSD, Tukey's LSD, and Bonferroni tests, as follows:

Fischer's group 1: primary effluent, digested sludge, digester supernatant;

Tukey's group 1: same as Fischer's, plus secondary effluent, thickener overflow and BFP filtrate;

Bonferroni's group 1: same as Tukey's, plus influent;

Fischer's group 2: influent, primary effluent, secondary effluent, thickener overflow, digested sludge, BFP filtrate;

Tukey's group 2: same as Fischer's, plus activated sludge and thickened sludge;

Bonferroni's group 2: same as Tukey's;

Fischer's group 3: influent, activated sludge and thickened sludge;

Tukey's group 3: none;

Bonferroni's group 3: none

In summary, different results can be obtained depending on the choice of the test used to make pairwise comparisons between means once ANOVA rejected the hypothesis that the means of all samples were equal. Fischer's LSD was chosen in this study because it was the test that rejected equality with smaller difference between the means.

4.6.4 *Limitations of the Mass Balance*

In order to perform mass balances, it was necessary to know (1) flowrates, (2) suspended solids concentrations, and (3) parameters such as yield coefficient, biodegradability, and endogenous decay. As described in Appendix F, the sources of data were (1) records from the plant, (2) analyses in samples collected for this study, and (3) literature. Each of these sources contained uncertainties. Records from the plant were incomplete, the number of samples analyzed were limited, and values in the literature varied within a certain range. In addition, the equations used to calculate the concentrations of suspended solids in activated sludge and anaerobic digestion processes were based on assumptions that might oversimplify the complexity of the reactions taken place. For this reason, results obtained from the mass balance calculations were only approximations of the true values.

Chapter 5: Conclusions and Recommendations

Extracellular polymers produced by bacteria constitute part of the solids pool found in wastewater and sludge treatment plants. They have important roles in the processes of flocculation, sludge dewatering, and trace metals adsorption in treatment systems. In addition, the presence of biopolymers in secondary effluents is of concern for reasons ranging from toxicity to soil clogging in aquifer recharge systems.

The literature review section identified lack of knowledge with respect to quantitative and qualitative characterization of biopolymers within wastewater and sludge treatment plants. Biopolymers have been more frequently studied in activated sludge, but, even there, their physical and chemical characterizations are incomplete. Fewer investigations have been made in anaerobic digested sludge, and essentially nothing is known about the composition of polymers in influent and in other locations at treatment plants.

This research was designed to provide a more complete characterization of biopolymers in a full-scale wastewater and sludge treatment plant. Nine main locations within the treatment plant were sampled three times during the months of June, July and August of 1998, with an additional collection from selected locations at the beginning of October. The characterization of biopolymers included analyses of carbohydrate, protein, RNA, DNA, humic acids, total organic carbon, molecular weight, ethanol insoluble matter and five trace metals. Several biopolymer extraction methods and analytical procedures for chemical analyses were considered and evaluated. An existing biopolymer extraction method was modified to remove non-biopolymer components from the extract. A procedure for humic acids estimation in the presence of protein was developed.

This investigation showed that the organic fraction of extracellular polymers in wastewater and sludge treatment is composed by protein, carbohydrate, RNA, DNA, and humic acids. Protein was found to be the major biopolymer component in all locations, comprising approximately 50 % by weight of the polymer. The second major compound was RNA. Protein and RNA are also the two major cell constituents, suggesting that intracellular material, either through excretion or lysis, forms part of the biopolymers. Humic acids, which are produced as a result of organic matter decomposition, were also found to be a polymer constituent. In addition to organic compounds, an inorganic fraction also was present in biopolymers, comprising 10 % to 20 % of their weight.

Analysis of Variance rejected the hypothesis that the mean weight ratios of solids-bound biopolymer carbohydrate, protein, RNA and TOC to volatile suspended solids were the same in all locations at the treatment plant. Fischer's Least Significant Difference test rejected the hypothesis that the weight ratios of capsular biopolymer TOC to volatile suspended solids of activated sludge and secondary effluent were the same. On the other hand, Fischer's LSD test did not reject the hypothesis that the weight ratios of capsular TOC to VSS of secondary effluent, digested sludge and digester supernatant were the same. Also, the hypothesis that the weight ratios of capsular TOC to VSS of primary effluent, secondary effluent, digested sludge and belt filter press filtrate/washwater were the same was not rejected. This might be an indication that particles from the sludge treatment processes are being carried out into the secondary effluent.

Although the hypothesis of equal weight ratios of capsular biopolymer constituents to volatile suspended solids at various locations was rejected, ANOVA didn't reject the hypothesis that the fractions of each chemical compound in the biopolymers at six different locations were the same. This result suggests that,

although the amounts of solids-bound biopolymer change, their composition remains the same throughout the treatment plant.

Protein also was the major constituent of slime biopolymers, but the ratio of protein to carbohydrate in slime was lower than the ratio in capsular biopolymer. The concentration of slime constituents was higher than the concentration of capsular constituents in samples with low suspended solids concentrations, such as influent and secondary effluent, but were lower in samples with higher suspended solids concentrations, such as activated, thickened and digested sludges.

X-ray analyses showed that the elemental composition of capsule did not change in different locations of the treatment plant, while some variations were detected in slime. Phosphorus and sulfur were the elements with higher peaks, respectively, for solids-bound and loosely-bound biopolymers.

Biopolymers molecular weight distributions were measured by size-exclusion chromatography. The chromatograms showed the presence of molecules with sizes greater than 2×10^6 dalton, and smaller than 1.5×10^5 dalton. Separation of molecules between these two sizes was not accomplished. The higher molecular weight fraction is in accordance with the property by which biopolymers bridge negatively charged particles, a mechanism that leads to floc formation.

Flowrate and total and volatile suspended solids mass balances were performed using data provided by the plant, collected for this research, and taken from the literature. It was estimated that the return flows from sludge treatment processes – thickener overflow, digester supernatant, and belt filter press – constituted approximately 9 % and 13 % of the influent flowrate and total suspended solids load, respectively.

Capsular biopolymers mass balances showed net production of protein, carbohydrate, RNA, and humic acids in the activated sludge process. The productions

varied in the range of 25 % to 40 % of the loads coming from primary effluent. On the other hand, during anaerobic digestion, 60 % to 70 % of the capsular protein, carbohydrate, RNA, DNA, and humic acids present in the anaerobic digester feed were destroyed. This result suggests that fractions of capsular carbohydrate, protein, DNA, RNA, and humic acids are resistant or are not available for biodegradation, at least under the conditions prevailing in the digester.

Biopolymer mass balance in the primary sedimentation tanks indicated that particles from primary sludge should hold more biopolymers per unit of volatile suspended solids than particles from primary effluent. However, this hypothesis was not confirmed in one measurement made with primary sludge capsular biopolymers. The measured weight ratios of biopolymer constituents to volatile suspended solids in primary sludge were about the same as the ratios in primary effluent biopolymers.

The trace metals copper, zinc, lead, cadmium and chromium were detected in capsular biopolymers. The trace metals content of capsular biopolymer was greater in wastewater than in any other location of the treatment plant. This finding could not be correlated with biopolymer composition, or the ratios between the total mass of individual metals in samples to mass of capsular biopolymers.

Copper and zinc were present in slime biopolymers, while lead, cadmium and chromium were below their detection limits. Trace metals associated with slime represented less than 0.1 % of the metals in samples with high suspended solids concentrations, such as thickened and digested sludge. In samples with low suspended solids concentrations, the percentage of metals associated with slime reached 45 %. Trace metals associated with capsular biopolymers represented less than 10 % of the metals content of samples.

Mass balances for metals in samples and capsular biopolymers were not achieved. Input and output loads of trace metals to each treatment process showed

differences in the range of zero to 70 %, and zero to 50 %, for metals in samples and metals associated with capsular biopolymers, respectively.

A major limitation in this research was the small number of samples collected in each location. A concern exists with respect to how well the three samples that were collected in each location represented the characteristics of the wastewater or sludge during the three-month period in which the samples were collected. Some of the variation that could have occurred in the characteristics of the samples were incorporated into a confidence interval for the mean. Also, the application of the Two-Factor Analysis of Variance to the data showed that the sampling period in which the samples were collected was not significantly related to the analytical values measured. A recommendation for future research would be to monitor biopolymer composition in a more extended period of time than used in this research, with more frequent sampling. Such a program would increase the confidence level for the results.

It would be advantageous to have a more efficient procedure than batch centrifugation to concentrate solids from those sites with low concentrations of suspended solids. This is especially the case for secondary effluent, where suspended solids concentrations in the range 2 to 3 mg/l demanded great volumes of sample, as well as long periods of centrifugation in order to collect a minimum amount of solids for biopolymer extraction. One method that could be employed is continuous centrifugation.

Very little information exists about lipids in biopolymers. Gravimetric methods evaluated in this study were deemed unsuitable for lipid analysis in biopolymers due to the minimum weight of lipids required. A colorimetric method tested showed low sensitivity and the use of a compound as standard that was not meaningful for biopolymers. It is recommended that chromatographic techniques, such as gas-liquid

chromatography, be investigated as alternative methods to analyze lipids in biopolymers.

Some possibilities for future research on biopolymers include:

(1) Monitor biopolymer quantity and composition over an extended period of time, with more frequent sampling;

(2) Develop a model relating biopolymer composition and mean cell residence time in activated sludge and anaerobic digester processes;

(3) Investigate the partitioning of trace metals between capsule and non-capsule in suspended solids and between slime and non-slime in dissolved solids;

(4) Prepare adsorption isotherms for trace metals in biopolymers extracted from different locations at the treatment plant;

(5) Study flocculative properties of biopolymers in different locations at the treatment plant;

(6) Investigate changes in biopolymer composition and quantity with shifts in microbial population equilibrium in activated sludge;

(7) Investigate methods to reduce sand or soil clogging by biopolymers resulting from application of secondary effluents to aquifer recharge fields;

(8) Investigate toxic effects associated with the presence of biopolymers in secondary effluents;

(9) Investigate sludge dewatering properties with changes in biopolymer composition and quantity;

(10) Investigate the reasons for the apparent trace metal desorption from capsular biopolymer as wastewater enters the treatment plant;

(11) Study biopolymer composition in biological treatment of industrial wastes, and the relationship between composition, flocculation, and dewaterability of the sludges;

(12) Investigate the trace metals content of biopolymers using a calcium specific chelate to extract capsular biopolymers, and compare the results with biopolymers extracted with the cation exchange resin;

(13) Investigate the apparent preferential settling of particles holding more polymers in relation to particles with less polymers.

APPENDIX A

MASS OF VOLATILE SUSPENDED SOLIDS FROM WHICH SOLIDS-BOUND BIOPOLYMERS WERE EXTRACTED

EXTRACTION BUFFER VOLUME

Table A.1 description: Table A.1 presents the quantities of volatile suspended solids that were used for extraction of the solids-bound biopolymers. For example, 1,444 mg of volatile suspended solids were used for extraction of capsular biopolymers in the activated sludge sample that was part of the first set of samples.

The period encompassing each set was (see also Table 3.1) :

First Set: June 04 to June 19, 1998;

Second Set: June 29 to July 13, 1998;

Third Set: August 06, August 20, 1998;

Fourth Set: October 2, 1998.

Table A.1 Mass of Volatile Suspended Solids From Which Solids-Bound Biopolymers Were Extracted (mg)

Location	First	Second	Third	Fourth
Influent	1789	973	1927	
Primary Effluent	570	752	1103	
Primary Sludge			1955	
Activated Sludge	1444	1760	1858	1544
Secondary Sludge				2940
Secondary Effluent	32	44	15.8	
Thickened Sludge	2259	2393	2450	
Digested Sludge	2180	3921	3356	
Thick. Overflow	614	992	880	
An Dig Supernatant	1732	1865	1649	
BFP Filtrate	1139	1803	1869	

Table A.2 description: In solids-bound biopolymers extraction, the sample volatile suspended solids were separated by centrifugation, and then resuspended in an extraction buffer. The volume of the buffer was less than the original volume from which the solids had been separated. This was a way to concentrate biopolymers in order to have the chemical compounds in the biopolymers in quantities above the limit of detection for the analytical methods that were being used.

Table A.2 - Extraction Buffer Volume (ml)

Location	First	Second	Third	Fourth
Influent	300	230	300	
Primary Effluent	300	300	250	
Primary Sludge			300	
Activated Sludge	400	300	300	300
Secondary Sludge				300
Secondary Effluent	100	42	35	
Thickened Sludge	300	300	300	
Digested Sludge	300	300	300	
Thick. Overflow	250	250	250	
An Dig Supernatant	300	300	300	
BFP Filtrate	300	300	300	

APPENDIX B

MUTUAL INTERFERENCE OF PROTEIN AND HUMIC ACIDS

Table B.1 description: Table B.1 presents the absorbancies of standards solutions of protein and humic acids that were subjected to the Folin-Lowry method for protein analysis. Table B.1 shows that, in addition to protein, humic acids also react with the Folin-Lowry reagent.

Table B.1 Absorbance of Protein and Humic Acids

Standard	Absorbance	
(mg/l)	Protein	Humic Acids
0	0.026	0.029
25	0.104	0.071
50	0.196	0.107
75	0.247	0.148
100	0.308	0.177
125	0.377	0.216
150	0.443	0.248
175	0.484	0.277
200	0.543	0.290
225	0.573	0.335
250	0.638	0.366

Table B.2 description: Table B.2 shows the mutual interference that occurs when protein and humic acids are present in the same sample. The first two columns show the concentrations of standards that were prepared. For example, the second standard had 25 mg/l protein and 225 mg/l humic acids; the seventh standard had 150 mg/l protein and 100 mg/l humic acids. The third column in Table B.2 shows the sum of the individual absorbancies of protein and humic acids, which was presented in Table B.1. For example, 25 mg/l protein had absorbance of 0.104, while 225 mg/l humic acids had absorbance of 0.335. The sum equals 0.439. The fourth column in Table B.2 presents the values of the absorbancies that were actually measured for the mixtures of protein and humic acids. The absorbancies of the mixtures were a fraction only of the absorbancies that were measured when the concentrations of protein and humic acids were measured separately. For example, the absorbance of the mixture 25 mg/l protein with 225 mg/l humic acids was 0.412 instead of 0.439, which had been measured when 25 mg/l protein and 225 mg/l humic acids were separated. The last column in Table B.2 shows the ratio between the values in columns 4 and 3. The information provided by Table B.2 was used to quantify the presence of humic acids in biopolymers (see Section 3.3.3).

Table B.2 Absorbance of Mixtures of Protein and Humic Acids

Standards (Mixture)		Absorbance		Ratio
Protein	Humic Acids	Sum of Individuals	Measured	
25	225	0.439	0.412	0.939
50	200	0.486	0.412	0.848
75	175	0.524	0.443	0.845
100	150	0.556	0.498	0.896
125	125	0.593	0.527	0.889
150	100	0.620	0.550	0.887
175	75	0.632	0.582	0.921
200	50	0.650	0.598	0.920
225	25	0.644	0.624	0.969

APPENDIX C

SOLIDS CONCENTRATION, pH, AND CONDUCTIVITY VALUES

Tables description: Tables C.1, C.2, and C.3 present, respectively, the concentration of solids, pH and conductivity values measured at the locations where samples were collected at the Ithaca Wastewater and Sludge Treatment Plant. The dates each sample were collected are presented in Table 3.1 of Chapter 3. The period encompassing each set was:

First Set: June 04 to June 19, 1998;

Second Set: June 29 to July 13, 1998;

Third Set: August 06, August 20, 1998;

Fourth Set: October 2, 1998.

The acronyms in Table C.1 have the following meaning:
TS = total solids; FTS = fixed total solids; VTS = volatile total solids; TSS = total suspended solids; FSS = fixed suspended solids; VSS = volatile suspended solids; TDS = total dissolved solids; FDS = fixed dissolved solids; VDS = volatile dissolved solids

The sludge volume index values for activated sludge were 76.4 ml/g (first set), 64.3 ml/g (second set), 161 ml/g (third set), and 170 ml/g (fourth set).

Table C.1 Solids Concentration in Samples (Units: mg/l)
(First Set of Samples)

Sample	TS	FTS	VTS	TSS	FSS	VSS	TDS	FDS	VDS
Influent	578	383	195	101	17	84	477	366	111
Primary Effluent	539	391	148	58	14	44	481	377	104
Primary Sludge									
Activated Sludge	1,731	793	938	1,178	376	802	553	417	136
Secondary Sludge									
Secondary Effluent	516	397	119	3.0	0.9	2.1	513	396	117
Thickened Sludge	39,395	10,245	29,150	37,336	9,097	28,239	2,059	1,148	911
Digested Sludge	42,047	19,525	22,522	39,021	17,218	21,803	3,026	2,307	719
Thickener Overflow	661	458	203	73	18	55	588	440	148
Digester Supernatant	7,663	3,180	4,483	6,010	2,161	3,849	1,653	1,019	634
BFP Filtrate	2,132	1,438	694	1,176	543	633	956	895	61

Table C.1 Solids Concentration in Samples (Units: mg/l)
(Second Set of Samples)

Sample	TS	FTS	VTS	TSS	FSS	VSS	TDS	FDS	VDS
Influent	560	314	246	123	19	104	437	295	142
Primary Effluent	503	380	123	54	15	39	449	365	84
Primary Sludge									
Activated Sludge	1,476	662	814	933	281	652	543	381	162
Secondary Sludge									
Secondary Effluent	540	384	156	2.3	0.1	2.2	538	384	154
Thickened Sludge	47,654	12,356	35,298	45,055	10,874	34,181	2,599	1,482	1,117
Digested Sludge	47,208	20,372	26,836	45,607	19,464	26,143	1,601	908	693
Thickener Overflow	613	385	228	79	15	64	534	370	164
Digester Supernatant	2,862	1,369	1,493	1,483	353	1,130	1,379	1,016	363
BFP Filtrate	1,360	891	469	442	227	215	918	664	254

Table C.1 Solids Concentration in Samples (Units: mg/l)
(Third Set of Samples)

Sample	TS	FTS	VTS	TSS	FSS	VSS	TDS	FDS	VDS
Influent	953	473	480	197	36	161	756	437	319
Primary Effluent	613	442	171	79	20	59	534	422	112
Primary Sludge	1,758	885	873	1,127	403	724	631	968	149
Activated Sludge	1,455	633	822	931	243	688	524	390	134
Secondary Sludge									
Secondary Effluent	494	387	107	2.1	0.7	1.3	492	386	106
Thickened Sludge	25,961	6,841	19,120	23,784	5,654	18,130	2,177	1,187	990
Digested Sludge	58,774	24,608	34,166	56,859	23,299	33,560	1,915	1,309	606
Thickener Overflow	692	512	180	104	23	81	588	489	99
Digester Supernatant	2,826	1,594	1,232	1,276	360	916	1,550	1,234	316
BFP Filtrate	2,108	1,371	737	1,183	560	623	925	811	114

Table C.1 Solids Concentration in Samples (Units: mg/l)
(Fourth Set of Samples)

Sample	TS	FTS	VTS	TSS	FSS	VSS	TDS	FDS	VDS
Influent									
Primary Effluent									
Primary Sludge									
Activated Sludge				1,178	287	858			
Secondary Sludge				4,352	1,085	3,267			
Secondary Effluent									
Thickened Sludge									
Digested Sludge									
Thickener Overflow									
Digester Supernatant									
BFP Filtrate									

Table C.2 pH

Sample	First Set	Second Set	Third Set
Influent	7.5	7.4	7.1
Primary Effluent	7.2	7.4	-
Primary Sludge			6.9
Activated Sludge	7.0	7.1	6.8
Secondary Sludge			
Secondary Effluent	7.1	7.1	7.0
Thickened Sludge	6.1	5.7	5.9
Digested Sludge	6.9	7.2	7.4
Thickener Overflow	7.6	7.7	7.2
Digester Supernatant	7.8	7.7	7.7
BFP Filtrate	7.3	7.1	7.3

pH was not measured in activated and secondary sludges samples collected as the fourth set.

Table C.3 Conductivity (unit: μS/cm)

Sample	First Set	Second Set	Third Set
Influent	752	755	1,020
Primary Effluent	802	843	-
Primary Sludge			825
Activated Sludge	732	728	720
Secondary Sludge			
Secondary Effluent	742	797	760
Thickened Sludge	1,520	2,210	2,510
Digested Sludge	7,760	7,790	8,110
Thickener Overflow	943	917	957
Digester Supernatant	6,390	8,200	6,300
BFP Filtrate	2,850	2,890	3,810

Conductivity was not measured in activated and secondary sludges samples collected as the fourth set.

APPENDIX D

LIMITS OF DETECTION OF ANALYTICAL METHODS

Table D.1 description: Table D.1 presents the limits of detection of the analytical methods. The limit of detection was calculated using the following equation (see also Section 3.4):

$$C_{min} = \frac{A_{min} - \overline{A}_{blank}}{m}$$

where, C_{min} = minimum distinguishable concentration,

A_{min} = minimum distinguishable absorbance,

$\overline{A}_{blank}$ = blank mean absorbance,

m = slope of the calibration curve

The minimum distinguishable absorbance is given by the blank mean absorbance plus three times the standard deviations of the mean (Skoog and Leary, 1992). The blank mean absorbance is the mean absorbance of five blanks. For example, the limit of detection for carbohydrate in the first set was calculated as follows:

Blank 1 absorbance: 0.003

Blank 2 absorbance: - 0.004

Blank 3 absorbance: 0.004

Blank 4 absorbance: 0.015

Blank 5 absorbance: 0.005

The mean and standard deviation for the blank absorbances were 0.0046 and 0.0068, respectively. Hence, the minimum distinguishable absorbance was 0.0046 + 3 x 0.0068 = 0.025. Since the slope of the calibration curve was 0.0111, the minimum distinguishable concentration was (0.025 – 0.0046)/0.0111 = 1.9 mg/l.

The limit of detection in each set changed because absorbancies of blanks varied every time new batches of samples were prepared. Each batch encompassed one set of samples.

Table D.1 Limits of Detection (mg/l)

Compound	First	Second	Third	Fourth	Mean
Carbohydrates	1.9	0.6	1.0	0.4	0.9
Humic Acids	0.9	5.8	3.9	1.0	2.9
RNA	2.8	1.5	2.1	6.2	3.1
DNA	15.4	6.1	10.3	11.4	10.8
TOC	1.2	3.0	1.3	1.0	1.6

Table D.2 to D.6 Description: For solids-bound biopolymers, unit mass limits of detection were calculated. This was possible through use of information contained in Tables A.1, A.2 and D.1. Considering again the first set of carbohydrates as an example. The limit of detection was 1.9 mg/L; the mass of solids used to extract solids-bound biopolymers was 1,789 mg VSS; the volume of buffer used to resuspend the solids was 300 ml. The unit mass limit of detection was [(1.9 mg/L) x (0.3 L)]/(1.789 g VSS) = 0.3 mg carb/g VSS.

Table D.2 Unit Mass Limits of Detection - Carbohydrate (Unit: mg carbohydrate/g volatile solids)

Location	First	Second	Third	Fourth
Influent	0.3	0.1	0.1	
Primary Effluent	1.0	0.2	0.2	
Primary Sludge			0.1	
Activated Sludge	0.5	0.1	0.2	0.1
Secondary Sludge				0.0
Secondary Effluent	5.8	0.6	2.1	
Thickened Sludge	0.2	0.1	0.1	
Digested Sludge	0.3	0.0	0.1	
Thick. Overflow	0.8	0.2	0.3	
An Dig Supernatant	0.3	0.1	0.2	
BFP Filtrate	0.5	0.1	0.2	

Table D.3 Unit Mass Limits of Detection - Humic Acids (Unit: mg HA/g volatile solids)

Location	First	Second	Third	Fourth
Influent	0.2	1.4	0.6	
Primary Effluent	0.5	2.3	0.9	
Primary Sludge			0.6	
Activated Sludge	0.2	1.0	0.6	0.2
Secondary Sludge				0.1
Secondary Effluent				
Thickened Sludge	0.1	0.7	0.5	
Digested Sludge	0.1	0.4	0.3	
Thick. Overflow	0.4	1.5	1.1	
An Dig Supernatant	0.2	0.9	0.7	
BFP Filtrate	0.2	1.0	0.6	

Table D.4 Unit Mass Limits of Detection - Ribonucleic Acid (Unit: mg RNA/g volatile solids)

Location	First	Second	Third	Fourth
Influent	1.3	0.3	0.3	
Primary Effluent	3.9	0.5	0.5	
Primary Sludge			0.3	
Activated Sludge	2.1	0.2	0.3	1.3
Secondary Sludge				0.7
Secondary Effluent	8.8	1.3	4.6	
Thickened Sludge	1.0	0.2	0.3	
Digested Sludge	1.0	0.1	0.2	
Thick. Overflow	3.0	0.3	0.6	
An Dig Supernatant	1.3	0.2	0.4	
BFP Filtrate	2.0	0.2	0.3	

Table D.5 Unit Mass Limits of Detection – Deoxyribonucleic Acid (Unit: mg DNA/g VSS)

Location	First	Second	Third	Fourth
Influent	2.6	1.4	1.6	
Primary Effluent	8.1	2.4	2.3	
Primary Sludge			1.6	
Activated Sludge	4.3	1.0	1.7	2.2
Secondary Sludge				1.2
Secondary Effluent	48.1	5.8	22.9	
Thickened Sludge	2.0	0.8	1.3	
Digested Sludge	2.1	0.5	0.9	
Thick. Overflow	6.3	1.5	2.9	
An Dig Supernatant	2.7	1.0	1.9	
BFP Filtrate	4.1	1.0	1.7	

Table D. 6 Unit Mass Limits of Detection – Total Organic Carbon (Unit: mg TOC/g VSS)

Location	First	Second	Third	Fourth
Influent	0.2	0.7	0.2	
Primary Effluent	0.6	1.2	0.3	
Primary Sludge			0.2	
Activated Sludge	0.3	0.5	0.2	0.2
Secondary Sludge				0.1
Secondary Effluent	3.6	2.8	2.8	
Thickened Sludge	0.2	0.4	0.2	
Digested Sludge	0.2	0.2	0.1	
Thick. Overflow	0.5	0.8	0.4	
An Dig Supernatant	0.2	0.5	0.2	
BFP Filtrate	0.3	0.5	0.2	

APPENDIX E

CHEMICAL ANALYSES

SOLIDS-BOUND BIOPOLYMERS (CAPSULE)

LOOSELY-BOUND BIOPOLYMERS (SLIME)

Tables E.1 to E.6 description: the values in Tables E.1 to E.6 were calculated using the following equation:

[(chemical concentration in buffer x buffer volume)/mass of VSS used in extraction]

For example, the concentration of carbohydrate in the first influent sample was 35.5 mg/L buffer. The buffer volume was 300 mL, and the mass of VSS used was 1.789 g. The content of carbohydrate was [(35.5 x 0.30/1.789] = 5.9 mg carb/g VSS.

Mass of VSS used and buffer volumes are presented in Tables A.1 and A.2 of Appendix A. The periods comprising each set are described in Appendix A.

Tables E.7 to E.12 description: the values in Tables E.7 to E.12 were calculated by multiplying the values from Tables E.1 to E.6 by the concentrations of VSS presented in Tables C.1 to C.4 of Appendix C. For example, the concentration of volatile suspended solids in the influent, first set, was 84 mg/l. Thus, the concentration of solids-bound biopolymer in the influent was 5.9 mg carb/g VSS x 84 x 10^{-3} g/L = 0.5 mg carb/L.

Tables E.13 to E.18 description: the values in Tables E.13 to E.18 are the concentrations of chemical compounds in loosely-bound biopolymers (slime).

Table E.1 Carbohydrate Content of Solids-Bound Biopolymer (Unit: mg carbohydrate/g volatile suspended solids)

Location	First	Second	Third	Fourth
Influent	5.9	7.5	6.1	
Primary Effluent	5.4	5.1	3.7	
Primary Sludge			4.0	
Activated Sludge	7.1	8.3	1.3	7.7
Secondary Sludge				7.9
Secondary Effluent	7.8	4.5	3.7	
Thickened Sludge	6.9	7.6	8.4	
Digested Sludge	4.2	5.2	5.2	
Thick. Overflow	4.2	6.5	6.1	
An Dig Supernatant	3.7	2.2	3.8	
BFP Filtrate	5.9	5.0	4.7	

Table E.2 Protein Content of Solids-Bound Biopolymers (Unit: mg protein/g volatile suspended solids)

Location	First	Second	Third	Fourth
Influent	29.8	27.7	21.0	
Primary Effluent	16.6	16.8	16.4	
Primary Sludge			16.6	
Activated Sludge	30.8	29.7	5.6	23.0
Secondary Sludge				25.7
Secondary Effluent	27.1	19.4	25.7	
Thickened Sludge	26.0	30.6	33.4	
Digested Sludge	16.4	17.1	19.2	
Thick. Overflow	17.8	31.2	20.4	
An Dig Supernatant	16.4	10.7	16.7	
BFP Filtrate	24.4	16.5	22.1	

Table E.3 Humic Acids Content of Solids-Bound Biopolymer (Unit: mg HA/g volatile suspended solids)

Location	First	Second	Third	Fourth
Influent	6.2	15.7	6.8	
Primary Effluent	9.0	3.4	< 0.9	
Primary Sludge			5.9	
Activated Sludge	8.8	11.8	0.3	3.9
Secondary Sludge				3.3
Secondary Effluent				
Thickened Sludge	5.2	5.2	7.8	
Digested Sludge	3.4	6.1	6.4	
Thick. Overflow	3.7	6.4	< 1.1	
An Dig Supernatant	6.6	6.4	5.4	
BFP Filtrate	3.7	8.7	11.6	

Table E.4 Ribonucleic Acid Content of Solids-Bound Biopolymer (Unit: mg RNA/g volatile suspended solids)

Location	First	Second	Third	Fourth
Influent	7.8	7.2	5.9	
Primary Effluent	6.2	5.2	5.6	
Primary Sludge			4.7	
Activated Sludge	14.0	9.2	2.5	8.3
Secondary Sludge				8.3
Secondary Effluent	< 8.8	7.2	5.1	
Thickened Sludge	11.9	8.2	11.0	
Digested Sludge	10.0	6.0	6.7	
Thick. Overflow	8.9	8.6	6.8	
An Dig Supernatant	7.2	3.0	6.9	
BFP Filtrate	6.2	5.9	6.9	

Table E.5 Deoxyribonucleic Acid Content of Solids-Bound Biopolymer (Unit: mg DNA/g volatile suspended solids)

Location	First	Second	Third	Fourth
Influent	4.2	5.5	2.6	
Primary Effluent	< 8.1	< 2.4	< 2.3	
Primary Sludge			5.1	
Activated Sludge	5.2	1.5	< 1.7	2.5
Secondary Sludge				2.2
Secondary Effluent	< 48.1	< 5.8	< 22.9	
Thickened Sludge	3.2	3.6	3.3	
Digested Sludge	< 2.1	1.5	2.8	
Thick. Overflow	< 6.3	< 1.5	< 2.9	
An Dig Supernatant	< 2.7	< 1.0	2.5	
BFP Filtrate	< 4.0	< 1.0	3.4	

Table E.6 Total Organic Carbon Content of Solids-Bound Biopolymer (mg TOC/g volatile suspended solids)

Location	First	Second	Third	Fourth
Influent	25.0	28.7	26.9	
Primary Effluent	24.0	18.3	16.2	
Primary Sludge			16.6	
Activated Sludge	24.0	26.0	5.0	19.9
Secondary Sludge				21.0
Secondary Effluent	17.1	12.8	16.0	
Thickened Sludge	23.2	27.5	28.8	
Digested Sludge	16.4	16.0	18.1	
Thick. Overflow	22.2	28.8	20.7	
An Dig Supernatant	15.6	11.4	14.2	
BFP Filtrate	23.3	15.1	21.3	

Table E.7 Solids-Bound Biopolymer Carbohydrate Concentration (Unit: mg/L, except secondary effluent, µg/L)

Location	First	Second	Third	Fourth
Influent	0.5	0.8	1.0	
Primary Effluent	0.2	0.2	0.2	
Primary Sludge			2.9	
Activated Sludge	5.7	5.4	0.9	6.6
Secondary Sludge				25.8
Secondary Effluent	16.4	9.9	5.0	
Thickened Sludge	194.8	259.8	152.3	
Digested Sludge	91.6	135.9	174.5	
Thick. Overflow	0.2	0.4	0.5	
An Dig Supernatant	14.2	2.5	3.5	
BFP Filtrate	3.7	1.1	2.9	

Table E.8 Solids-Bound Biopolymers Protein Concentration (Unit: mg/l, except secondary effluent, µg/L)

Location	First	Second	Third	Fourth
Influent	2.5	2.9	3.4	
Primary Effluent	0.7	0.7	1.0	
Primary Sludge			12.0	
Activated Sludge	24.7	19.4	3.9	19.7
Secondary Sludge				84.0
Secondary Effluent	56.9	42.7	34.4	
Thickened Sludge	734.2	1045.9	605.6	
Digested Sludge	357.6	447.0	644.4	
Thick. Overflow	1.0	2.0	1.7	
An Dig Supernatant	63.1	12.1	15.3	
BFP Filtrate	15.4	3.5	13.8	

Table E.9 Solids-Bound Biopolymers Humic Acids Concentration (Unit: mg/L)

Location	First	Second	Third	Fourth
Influent	0.5	1.6	1.1	
Primary Effluent	0.4	0.1	0.0	
Primary Sludge			4.3	
Activated Sludge	7.1	7.7	0.2	3.3
Secondary Sludge				10.8
Secondary Effluent	-	-	-	
Thickened Sludge	146.8	177.7	141.4	
Digested Sludge	74.1	159.5	214.8	
Thick. Overflow	0.2	0.4	0.0	
An Dig Supernatant	25.4	7.2	4.9	
BFP Filtrate	2.3	1.9	7.2	

Table E.10 Solids-Bound Biopolymers Ribonucleic Acids Concentration (Unit: mg/L, except secondary effluent, µg/L)

Location	First	Second	Third	Fourth
Influent	0.7	0.7	0.9	
Primary Effluent	0.3	0.2	0.3	
Primary Sludge			3.4	
Activated Sludge	11.2	6.0	1.7	8.1
Secondary Sludge				27.1
Secondary Effluent	9.2	15.8	6.8	
Thickened Sludge	336.0	280.3	199.4	
Digested Sludge	218.0	156.9	224.9	
Thick. Overflow	0.5	0.6	0.6	
An Dig Supernatant	27.7	3.4	4.7	
BFP Filtrate	3.9	1.3	4.3	

Table E.11 Solids-Bound Biopolymers Deoxyribonucleic Acid Concentration (Unit: mg/L, except secondary effluent, µg/L)

Location	First	Second	Third	Fourth
Influent	0.4	0.6	0.4	
Primary Effluent	< 15.4	< 6.1	< 10.3	
Primary Sludge			3.7	
Activated Sludge	4.2	1.0	< 10.3	2.1
Secondary Sludge				7.2
Secondary Effluent	< 15.4	< 6.1	< 10.3	
Thickened Sludge	90.4	123.1	59.8	
Digested Sludge	24.0	39.2	94.0	
Thick. Overflow	< 15.4	< 6.1	< 10.3	
An Dig Supernatant	5.0	0.6	2.3	
BFP Filtrate	1.3	0.1	2.1	

Table E.12 Solids-Bound Biopolymers Total Organic Carbon Concentration (Unit: mg/L, except secondary effluent, µg/L)

Location	First	Second	Third	Fourth
Influent	2.1	3.0	4.3	
Primary Effluent	1.1	0.7	1.0	
Primary Sludge			12.0	
Activated Sludge	19.2	17.0	3.6	17.1
Secondary Sludge				68.6
Secondary Effluent	35.9	28.2	21.4	
Thickened Sludge	655.1	940.0	522.1	
Digested Sludge	357.6	418.3	607.4	
Thick. Overflow	1.2	1.8	1.7	
An Dig Supernatant	60.0	12.9	13.0	
BFP Filtrate	14.7	3.2	13.3	

Table E.13 Carbohydrate in Loosely-Bound Biopolymer (mg/l)

Location	First	Second	Third	Fourth
Influent	2.4	2.3	6.2	
Primary Effluent	< 1.9	3.5	2.2	
Primary Sludge			2.0	
Activated Sludge	< 1.9	2.7	< 1.0	2.8
Secondary Sludge				
Secondary Effluent	< 1.9	2.8	< 1.0	
Thickened Sludge	38.6	51.8	28.3	
Digested Sludge	8.7	16.3	30.5	
Thick. Overflow	< 1.9	2.4	2.7	
An Dig Supernatant	13.6	10.7	11.6	
BFP Filtrate	3.5	2.5	2.7	

Table E.14 Protein in Loosely-Bound Biopolymer (mg/l)

Location	First	Second	Third	Fourth
Influent	8.8	3.9	7.9	
Primary Effluent	2.3	1.5	3.3	
Primary Sludge			4.0	
Activated Sludge	2.8	1.9	1.4	1.6
Secondary Sludge				
Secondary Effluent	2.8	1.8	2.3	
Thickened Sludge	54.3	66.9	50.2	
Digested Sludge	25.4	54.7	116.4	
Thick. Overflow	6.1	2.4	5.4	
An Dig Supernatant	100.6	53.4	43.8	
BFP Filtrate	7.4	4.2	7.0	

Table E.15 Humic Acids in Loosely-Bound Biopolymer (mg/l)

Location	First	Second	Third	Fourth
Influent	< 0.9	< 5.8	6.5	
Primary Effluent	< 0.9	< 5.8	< 3.9	
Primary Sludge			< 3.9	
Activated Sludge	< 0.9	< 5.8	< 3.9	< 1.0
Secondary Sludge				
Secondary Effluent	< 0.9	< 5.8	< 3.9	
Thickened Sludge	< 0.9	19.0	< 3.9	
Digested Sludge	< 0.9	11.2	4.5	
Thick. Overflow	< 0.9	< 5.8	< 3.9	
An Dig Supernatant	< 0.9	< 5.8	< 3.9	
BFP Filtrate	< 0.9	< 5.8	< 3.9	

Table E.16 Ribonucleic Acid in Loosely-Bound Biopolymer (mg/l)

Location	First	Second	Third	Fourth
Influent	< 2.8	< 1.5	5.2	
Primary Effluent	< 2.8	< 1.5	< 2.1	
Primary Sludge				
Activated Sludge	< 2.8	< 1.5	< 2.1	< 6.2
Secondary Sludge				
Secondary Effluent	< 2.8	< 1.5	< 2.1	
Thickened Sludge	70.8	42.4	29.4	
Digested Sludge	14.7	25.8	35.3	
Thick. Overflow	< 2.8	< 1.5	2.5	
An Dig Supernatant	23.5	12.4	19.7	
BFP Filtrate	5.1	3.8	10.9	

Table E.17 Deoxyribonucleic Acid in Loosely-Bound Biopolymer (mg/l)

Location	First	Second	Third	Fourth
Influent	< 15.4	< 6.1	< 10.3	
Primary Effluent	< 15.4	< 6.1	< 10.3	
Primary Sludge				
Activated Sludge	< 15.4	< 6.1	< 10.3	< 11.4
Secondary Sludge				
Secondary Effluent	< 15.4	< 6.1	< 10.3	
Thickened Sludge	16.8	14.3	22.3	
Digested Sludge	< 15.4	< 6.1	< 10.3	
Thick. Overflow	< 15.4	< 6.1	< 10.3	
An Dig Supernatant	< 15.4	< 6.1	< 10.3	
BFP Filtrate	< 15.4	< 6.1	< 10.3	

Table E.18 Total Organic Carbon in Loosely-Bound Biopolymer (mg/l)

Location	First	Second	Third	Fourth
Influent	5.0	7.3	15.5	
Primary Effluent	3.6	3.7	2.9	
Primary Sludge			4.0	
Activated Sludge	2.6	3.9	< 1.3	2.5
Secondary Sludge				
Secondary Effluent	6.0	6.0	< 1.3	
Thickened Sludge	73.2	95.1	82.9	
Digested Sludge	28.8	59.1	117.0	
Thick. Overflow	5.0	4.4	7.0	
An Dig Supernatant	80.0	51.6	49.1	
BFP Filtrate	9.0	5.5	10.2	

APPENDIX F

DEMONSTRATION OF CALCULATIONS USED TO PERFORM FLOWRATE AND SUSPENDED SOLIDS MASS BALANCES AT THE ITHACA WASTEWATER AND SLUDGE TREATMENT PLANT FIRST ITERATION

1. Introduction

This appendix describes the initial values and the first iteration of calculations of flowrates and the mass balances for total and volatile suspended solids within the Ithaca Wastewater and Sludge Treatment Plant for the average conditions of the months of June, July and August 1998. As explained below, the numerical values resulting from the first iteration were not the final values. The purpose of this appendix is to show how the mass balances were prepared, the equations used, data required, and assumptions made.

Mass balances were prepared for each of the following treatment systems: primary sedimentation tanks, degritter, activated sludge process, thickeners, anaerobic digesters, and belt filter presses. As explained in Section 4.4.1, these processes function as a system, with the output of one process being the input of another process. In addition, the wastewater and sludge treatment processes work as a feedback system; wastewater treatment processes generate sludges which are sent to the sludge treatment processes; the latter generate return flows to the wastewater treatment processes. This feedback system required that the mass balances for each process had to be repeated until a steady-state condition was achieved. The criteria used to define the achievement of a steady-state condition was that the difference in the numerical values of suspended solids loads and flowrates between two consecutive iterations for any given process fell below one percent.

The data records from the plant were insufficient for mass balances calculations. For example, no data existed with respect to flowrates in the thickeners overflows, digester supernatant, and belt filter press filtrate and washwater. These flowrates were an essential portion of the mass balance on the primary sedimentation tanks. In the first iteration, in order to solve the system of equations for the mass balance on the primary sedimentation tanks, it was necessary to assume values for the

flowrates of the anaerobic digester supernatant and overflow from thickeners. The values assumed were zero (an estimation of the combined flowrate of filtrate and washwater was possible). The solution of the mass balance for the primary sedimentation tanks allowed the estimations of primary effluent and primary sludge flowrates and suspended solids loads. The latter loads, together with waste activated sludge and chemical sludge loads allowed the calculation of mass balances for the sludge treatment processes. The solution of the mass balances for the sludge treatment processes included the loads of suspended solids in the return flows (overflow, supernatant, and filtrate/washwater). Then, in the second iteration, the primary sedimentation tanks mass balance was recalculated to include the updated information on the loads of suspended solids in the return flows. As explained above, this iterative process to calculate mass balance was repeated until the difference between the flowrates and the loads of suspended solids in consecutive iterations fell below one percent.

In the calculations for mass balances, the numbers were kept as they appeared in the calculations, without consideration to the number of significant digits. As it will be seen in the next sections, the flowrate of the grit slurry was approximately 50 gal/d, but contained a load of suspended solids greater than the load of SS in secondary effluent, with a flowrate of about 6,300,000 gal/d. Rounding a number in the range of the secondary effluent flowrate would mean to consider numbers in the range of the grit slurry flowrate as not significant. Consideration of all input to, and output from, the treatment processes without consideration of their numerical values also helped to have a better understanding of the functioning of the plant.

Ultimately, the mass balance allowed an estimation of flowrates and suspended solids loads in all processes within the wastewater and sludge treatment plant. This information was used to perform a biopolymers mass balance, which was the final

goal. The biopolymers mass balance allowed observations regarding the fate and transformation of capsule and slime within the wastewater and sludge treatment plant.

2. Data Requirements

Data required to prepare the mass balances were gathered from three sources: (1) the Ithaca plant records, (2) the analyses of samples collected for this research, and (3) the literature.

2.1 *Data Provided by the Ithaca Wastewater and Sludge Treatment Plant Records*

The following data from the plant records were averages for the months of June, July and August 1998.

2.1.1 *Raw Wastewater*

Total suspended solids concentration: X_T^W = 142 mg/l

Ratio of volatile to total suspended solids: 0.82

Five-day biochemical oxygen demand concentration: BOD_5 = 140 mg/l

Total phosphorus concentration: 2.9 mg/l

2.1.2 *Primary Effluent*

Total suspended solids concentration: X_T^{PE} = 79 mg/l

Ratio volatile to total suspended solids: 0.76

Five-day biochemical oxygen demand concentration: BOD_5 = 79 mg/l

2.1.3 *Activated Sludge*

Mixed liquor volatile suspended solids concentration: X_V = 850 mg/l

Mean cell residence time: θ_C^{AS} = 8.8 d

2.1.4 *Secondary Effluent*

Flowrate: Q_{SE} = 6,203,333 gal/d

Total suspended solids concentration: X_T^{SE} = 2.5 mg/l

Five-day biochemical oxygen demand concentration: BOD_5 = 12 mg/l

Total phosphorus concentration: 0.60 mg/l

2.1.5 *Anaerobic Digester*

Mean cell residence time: θ_C^{Dig} = 28 d

Percent of total solids in digested sludge: 4 %

2.1.6 *Dewatered Sludge*

Percentage of total solids in wet sludge cake: 21 %

Daily weight of dry total solids in sludge cake: 6,150 lb/d (used only in the first iteration)

Ratio of volatile to total solids: 0.54

2.1.7 *Chemicals Used*

Ferrous sulfate: 268 lb/d, applied at 6 % solution

Synthetic polymer: 16 lb per ton of digested sludge total solids, applied at 0.6 % solution.

2.1.8 *Other Data*

Total suspended solids in recycle sludge: 2,850 mg/l

Total solids in septage: 1,351 lb/d

Percent of total solids in septage: 2.04 %

Ratio of volatile to total solids in septage: 0.69

Percent of total solids in thickened sludge: 3.8 %

2.2 *Data Obtained from Samples Collected for this Study*

The following data were the averages of three measurements in samples collected during June, July and August 1998, except the primary sludge datum, which was measured only once.

Total suspended solids concentration in primary sludge, after the degritter: 1,127 mg/l

Total and volatile suspended solids concentrations in thickener overflow: 85 mg/l and 67 mg/l, respectively

Total and volatile suspended solids concentrations in anaerobic digester supernatant: 2,923 mg/l and 1,965 mg/l, respectively

Total and volatile suspended solids concentration in filtrate with washwater from belt filter press: 934 mg/l and 490 mg/l, respectively

2.3 *Data Used from the Literature*

The following data were taken from the literature:

BOD rate constant: $k_1 = 0.30\ d^{-1}$ (Sawyer et al., 1994; Metcalf and Eddy, 1991)

Specific decay rate of microorganisms in the activated sludge process: $b^{AS} = 0.1\ d^{-1}$ (Gossett and Belser, 1982)

Specific decay rate of microorganisms in the anaerobic digestion process: $b^{DIG} = 0.03\ d^{-1}$ (McCarty, 1975)

Biodegradable fraction of wastewater volatile suspended solids: $f_d^W = 0.65$ (WEF, 1998)

Biodegradable fraction of active organisms (X_a) in the activated sludge and anaerobic digestion processes: $f_d = 0.8$ (McCarty, 1975)

Half-velocity constant, activated sludge: $K_S = 70$ mg/l BOD_L (Metcalf and Eddy,

1991);

Half-velocity constant, anaerobic digester: K_C = 1.8 g/l BOD_L (O'Rourke, 1968, cited by Gossett and Belser, 1982)

Maximum specific rate of substrate utilization, activated sludge: k^{AS} = 5 mg BOD_L/g X_a.day (Metcalf and Eddy, 1991)

Yield coefficient for volatile acid-using methane bacteria: 0.04 g X_a/g BOD_L (O'Rourke, 1968, cited by Gossett and Belser, 1982);

Maximum specific rate of substrate utilization for volatile acid-using methane bacteria: k^{DIG} = 6.67 g BOD_L/g X_a.day (O'Rourke, 1968, cited by Gossett and Belser, 1982);

Belt Filter Press suspended solids capture efficiency: 95 % (Metcalf and Eddy, 1991);

Density of fixed (mineral) suspended solids: e_F = 2.5 g/cm^3 (Metcalf and Eddy, 1991);

Density of volatile suspended solids: e_V = 1.0 g/cm^3 (Metcalf and Eddy, 1991);

Soluble fraction of phosphorus in raw wastewater: 0.65 (WEF, 1998)

3. Establishment of Initial Values for Flowrates and Total and Volatile Suspended Solids Loads in Return Flows from Sludge Treatment Processes

Return flows from sludge treatment processes were composed of thickener overflow, anaerobic digester supernatant, and combined filtrate/washwater from the belt filter press (Figure 4.19). Equations (F.1) and (F.2) describe the flowrates and total suspended solids loads of the return flows.

$$Q_{RF} = Q_{OVFL} + Q_{SUP} + Q_{FW} \tag{F.1}$$

$$Q_{RF} \cdot X_T^{RF} = Q_{OVFL} \cdot X_T^{OVFL} + Q_{SUP} \cdot X_T^{SUP} + Q_{FW} \cdot X_T^{FW} \tag{F.2}$$

where Q and X represent flowrate and suspended solids concentration, respectively; the subscripts and superscripts T, RF, OVFL, SUP, FW represent total, return flows, overflow, supernatant, and combined filtrate/washwater, respectively..

Return flows from sludge treatment processes were input to wastewater treatment processes. For this reason, in order to perform mass balances in the wastewater treatment processes, knowledge of flowrates and suspended solids loads in return flows was required. However, the flowrates of two components of the return flows were unknown, and initial values had to be assumed. The initial values established for thickener overflow and anaerobic digester supernatant flowrates were zero. Because suspended solids load is given by the product between flowrate and concentration, the initial values for suspended solids loads of overflow and supernatant also were zero.

An estimation of the flowrate, total and volatile suspended solids loads of the third component of the return flows, the combined filtrate and washwater from the belt filter press, was possible using the following information: (a) weight of total suspended solids in the dewatered sludge cake; (b) belt filter press suspended solids capture efficiency; (c) concentration of TSS in the combined filtrate with washwater; (d) ratio VSS:TSS in the combined filtrate with washwater. Assuming the belt filter press had 95 % suspended solids capture efficiency, the load of total suspended solids in the combined filtrate and washwater was (0.05/0.95)·6,150 lb/d = 324 lb/d, where 6,150 was the load of total suspended solids in the sludge cake. The average total suspended solids concentration in the combined filtrate and washwater, measured in samples collected for this study, was 934 mg/l. The combined flowrate was calculated by dividing TSS load by TSS concentration,

$$\text{Combined flowrate} = \frac{324\frac{lb}{d}\cdot 0.4536\cdot 10^{6}\frac{mg}{lb}}{934\frac{mg}{l}} = 157,349\frac{l}{d} = 41,567\frac{gal}{d}$$

The volatile suspended solids load in the combined filtrate and washwater was 0.54 x 324 = 175 lb/d. Substituting the corresponding values in Equations (4.1) and (4.2),

$$Q_{RF} = 0 + 0 + 41{,}567 = 41{,}567 \frac{gal}{d}$$

$$Q_{RF} \cdot X_T^{RF} = (0) \cdot (85) + (0) \cdot (2{,}923) + (41{,}567) \cdot (934) \cdot (8.3456 \cdot 10^{-6}) = 324 \frac{lb}{d}$$

4. Flowrates and Mass Balances for Total and Volatile Suspended Solids on the Primary Sedimentation Tanks

Figure F.1 presents the input to, and output from, the primary sedimentation tanks. The flowrate and total suspended solids mass balances are:

$$Q_W + Q_{RF} + Q_{FeSO_4} = Q_{PE} + Q_{PSG} \qquad \text{(F.3)}$$

$$Q_W \cdot X_T^W + Q_{RF} \cdot X_T^{RF} + Q_{FeSO_4} \cdot X_T^{FeSO_4} = Q_{PE} \cdot X_T^{PE} + Q_{PSG} \cdot X_T^{PSG} \qquad \text{(F.4)}$$

where the subscripts and superscripts W, $FeSO_4$, PE, PSG stand for wastewater, ferrous sulfate, primary effluent and primary sludge with grit, respectively.

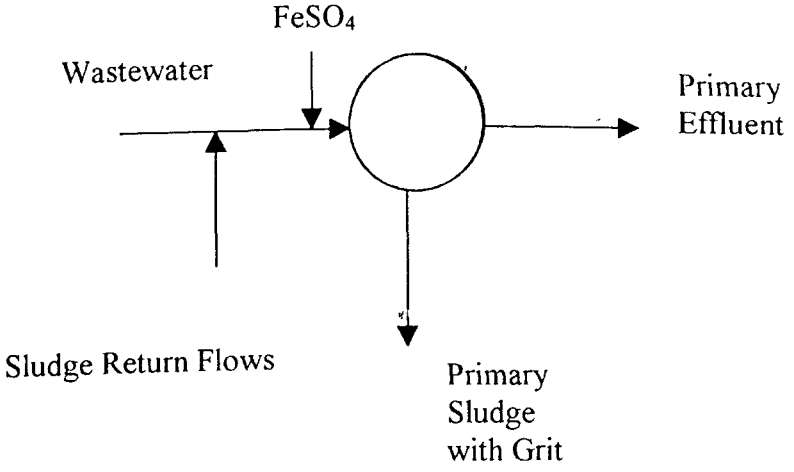

Figure F.1 Input to, and Output from, the Primary Sedimentation Tanks

In Equation (F.3), primary effluent and primary sludge with grit flowrates were unknowns, while raw wastewater and ferrous sulfate flowrates were estimated from

available information. The initial value for the return flows flowrate was established in Section 3. In Equation (F.2), total suspended solids concentrations (TSS) in raw wastewater, primary effluent, and ferrous sulfate were known, while TSS concentration in primary sludge with grit was estimated using data for TSS concentration in primary sludge without grit. TSS in return flows were established by the initial values presented in Section 3. In the following sections, data for each component of the primary sedimentation tank mass balance are presented.

4.1 Raw Wastewater Flowrate and Suspended Solids Loads

4.1.1 *Suspended Solids Concentrations*

Total suspended solids: 142 mg/l;

Volatile suspended solids: 0.82 x 142 mg/l = 116 mg/l

4.1.2 *Flowrate*

The raw wastewater flowrate is not directly measured at the Ithaca plant. It was estimated using Equation (F.5)

$$Q_W = Q_{SE} + Q_{Cake} + Q_{Grit} - Q_{FeSO_4} - Q_{Sept} - Q_{Poly} - Q_{Wash} \qquad \text{(F.5)}$$

where Q_{SE}, Q_{Cake}, Q_{Grit}, Q_{FeSO_4}, Q_{Sept}, Q_{Poly}, and Q_{Wash} are, respectively, secondary effluent, dewatered sludge cake, grit removal, ferrous sulfate, septage, synthetic polymer, and belt filter press washwater flowrates.

a) Secondary effluent flowrate

The average secondary effluent flowrate during the months of June, July and August was 6,203,333 gal/d.

b) Dewatered sludge cake daily volume

The daily volume of dewatered sludge cake was calculated using Equation (F.6) (Metcalf and Eddy, 1991)

$$V = \frac{W_S}{e_W \cdot \rho \cdot f_m} \tag{F.6}$$

where V = daily volume of sludge cake

e_W = density of water

ρ = specific gravity of sludge cake (dimensionless)

f_m = mass fraction of total suspended solids in sludge cake

W_S = weight of dry total suspended solids in sludge cake

(It was assumed that total suspended solids in sludge cake was equivalent to total solids, which is what is measured at the plant).

The specific gravity of sludge cake was calculated by dividing the sludge cake mass density by the water mass density. The mass density of the sludge cake was calculated using Equation (F.7)

$$\frac{1}{e} = \frac{f_m}{e_P} + \frac{(1-f_m)}{e_L} \tag{F.7}$$

where e, e_P, e_L are respectively, the mass densities of the sludge cake, suspended solids in sludge cake and water. The density of sludge cake solids was calculated using Equation (F.8)

$$\frac{1}{e_P} = \frac{f_F}{e_F} + \frac{f_V}{e_V} \quad \rightarrow \quad \frac{1}{e_P} = \frac{(1-f_V)}{e_F} + \frac{f_V}{e_V}. \tag{F.8}$$

where f_F, f_V, e_F, e_V are, respectively, the mass fractions and densities of fixed and volatile suspended solids. Substituting $f_V = 0.54, e_F = 2.5\,g/cm^3, e_V = 1.0\,g/cm^3$ into Equation (F.8),

$$e_P = \left[\frac{(1-0.54)}{2.5} + \frac{0.54}{1.0}\right]^{-1} = 1.3812\frac{g}{cm^3}$$

Substituting $e_P, e_W = 1.0\,g/cm^3, f_m = 0.21$ into Equation (F.7),

$$e_{Cake} = \left[\frac{0.21}{1.3812} + \frac{(1-0.21)}{1.0}\right]^{-1} = 1.0615\frac{g}{cm^3}$$

Substituting $W_S = 6,150\,lb/d, e_W, e_{Cake}, and\ f_m$ into Equation (F.6),

$$\text{Daily volume of sludge cake} = \frac{6,150\frac{lb}{d}\cdot 0.4536\frac{kg}{lb}}{1.0\frac{kg}{l}\cdot 1.0615\cdot 0.21} = 12,514\frac{l}{d} = 3,306\frac{gal}{d}$$

c) Daily volume of grit

Grit solids content varies from 35 to 80 %, with volatile fraction from 1 to 55 % (Droste, 1998). The weight of solids removed daily was 500 lb/d. Assuming a 80 % solids content, and 40 % volatile solids, the density of grit solids was calculated using Equation (F.8).

$$\frac{1}{e_P} = \frac{0.6}{2.5} + \frac{0.4}{1.0} = 0.640 \rightarrow e_P = 1.5625\frac{g}{cm^3} \text{ (grit solids)}$$

The density of grit was calculated using Equation (F.7).

$$\frac{1}{e_{Grit}} = \frac{f_m}{e_S} + \frac{(1-f_m)}{e_W} = \frac{0.8}{1.5625} + \frac{0.2}{1.0} \rightarrow e_{Grit} = 1.4025\frac{g}{cm^3}$$

Substituting the corresponding values into Equation (F.6),

$$\text{Daily volume of grit} = \frac{500\frac{lb}{d}\cdot 0.4536\frac{kg}{lb}}{1.0\frac{kg}{l}\cdot 1.4045\cdot 0.80} = 202\frac{l}{d} = 53\frac{gal}{d};$$

d) Daily Volume of ferrous sulfate

The quantity of ferrous sulfate used per day was 268 lb/d, applied in 6 % solution. The daily volume of ferrous sulfate was

$$Q_{FeSO_4} = \frac{268\,{}^{lb}/_{d} \cdot 0.4536\,{}^{kg}/_{lb}}{\frac{6\,kg\,FeSO_4}{100\,kg\,H_2O} \cdot 1\frac{kg\,H_2O}{l}} = 2{,}026\frac{l}{d} = 535\frac{gal}{d}$$

e) Daily volume of septage

The density of septage solids was calculated using Equation (F.8)

$$\frac{1}{e_P} = \frac{(1-0.69)}{2.5} + \frac{0.69}{1.0} = 0.8140 \rightarrow e_P = 1.2285\frac{g}{cm^3}$$

Substituting the corresponding values into Equation (F.7)

$$e_{Sept} = \left[\frac{0.0204}{1.2285} + \frac{(1-0.0204)}{1.0}\right]^{-1} = 1.0038\frac{g}{cm^3}$$

The daily volume of septage was calculated substituting the corresponding values into Equation (F.6):

$$\text{Daily volume of septage} = \frac{1{,}351\frac{lb}{d} \cdot 0.4536\frac{kg}{lb}}{1.0\frac{kg}{l} \cdot 1.0038 \cdot 0.0204 \cdot} = 29{,}926\frac{l}{d} = 7{,}906\frac{gal}{d}$$

f) Daily volume of synthetic polymer

The application rate of synthetic polymer was 16 lb per ton of digested sludge total solids, applied in 0.6 % solution. Assuming the belt filter press had 95 % suspended solids capture efficiency (Metcalf and Eddy, 1991), the dry weight of total suspended solids in digested sludge was 6,150/0.95 = 6,474 lb/d. The quantity of synthetic polymer was (16/2000) x 6,474 = 52 lb/d.

$$\text{Daily volume of polymer} = \frac{52\frac{lb}{d} \cdot 0.4536\frac{kg}{lb}}{\frac{0.6\,kg\,polymer}{100\,kg\,solution} 1.0\frac{kg}{l}} = 3{,}931\frac{l}{d} = 1{,}038\frac{gal}{d}$$

g) Daily volume of washwater

The daily volume of washwater was estimated from information relative to the percentage of total suspended solids in thickened digested sludge and dewatered sludge cake, ratio VSS to TSS in digested sludge, and concentration of total suspended solids in the combined flows of filtrate and washwater.

The density of digested sludge solids was calculated using Equation (F.8)

$$e_P = \left[\frac{(1-0.54)}{2.5} + \frac{0.54}{1.0}\right]^{-1} = 1.3812 \frac{g}{cm^3}$$

The density of thickened digested sludge was calculated using Equation (F.7), with the concentration of total suspended solids in thickened digested sludge being 4%.

$$e_{DigSl} = \left[\frac{0.04}{1.3812} + \frac{(1-0.04)}{1.0}\right]^{-1} = 1.0112 \frac{g}{cm^3}$$

The weight of total suspended solids in thickened digested sludge was 6,150/0.95 = 6,474 lb/d, assuming a 95 % suspended solids capture efficiency in the belt filter press. Substituting the corresponding values into Equation (F.6),

$$\text{Daily volume of digested sludge} = \frac{6{,}474 \frac{lb}{d} \cdot 0.4536 \frac{kg}{lb}}{1.0 \frac{kg}{l} \cdot 1.0112 \cdot 0.04} = 72{,}601 \frac{l}{d} = 19{,}179 \frac{gal}{d}$$

Filtrate flowrate = daily volume of thickened digested sludge + daily volume of synthetic polymer – daily volume of dewatered sludge cake = 19,179 + 1,038 – 3,306 = 16,911 gal/d

The measured concentration of total suspended solids in the combined flow of filtrate and washwater was 934 mg/l; the load of solids in the combined flow was 6,474 x 0.05 = 324 lb/d (assuming a suspended solids capture by the belt filter press of 95 %). The combined flowrate of filtrate and washwater was

$$\text{Combined flowrate} = \frac{324\frac{lb}{d}\cdot 0.4536\cdot 10^{6}\frac{mg}{lb}}{934\frac{mg}{l}} = 157,349\frac{l}{d} = 41,567\frac{gal}{d}$$

Washwater flowrate = combined flowrate – filtrate flowrate = 41,567 – 16,911 =

= 24,656 gal/d

The value of raw wastewater flowrate was estimated by substituting the values of flowrates of secondary effluent, sludge cake, grit, ferrous sulfate, septage, synthetic polymer, and washwater in Equation (F.5)

$Q_W = Q_{SE} + Q_{Cake} + Q_{Grit} - Q_{FeSO_4} - Q_{Sept} - Q_{Poly} - Q_{Wash}$ = 6,203,333 + 3,306 + 53 – 535 - 7,906 – 1,038 – 24,656 = 6,172,557 gal/d

4.1.3 *Total and Volatile Suspended Solids Loads in Raw Wastewater*

The loads of total and volatile suspended solids in raw wastewater were calculated by multiplying the respective concentrations by the flowrate.

Load of total suspended solids in raw wastewater = 142 mg/l x 6,172,557 gal/d x (8.3454×10^{-6}) = 7,315 lb/d

Load of volatile suspended solids in raw wastewater = 0.82 x 7,315 = 5,998 lb/d

4.2 Sludge Return Flows Flowrate and Suspended Solids Loads

The second component of the primary sedimentation tanks mass balance was sludge return flows. The initial values of flowrate and suspended solids loads were described in Section 3.

4.3 Ferrous Sulfate Flowrate and Suspended Solids Loads

Ferrous sulfate was dissolved in water prior to application. The suspended solids concentration in solution was zero.

Ferrous sulfate total and volatile suspended solids loads = zero

Ferrous sulfate flowrate = 535 gal/d (Section 4.1.2.d)

4.4 Primary Effluent Flowrate and Suspended Solids Loads

The flowrate of primary effluent was unknown. The total suspended solids concentration was 79 mg/l. The volatile suspended solids was 0.76 x 79 = 60 mg/l.

4.5 Primary Sludge with Grit Flowrate and Suspended Solids Loads

Both flowrate and suspended solids concentrations were unknown. In order to solve the system of Equations (F.3) and (F.4), an estimation of the suspended solids concentration had to be made. This estimation was 1,380 mg/l; it was made based on the suspended solids concentration in primary sludge without grit (1,127 mg/l), and the quantity of grit removed each day (500 lb/d). The concentration of suspended solids in primary sludge is very dilute in this plant because sludge blankets are not kept in the primary sedimentation tanks (Denmark, 1998). Low suspended solids concentration is a requirement for the proper operation of the degritter (Metcalf and Eddy, 1991).

The data presented in Sections 4.1 through 4.5 were substituted into Equations (F.3) and (F.4), and solved for the flowrates of primary effluent and primary sludge with grit.

$$6{,}172{,}557 + 41{,}567 + 535 = Q_{PE} + Q_{PSG}$$

$$(6{,}172{,}557)\cdot(142)+(41{,}567)\cdot(934)+(535)\cdot(0)=Q_{PE}\cdot(79)+Q_{PSG}\cdot(1380)$$

The solutions of these equations are Q_{PE} = 5,888,473 gal/d and Q_{PSG} = 326,186 gal/d. The loads of suspended solids in primary effluent and primary sludge with grit were calculated by multiplying the suspended solids concentrations by the respective flowrates.

Load of total suspended solids in primary effluent = 79 mg/l x 5,888,473 gal/d x

x (8.3454×10^{-6}) = 3882 lb/d;

Load of volatile suspended solids in primary effluent = 0.76 x 3882 = 2950 lb/d;

Load of total suspended solids in primary sludge with grit = 1,380 mg/l x

x 326,186 gal/d x (8.3454×10^{-6}) = 3,757 lb/d;

Load of volatile suspended solids in primary sludge with grit = load from wastewater + load from return flow - load primary effluent = 5,998 + 175 – 2,950 = 3,223 lb/d

Figure F.2 presents the mass balance for suspended solids on the primary sedimentation tanks. The values in this mass balance resulted from the calculations of the first iteration. These values were updated when mass balances on the sludge treatment processes provided improved flowrate and load estimations for the sludge return flows.

5. Flowrates and Mass Balances for Total and Volatile Suspended Solids on the Degritter

The flowrate in primary sludge without grit was calculated by subtracting the daily volume of grit from the flowrate of primary sludge with grit.

Primary sludge without grit flowrate = 326,186 – 53 = 326,133 gal/d

The load in primary sludge without grit was calculated by subtracting the daily load of grit removed from the load of suspended solids in primary sludge with grit.

Load of total suspended solids in primary sludge without grit = 3,757 – 500 =

= 3,257 lb/d;

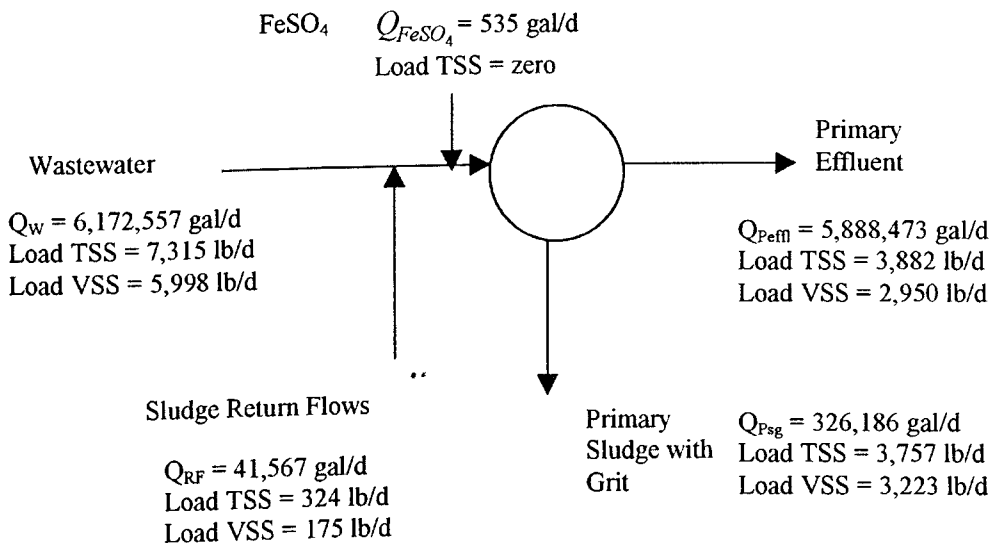

Figure F.2 Flowrates and Mass Balance for Suspended Solids on the Primary Sedimentation Tank

Load of volatile suspended solids in primary sludge without grit = 3,223 – 200 =

= 3,023 lb/d;

Concentration of total suspended solids in primary sludge without grit =

= 3,257 lb/d x (0.4536 x 10^6) mg/lb/(326,133 gal/d x 3.7854 l/gal) = 1,197 mg/l; the concentration of total suspended solids measured in one sample of primary sludge without grit was 1,127 mg/l. The difference between the calculated and measured concentrations was considered acceptable. Figure F.3 presents the mass balance for suspended solids on the degritter, after the first iteration.

6. Production of Chemical Sludge

Phosphorus present in raw wastewater at the Ithaca treatment plant is removed from the liquid phase by four mechanisms: (1) a fraction of particulate phosphorus settles in the primary sedimentation tank; (2) a fraction of soluble phosphorus is incorporated into new biomass formed in the activated sludge process, and removed

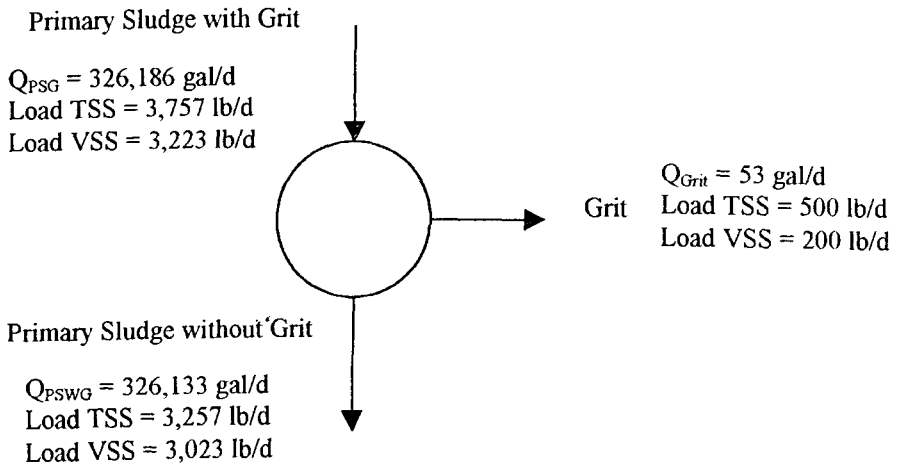

Figure F.3 Flowrates and Mass Balance for Suspended Solids on the Degritter

by settling in the secondary sedimentation tank; (3) a fraction of colloidal and particulate phosphorus that escaped sedimentation in the primary clarifier may be incorporated into activated sludge flocs, and be removed in the secondary sedimentation tank (4) a fraction of soluble phosphorus is removed by chemical precipitation through reaction with ferric ions.

This section estimates the quantity of sludge formed by chemical precipitation.

Ferrous sulfate ($FeSO_4$) is applied at 6 % solution in the primary sedimentation tanks. According to the plant operator (Denmark, 1998), iron reacts with phosphorus in the aeration tanks, where ferrous ions are oxidized to ferric ions. The Fe^{+3} reacts first with phosphate ions; in cases in which an excess Fe^{+3} is present after the reaction with phosphate ions, the excess Fe^{+3} reacts with hydroxide ions. Equations (F.9) and (F.10) represent the reactions between Fe^{+3} with phosphate and hydroxide ions (U.S.EPA, 1987).

$$Fe^{+3} + PO_4^{-3} = FePO_4\downarrow \qquad \text{(F.9)}$$

$$Fe^{+3} + 3OH^- = Fe(OH)_3\downarrow \qquad \text{(F.10)}$$

The mole ratio of Fe to P is 1:1 (Equation F.9), or

$$\frac{55.85 g\,Fe}{31 g\,P} = 1.8 \frac{g\,Fe}{g\,P}$$

The daily quantity of $FeSO_4$ applied was 268 lb/d. The quantity of Fe in 268 lb/d $FeSO_4$ is

$$\frac{55.85 g\,Fe}{55.85 g\,Fe + 32 g\,S + (4 \cdot 16 g\,O)} \cdot 268 \frac{lb}{d} = 99 \frac{lb}{d} Fe,$$

where 55.85 g, 32 g and 16 g are the atomic weights of iron, sulfur and oxygen, respectively. The maximum daily quantity of phosphorus that could be removed by chemical precipitation was 99/1.8 = 55 lb/d P.

The average phosphorus concentration in raw wastewater was 2.9 mg/l. The concentration of phosphorus in the primary effluent was not known. Assuming that the fraction of soluble phosphorus in raw wastewater was 65 % (WEF, 1998), the phosphorus concentration in primary effluent was estimated as 2.9 – 0.45 x (0.35 x x 2.9) = 2.44 mg/l, where 0.45 was the fraction of suspended solids removed in the primary sedimentation tanks. Since the secondary effluent phosphorus concentration was 0.60 mg/l, and the flowrate of primary effluent in the first iteration was 5,888,473 gal/d, the daily load of phosphorus removed by chemical precipitation and the activated sludge process was (2.44 – 0.60) mg/l x 5,888,473 gal/d x (8.3454×10^{-6}) = 90.4 lb/d. Since chemical precipitation could remove only 55 lb/d, the difference 90.4 – 55 = 35.4 lb/d P was assumed to be removed by the combination of biomass uptake, incorporation into floc and sedimentation.

The daily quantity of chemical sludge formed was:

$$\text{Chemical sludge} = \frac{55.85 g\,Fe + 31 g\,P + (4 \cdot 16 g\,O)}{55.85 g\,Fe} \cdot 99 \frac{lb}{d} Fe = 268 \frac{lb}{d} FePO_4.$$

Because there was not an excess of Fe^{+3}, no iron hydroxide sludge was considered to be formed.

7. Flowrates and Mass Balances for Total and Volatile Suspended Solids on the Activated Sludge Process

In the activated sludge process, organic waste is introduced into a reactor and oxidized by an aerobic bacterial culture maintained in suspension (Metcalf and Eddy, 1991). A portion of the organic waste is synthesized into bacterial protoplasm, while another portion is used for energy. There is a transfer of electrons from the organic waste to oxygen; the organic waste is an *electron donor*, while oxygen is an *electron acceptor* (Sawyer et al., 1994). The energy released in the oxidation reaction is used for cell maintenance and synthesis. The bacterial metabolism in the activated sludge process is classified as chemoheterotrophic, because the carbon source used for cell synthesis comes from organic carbon, and the energy is obtained by chemical oxidation reaction. Figure F.4 depicts the schematic diagram of bacterial metabolism in the activated sludge process.

In order to develop a mass balance for the activated sludge process, it was necessary to determine the concentrations of suspended solids in the aeration tanks, which required an estimation of the yield coefficient for the process. These topics are addressed in the following sections.

7.1 *Estimation of the Yield Coefficient*

The estimation of the yield coefficient is important for determination of the total biomass produced in the activated sludge process. The yield coefficient is defined as the ratio between the mass of cells formed to the mass of organic matter used when

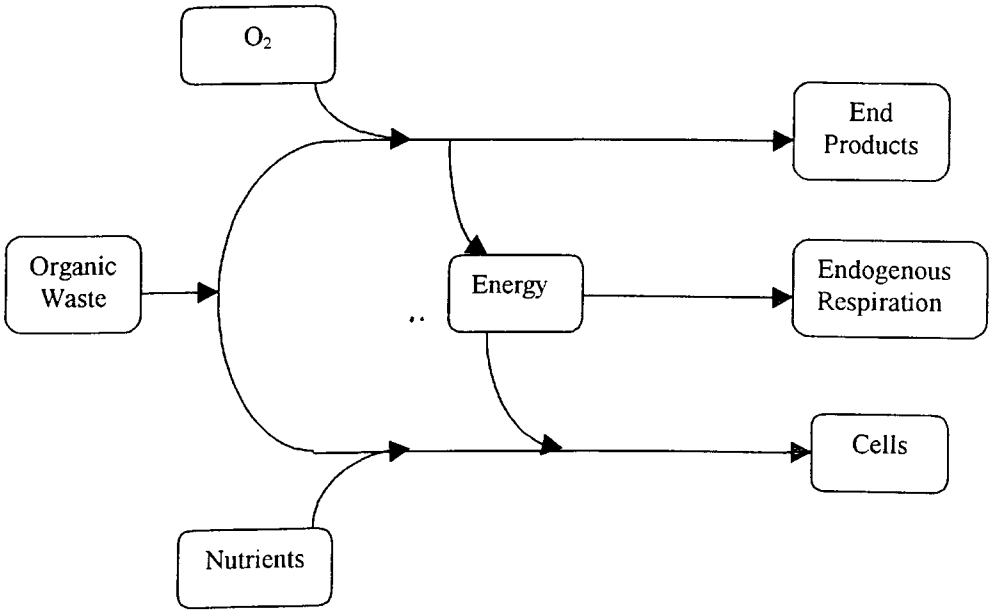

Figure F.4 Schematic Representation of Bacterial Metabolism in Activated Sludge Process (Adapted from Metcalf and Eddy, 1991 and Sawyer et al., 1994)

endogenous respiration is negligible in relation to cell synthesis (McCarty, 1971, Metcalf and Eddy, 1991) (Equation (F.11)).

$$Y = \frac{mg\ X_a\ formed}{mg\ BOD_L\ used} \qquad \text{(F.11)}$$

where X_a is active biomass. Yield coefficients values can be estimated from reported values, from experimental measurements with the waste being studied, or from thermodynamics principles (McCarty, 1975). The yield coefficient for this study was estimated using the thermodynamics concepts described in McCarty (1971,1975).

McCarty (1971) proposed a method to predict yield coefficient based on the free energies of reaction for waste oxidation and cell synthesis. The energy released from oxidation of a substrate is transferred in two steps: (1) energy from the substrate source to an energy carrier, such as ADP, forming ATP; (2) energy from ATP to a

reaction for cell synthesis or maintenance, with ATP being hydrolyzed to ADP. In each of the transfers, loss of energy occurs. The model is illustrated in Figure F.5.

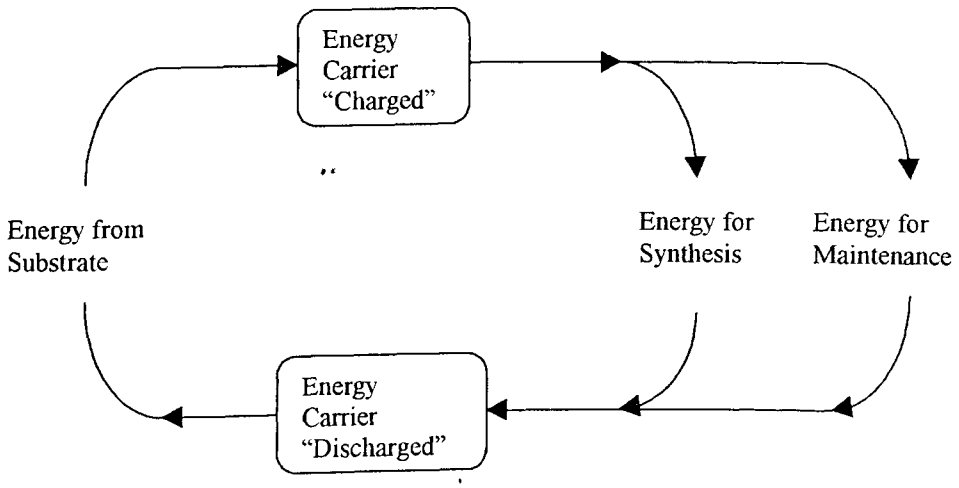

Figure F.5 Energy Transport to Biological Synthesis and Maintenance by Way of an Energy Carrier (Source: McCarty, 1971)

McCarty (1971) suggested that the proportions of the organic waste that are used for synthesis and energy be evaluated in terms of *electron equivalents* (eeq). The number of electron equivalents in a unit mass of cells and BOD_L can be determined from the half-reactions for bacterial cell synthesis and electron acceptors (reactions 1 and 3, respectively, in Table 6.4, Sawyer et al., 1994).

Half-reaction 1: $\frac{1}{5}CO_2 + \frac{1}{20}HCO_3 + \frac{1}{20}NH_4^+ + H^+ + e^- = \frac{1}{20}C_5H_7O_2N + \frac{9}{20}H_2O$

where $C_5H_7O_2N$ is assumed to represent the composition of cells formed. Thus, one electron equivalent of cells is equal to 1/20 mol $C_5H_7O_2N$. Since one mol of $C_5H_7O_2N$ has molecular weight of 113 g, one eeq of cells equals 113 g/20 = 5.65 g cells.

Half-reaction 3: $\frac{1}{4}O_2 + H^+ + e^- = \frac{1}{2}H_2O$

One electron equivalent of BOD_L is equal to (1/4) O_2. Since one mol of O_2 has molecular weight of 32 g, one eeq of BOD_L equals 32 g/4 = 8 g BOD_L.

Defining a_e as a yield coefficient based on electron equivalents, with units electron equivalents of cells synthesized per electron equivalent of electron donor used, Equation (F.11) can be written as Equation (F.12)

$$Y = \frac{5.65\,g\,X_a}{eeq\,X_a} \cdot \frac{1}{\frac{8\,g\,BOD_L}{eeq\,BOD_L}} \frac{eeq\,X_a\,formed}{eeq\,BOD_L\,used} = \frac{5.65 \cdot a_e}{8}, \qquad \text{(F.12)}$$

Equation (F13) relates a_e with A, the number of electron equivalents of electron donor converted for energy per electron equivalent of cells synthesized (McCarty, 1975),

$$A = \frac{1 - a_e}{a_e} \qquad \text{(F.13)}$$

Equation (F.13) can be rearranged to give $a_e = \frac{1}{1+A}$, which can be substituted in Equation (F.12) to give

$$Y = \frac{5.65}{8} \cdot \frac{1}{(1+A)} \qquad \text{(F.14)}$$

From thermodynamics considerations, McCarty (1971) established that A is related to the free energy required for synthesis of one eeq of cells, the free energy per eeq of substrate converted for energy, and the efficiency of energy transfer according to Equation (F.15)

$$A = \frac{-\Delta G_S}{k \cdot \Delta G_r} \qquad \text{(F.15)}$$

where ΔG_S = carrier (ATP) free energy required for synthesis of one eeq of cells from the carbon and nitrogen sources;

ΔG_r = free energy released per eeq of substrate converted for energy;

k = efficiency of energy transfer to and from the energy carrier (ATP)

McCarty (1971) postulated that the energy required to convert a carbon source into cells (ΔG_S) consists of three energy fractions (Equation (F.16)),

$$\Delta G_S = \frac{\Delta G_P}{k^m} + \Delta G_C + \frac{\Delta G_n}{k} \qquad \text{(F.16)}$$

(1) Energy required to convert the carbon source to an intermediate compound, pyruvate. Depending on the oxidation state of the carbon source, such conversion releases energy to the cell (e.g. conversion of carbohydrate to pyruvate) or requires energy (e.g., conversion of CO_2 to pyruvate). This fraction is represented by the term $\Delta G_P/k^m$ in Equation (F.16). The value of m is + 1 if ΔG_P is positive, and – 1 if it is negative.

(2) Energy required to reduce an oxidized nitrogen source to the ammonia level (e.g. nitrate to ammonia) prior to synthesis into cellular material. This fraction is represented by the term $\Delta G_n/k$ in Equation (F.16). When ammonia is the nitrogen source, this term is zero.

(3) Energy required to convert the intermediate carbon source (pyruvate) and ammonia into cellular material, ΔG_C. This energy equals 7.5 kcal/eeq of cells (McCarty, 1971).

Figure F.6 shows the representation of the three energy requirements for cell synthesis.

The values for ΔG_r, ΔG_P, ΔG_n are calculated from established standard free energy of reactions [$\Delta G^0(W)$] (McCarty, 1971). A value for ΔG_r is obtained by subtracting the standard free energy for the half-reaction of the electron acceptor from the standard free energy for the half-reaction of the electron donor. The value of ΔG_P is obtained by subtracting the standard free energy for the half-reaction of pyruvate from the standard free energy for the half-reaction of electron donor. Finally, if nitrate

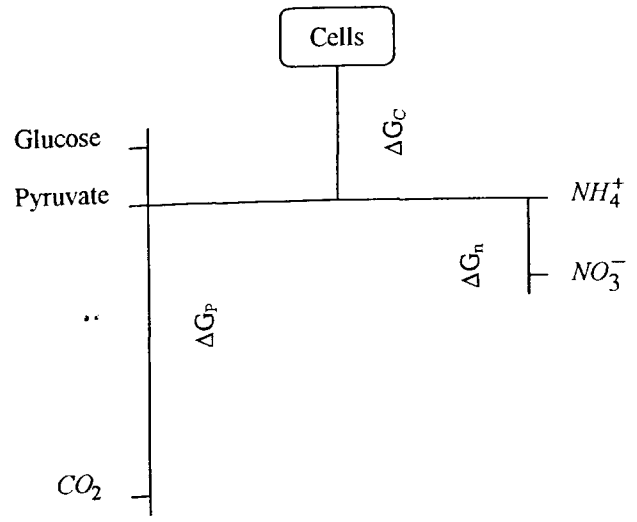

Figure F.6 Energy Requirements for Cell Synthesis from Carbon and Nitrogen Sources (Source: McCarty, 1971)

is the nitrogen source, the value of ΔG_n is obtained by subtracting the standard free energy for the half-reaction of nitrate from the standard free energy for the half-reaction of ammonia.

In the activated sludge process at the Ithaca Wastewater and Sludge Treatment Plant, the electron donor, electron acceptor, and nitrogen source are, respectively, wastewater, oxygen, and ammonia. The composition of wastewater was taken as $C_{10}H_{19}O_3N$, as assumed by Sawyer et al. (1994). The intermediate compound in the conversion of the carbon source to cells was pyruvate.

The standard free energies for the half-reactions were those presented in McCarty (1975).

ΔG_r = standard free energy of the half-reaction electron donor – standard free energy of the half-reaction electron acceptor

Half-reaction electron donor:

$$\frac{1}{50}C_{10}H_{19}O_3N + \frac{9}{25}H_2O = \frac{9}{50}CO_2 + \frac{1}{50}NH_4^+ + \frac{1}{50}HCO_3^- + H^+ + e^-;$$

$\Delta G^0(w)$ = -7.6 kcal/eeq;

Half-reaction electron acceptor:

$$\frac{1}{2}H_2O = \frac{1}{4}O_2 + H^+ + e^-;$$

$\Delta G^0(w)$ = +18.675 kcal/eeq;

Overall:

$$\frac{1}{50}C_{10}H_{19}O_3N + \frac{1}{4}O_2 = \frac{9}{50}CO_2 + \frac{1}{50}NH_4^+ + \frac{1}{50}HCO_3^- + \frac{7}{50}H_2O$$

ΔG_r = -7.6 - 18.675 = -26.275 kcal/eeq

ΔG_P = standard free energy of the half-reaction electron acceptor – standard free energy half-reaction pyruvate

Half-reaction electron donor:

$$\frac{1}{50}C_{10}H_{19}O_3N + \frac{9}{25}H_2O = \frac{9}{50}CO_2 + \frac{1}{50}NH_4^+ + \frac{1}{50}HCO_3^- + H^+ + e^-;$$

$\Delta G^0(w)$ = -7.6 kcal/eeq;

Half-reaction pyruvate:

$$\frac{1}{10}CH_3COCOO^- + \frac{2}{5}H_2O = \frac{1}{5}CO_2 + \frac{1}{10}HCO_3^- + H^+ + e^-;$$

$\Delta G^0(w)$ = - 8.545 kcal/eeq;

Overall:

$$\frac{1}{50}C_{10}H_{19}O_3N + \frac{1}{50}CO_2 + \frac{4}{50}HCO_3^- = \frac{1}{10}CH_3COCOO^- + \frac{1}{50}NH_4^+ + \frac{1}{25}H_2O$$

ΔG_P = -7.6 - (-8.545) = 0.945 kcal/eeq;

ΔG_n = zero, since ammonia was the nitrogen source.

McCarty (1971) stated that the efficiencies of energy transfer for both aerobic and anaerobic cultures range from 40 to 80 %, with 60 % average. The value of k in Equations (F.15) and (F.16) was assumed to be 0.6. Substituting the corresponding values in Equation (F.16),

$$\Delta G_S = \frac{0.945}{(0.6)^1} + 7.5 + 0 = 9.075 \frac{kcal}{eeq}$$

Substituting the corresponding values in Equation (F.15),

$$A = \frac{-9.075 \frac{kcal}{eeq}}{0.6 \cdot \left(-26.275 \frac{kcal}{eeq}\right)} = 0.5756 \frac{eeq\ substrate\ converted\ for\ energy}{eeq\ cells\ synthesized}$$

Substituting the value of A in Equation (F.13),

$$Y = \frac{5.65}{8} \cdot \frac{1}{(1+A)} = \frac{5.65 g X_a}{8 g BOD_L} \cdot \frac{1}{(1+0.5756)} = 0.45 \frac{g X_a\ formed}{g\ BOD_L\ used}$$

The calculated yield coefficient was within the range presented by Metcalf and Eddy (1991) for activated sludge process treating domestic wastewater, 0.4 – 0.8 mg X_a formed/mg BOD_5 used.

7.2 Concentrations of Total and Volatile Suspended Solids in the Aeration Tanks

The total and volatile suspended solids concentration values were used in the estimation of the amount of waste activated sludge to be treated in the sludge treatment processes. The volatile suspended solids concentration in the aeration tanks are formed by three fractions: (1) active VSS, which represents the microorganisms that are alive and degrading the organic matter; (2) nonviable, inert VSS, which are non-biodegradable residuals of organic matter decomposition; (3) nonviable,

biodegradable VSS. Equations (F.17), (F.18), (F.19) and (F.20) describe, respectively, the concentrations of active VSS, inert VSS, total VSS, and TSS in the aeration tanks. These equations were developed from suspended solids mass balances on the aeration tanks and secondary sedimentation tanks.

$$X_a = \frac{\theta_C^{AS}}{\theta^{AS}} \frac{Y \cdot (S_{PE} - S)}{1 + b^{AS} \cdot \theta_C^{AS}} \qquad (F.17)$$

$$X_i = \frac{\theta_C^{AS}}{\theta^{AS}} (1 - f_d^{PE}) \cdot X_V^{PE} + (1 - f_d^{AS}) \cdot b^{AS} \cdot X_a \cdot \theta_C^{AS} \qquad (F.18)$$

$$X_V = \frac{\theta_C^{AS}}{\theta^{AS}} \left[X_V^{PE} (1 - f_d^{PE}) + \frac{Y(S_{PE} - S)}{1 + b^{AS} \theta_C^{AS}} \left[1 + (1 - f_d^{AS}) b^{AS} \theta_C^{AS} \right] \right] \qquad (F.19)$$

$$X_T = \frac{\theta_C^{AS}}{\theta_C} \left[X_F^{PE} + X_{CSI} + X_V^{PE} (1 - f_d^{PE}) + \frac{Y(S_{PE} - S)}{0.9 \cdot (1 + b^{AS} \theta_C^{AS})} \left[1 + (1 - f_d^{AS}) b^{AS} \theta_C^{AS} \right] \right] \qquad (F.20)$$

where θ^{AS} = hydraulic detention time;

S_{PE} = Total BOD_L of primary effluent, to be determined;

S = Soluble BOD_L of secondary effluent, to be determined;

f_d^{PE} = biodegradable fraction of primary effluent volatile suspended solids, assumed to be the same as the biodegradable fraction of wastewater VSS, 0.65 (Equation F.33, Section 9.1);

X_V^{PE} = concentration of VSS in primary effluent, 60 mg/l;

f_d^{AS} = biodegradable fraction of active VSS formed in the activated sludge process, 0.8;

X_F^{PE} = concentration of fixed suspended solids in primary effluent, 19 mg/l;

X_{CSI} = concentration of chemical sludge, calculated by dividing the load of chemical sludge (268 lb/d) by primary effluent flowrate (5,887,539 gal/d); X_{CSI} = 5.45 mg/l

In the development of Equation (F.17), it was assumed that the contribution of active organisms from the primary effluent was negligible. In Equations (F.18) and (F.19), it was assumed that hydrolysis was fast enough to convert all the nonviable, biodegradable volatile suspended solids into soluble form. Equation (F.18) shows that inert organic compounds in activated sludge have two sources: (1) residuals from the decomposition of volatile suspended solids from primary effluent, and (2) residuals from decomposition of active volatile suspended solids. The inorganic (mineral) solids in the activated sludge process were formed by three sources (Equation F.20): (1) fixed suspended solids in primary effluent; (2) chemical sludge, and (3) inorganic fraction of biomass, assumed to be 10 % of the dry weight (McCarty, 1971).

The theoretical soluble BOD_L substrate concentration S in Equations (F.17), (F.19), and (F.20) was given by Equation (F.21) (Metcalf and Eddy, 1991).

$$S = \frac{K_s \cdot \left(1 + b^{AS} \cdot \theta_C^{AS}\right)}{\theta_C^{AS} \cdot \left(Y \cdot k^{AS} - b^{AS}\right) - 1} \qquad \text{(F.21)}$$

where K_S = half-velocity constant

k^{AS} = maximum specific rate of substrate utilization (mg substrate used/mg active volatile suspended solids per day);

Substituting K_S = 70 mg/l BOD_L, k^{AS} = 5 mg BOD_L/g X_a·day, b^{AS} = 0.1 day^{-1}, θ_C^{AS} = 8.8 days, $Y = 0.45\ mg\ X_a / mg\ BOD_L$ in Equation (F.21),

$$S = \frac{70 \cdot (1 + 0.1 \cdot 8.8)}{8.8 \cdot (0.45 \cdot 5.0 - 0.1) - 1} = 7.3 \frac{mg\ BOD_L}{l}$$

The measured average BOD_5 in secondary effluent measured in the plant was 12 mg/l (soluble and particulate). The ultimate BOD of the secondary effluent was calculated using Equation (F.22) (Sawyer et al., 1994), assuming a BOD rate constant of 0.30 day^{-1} (Sawyer et al., 1994)

$$BOD_L = \frac{BOD_5}{1 - e^{-k \cdot t}} \tag{F.22}$$

$$BOD_L = \frac{12}{1 - e^{-(0.3) \cdot 5}} = 15 \frac{mg}{l}$$

Activated sludge biomass was assumed to have composition $C_5H_7O_2N$ (Sawyer et al., 1994). The oxidation of $C_5H_7O_2N$ (113 g) requires 5 moles O_2 (160 g) (Equation F.23), or 1.42 mg BOD_L/mg X_a.

$$C_5H_7O_2N + 5O_2 = 5CO_2 + 2H_2O + NH_3 \tag{F.23}$$

An estimation of the BOD_L of volatile suspended solids in secondary effluent was calculated by multiplying the volatile suspended solids concentration in secondary effluent by 1.42 mg BOD_L/mg X_a.

$$2.5 \frac{mg\ TSS}{l} \cdot 0.75 \frac{mg\ VSS}{mg\ TSS} \cdot 1.42 \frac{mg\ O_2}{mg\ VSS} \approx 3 \frac{mg\ BOD_L}{l}.$$

In this calculation it was assumed that all volatile suspended solids were 100 % biodegradable. The soluble BOD_L in secondary effluent was estimated by the difference between total and volatile suspended solids BOD_L, 15 – 3 = 12 mg/l. This value was used in Equations (F.17), (F.18), and (F.20) instead of the theoretical value calculated using Equation (F.21). The BOD_L of primary effluent, S_{PE} was calculated using Equation (F.22), with the 5-day BOD and rate constant equal to 79 mg/l, and of 0.30 day^{-1}, respectively.

Substituting the corresponding values in Equations (F.17), (F.18), and (F.19), the values of X_a, X_i, and X_V were:

$$X_a = \frac{8.8}{0.24} \cdot \frac{0.45 \cdot (102 - 12)}{1 + 0.1 \cdot 8.8} = 790 \frac{mg}{l}$$

$$X_i = \frac{8.8}{0.24} \cdot (1 - 0.65) \cdot 60 + (1 - 0.8) \cdot 0.1 \cdot 790 \cdot 8.8 = 910 \frac{mg}{l}$$

$$X_V = \frac{8.8}{0.24} \cdot \left[(1-0.65) \cdot 60 + \frac{0.45 \cdot (102-12)}{1+0.1 \cdot 8.8} [1+(1-0.8) \cdot 0.1 \cdot 8.8 \right] = 1,700 \frac{mg}{l}$$

The hydraulic detention time used in these equations was 0.24 d, which was taken from the plant's records. The concentration of volatile suspended solids calculated using Equation (F.19) was twice the measured concentration in the aeration tank, which was 850 mg/l. Many assumptions had to be made to estimate parameters of Equation (F.19), such as the composition of primary effluent volatile suspended solids ($C_{10}H_{19}O_3N$), the composition of biomass formed in activated sludge ($C_5H_7O_2N$), the biodegradable fractions of primary effluent VSS and biomass, and the specific decay rate of biomass. Although all parameters used in Equation (F.19) were within the range cited in the literature for domestic waste, there was a 100 % difference between the predicted value by the model and the measured value in samples.

The excess organic biological sludge produced in the activated sludge process is given by Equation (F.24).

$$\text{Excess organic biological sludge} = \frac{X_V \cdot V}{\theta_C^{AS}} = \frac{X_V \cdot (\theta^{AS} \cdot Q_{PE})}{\theta_C^{AS}} \qquad \text{(F.24)}$$

Two alternatives were considered for evaluating the variables in Equation (F.24). The first alternative was to consider the data measured at the plant – X_V = 850mg/l, $\theta^{AS} = 0.24d$, $\theta_C^{AS} = 8.8d$. In this case it was unnecessary to use Equation (F.19) to calculate the volatile suspended solids concentration to be used in Equation (F.24). The second alternative was to use two variables measured at the plant, and allow the third variable to be evaluated using Equation (F.19). In the second alternative, X_V = 850 mg/l, θ_C^{AS} = 8.8 d, but $\theta^{AS} = 0.48d$ (by solving Equation (F.19) for θ^{AS}). The quantity of excess sludge of the second alternative was twice as much as the quantity of sludge calculated using the first alternative. The use of variables with values of the

second alternative produced an estimation of the quantity of sludge that approximated better the quantity of sludge measured after sludge dewatering. For this reason, the values of the second alternative were used to estimate the excess quantity of biological sludge. Substituting the corresponding values into Equation (F.24),

Excess organic biological sludge =

$$\frac{850\frac{mg}{l}\cdot 0.48d\cdot 5{,}888{,}473\frac{gal}{d}\cdot(8.3454\cdot 10^{-6})}{8.8d}=2{,}278\frac{lb}{d}$$

Equations (F.17) and (F.18) were recalculated using θ^{AS} = 0.48 d, resulting in X_a = 395 mg/l and X_i = 455 mg/l.

The concentration of total suspended solids in the aeration tank was calculated by substituting the corresponding values in Equation (F.20), resulting X_T = 1,349 mg/l. The excess quantity of waste activated sludge and chemical sludge was

$$\frac{1{,}349\frac{mg}{l}\cdot 0.48d\cdot 5{,}888{,}473\frac{gal}{d}\cdot(8.3454\cdot 10^{-6})}{8.8d}=3{,}616\frac{lb}{d}$$

The total quantity of secondary sludge was formed by two parts: (a) chemical sludge, 268 lb/d, and (2) waste activated sludge, 3,616 – 268 = 3,348 lb/d. The ratio VSS:TSS in the sludge was 2,278/3,616 = 0.63. This ratio was lower than the measured ratio in samples collected in the aeration tank, which was 0.71. This observation suggests that some chemical sludge might be forming and settling in the primary clarifier, or the chemical sludge was not completely mixed in the aeration tanks, at the time measurements were made.

In the first iteration of calculations, the quantity of sludge sent to the thickeners was equal to 3,616 lb/d minus the quantity of total suspended solids lost in the secondary effluent. The flowrates of waste secondary sludge and secondary effluent

were determined from a mass balance on the secondary sedimentation tanks (Figure F.7).

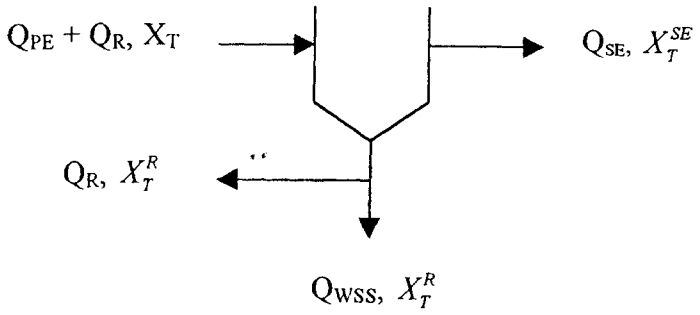

Figure F.7 Input to, and Output from, the Secondary Sedimentation Tank

A mass balance for total suspended solids on the secondary sedimentation tank was written in the form of Equation (F.25).

$$(Q_{PE}+Q_R)\cdot X_T = Q_R \cdot X_T^R + Q_{WSS}\cdot X_T^R + Q_{SE}\cdot X_T^{SE} \quad \text{(F.25)}$$

where $Q_R = R \cdot Q_{PE}$ (F.26)

Q_{PE} = primary effluent flowrate, 5,888,473 gal/d

Q_R = recycle flowrate

Q_{SE} = secondary effluent flowrate

Q_{WSS} = waste secondary sludge flowrate (activated and chemical sludges)

X_T = total suspended solids concentration in the aeration tank, 1349 mg/l

X_T^{SE} = total suspended solids concentration in secondary effluent, 2.5 mg/l

X_T^R = total suspended solids concentration in recycle sludge, 2850 mg/l

$$Q_{SE} = Q_{PE} - Q_W \quad \text{(F.27)}$$

$$R = \frac{1-\frac{\theta^{AS}}{\theta_C^{AS}}}{\frac{X_T^R}{X_T}-1} \quad \text{(F.28)}$$

A value R = 0.85 was obtained by substituting θ^{AS} = 0.48 d, θ_C^{AS} = 8.8 d, X_T = 1,349 mg/l, and X_T^R = 2,850 mg/l in Equation (F.28). Substituting R into Equation (F.26), Q_R = 0.85 x 5,888,473 = 5,003,508 gal/d. Substituting the corresponding values and Equation (F.27) into Equation (F.25),

$$(5,888,473+5,003,508)\cdot 1,349=(5,003,508)\cdot 2,850+Q_{WSS}\cdot 2,850$$
$$+(5,888,743-Q_{WSS})\cdot 2.$$

which has as solution Qwss = 146,993 gal/d. Hence, Q_{SE} = 5,888,473 – 146,993 = = 5,741,480 gal/d. These values, as stated before, were updated in subsequent iterations.

According to the plant's records, secondary effluent and waste secondary sludge flowrates were, respectively, 6,203,333 and 148,500 gal/d. As mass balances were repeated in further iterations, secondary effluent flowrate converged to the same value reported in the plant's records.

The loads of suspended solids in waste secondary sludge (waste activated sludge plus chemical sludge) and secondary effluent were calculated by multiplying the suspended solids concentrations by the respective flowrates.

Load of waste activated and chemical sludge to the thickeners = 2,850 mg/l x 146,993 gal/d x (8.3454 x 10^{-6}) = 3,496 lb/d;

Load of volatile suspended solids to the thickeners = 0.63 x 3,496 = 2,202 lb/d;

Load of suspended solids in secondary effluent = 2.5 mg/l x 5,741,780 gal/d x (8.3454 x 10^{-6}) = 120 lb/d;

Load of volatile suspended solids in secondary effluent = 0.63 x 120 = 75 lb/d

Total load of suspended solids = 3,496 + 120 = 3,616 lb/d

Figure F.8 presents the preliminary mass balance for suspended solids and flowrates on the activated sludge process. These values correspond to the calculations

of the first iteration. These values were updated as the flowrates and suspended solids concentrations from sludge treatment processes were calculated.

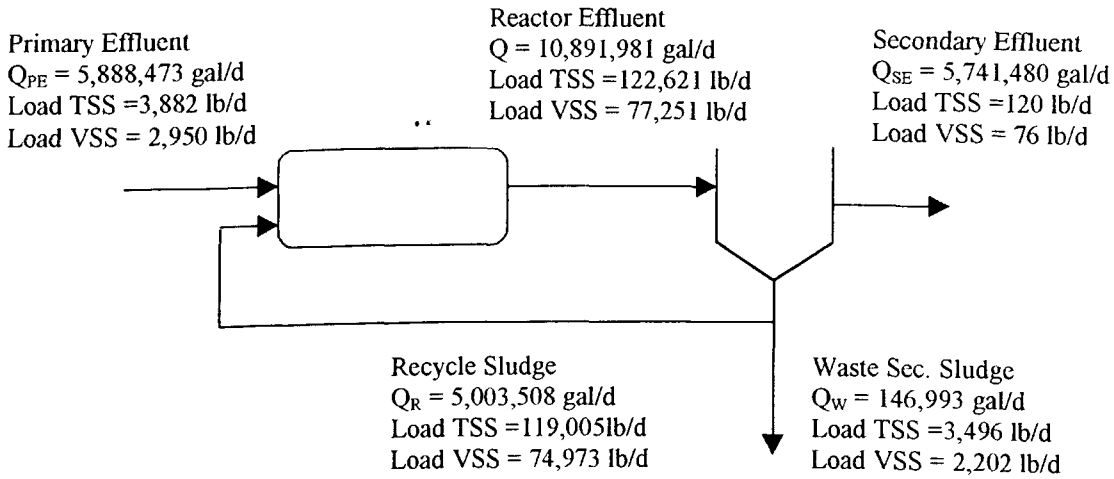

Figure F.8 Flowrates and Mass Balance for Suspended Solids on the Activated Sludge Process

8. Flowrates and Mass Balances for Total and Volatile Suspended Solids on the Thickeners

Table F.1 presents the loads of suspended solids and flowrates in primary sludge without grit and waste secondary sludge. The latter was formed by waste activated sludge and chemical sludge.

The ratio between volatile and total suspended solids in the thickener feed was 5,225/6,763 = 0.774. This value was higher than the observed ratio in the thickened sludge, which was 0.74. Eastman and Fergusson (1981) suggested that solubilization can occur in sludge thickeners, after having measured a high hydrolysis rate (8.0 d^{-1}) in primary sludge. Hydrolysis, however, was not considered in the mass balance calculations for the thickeners. Figure F.9 presents the input to, and output from, the thickeners.

Table F.1 Flowrates and Suspended Solids Loads in Primary and Waste Secondary Sludges

Source	Unit	Primary Sludge	Waste Sec. Sludge	Total
Total Susp. Solids	lb/d	3,257	3,496	6,753
Volatile Susp. Solids	lb/d	3,023	2,202	5,225
Fixed Susp. Solids	lb/d	234	1,294	1,528
Flowrate	gal/d	326,133	146,993	473,126

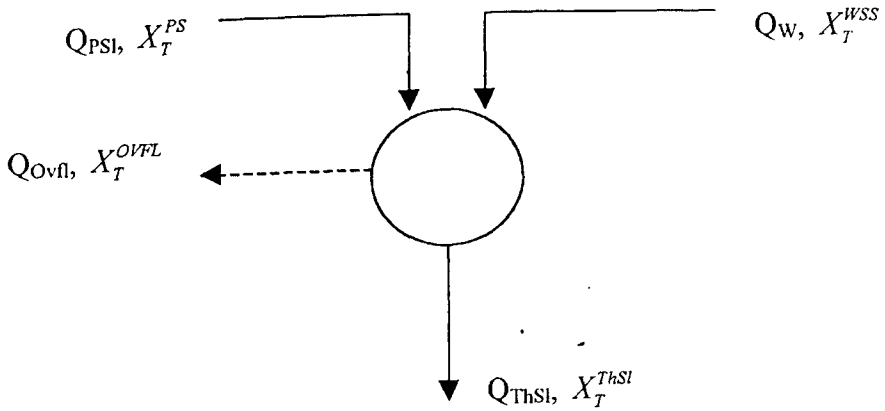

Figure F.9 Input to, and Output from, the Thickeners

Thickened sludge flowrate was estimated using Equation (F.6). In order to solve this equation, it was necessary to know (1) the total load of suspended solids, (2) the density of thickened sludge, and (3) the mass fraction of total suspended solids in thickened sludge (f_m = 0.038). The density of the thickened sludge particles was calculated using Equation (F.8)

$$e_P = \left[\frac{(1-0.774)}{2.5} + \frac{0.774}{1.0}\right]^{-1} = 1.1569 \frac{g}{cm^3}$$

The density of thickened sludge was calculated by substituting the corresponding values in Equation (F.7).

$$e_{ThSl} = \left[\frac{0.038}{1.1569} + \frac{(1-0.038)}{1.0} \right]^{-1} = 1.0052 \frac{g}{cm^3}$$

A mass balance for total suspended solids on the thickener is given by Equation (F.29). The load of total suspended solids in thickened sludge was unknown because the load of total suspended solids in the thickener overflow was unknown (Equation F.30). The concentration of total suspended solids in the overflow was known, but not the flowrate. Equation (F.31) describes the flowrate balance on the thickener.

$$L_T^{ThSl} = L_T^{PS} + L_T^{WSS} - L_T^{OVFL} \quad \text{(F.29)}$$

$$L_T^{OVFL} = Q_{OVFL} \cdot X_T^{OVFL} \quad \text{(F.30)}$$

$$Q_{OVFL} = Q_{PS} + Q_{WSS} - Q_{ThSl} \quad \text{(F.31)}$$

In Equations (F.29) to (F.31), Q and L stand for flowrate and load of total suspended solids, respectively; the subscripts and superscripts ThSl, PS, WSS, OVFL mean thickened sludge, primary sludge, waste secondary sludge, and overflow, respectively. In order to solve Equation (F.6) for the daily volume of thickened sludge, it initially was assumed that the load of total suspended solids in overflow was zero. Substituting the corresponding values in Equation (F.6),

$$Q_{ThSl} = \frac{6,753\, {lb}/{d} \cdot 0.4536\, {kg}/{lb}}{1.0\, {kg}/{l} \cdot 1.0052 \cdot 0.038} = 80,191\, {l}/{d} = 21,184\, {gal}/{d}$$

The overflow flowrate was calculated by substituting the corresponding values in Equation (F.31),

Q_{OVFL} = 326,133 + 146,993 – 21,184 = 451,942 gal/d

The load of total suspended solids in overflow was calculated using Equation (F.30)

$$L_T^{OVFL} = 85 \text{ mg/l x } 451{,}942 \text{ gal/d x } (8.3454 \text{ x } 10^{-6}) = 321 \text{ lb/d},$$

where 85 mg/l was the average concentration of total suspended solids in the thickener overflow measured in three samples. The flowrate of thickened sludge was recalculated by subtracting 321 lb/d from 6,753 lb/d in the term corresponding to the load of total suspended solids of thickened sludge in Equation (F.6),

$$Q_{ThSl} = \frac{(6{,}753 - 321)\,{}^{lb}/_{d} \cdot 0.4536\,{}^{kg}/_{lb}}{1.0\,{}^{kg}/_{l} \cdot 1.0052 \cdot 0.038} = 76{,}383\,{}^{l}/_{d} = 20{,}179\,{}^{gal}/_{d}$$

The overflow flowrate was recalculated using Equation (F.31),

$$Q_{OVFL} = 326{,}133 + 146{,}993 - 20{,}179 = 452{,}947 \text{ gal/d}$$

The load of total suspended solids in overflow was recalculated using Equation (F.30)

$$L_T^{OVFL} = 85 \text{ mg/l x } 452{,}947 \text{ gal/d x } (8.3454 \text{ x } 10^{-6}) = 321 \text{ lb/d}.$$

Because the calculated load of total suspended solids in thickener overflow remained the same, additional correction for the load of total suspended solids in thickened sludge was not necessary. Figure F.10 presents the flowrates and the suspended solids mass balance for the thickeners in the first iteration of calculations.

Table F.2 presents the flowrates and suspended solids loads in thickened sludge and overflow. It was assumed that the composition of suspended solids in overflow and thickened sludge were proportional to suspended solids loads of primary sludge and waste secondary sludge. The composition of thickened sludge was taken into consideration in the estimation of the organic load in the anaerobic digester feed.

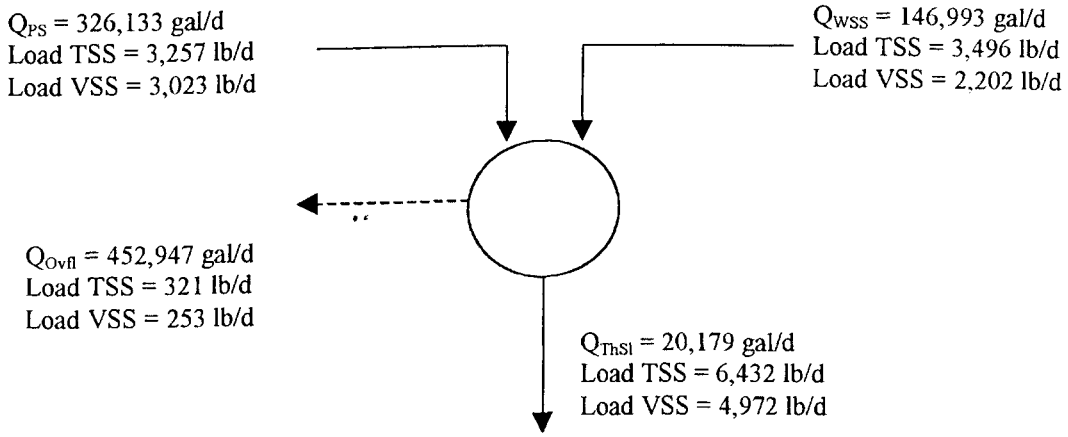

Figure F.10 Flowrates and Mass Balance for Suspended Solids on the Thickeners

Table F.2 Thickened Sludge and Thickener Overflow Flowrates and Solids Loads

Characteristic	Source	Thickened Sludge	Thickener Overflow
Total Solids	Primary Sludge	3,102	155
	Waste Sec. Sludge	3,330	166
	Total	6,432	321
Volatile Solids	Primary Sludge	2,876	146
	Waste Sec. Sludge	2,096	107
	Total	4,972	253
Fixed Solids	Primary Sludge	226	9
	Waste Sec. Sludge	1,234	59
	Total	1,460	68
Flowrates	Primary Sludge	13,909	312,216
	Waste Sec. Sludge	6,270	140,731
	Total	20,179	452,947

9. Flowrates and Mass Balances for Total and Volatile Suspended Solids on the Anaerobic Digesters

In anaerobic digestion, biodegradable organic substrate is decomposed in the absence of oxygen, with formation of end products such as methane and carbon

dioxide gases. A fraction of the organic substrate is used for cell synthesis, but the growth yield is lower than the yield observed in aerobic systems (Metcalf and Eddy, 1991). The anaerobic digesters at the Ithaca Wastewater and Sludge Treatment Plant are comprised of two tanks, the primary, used mostly for digestion, and the secondary, used mostly for sludge thickening. In the following sections, the terms digested sludge, thickened digested sludge, and supernatant mean, respectively, sludge that had been digested but not thickened, digested sludge that had been thickened, and digested sludge supernatant that had been formed as a result of sludge thickening.

9.1 Anaerobic Digester Feed Composition

Anaerobic digester feed was composed by thickened sludge and septage. Septage density and flowrate were calculated in Section 4.1.2. Table F.3 presents the flowrates and suspended solids loads, per source, in the anaerobic digester feed.

Table F.3 Flowrates and Suspended Solids Loads in Anaerobic Digester Feed (Units: flowrate, gal/d; load, lb/d)

Characteristic	Primary Sludge	WSS	Septage	Total
Total Solids	3,102	3,330	1,351	7,783
Volatile Solids	2,876	2,096	928	5,900
Fixed Solids	226	1,234	423	1,883
Flowrate	13,909	6,270	7,906	28,085

The mass balance on the anaerobic digester required the estimation of the concentration of volatile suspended solids in the digested sludge, before thickening. In order to estimate the concentration of VSS in the digested sludge, it was necessary to estimate the BOD_L in the digester feed. Equation (F.32) was used to calculate the load of BOD_L in the digester feed.

$$\text{Load } BOD_L = L_V^{PS} \cdot f_d^{PS} \cdot O^{PS} + L_V^{WSS} \cdot f_d^{WSS} \cdot O^{WSS} \cdot \frac{X_a^{WSS}}{X_V^{WSS}} + L_V^{Sept} \cdot f_d^{Sept} \cdot O^{Sept} \qquad \text{(F.32)}$$

In Equation (F.32), L_V represents the load of volatile suspended solids, with the superscripts PS, WSS, and Sept meaning primary sludge, waste secondary sludge, and septage; f_d represents the fraction of the volatile suspended solids that was biodegradable, and O represents the mass of O_2 required to oxidize a unit mass of volatile suspended solids. The term X_a^{WSS} / X_V^{WSS} is present in Equation (F.32) because the only biodegradable volatile suspended solids in waste secondary sludge was active volatile suspended solids. In the activated sludge process, it was assumed that all nonviable biodegradable volatile suspended solids were made soluble in the aeration tank. From Section 7.2, the ratio X_a^{WSS} / X_V^{WSS} was equal to 395/850 = 0.465.

The biodegradable fractions of primary sludge and septage volatile suspended solids were assumed to be the same as for raw wastewater volatile suspended solids. This fraction was estimated from data described in the publication *Design of Municipal Wastewater Treatment Plants* (WEF, 1998). Typical 5-day BOD and COD of particulate solids in domestic wastewater were cited as 135 mg/l and 260 mg/l, respectively. Assuming a BOD rate constant of 0.30 d^{-1} (Sawyer et al., 1994), the BOD_L corresponding to BOD_5 of 135 mg/l was calculated using Equation (F.22),

$$BOD_L = \frac{BOD_5}{1 - e^{-k \cdot t}} = \frac{135}{1 - e^{-(0.3) \cdot 5}} = 174 \frac{mg}{l}$$

The biodegradable fraction of raw wastewater volatile suspended solid was calculated using Equation (F.33)

$$f_d^W = \frac{BOD_L}{COD} = \frac{174}{260} = 0.67 \qquad \text{(F.33)}$$

The value of f_d^W was approximated as 0.65. The biodegradable fraction of volatile suspended solids in waste secondary sludge, which was the biodegradable fraction of active volatile suspended solids, was assumed to be 0.8 (McCarty, 1971).

In order to determine O^{PS}, O^{WSS}, and O^{Sept} in Equation (F.32), it was necessary to assume a composition for volatile suspended solids in primary sludge, septage, and biomass in the waste secondary sludge. The composition of volatile suspended solids in primary sludge and septage were assumed to be the same as VSS in raw wastewater and the composition of biomass in waste secondary sludge was assumed to be the same as the composition of active VSS in activated sludge. The compositions of wastewater VSS and active VSS were assumed to be, respectively, $C_{10}H_{19}O_3N$ and $C_5H_7O_2N$ (Sawyer et al., 1994). The quantities of oxygen required to oxidize one mol of $C_{10}H_{19}O_3N$ and $C_5H_7O_2N$ were calculated using Equation (F.34) (Sawyer et al., 1994)

$$C_nH_aO_bN_c+\left(n+\frac{a}{4}-\frac{b}{2}-\frac{3}{4}\cdot c\right)O_2 \rightarrow nCO_2+\left(\frac{a}{2}-\frac{3}{2}\cdot c\right)H_2O+cNH_3 \qquad \text{(F.34)}$$

Substituting the corresponding values in Equation (F.34)

$$C_{10}H_{19}O_3N+12.5O_2 \rightarrow 10CO_2+8H_2O+NH_3$$

$$C_5H_7O_2N+5O_2 \rightarrow 5CO_2+2H_2O+NH_3$$

The oxidation of one mol $C_{10}H_{19}O_3N$ (201 g) requires 12.5 moles O_2 (400 g), or

$$\frac{400\,g\,O_2}{201\,g\,VSS}=1.99\,\frac{g\,O_2}{g\,VSS}.$$

The oxidation of one mol $C_5H_7O_2N$ (113 g) requires 5 moles O_2 (160 g), or

$$\frac{160\,g\,O_2}{113\,g\,VSS}=1.42\,\frac{g\,O_2}{g\,VSS}.$$

The load of BOD_L in the digester feed was calculated by substituting the corresponding values into Equation (F.32),

$$\text{Load } BOD_L = 2{,}876\frac{lb}{d}\cdot 0.65\cdot 1.99 + 2{,}096\cdot 0.8\cdot 1.42\cdot 0.465 + 928\cdot 0.65\cdot 1.99 = 6{,}028\frac{lb}{d}$$

Equation (F.35) was used to estimate the load of COD in the digester feed. This equation is similar to Equation (F.32), omitting the terms for the biodegradability of the volatile suspended solids.

$$\text{Load } COD = L_V^{PS}\cdot O^{PS} + L_V^{WSS}\cdot O^{WSS} + L_V^{Sept}\cdot O^{Sept} \qquad \text{(F.35)}$$

$$\text{Load } COD = 2{,}876\cdot 1.99 + 2{,}096\cdot 1.42 + 928\cdot 1.99 = 10{,}546\frac{lb}{d}$$

The ratio of BOD_L/COD in digester feed VSS was 6,028/10,546 = 0.572

The contribution of soluble BOD_L was estimated to be negligible in comparison with the particulate BOD_L load in the digester feed. If no hydrolysis took place in the thickeners, the contribution of soluble BOD_L from waste secondary sludge was given by the product between the activated sludge process effluent BOD_L (12 mg/l) and the flowrate contributed to the digester feed by the waste secondary sludge (6,270 gal/d, Table F.3).

Load soluble BOD_L from waste secondary sludge = 12 mg/l x 6,270 gal/d x

x (8.3454×10^{-6}) = 0.6 lb/d.

The concentration of soluble BOD_L in primary sludge was assumed to be equal to the soluble BOD_L concentration in wastewater.

Soluble BOD_L concentration = Total BOD_L concentration – Particulate BOD_L concentration

Total BOD_L in wastewater was calculated using Equation (F.22), with BOD rate constant of 0.30 d^{-1},

Wastewater total $BOD_L = \frac{BOD_5}{1-e^{-k \cdot t}} = \frac{140}{1-e^{-(0.3)\cdot 5}} = 180 \frac{mg}{l}$, where 140 mg/l was the 5-day BOD in wastewater. The wastewater particulate BOD_L was estimated using Equation (F.36),

$$\text{Wastewater particulate } BOD_L = X_V^W \cdot f_d^W \cdot O^W \qquad \text{(F.36)}$$

with the variables having the same meaning as the variables in Equation (F.32). The concentration of volatile suspended solids in wastewater was 142 mg/l x 0.82 = = 116 mg/l. Substituting the corresponding values in Equation (F.36),

$$\text{Wastewater particulate } BOD_L = 116 \frac{mg\,VSS}{l} \cdot 0.65 \cdot 1.99 \frac{mg\,O_2}{mg\,VSS} = 150 \frac{mg}{l}$$

Wastewater Soluble BOD_L = 180 – 150 = 30 mg/l.

Contribution of soluble BOD_L in primary sludge to the BOD_L of digester feed = = 30 mg/l x 13,909 gal/d x (8.3454×10^{-6}) = 3.5 lb/d

The calculation of soluble BOD_L in primary sludge also assumed that no hydrolysis of particulate matter took place in the primary sedimentation tanks and thickeners.

The load of BOD_L in soluble septage could not be estimated because there was no datum for the total BOD_L of septage. The load of soluble BOD_L in septage was ignored. The error in neglecting this load was expected to be minimum because (1) the soluble BOD_L was a very small fraction of the total BOD_L in the digester feed, and (2) the flowrate of septage was approximately 30 % of the total flowrate.

Total soluble BOD_L in digester feed = 0.6 + 3.5 = 4.1 lb/d (excluding septage soluble BOD_L).

Total BOD_L in digester feed = particulate BOD_L + soluble BOD_L = 6,028 + 4.1 = = 6,032 lb/d

9.2 Overall Yield Coefficient for Anaerobic Digestion

The yield coefficient for anaerobic digestion was calculated using the thermodynamics principles discussed in Section 7.1. Equations (F.14) through (F.16), rewritten below, were solved to estimate the yield coefficient.

$$Y = \frac{5.65}{8} \cdot \frac{1}{1+A} \qquad \text{(F.14)}$$

$$A = \frac{-\Delta G_S}{k \cdot \Delta G_r} \qquad \text{(F.15)}$$

$$\Delta G_S = \frac{\Delta G_p}{k^m} + \Delta G_C + \frac{\Delta G_n}{k} \qquad \text{(F.16)}$$

The following assumptions were made for the solution of Equations (F.14) through (F.16):

Electron donor: $C_{10}H_{19}O_3N$;

Electron acceptor: CO_2;

Intermediate compound in the conversion of the carbon source to cells: pyruvate;

Nitrogen source for cell synthesis: ammonia;

The composition of the electron donor was assumed to be the composition of volatile suspended solids in wastewater ($C_{10}H_{19}O_3N$), although part of the digester feed was composed by waste activated sludge ($C_5H_7O_2N$). This assumption was necessary because the free energy for oxidation of $C_5H_7O_2N$ is not known (McCarty, 1975, Sawyer et al., 1994). The free energies for the oxidative and reductive half-reactions are presented below.

ΔG_r = standard free energy of the half-reaction electron donor – standard free energy of the half-reaction electron acceptor;

Half-reaction electron donor:

$$\frac{1}{50}C_{10}H_{19}O_3N + \frac{9}{25}H_2O = \frac{9}{50}CO_2 + \frac{1}{50}NH_4^+ + \frac{1}{50}HCO_3^- + H^+ + e^-;$$

$\Delta G^0(w)$ = -7.6 kcal/eeq;

Half-reaction electron acceptor:

$$\frac{1}{8}CH_4 + \frac{1}{4}H_2O = \frac{1}{8}CO_2 + H^+ + e^-;$$

$\Delta G^0(w)$ = -5.763 kcal/eeq;

ΔG_r = -7.6 - (-5.763) = -1.837 kcal/eeq

ΔG_P = standard free energy of the half reaction electron donor – standard free energy of the half reaction pyruvate;

Half-reaction pyruvate:

$$\frac{1}{10}CH_3COCOO^- + \frac{2}{5}H_2O = \frac{1}{5}CO_2 + \frac{1}{10}HCO_3^- + H^+ + e^-;$$

$\Delta G^0(w)$ = -8.545 kcal/eeq;

ΔG_P = -7.6 - (-8.545) = 0.945 kcal/eeq;

ΔG_C = 7.5 kcal (McCarty, 1975);

ΔG_n = 0, because the nitrogen source was ammonia

Substituting ΔG_P, ΔG_C, ΔG_n, and k values in Equation (F.16),

$$\Delta G_S = \frac{0.945}{0.6^1} + 7.5 + 0 = 9.075 \frac{kcal}{eeq}$$

Substituting the values for ΔG_r, ΔG_S, and k in Equation (F.15)

$$A = \frac{-9.075}{0.6 \cdot (-1.837)} = 8.23 \frac{eeq\ substrate\ to\ energy}{eeq\ cells\ formed}$$

Substituting the value for A in Equation (F.14)

$$\text{Yield coefficient} = \frac{5.65}{8 \cdot (1 + A)} = \frac{5.65}{8 \cdot (1 + 8.23)} = 0.0765 \frac{gVSS\ formed}{g\ BOD_L\ used}$$

The calculated value of the overall yield coefficient, 0.0765 mg VSS/mg BOD_L, was within the range cited by Metcalf and Eddy (1991) for anaerobic digestion of domestic sludge (0.040 – 0.100 mg VSS/mg BOD_5).

9.3 Stoichiometry of Anaerobic Digestion

The stoichiometry of anaerobic digestion was used to estimate the volume of gas produced and the reduction in sludge volume during digestion. The overall reaction for anaerobic digestion was written in the form of half-reactions, as described in Sawyer et al. (1994),

$$R = f_s \cdot R_c + f_e \cdot R_a - R_d \qquad \text{(F.37)}$$

$$f_s + f_e = 1 \qquad \text{(F.38)}$$

where R = overall reaction

R_d = half-reaction for electron donor

R_a = half-reaction for electron acceptor

R_c = half-reaction for synthesis of bacterial cells

f_e = fraction of electron donor used for energy

f_s = fraction of electron donor used for synthesis

Half-reactions for electron donor, electron acceptor, and synthesis are presented in Table 6.4 in Sawyer et al. (1994):

R_d = half-reaction for electron donor (domestic wastewater):

$$\frac{9}{50}CO_2 + \frac{1}{50}NH_4^+ + \frac{1}{50}HCO_3^- + H^+ + e^- = \frac{1}{50}C_{10}H_{19}O_3N + \frac{9}{25}H_2O\ ;$$

R_a = half-reaction for electron acceptor (CO_2):

$$\frac{1}{8}CO_2 + H^+ + e^- = \frac{1}{8}CH_4 + \frac{1}{4}H_2O$$

R_c = half-reaction for synthesis of bacterial cells (ammonia as a nitrogen source):

$$\frac{1}{5}CO_2+\frac{1}{20}HCO_3^-+\frac{1}{20}NH_4^++H^++e^-=\frac{1}{20}C_5H_7O_2N+\frac{9}{20}H_2O$$

Equation (F.39) relates the fraction of electron donor converted to synthesis with cell yield coefficient based on electron equivalents, a_e, specific decay rate, b, the mean cell residence time, θ_C, and the biodegradable fraction of active organisms, f_d (McCarty, 1975).

$$f_s=a_e\cdot\left(1-\frac{f_d\cdot b^{DIG}\cdot\theta_C^{DIG}}{1+b^{DIG}\cdot\theta_C^{DIG}}\right)=\left(\frac{1}{1+A}\right)\cdot\left(1-\frac{f_d\cdot b^{DIG}\cdot\theta_C^{DIG}}{1+b^{DIG}\cdot\theta_C^{DIG}}\right) \quad \text{(F.39)}$$

Substituting $A = 8.23\ \frac{eeq\ substrate converted\ to\ energy}{eeq\ cells\ formed}$, $f_d = 0.8$, $b^{DIG} = 0.03\ d^{-1}$, $\theta_C^{DIG}=28d$ in Equation (F.39),

$$f_s=\left(\frac{1}{1+8.23}\right)\cdot\left(1-\frac{0.8\cdot0.03\cdot28}{1+0.03\cdot28}\right)=0.0688,\ \text{and}\ f_e=1-f_s=1-0.0688=0.9312$$

Substituting f_e, and f_s, in Equation (F.37), $R=0.0688\cdot R_c+0.9312\cdot R_a-R_d$

$$0.0688\cdot R_c: 0.0138CO_2+0.0034HCO_3^-+0.0034NH_4^++0.0688H^++0.0688e^-=$$
$$=0.0034C_5H_7O_2N+0.0310H_2O$$

$$0.9312\cdot R_a: 0.1164CO_2+0.9312H^++0.9312e^-=0.1164CH_4+0.2328H_2O$$

$$-R_d: \frac{1}{50}C_{10}H_{19}O_3N+\frac{9}{25}H_2O=\frac{9}{50}CO_2+\frac{1}{50}NH_4^++\frac{1}{50}HCO_3^-+H^++e^-$$

$$R:\ 0.02C_{10}H_{19}O_3N+0.0962H_2O=$$
$$=0.0498CO_2+0.1164CH_4+0.0166NH_4^++0.0166HCO_3^-+0.0034C_5H_7O_2N$$

The overall reaction R was normalized by multiplying R by 50,

$$C_{10}H_{19}O_3N+4.81H_2O=2.49CO_2+5.82CH_4+0.83NH_4^++0.83HCO_3^-+0.17C_5H_7O_2N \quad \text{(F.40)}$$

Using information presented in Equation (F.40), the volume of methane produced during anaerobic digestion was calculated with Equation (F.41),

$$\frac{5.82\,moles\,CH_4}{400\,g\,BOD_L}\cdot(S_{Feed}-S)\frac{g\,BOD_L}{l}\cdot Q_{Feed}\cdot 22.4\frac{l}{mol\,CH_4} \qquad (F.41)$$

where 400 g BOD_L is equivalent to one mol of $C_{10}H_{19}O_3N$ (molecular weight 201 g) (Equation F.34), S_{Feed} is the concentration of BOD_L in the digester feed, S is the soluble BOD_L in the digester effluent, Q_{Feed} is the digester feed flowrate, and 22.4 is the conversion factor from moles to liters of methane.

From Table F.3, digester feed flowrate Q_{Feed} = 28,085 gal/d;

BOD_L load in digester feed = 6,032 lb/d; $S_{Feed}=\dfrac{6{,}032\frac{lb}{d}\cdot 0.4536\cdot 10^3\frac{g}{lb}}{28{,}085\frac{gal}{d}\cdot 3.7854\frac{l}{gal}}=25.736\frac{g}{l}$

The effluent soluble BOD_L was calculated using Equation (F.42) (Gossett and Belzer, 1982)

$$S=\frac{K_C\cdot\left(1+b^{DIG}\cdot\theta_C^{DIG}\right)}{\theta_C^{DIG}\cdot\left(Y_a\cdot k_a-b^{DIG}\right)-1} \qquad (F.42)$$

where K_C = half-velocity constant, anaerobic digester, 1.8 g/l BOD_L

Y_a = yield coefficient for volatile-acid using methane bacteria, $0.04\frac{g\,X_a}{g\,BOD_L}$

k_a = maximum specific substrate utilization rate for volatile-acid-using methane bacteria, $6.67\frac{g\,BOD_L}{g\,X_a\cdot d}$

Substituting the corresponding values in Equation (F.42),

$$S=\frac{1.8\cdot(1+0.03\cdot 28)}{28\cdot(0.04\cdot 6.67-0.03)-1}=0.59\frac{g\,BOD_L}{l}$$

Substituting the corresponding values in Equation (F.41),

$$\frac{5.82\,moles\,CH_4}{400\,g\,BOD_L}\cdot(25.736-0.590)\frac{g\,BOD_L}{l}\cdot 28,085\frac{gal}{d}\cdot 3.7854\frac{l}{gal}\cdot 22.4\frac{l}{mol\,CH_4}=$$

$$= 871,300\frac{l}{d} = 230,173\frac{gal}{d} = 30,757\frac{ft^3}{d}$$

The measured average methane production during June, July and August 1998 was 32,401 ft^3/d.

The carbon dioxide production in anaerobic digestion was also estimated using information from the stoichiometric reaction (Equation F.40)

$$\frac{2.49\,moles\,CH_4}{400\,g\,BOD_L}\cdot(25.736-0.590)\frac{g\,BOD_L}{l}\cdot 28,085\frac{gal}{d}\cdot 3.7854\frac{l}{gal}\cdot 22.4\frac{l}{mol\,CH_4}=$$

$$= 372,772\frac{l}{d} = 98,476\frac{gal}{d} = 13,159\frac{ft^3}{d}$$

The percentage of CH_4 and CO_2 in digester gas was

$$\%\,CH_4 = \frac{30,757}{(13,159+30,757)}\cdot 100 = 70\%, \text{ and } \%\ CO_2 = 30\ \%$$

9.4 Total and Volatile Suspended Solids Loads in Digested Sludge, Thickened Digested Sludge, and Supernatant

Equation (F.19), rewritten below with the superscripts for digestion, was used to estimate the concentration of volatile suspended solids in digested sludge

$$X_V = \frac{\theta_C^{DIG}}{\theta^{DIG}}\left[X_V^{Feed}\left(1-f_d^{Feed}\right)+\frac{Y(S_{Feed}-S)}{1+b^{DIG}\theta_C^{DIG}}\left[1+(1-f_d)b^{DIG}\theta_C^{DIG}\right]\right]$$

where $\theta_C^{DIG} = \theta^{DIG} =$ 28 days. It was assumed that all digestion occurred in the primary digester tank while digested sludge thickening and supernatant formation occurred in the secondary tank. The residence time in the primary digester tank was 28 days;

X_V^{Feed} = volatile suspended solids concentration in anaerobic digester feed =

$$= \frac{5{,}900\frac{lb}{d}\cdot 0.4536\cdot 10^3\frac{g}{lb}}{28{,}085\frac{gal}{d}\cdot 3.7854\frac{l}{gal}} = 25.173\frac{g}{l}$$

f_d^{Feed} = biodegradable fraction of volatile suspended solids in feed. This fraction was calculated using Equation (F.43)

$$f_d^{Feed} = \frac{\left(L_V^{PS}\cdot f_d^{PS}\right)+\left(L_V^{WSS}\cdot f_d^{WSS}\cdot\frac{X_a^{WSS}}{X_V^{WSS}}\right)+\left(L_V^{Sept}\cdot f_d^{Sept}\right)}{L_V^{PS}+L_V^{WSS}+L_V^{Sept}} \qquad \text{(F.43)}$$

where the variables were the same as defined for Equation (F.32). The volatile suspended solids loads for Equation (F.43) were presented in Table F.3, while the biodegradable fractions of volatile suspended solids of primary sludge, biomass in waste secondary sludge, and septage were, respectively, 0.65, 0.8, and 0.65 (Section 9.1). Substituting the corresponding values in Equation (F.43),

$$f_d^{Feed} = \frac{(2{,}876\cdot 0.65)+\left(2{,}096\cdot 0.8\cdot\frac{395}{850}\right)+(928\cdot 0.65)}{2{,}876+2{,}096+928} = 0.551$$

The overall yield coefficient for Equation (F 19) was Y = 0.0765 mg VSS/g BOD_L (Section 9.2). The biodegradable fraction of biomass in anaerobic digestion was assumed to be the same as the biodegradable fraction of biomass in aerobic systems, f_d = 0.8 (McCarty, 1975). Substituting the corresponding values in Equation (F.19),

$$X_V = \frac{28}{28}\left[25.173\cdot(1-0.551)+\frac{0.0765(25.736-0.590)}{1+0.03\cdot 28}\left[1+(1-0.8)\cdot 0.03\cdot 28\right]\right] =$$

$$= 11.303 + 1.221 = 12.524\frac{g}{l}$$

From the solution of Equation (F.19), the digested sludge volatile suspended solids were formed by residual VSS of the digester feed that were not degraded during digestion (11.303 g/l), and by new biomass formed (1.221 g/l). The percent reduction of VSS in anaerobic digester was [(25.173-12.524)/25.173] x 100 = 50.25 %. The mass of VSS destroyed was 0.5025 x 5,900 lb/d = 2,965 lb/d, where 5,900 lb/d was the volatile solids load in the digester feed (Table F.3).

The concentration of total suspended solids in anaerobic digester was calculated using Equation (F.20), rewritten below with digestion notation.

$$X_T = \frac{\theta_C^{DID}}{\theta^{DIG}}\left[X_F^{Feed} + X_V^{Feed}\left(1 - f_d^{Feed}\right) + \frac{Y\left(S_{Feed} - S\right)}{0.9 \cdot \left(1 + b^{DIG}\theta_C^{DIG}\right)}\left[1 + \left(1 - f_d\right) b^{DIG}\theta_C^{DIG}\right]\right]$$

where X_F^{Feed} = concentration of fixed suspended solids in the digester feed, which included the chemical sludge formed by precipitation of ferric phosphate in the activated sludge process:

$$X_F^{Feed} = \frac{1{,}883\frac{lb}{d} \cdot 0.4536 \cdot 10^3 \frac{g}{lb}}{28{,}085\frac{gal}{d} \cdot 3.7854\frac{l}{gal}} = 8.034\frac{g}{l}$$

Substituting the corresponding values in Equation (F.20),

$$X_T = \frac{28}{28}\left[8.034 + 25.173 \cdot (1 - 0.551) + \frac{0.0765(25.736 - 0.590)}{0.9 \cdot (1 + 0.03 \cdot 28)}\left[1 + (1 - 0.8) \cdot 0.03 \cdot 28\right]\right] =$$

$$= 8.034 + 11.303 + 1.357 = 20.694\frac{g}{l}$$

The ratio VSS:TSS in digested sludge was 12.524/20.694 = 0.605. The observed ratio in three samples of digested sludge collected during June, July and August was 0.58. The ratio in the plant's records was 0.54. The term 1.357 g/l represented new biomass composed by volatile fraction (1.221 g/l) and an inorganic fraction (0.136 g/l). The concentration of fixed suspended solids in digested sludge

was 8.034 + 0.136 = 8.170 g/l. The load of fixed suspended solids in digested sludge was (8.170/8.034) x 1,883 lb/d = 1,915 lb/d. The increase in FSS was 1915 – 1883 = 32 lb/d.

The load of volatile suspended solids in digested sludge was 5,900 – 2,965 = 2935 lb/d, where 5,900 lb/d was the value for volatile suspended solids in the digester feed (Table F.3), and 2,965 lb/d was the amount of volatile solids destroyed during digestion. The load of total suspended solids in the digested sludge was 2,935/0.605 = 4,851lb/d.

The daily volume of sludge feed was decreased by the volume of volatile suspended solids and water that were destroyed during digestion. The load of VSS that was destroyed was 2,965 lb/d = 1,345 kg/d. Assuming a volatile suspended solids density of 1.0 g/cm^3 (Metcalf and Eddy, 1991), the volume of VSS destroyed was 1,345 l/d = 355 gal/d. The volume of water destroyed each day was estimated using information from the stoichiometric equation for anaerobic digestion (Equation F.40), and Equation (F.44)

Volume water destroyed each day =

$$= \frac{4.81 moles\ H_2O}{1.0 mol\ C_{10}H_{19}O_7N} \cdot \left(X_V^{Feed} - X_V\right) \cdot Q_{Feed} \cdot 18 \frac{g}{mol\ H_2O} \cdot 10^{-3} \frac{kg}{g} \cdot 1.0 \frac{l}{kg} \qquad \text{(F.44)}$$

Substituting the corresponding values in Equation (F.44),

$$\frac{4.81 moles\ H_2O}{201 gVSS} \cdot (25.173 - 12.524) \frac{g}{l} \cdot 28{,}085 \frac{gal}{d} \cdot 3.7854 \frac{l}{gal} \cdot 18 \cdot 10^{-3} \frac{Kg}{mol\ H_2O} \cdot 1.0 \frac{l}{kg} = 579 \frac{l}{d} = 153 \frac{gal}{d}$$

Total volume reduction = 153 + 355 = 508 gal/d. The volume of digested sludge was 28,085 – 508 = 27,577 gal/d.

Digested sludge is concentrated in the secondary digestion tank, with formation of thickened digested sludge and supernatant. Equations (F.45) through (F.47) present the flowrate and total suspended solids mass balance in the anaerobic digesters.

$$L_T^{ThDS} = L_T^{Feed} - L_t^{Dest} - L_T^{Sup} \tag{F.45}$$

$$L_T^{Sup} = Q_{Sup} \cdot X_T^{Sup} \tag{F.46}$$

$$Q_{Sup} = Q_{Feed} - Q_{Dest} - Q_{ThDS} \tag{F.47}$$

where the subscripts and superscripts ThDS and Sup mean thickened digested sludge and supernatant, respectively. The thickened digested sludge, Q_{ThDS}, was calculated using Equation (F.6). From the plant's records, the average percentage of total solids in thickened digested sludge was four percent. The densities of thickened digested sludge solids and thickened digested sludge were calculated using Equations (F.8) and (F.7), respectively.

$$e_P = \left[\frac{(1-0.605)}{2.5} + \frac{0.605}{1.0}\right]^{-1} = 1.3106 \frac{g}{cm^3}$$

$$e_{ThDS} = \left[\frac{0.040}{1.3106} + \frac{(1-0.040)}{1.0}\right]^{-1} = 1.0096 \frac{g}{cm^3}$$

Initially, it was assumed that the load of total suspended solids in supernatant, L_T^{Sup}, was zero in Equation (F.45). Hence, the load of thickened digested sludge, L_T^{ThDS}, was equal to 4,851 lb/d. Substituting the corresponding values in Equation (F.6), the daily volume of thickened digested sludge at 4 % total solids was

$$Q_{ThDS} = \frac{4{,}851\,{}^{lb}/_{d} \cdot 0.4536\,{}^{kg}/_{lb}}{1.0\,{}^{kg}/_{l} \cdot 1.0096 \cdot 0.040} = 54{,}486 \frac{l}{d} = 14{,}394 \frac{gal}{d}$$

The supernatant flowrate was calculated using Equation (F.47),

Supernatant flowrate = 28,085 - 508 - 14,394 gal/d = 13,183 gal/d. Since the average measured concentration of total suspended solids in digester supernatant was 2,923 mg/l, from Equation (F.46), the load of total suspended solids in supernatant was 2,923 mg/l x 13,183 gal/d x (8.3454×10^{-6}) = 322 lb/d. This value was substituted into Equation (F.45), and a new value of L_T^{ThDS} was obtained. The thickened digested sludge flowrate was recalculated using the new value of L_T^{ThDS} in Equation (F.6)

$$Q_{ThDS} = \frac{(4{,}851-322)\,{}^{lb}/_{d} \cdot 0.4536\,{}^{kg}/_{lb}}{1.0\,{}^{kg}/_{l} \cdot 1.0096 \cdot 0.040} = 50{,}869\,{}^{l}/_{d} = 13{,}438\,{}^{gal}/_{d}$$

The supernatant flowrate was recalculated as 28,085 - 508 - 13,438 = 14,139 gal/d; Using Equation (F.46), the supernatant load of TSS was recalculated as 2,923 mg/l x 14,139 gal/d x (8.3454×10^{-6}) = 345 lb/d; the thickened digested sludge flowrate was recalculated to account for the new value of the TSS load in supernatant,

$$Q_{ThDS} = \frac{(4{,}851-345)\,{}^{lb}/_{d} \cdot 0.4536\,{}^{kg}/_{lb}}{1.0\,{}^{kg}/_{l} \cdot 1.0096 \cdot 0.040} = 50{,}611\,{}^{l}/_{d} = 13{,}370\,{}^{gal}/_{d}$$

The supernatant flowrate was again recalculated as 28,085 - 508 – 13,370 =

= 14,207 gal/d

The supernatant load was recalculated as 2,923 mg/l x 14,207 gal/d x (8.3454×10^{-6}) = 347 lb/d. The thickened digested sludge flowrate was recalculated as

$$Q_{ThDS} = \frac{(4{,}851-347)\,{}^{lb}/_{d} \cdot 0.4536\,{}^{kg}/_{lb}}{1.0\,{}^{kg}/_{l} \cdot 1.0096 \cdot 0.040} = 50{,}589\,{}^{l}/_{d} = 13{,}364\,{}^{gal}/_{d}$$

Supernatant flowrate = 28,085 - 508 - 13,364 = 14,213 gal/d

Load of TSS in supernatant = 2,923 mg/l x 14,213 gal/d x (8.3454×10^{-6}) = 347 lb/d

Because the calculated load of TSS in digester supernatant remained the same, additional correction for the load of total suspended solids in thickened digested

sludge was not necessary. The load of volatile suspended solids in digester supernatant was calculated by multiplying the ratio of VSS:TSS by the load of TSS.

Load of VSS in supernatant = 0.605 x 347 = 210 lb/d

Figure F.11 presents the mass balance for suspended solids on the anaerobic digesters, at the end of the first iteration of calculations. These results were modified in subsequent iterations.

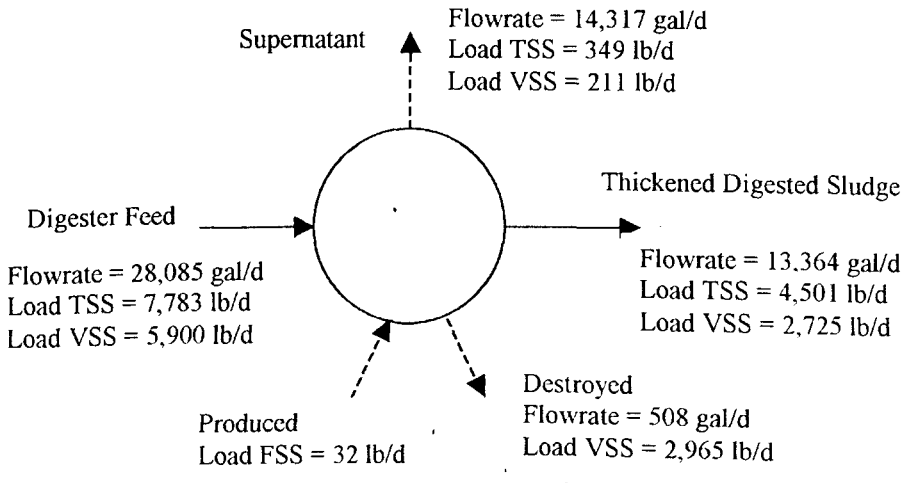

Figure F.11 Flowrates and Mass Balance for Suspended Solids on the Anaerobic Digesters

10. Flowrates and Mass Balances for Total and Volatile Suspended Solids on the Belt Filter Press

The thickened digested sludge, with an average of 4 % total suspended solids was dewatered in a belt filter press, resulting in a sludge cake with approximately 21 % totalsuspended solids. Before entering the BFP, the sludge was conditioned by application of a synthetic polymer at 16 lb per ton of TSS.

At the beginning of this appendix, the initial conditions for the return flows (Section 3), and flowrates for sludge cake, synthetic polymer, and washwater (Section

4, categories 4.1.2.b, 4.1.2.f, and 4.1.2.g, respectively) were established using values taken from the plant's records for the weight of total suspended solids and the ratio of VSS:TSS in dewatered sludge cake. These values were modified to the values calculated in Section 9 for the weight of total suspended solids, ratio of VSS:TSS, and flowrate in the thickened digested sludge.

The flowrate, total and volatile suspended solids mass balances on the belt filter press are described by Equations (F.48) through (F.53) (see also Figure F.12).

$$Q_{ThDS} + Q_{Poly} + Q_{Wash} = Q_{FW} + Q_{Cake} \tag{F.48}$$

$$Q_{ThDS} + Q_{Poly} = Q_{Filt} + Q_{Cake} \tag{F.49}$$

$$Q_{FW} = Q_{Filt} + Q_{Wash} \tag{F.50}$$

$$L_T^{ThDS} = L_T^{Cake} + L_T^{FW} \tag{F.51}$$

$$L_V^{ThDS} = L_V^{Cake} + L_V^{FW} \tag{F.52}$$

$$L_T^{FW} = Q_{FW} \cdot X_T^{FW} \tag{F.53}$$

where L represents load of suspended solids, and is given by the product between flowrate and suspended solids concentration.

The quantity of synthetic polymer added to thickened digested sludge was:

$$\text{Polymer added} = \frac{16\,lb\ polymer}{2{,}000\,lb\ TSS} \cdot 4{,}501 \frac{lb\ TSS}{d} = 36 \frac{lb}{d}$$

The polymer was applied in 0.6 % solution. The daily volume of polymer solution applied was:

$$\text{Daily volume} = \frac{36 \frac{lb}{d} \cdot 0.4536 \frac{kg}{lb}}{\frac{0.6\,kg\ polymer}{100\,kg\ solution} \cdot 1.0 \frac{kg}{l}} = 2{,}722 \frac{l}{d} = 719 \frac{gal}{d}$$

In order to estimate the flowrate in the filtrate and washwater, it was necessary to assume that the belt filter press had a given efficiency in the capture of suspended solids of the thickened digested sludge. A 95 % efficiency was assumed (Metcalf and

Eddy, 1991). The suspended solids loads in dewatered cake was estimated as 0.95 x 4,501 lb/d = 4,276 lb/d; the load of suspended solids in the filtrate/washwater was estimated as 4,501 – 4,276 = 225 lb/d.

The daily volume of dewatered sludge cake was estimated using Equation (F.6). The density of sludge cake solids was calculated by substituting $f_V = 0.605$, $e_F = 2.5 g/cm^3$, and $e_V = 1.0 g/cm^3$ into Equation (F.8),

$$e_P = \left[\frac{(1-0.605)}{2.5} + \frac{0.605}{1.0}\right]^{-1} = 1.3106 \frac{g}{cm^3}$$

The percentage of total solids in the sludge cake was 21 %. The density of the sludge cake was calculated using Equation (F.7)

$$e_{Cake} = \left[\frac{0.21}{1.3106} + \frac{(1-0.21)}{1.0}\right]^{-1} = 1.0524 \frac{g}{cm^3}$$

Substituting the corresponding values into Equation (F.6),

$$Q_{Cake} = \frac{4,276 \, {}^{lb}/_{d} \cdot 0.4536 \, {}^{kg}/_{lb}}{1.0 \, {}^{kg}/_{l} \cdot 1.0524 \cdot 0.21} = 8,776 \, {}^{l}/_{d} = 2,318 \, {}^{gal}/_{d}$$

The flowrate of BFP filtrate was calculated by substituting the values of the flowrates for thickened digested sludge, polymer, and sludge cake into Equation (F.49).

Filtrate flowrate = 13,364 + 719 - 2,318 = 11,764 gal/d. The concentration of total suspended solids measured in the combined flowrates of filtrate and washwater was 934 mg/l. Using Equation (F.53), the flowrate of the combined filtrate and washwater was:

$$Q_{FW} = \frac{225\frac{lb}{d} \cdot 0.4536\frac{kg}{lb}}{934 \cdot 10^{-6}\frac{kg}{l}} = 109,270\frac{l}{d} = 28,866\frac{gal}{d}$$

The washwater flowrate was calculated by substituting the corresponding values into Equation (F.50)

Q_{Wash} = 28,866 – 11,764 = 17,102 gal/d

Figure F.12 presents the flowrates and suspended solids mass balances on the belt filter press. The average daily weight of dry total solids in dewatered sludge cake, according to the plant's records, was 6,150 lb/d. The estimated weight of total solids in sludge cake at the end of the first iteration, 4,276 lb/d, represented 70 % of the value recorded at the plant, which was considered acceptable.

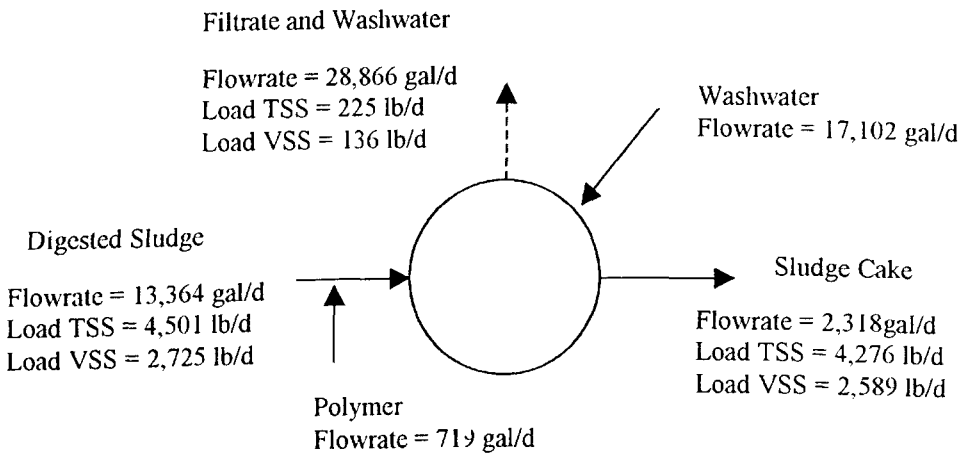

Figure F.12 Flowrates and Mass Balances for Suspended Solids on the Belt Filter Press

11. Summary

This appendix demonstrated the calculations used to perform the first iteration of the mass balances for suspended solids and flowrates on the wastewater and sludge treatment processes. In order to perform these calculations, it was necessary to establish initial values for suspended solids loads and flowrates for the return flows.

At the end of the first iteration, new values for the return flows were obtained. These values were used as input to perform the mass balances of the second iteration.

Table F.4 presents the summary of suspended solids in thickener overflow, anaerobic digester supernatant, and combined filtrate/washwater from belt filter press, at the end of the first iteration of calculations. Figure F.13 shows the flowrates and suspended solids loads for each process of the treatment plant after the first iteration. The values in the dashed box on the arrow coming from the sludge processing facilities were the values of the initial conditions for the return flows. The values in the solid box were products of the first iteration, and were used in the second iteration. The calculations for mass balances on the primary sedimentation tanks and activated sludge process were repeated using the loads from Table F.4; new values for sludge quantities were used as input to mass balances in the thickeners, anaerobic digesters and belt filter press. This process was repeated until the differences in suspended solids loads and flowrates between consecutive iterations fell below one percent, which occurred after the fourth iteration.

Table F.4 Flowrates and Suspended Solids Loads and Concentrations in Return Flows from Sludge Treatment Processes (End of First Iteration)

Source	Flowrate	Total Solids		Volatile Solids		Fixed Solids	
		Conc.	Load	Conc.	Load	Conc.	Load
	(gal/d)	(mg/l)	(lb/d)	(mg/l)	(lb/d)	(mg/l)	(lb/d)
Thickener Overflow	452,947	85	321	67	253	18	68
Digester Supernatant	14,213	2,923	347	1,770	210	1,153	137
BFP Filtrate + Washwater	28,865	934	225	565	136	369	89
Total	496,025	216	893	145	599	71	294

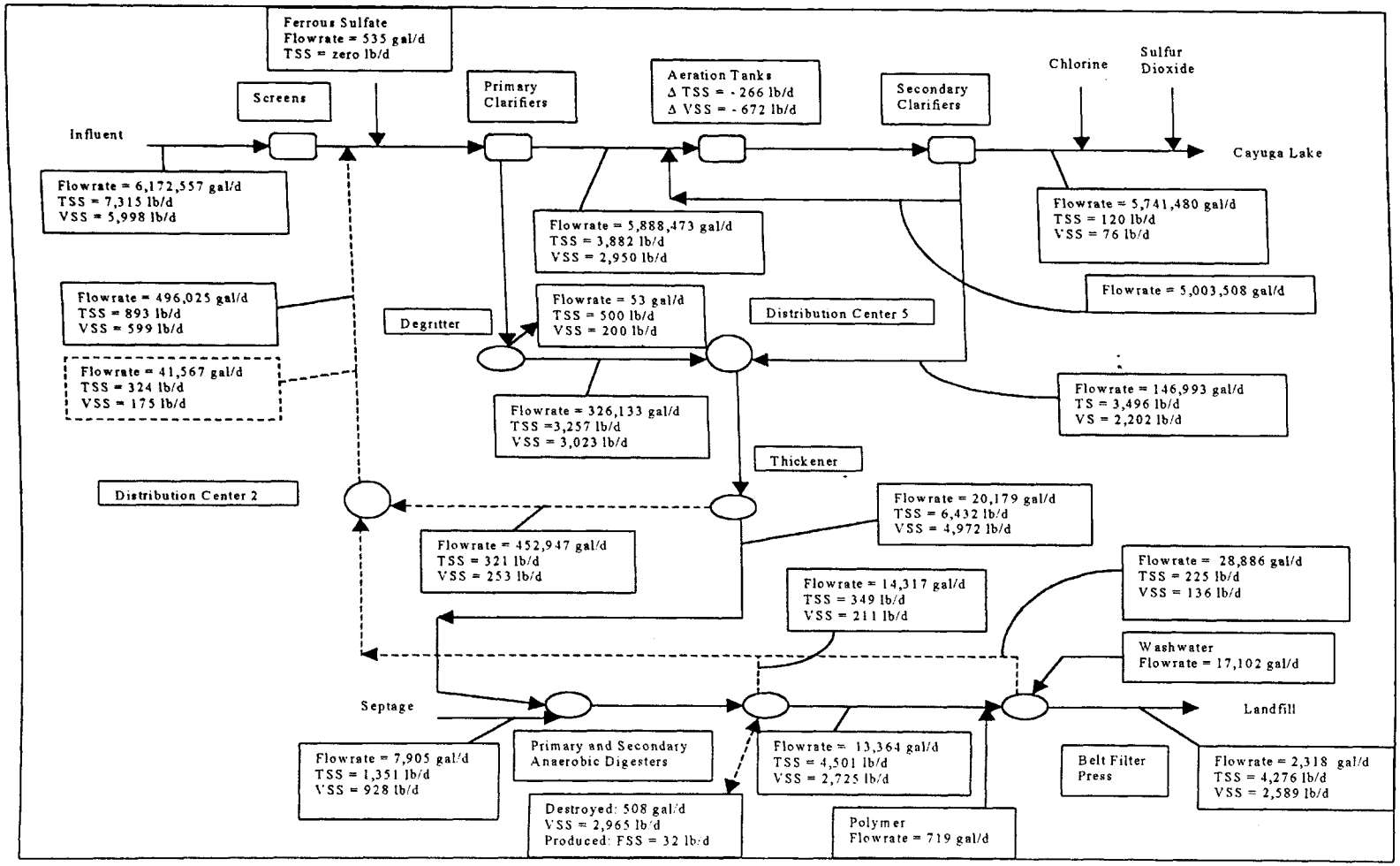

Figure F.13 Flowrates and Suspended Solids Loads in Wastewater and Sludge Treatment Processes at the Ithaca Wastewater and Sludge Treatment Plant (First Iteration)

APPENDIX G

TRACE METALS LIMITS OF DETECTION

Table G.1 description: detection limits were the ones suggested by the Standard Methods for the Examination of Water and Wastewater (APHA et al., 1995), except zinc, flame emission and zinc, graphite furnace. The detection limit of 5 μg/L for zinc using flame emission was not attainable; no detection limit for zinc using graphite furnace is suggested in Standard Methods (APHA et al., 1995). In both cases, the procedure described in Appendix D was used.

Table G.2 to G.6 description: the unit mass limits of detection for trace metals were calculated by multiplying the limit of detection by extraction buffer volume, and dividing the product by the mass of volatile suspended solids used for extraction. For example, for copper, influent, first set, the unit mass limit of detection is given by [(1.0 μg/L x 0.3 L)]/1.789 g = 0.17 μg Cu/g VSS.

Table G.1 Trace Metals Limits of Detection (μg/l)

Metal	Technique	Detection Limit
Copper	Flame Emission	10.0
Copper	Graphite Furnace	1.0
Zinc	Flame Emission	35.0
Zinc	Graphite Furnace	7.5
Lead	Graphite Furnace	5.0
Cadmium	Graphite Furnace	0.5
Chromium	Graphite Furnace	5.0

Table G.2 Unit Mass Limits of Detection - Copper
(Unit: µg copper/g volatile suspended solids)

Location	First	Second	Third	Fourth
Influent	0.17	0.24	0.16	
Primary Effluent	0.53	0.40	0.23	
Primary Sludge			0.15	
Activated Sludge	0.28	0.17	0.16	0.19
Secondary Sludge				0.10
Secondary Effluent	3.12	0.96	2.21	
Thickened Sludge	0.13	0.13	0.12	
Digested Sludge	0.14	0.08	0.09	
Thick. Overflow	0.41	0.25	0.28	
An Dig Supernatant	0.17	0.16	0.18	
BFP Filtrate	0.26	0.17	0.16	

Table G.3 Unit Mass Limits of Detection - Zinc
(Unit: µg zinc/g volatile suspended solids)

Location	First	Second	Third	Fourth
Influent	1.26	1.77	1.17	
Primary Effluent	3.95	2.99	1.70	
Primary Sludge			1.15	
Activated Sludge	2.08	1.28	1.21	1.46
Secondary Sludge				0.77
Secondary Effluent	23.44	7.16	16.61	
Thickened Sludge	1.00	0.94	0.92	
Digested Sludge	1.03	0.57	0.67	
Thick. Overflow	3.05	1.89	2.13	
An Dig Supernatant	1.30	1.21	1.36	
BFP Filtrate	1.98	1.25	1.20	

Table G.4 Unit Mass Limits of Detection - Lead
(Unit: µg lead/g volatile suspended solids)

Location	First	Second	Third	Fourth
Influent	0.84	1.18	0.78	
Primary Effluent	2.63	2.00	1.13	
Primary Sludge			0.77	
Activated Sludge	1.39	0.85	0.81	0.97
Secondary Sludge				0.51
Secondary Effluent	15.63	4.77	11.08	
Thickened Sludge	0.66	0.63	0.61	
Digested Sludge	0.69	0.38	0.45	
Thick. Overflow	2.04	1.26	1.42	
An Dig Supernatant	0.87	0.80	0.91	
BFP Filtrate	1.32	0.83	0.80	

Table G.5 Unit Mass Limits of Detection - Cadmium
(Unit: µg cadmium/g volatile suspended solids)

Location	First	Second	Third	Fourth
Influent	0.08	0.12	0.08	
Primary Effluent	0.26	0.20	0.11	
Primary Sludge			0.08	
Activated Sludge	0.14	0.09	0.08	0.10
Secondary Sludge				0.05
Secondary Effluent	1.56	0.48	1.11	
Thickened Sludge	0.07	0.06	0.06	
Digested Sludge	0.07	0.04	0.04	
Thick. Overflow	0.20	0.13	0.14	
An Dig Supernatant	0.09	0.08	0.09	
BFP Filtrate	0.13	0.08	0.08	

Table G.6 Unit Mass Limit of Detection - Chromium
(Unit: µg chromium/g volatile suspended solids)

Location	Second
Influent	1.18
Primary Effluent	2.00
Primary Sludge	
Activated Sludge	0.85
Secondary Sludge	
Secondary Effluent	4.77
Thickened Sludge	0.63
Digested Sludge	0.38
Thick. Overflow	1.26
An Dig Supernatant	0.80
BFP Filtrate	0.83

APPENDIX H

TRACE METALS ANALYSES

WHOLE SAMPLE

SOLIDS-BOUND BIOPOLYMERS

LOOSELY-BOUND BIOPOLYMERS

Tables H.1 to H.5 description: Tables H.1 to H.5 present the values of trace metals measured in whole samples.

Tables H.6 to H.10 description: Tables H.6 to H.10 present the unit mass of trace metals in solids-bound biopolymers. They were calculated by multiplying the concentration of the metal measured in the buffer extraction by the buffer volume, and dividing the product by the mass of volatile suspended solids used to extract the biopolymers. For example, copper, first set, was calculated by multiplying the concentration in the buffer, 459.9 µg/l by the buffer volume, 300 mL, and dividing by the mass of volatile suspended solids, 1.789 g. The result is 96.4 µg Cu/g VSS.

Tables H.11 to H.15 description: Tables H.11 to H.15 present the concentrations of trace metals in loosely-bound biopolymers.

Table H.1 Copper - Total (µg/l)

Location	First	Second	Third	Fourth
Influent	-	87.3	153.0	
Primary Effluent	42.5	25.3	48.4	
Primary Sludge			747.7	
Activated Sludge	646.7	487.7	589.4	589.4
Secondary Sludge				2,764.0
Thickened Sludge	20,711.7	20,423.8	17,833.1	
Digested Sludge	28,521.9	35,434.5	44,651.3	
Thick. Overflow	199.2	71.4	54.8	
An Dig Supernatant	4,924.4	742.6	1,042.0	
BFP Filtrate	822.6	575.6	2,134.2	

Table H.2 Zinc - Total (µg/l)

Location	First	Second	Third	Fourth
Influent	-	198.5	280.9	
Primary Effluent	75.6	50.0	57.5	
Primary Sludge			695.62	
Activated Sludge	625.2	527.7	-	571.5
Secondary Sludge				2355.3
Thickened Sludge	22,575.4	22,575.4	19,478.4	
Digested Sludge	34,719.7	40,893.8	47,258.3	
Thick. Overflow	197.9	42.5	57.4	
An Dig Supernatant	5,588.2	1,193.8	1,316.4	
BFP Filtrate	542.5	645.3	1,432.5	

Table H.3 Lead - Total (µg/l)

Location	First	Second	Third	Fourth
Influent	-	5.6	6.5	
Primary Effluent	< 5.0	< 5.0.	< 5.0	
Primary Sludge			60.7	
Activated Sludge	31.2	27.8	-	21.5
Secondary Sludge				52.8
Thickened Sludge	827.1	1,153.5	741.8	
Digested Sludge	1,155.2	1,519.1	1,824.3	
Thick. Overflow	13.3	< 5.0	< 5.0	
An Dig Supernatant	216.3	34.4	42.4	
BFP Filtrate	12.4	24.2	43.0	

Table H.4 Cadmium - Total (µg/l)

Location	First	Second	Third	Fourth
Influent		< 0.5	< 0.5	
Primary Effluent	< 0.5	< 0.5	< 0.5	
Primary Sludge			0.84	
Activated Sludge	2.04	0.90	-	1.11
Secondary Sludge				3.44
Secondary Effluent	..			
Thickened Sludge	35.84	37.67	26.68	
Digested Sludge	69.39	49.94	65.96	
Thick. Overflow	< 0.5	< 0.5	< 0.5	
An Dig Supernatant	10.22	1.40	1.15	
BFP Filtrate	0.77	0.97	2.14	

Table H.5 Chromium – Total (µg/L)

Location	Second
Influent	16.8
Primary Effluent	< 5.0
Primary Sludge	25.0
Activated Sludge	18.8
Secondary Sludge	61.8
Secondary Effluent	
Thickened Sludge	573.7
Digested Sludge	2,696.9
Thick. Overflow	< 5.0
An Dig Supernatant	31.9
BFP Filtrate	17.1

Table H.6 Copper in Solids-Bound Biopolymer
(Unit: μg copper/g volatile suspended solids)

Location	First	Second	Third	Fourth
Influent	96.4	108.7	67.1	
Primary Effluent	28.3	26.3	16.2	
Primary Sludge			40.3	
Activated Sludge	90.4	38.6	4.9	25.4
Secondary Sludge				26.8
Thickened Sludge	37.1	40.4	57.7	
Digested Sludge	15.6	33.9	49.6	
Thick. Overflow	36.6	34.9	20.6	
An Dig Supernatant	21.6	8.4	17.7	
BFP Filtrate	25.8	19.9	21.4	

Table H.7 Zinc in Solids-Bound Biopolymer
(Unit: μg zinc/g volatile suspended solids)

Location	First	Second	Third	Fourth
Influent	95.0	136.2	91.1	
Primary Effluent	55.3	69.4	26.4	
Primary Sludge			35.1	
Activated Sludge	32.3	36.1	8.1	27.9
Secondary Sludge				27.1
Thickened Sludge	25.5	36.0	48.7	
Digested Sludge	24.4	28.6	41.2	
Thick. Overflow	24.0	27.2	28.3	
An Dig Supernatant	15.9	10.8	21.1	
BFP Filtrate	30.6	20.7	27.8	

Table H.8 Lead in Solids-Bound Biopolymer
(Unit: μg lead/g volatile suspended solids)

Location	First	Second	Third	Fourth
Influent	6.2	6.2	3.1	
Primary Effluent	2.8	2.8	1.2	
Primary Sludge			3.4	
Activated Sludge	1.9	1.9	< 0.8	1.6
Secondary Sludge				1.3
Thickened Sludge	2.0	2.6	2.4	
Digested Sludge	0.9	1.3	1.7	
Thick. Overflow	2.4	3.4	2.5	
An Dig Supernatant	1.9	1.4	2.3	
BFP Filtrate	1.6	1.6	1.3	

Table H.9 Cadmium in Solids-Bound Biopolymer
(Unit: μg cadmium/g volatile suspended solids)

Location	First	Second	Third	Fourth
Influent	0.35	0.26	0.11	
Primary Effluent	< 0.26	< 0.20	< 0.11	
Primary Sludge			< 0.08	
Activated Sludge	< 0.14	0.24	< 0.08	0.16
Secondary Sludge				0.11
Thickened Sludge	< 0.07	0.06	0.07	
Digested Sludge	0.03	0.05	0.06	
Thick. Overflow	0.22	0.17	0.16	
An Dig Supernatant	0.09	0.11	0.10	
BFP Filtrate	0.14	0.11	0.10	

Table H.10 Chromium in Solids-Bound Biopolymer (Unit: µg chromium/g VSS)

Location	Second
Influent	2.2
Primary Effluent	< LD
Primary Sludge	-
Activated Sludge	< LD
Secondary Sludge	-
Thickened Sludge	1.2
Digested Sludge	1.1
Thick. Overflow	< LD
An Dig Supernatant	< LD
BFP Filtrate	1.7

Table H.11 Copper in Loosely-Bound Biopolymer (µg/l)

Location	First	Second	Third	Fourth
Influent	23.8	10.4	22.6	
Primary Effluent	8.0	5.8	9.0	
Primary Sludge			3.5	
Activated Sludge	-	5.25	-	4.06
Secondary Sludge				4.06
Thickened Sludge	41.4	11.5	11.4	
Digested Sludge	9.1	30.9	62.6	
Thick. Overflow	10.1	5.0	8.1	
An Dig Supernatant	41.3	12.4	10.3	
BFP Filtrate	9.6	8.3	6.3	

Table H.12 Zinc in Loosely-Bound Biopolymer (μg/l)

Location	First	Second	Third	Fourth
Influent	56.3	42.2	34.4	
Primary Effluent	32.8	-	22.0	
Primary Sludge	-	-	15.7	
Activated Sludge	-	33.0	-	17.0
Secondary Sludge				
Thickened Sludge	22.5''	18.6	22.1	
Digested Sludge	11.9	49.3	68.2	
Thick. Overflow	21.7	24.6	25.3	
An Dig Supernatant	45.7	56.2	40.9	
BFP Filtrate	17.6	19.1	34.1	

Table H.13 Lead in Loosely-Bound Biopolymer (μg/l)

Location	First	Second	Third	Fourth
Influent	< 5.0	< 5.0	< 5.0	
Primary Effluent	< 5.0	< 5.0	< 5.0	
Primary Sludge	-	-	< 5.0	
Activated Sludge	-	< 5.0	-	< 5.0
Secondary Sludge	-	-	-	
Thickened Sludge	< 5.0	< 5.0	< 5.0	
Digested Sludge	< 5.0	< 5.0	5.2	
Thick. Overflow	< 5.0	< 5.0	< 5.0	
An Dig Supernatant	< 5.0	< 5.0	< 5.0	
BFP Filtrate	< 5.0	< 5.0	< 5.0	

Table H.14 Cadmium in Loosely-Bound Biopolymer (μg/l)

Location	First	Second	Third	Fourth
Influent	< 0.5	< 0.5	< 0.5	
Primary Effluent	< 0.5	< 0.5	< 0.5	
Primary Sludge			< 0.5	
Activated Sludge	-	< 0.5	-	< 0.5
Secondary Sludge				
Thickened Sludge	< 0.5	< 0.5	< 0.5	
Digested Sludge	< 0.5	< 0.5	< 0.5	
Thick. Overflow	< 0.5	< 0.5	< 0.5	
An Dig Supernatant	< 0.5	< 0.5	< 0.5	
BFP Filtrate	< 0.5	< 0.5	< 0.5	

Table H.15 Chromium in Loosely-Bound Biopolymer (μg/L)

Location	Second
Influent	< 0.5
Primary Effluent	< 0.5
Primary Sludge	-
Activated Sludge	< 0.5
Secondary Sludge	-
Thickened Sludge	< 0.5
Digested Sludge	< 0.5
Thick. Overflow	< 0.5
An Dig Supernatant	< 0.5
BFP Filtrate	< 0.5

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