BACTERIAL EXTRACELLULAR POLYMERS AND FLOCCULATION OF ACTIVATED SLUDGES

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(ABSTRACT)

The extracellular polymers produced by bacteria play an important role in bacterial aggregation or bacterial flocculation in secondary waste treatment. The mechanisms responsible for this floc formation are thought to be polymer induced adsorption and interparticle bridging among bacterial cells or between bacterial cells and inorganic colloids. The efficiency of the processes following flocculation in the treatment line such as sedimentation, sludge thickening, and sludge dewatering depends on the extent of this bacterial flocculation.

In this research, sludge samples from under various substrate conditions were examined for type, molecular weight, physical charcteristics, and quantity of extracellular polymers so that the general characteristics of the various polymers could be established. An attempt was made to determine if a relationship exists between the state of bacterial aggregation and the polymer characteristics. This research also investigated the sludge physical

properties. The effect of various parameters such as pH, divalent cation (mixture and concentration), and mixing (period and intensity) on dewatering properties were studied.

A major goal of this study was to develop a flocculation model for activated sludge. This model could be used to determine if plants can increase the efficiency of waste treatment and sludge thickening and sludge dewatering processes.

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I. INTRODUCTION

Biological waste treatment processes, specifically activated sludge systems, have been widely applied to the treatment of both municipal and industrial wastewater for many years. The phenomena involved in the activated sludge process are substrate utilization and flocculation of micro-organisms. Substrate utilization is the first phase in which organic waste in the wastewater is utilized by micro-organisms under an aerobic environment. In this phase, new cells are synthesized and inert materials are released as by products. The flocculation of micro-organisms in the second phase and the sedimentation that follows are the most significant steps that determine the quality of effluent.

One of the major subjects being investigated in the research is in the area of flocculation and clarification. It is widely known that microbial cells agglomerate into small clumps and subsequently into sludge flocs by a natural bioflocculation process. Tenney and Stumm (1) and Busch and Stumm (2) are among the first researchers to propose that polymeric materials excreted by bacteria, or so-called bacterial extracellular polymers (BEP) or biopolymers are responsible for bioflocculation. These BEP act as a natural flocculating agent. At the present time, the mechanisms involved in this bioflocculation are believed to be

adsorption and bridging. However, the extent to which these BEP contribute to floc formation in activated sludge is still being debated by many researchers (2, 3, 4).

One approach to elucidating the applicable mechanisms is by determining the chemical characteristics of the BEP. It has been reported that the major composition of BEP is polysaccharide and its concentration and monomers may vary between different sludge samples. Using this approach a comparison of the molecular weight, quantity of BEP produced, and general characteristics of BEP of different biological treatment systems and different wastewater nutrient compositions is used in an attempt to evaluate the reasons for variation in sludge physical properties.

The other approach is to study the physical properties of BEP. At the present time, the physical properties of activated sludges and their relationship to extracellular polymers have not been widely studied. Adsorption characteristics of biopolymer are thought to depend on variables such as pH, divalent cation (mixture and concentration), and mixing (period and intensity) (5). These variables may have pronounced effects on the flocculation of activated sludge, however, little data exists relating the extent of bacterial extracellular polymeric adsorption to the physical properties of the flocs.

In the area of sludge disposal, operational cost, handling cost, and problems of process failures are some of the main

concerns. Recently several workers have tried to relate BEP to sludge thickening and dewatering properties (5, 6). Roberts and Olsson (6), Novak and Haugan (5), and Novak and Haugan (7) have demonstrated that BEP is a major factor in determining the amount of conditioning chemical required for improved sludge dewatering. An attempt to characterize the BEP both chemically and physically and the development of an adsorption isotherm may suggest mechanisms of bioflocculation and the role of BEP in sludge thickening and dewatering.

The first step in BEP characterization is the extraction of BEP from sludge floc. Once the BEP is extracted, the chemical and physical properties of the BEP can be studied. Many extraction methods have been proposed but it appears that there is no verifiable method for providing quantitative extraction of BEP (8). High-speed centrifugation, alkaline addition, and heat treatment are the most common methods for extraction of BEP.

It appears that legitimate questions remain as to the nature and significance of BEP in the activated sludge process. Among the more important questions are:

1. can bacterial extracellular polymer be reliably extracted from the activated sludge floc?,
2. to what extent does BEP contribute to bioflocculation?, and

3. is there a relationship between the chemical and physical characteristics of BEP and sludge thickening and dewatering properties?

In order to address these questions the following goals were formulated:

1. to determine the best method of BEP extraction,
2. to determine to the degree possible the relationship between the chemical composition of BEP and the adsorption properties of sludge samples,
3. to determine the relationship between the biopolymer characteristics and the sludge properties.

II. LITERATURE REVIEW

2.1 SLUDGE PROPERTIES

One of the most important process in the activated sludge wastewater treatment is sludge handling which includes stabilization, volume reduction, and ultimate disposal. Vesilind (9) estimated that sludge handling is responsible for about 30-40 percent of the capital cost, and about 50 percent of the operating cost of the treatment plant. The high cost of handling is associated with the large volume and relatively diluted sludge produced from the system. Normally the concentration of waste activated sludge from a wastewater treatment plant is between 0. 5 and 1. 5 percent solids.

The National Academy of Sciences (10) has estimated that the future combined sludge production in the U.S. will reach 10×10^6 metric tons in year 1990 (Figure 1). Fair and Imhoff (11) compared the volume of typical sludges produced from different wastewater treatment process and as shown in Table 1, activated sludge systems produces much higher sludge volume than raw primary sludge and trickling filter humus.

Because of this large volume of water, an efficient method to reduce sludge volumes prior to final disposal has been the concern of environmental engineers. Usually sludges posses several characteristics which directly inhibit the rate and

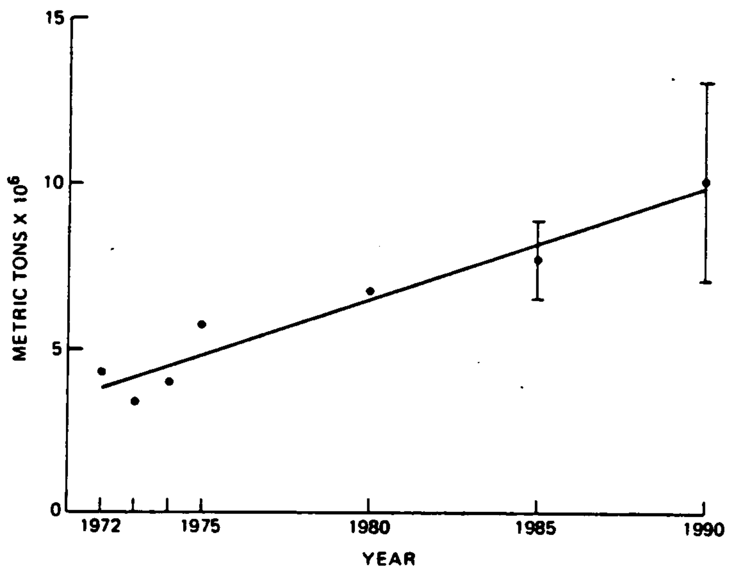

Figure 1. Estimates of present and future total sludge production in the U.S. (10)

Table 1

Comparison of sludge volume* production from different treatment systems (11)

Raw primary sludge	Trickling filter humus	Waste activated sludge
3530	530	14,600

* gallons (or cubic meter) of sludge produced per million gallons (or cubic meter) of wastewater treated.

performance (degree of clarification, thickening, and dewatering) achieved by any solid-liquid separation processes. Therefore, sludge conditioning is normally employed to improve the efficiency of sludge thickening and to increase the dewatering rates which would result in the increase of the solids concentration of the dewatered sludge.

Carberry and Englande (12) described the nature of sludge conditioning as:

> The presence of colloidal particles increases the compressibility of the sludges; therefore, colloids adversely affect all processes of sedimentation and dewatering. Most sewage sludges exhibit a net negative charge. This charge makes the particles tend to repulse each other and thus resist agglomeration in to larger particles. A large percentage of the sludge solids have a portion of bound water firmly attached to the particles. The retention of this water can result in a high water centent respectively low in cake solids after solid-liquid separation. The objective of sludge conditioning is to alter any of the properties outlined above so as to increase the ease with which water can be removed from the sludge.

The mechanisms behind the chemical sludge conditioning are described by Vesilind (13) as the neutralization of the charge and bridging of individual particles.

2.2 THE ROLE OF BIOPOLYMERS IN SLUDGE THICKENING AND DEWATERING

In the activated sludge treatment system, most of the operational problems and process failures are in the areas of sludge thickening and dewatering. The relationship

between bacterial extracellular polymer production and flocculation of activated sludge has been studied by several workers (2, 3, 4, 14, 15, 16). However, very few investigations have been conducted to study the role of BEP in sludge thickening and dewatering.

To study the characteristics of sludge, the sludge filtration rate is measured to determine the degree of dewatering rate. Several workers such as Parker et al. (17), Tenney et al. (18), Busch and Stumm (2), McKinney (19) and Tenney and Stumm (1) have agreed that filterability of a biological sludge is function of the degree of flocculation of that sludge. Tenney et al. (18) reported in their study that the dispersed microorganisms were associated with long filtration times when a vacuum was applied, while the well flocculated microorganisms produced short filtration times. They claimed that high molecular weight polysaccharides in the suspension acted as flocculants and served to structure the individual cells into a lattice or three-dimensional matrix. In the presence of a vacuum, this lattice would maintain some relative porosity for the withdrawal of water. Their conclusion was that the performance of sludge filtration is determined by the biopolymers present in the sludge. However, they found that those same biopolymers are not at all effective as conditioners of waste sludge for dewatering by vacuum filtration. This idea was later supported by Parker et al. (17).

Pitman (20) described a well flocculated sludge as having a low specific resistance to filtration and dewatering easily whereas a poorly flocculated sludge dewatered slowly. However Wu et al. (21) who studied the filterability of activated sludge in response to growth conditions reported that one of the reasons the filtering properties of activated sludge are poor is because of the overproduction of extracellular biopolymers that can produce a considerably higher surface charge around the sludge.

Chao and Keinath (22) suggested that normal sludge floc has a relatively smooth surface while filamentous and zoogleal bulking sludge surfaces contribute to sludge surface roughness. They also found that at high process loading intensity or lower sludge age the flocs had a smooth surface. Conversely under these conditions, there was a decrease in the production of biopolymers. When coupling their finding with Pitman's observations, it can be concluded that there is a relationship between the quantity of biopolymer produced and dewatering characteristics of the sludge.

It appears, based on a literature survey, that there is a relationship between the quantity of BEP produced and the dewatering characteristics of activated sludge. The presence of BEP tends to improve the sludge dewaterability. However, overproduction of BEP may result in poor dewatering.

2.3 THE ROLE OF BIOPOLYMERS IN SLUDGE CONDITIONING

Sludge conditioning is often a necessary step in a sludge dewatering process. However, our knowledge of sludge conditioning with regard to bacterial extracellular biopolymers is unclear. Roberts and Olsson (6) used cationic polyelectrolytes as conditioners to study the influence of supernatant colloidal particles on dewatering of activated sludge. They developed an adsorption model to describe the activated sludge flocs where floc matrix serves as the adsorbent and the adsorbate is comprised of natural anionic biopolymers. They used an elution technique to demonstrate that these colloidal biopolymers adsorb on the floc surface as a multilayer with the outermost layer of biopolymers in equilibrium with those in solution. The adsorbed biocolloids can be desorbed or dislodged from the floc surface by mixing. Cationic polymers added to the solution for sludge conditioning react primarily with unsorbed (or free) and desorbed anionic biopolymer rather than with the flocs. These biopolymers were shown later by Novak and Haugan (7) to determine the dewatering characteristics and chemical conditioning requirements of waste activated sludge.

The same authors (5) also described the nature of the activated sludge flocs as a matrix to which natural anionic colloids are weakly adsorbed. Upon mixing of the sludge conditioner and the activated sludge flocs, the biopolymers

are desorbed. The quantity of chemical conditioning required for sludge dewatering depends on the mixing intensity and consequently amount of desorbed bipolymer. When activated sludge is mixed intensively, large quantities of biopolymers are released and the results are the reduction of sludge filtration rates and the increase in chemical conditioning requirements.

Mixing time and solids concentration have also been shown by Novak and Haugan (7) in their later experiment as factors involved in sludge conditioning. They demonstrated that when mixing is not intense, polymer requirements are low and independent of the sludge solids concentraion. At higher sludge solids concentrations, more biocolloids can be released and higher polymers are required as mixing time increased. At the end they proposed an adsorption model for activated sludge flocs where total biopolymer is comprised of:

1. polymer incorporated into the sludge floc,
2. loosely adsorbed polymer, and
3. free or supernatant anionic particles.

2.4 BACTERIAL EXTRACELLULAR POLYMER

2.4.1 NATURE OF BACTERIAL EXTRACELLULAR POLYMER

There are two different thoughts about the origin of bacterial extracellular polymer. Pavoni et al. (16), believed that the majority of these biological polymers were autolytic products. However, Wilkinson (23) proposed that those polymers are produced by bacteria and serve as protective shield for the cell from harmful factors in the environment and possible has an advantage in the uptake of ions from the surrounding medium. The reason these contradictory ideas developed was because the method for polymer extraction used by Pavoni et al.. was inadequate for biopolymer harvesting. Centrifugation at 20,000xG as performed in their study has been shown by Novak and Haugan (8) not to strip polymers from cell surfaces. Their proposed compositional make-up of extracellular polymer, which was carbohydrate, protein, RNA, and DNA, was similar to the make-up of bacterial cells. The presence of bacterial cell components led to their conclusion that extracellular polymer is an autolytic product. However, the detection of cell constituents was probably the result of strong centrifugal force which is believed to disrupt bacterial cells and release cell components. Harris and Mitchell (15) even used centrifugation as a means to break up bacterial cells in

order to obtain lytic products or lytic polymers to use in their flocculation experiment.

Therefore, it is more likely that extracellular polymer is a biological secretion product of bacteria. Sandford and Conrad (24), and Gehr and Henry (25) classified extracellular material released by bacterial cells as capsular and slime. Friedman et al., (4) defined capsular material as the portion that adheres to the cell, whereas the slime is either loosely bound to the cell, or is totally free from it.

Wilkinson (23) further divided the capsular polymer into two forms, capsule and microcapsule. A capsule was defined as a covering layer with a definite external surface outside the cell wall. This layer can be seen by a light microscope, however, it is often difficult to distinguish where the cell wall ends and the capsule begins. A microcapsule has the same characteristics but a layer thickness of microcapsule is less than 200 μm while a capsule has a layer thickness of at least 200 μm.

2.4.2 BEP AND FLOCCULATION OF ACTIVATED SLUDGE

The early theory of flocculation was proposed by Butterfield (26) and Heukelekian and Littman (27). They reported that Zoogloeae bacteria which possess a gelatinous matrix was solely responsible for the flocculation in the activated sludge. However, Friedman and Dugan (28)

demonstrated that Zoogloeae and floc formation may not be directly related. They considered zoogloeae growth or zoogloeae formation as the production of a highly polymerized extracellular material analogous to a capsule, and flocculation as a clumping of cells probably resulting from cell surface attractions which may or may not involve physiochemical influences of capsular material.

Dienema and Zevenhuizen (4) pointed out that the percentage of floc-forming bacteria isolated from activated sludge lies between 10 and 20 percent of the total number of bacteria isolated. However, Harris and Mitchell (29) reported that the polymer produced by bacteria in activated sludge controls the flocculation process. Busch and Stumm (2) proposed that aggregation of microorganisms is effected by an interaction of polymers excreted by the microbial cell or exposed at their surface. They defined the so-called bioflocculation as:

> "an aggregation of cells resulting from specific adsorption of polymer and from bridging of polymers between cells."

They also reported that these biopolymers appear to contain functional surface groups that are primarily anionic and nonionic in most neutral pH ranges. The polymers produced by bacterial cells have flocculative properties similar to those of synthetic anionic and nonionic polyelectrolytes (2, 25). They are capable of destabilizing dispersions of bacteria (4) and inorganic colloids (or abiotic sols) (2, 25)

with negative surface potentials (29). Because of similar charges of the biopolymers (16), the bacteria (5), and the inorganic colloid surfaces (2), charge reduction or charge neutralization is not believed to be the primary mechanism of floc formation (16, 30, 31). In addition, the concentration of BEPs necessary for optimal aggragation has been shown to be in proportion to the total cellular surface area (15, 29). Therefore, Harris and Mitchell have concluded that BEP extend to adjacent bacterial or inorganic colloids and form a complex by hydrogen bonding or proton transfer (adsorption) (29) between bacterial or inorganic colloids with functional groups on two different polymers. Then molecular bridges (14, 16, 29,32) and alteration of the polymer charge occur (4). These mechanisms result in a three dimensional matrix that will subside from suspension under quiescent conditions (16).

2.5 EXTRACTION METHOD

Many chemical and physical extraction methods have been used to remove BEP from pure bacterial cultures, synthetic and actual activated sludge floc. As early as 1946, Martin (33) used centrifugation, dialyzation, and then precipitation to extract polysaccharides from a soil bacillus. Later, Dudman and Wilkinson (34) successfully extracted both slime and capsular polysaccharides from one of the

Aerobacter-Klebsilla strains. In their extractions, centrifugation was used for slime, and boiling and NaOH-extraction were used for capsular polysaccharides. To extract the capsular polysaccharide the sample was stirred with NaOH at room temperature for 30 minutes. In this method a formaldehyde solution was added to the cells sample prior to the addition of NaOH to reduce cell disruption.

Gaudy and Wolfe (35), Busch and Stumm (2), Sutherland (36), Nagahama et al. (37) , Gulas et al. (38), and Pavoni et al. (18) all used centrifugation to shear off polymeric material from bacterial cultures. However, Novak and Haugan(6) demonstrated that high speed centrifugation does not strip polymers from activated sludge floc. This demonstration is consistent with Brown and Lester (39), and Saunders (40). In Novak and Haugan's study, they used centrifugation at a series of different shear forces up to 18,000xG to extract biopolymers from activated sludge and compared the data to a control which was obtained from the supernatant of the sludge that was allowed to settle for two hours. The result showed no significant difference in polymer recovery between the control and the centrifuged samples. They also demonstrated that polymeric material could be released from the flocs by fill and draw elution with distilled water but this is a non-quantitative extraction method.

Besides centrifugation which has been proven to be an inadequate method, other methods such as boiling have been used. Wallen and Davis(41) extracted polymeric material from bacterial cultures isolated from activated sludge by using hot water and organic solvents (chloroform). The bacteria was identified as Zoogloea ramigera. Their photomicrographs showed a difference in floc surface between extracted and unextracted samples. Forster et al. (42) used a combination of heating and centrifugation on activated sludge samples. This method has been used by Kiff (43) and Takiguchi (44).

Extraction with organic solvents is one of the methods that has been used frequently by many researchers. Nishikawa and Kuriyama (45) used 2 % EDTA (ethylenediaminetetraacetic acid) to extract polymer from activated sludge obtained from a domestic sewage treatment plant after the solids concentration had been adjusted to 20,000 ppm. They (46) also extracted mucilage by mixing the activated sludge sample with acetone. The mixed sample was allowed to stand for a few hours, and then was mixed with 0.5 M Na_2CO_3. Furrah and Unz (47) extracted exocellular polymer from Zoogloea MP6 and 106 cultures and from activated sluge flocs, by blending samples with phosphate buffer and then precipitating the solubilized polymer with cetyltrimethylammonium bromide.

Acid extraction has been employed by some workers but it is not widely used with activated sludge samples. Sandford and Conrad (24) cultured slime-producing Aerobacter aerogenes

A3 (S1) and extracted their polysaccharides by centrifugation followed by pH adjustment of the supernatant to 2 with sulfuric acid.

Alkaline extraction such as extraction with ammonium hydroxide and sodium hydroxide have been frequently performed on activated sludge. Chao and Keinath (22) mixed one volume of sample with two volumes of 1 N ammonium hydroxide and the mixture was then blended for two minutes and centrifuged at 1,500 rev. /min. for twenty minutes. The measurement of polysaccharide was then made on the supernatant. Takiguchi (44) extracted mucilage in activated sludge with 1 N NaOH. Tezuka (48) cultured bacteria isolated from activated sludge and extracted highly viscous material from the pellet of bacterial cell flocs that resulted from centrifugation, by using 2 N NaOH.

In 1974, Carr and Ganczarczyk (31) compared nine different physical and chemical extraction methods using activated sludge obtained from a sewage treatment plant. Those methods were;

1. extraction by boiling or autoclaving,
2. extraction with trichloroacetic acid(TCA),
3. extraction with sulfuric acid at pH 2. ,
4. extraction by high-speed centrifugation,
5. extraction with the tetra sodium salt of EDTA,
6. acetone extraction,

7. ultrasound treatment,
8. alkaline extraction, and
9. extraction in boiling benzene and water.

They found that extractions by the use of sulfuric acid, high-speed centrifugation, and boiling benzene and water were not sufficient to remove the BEP from the activated sludge. The alkaline extraction was found to be too severe in the authors' opinion. They recommended the boiling water extraction for further studies on activated sludge extracellular material because of its simple routine and reasonable accuracy even though this method did not obtain the highest extraction yield among those nine methods. However, they also noted that at the time of their experiment, there was no reliable way of testing the accuracy of the methods being compared.

The selection of the most efficient method for extracting bacterial extracellular polymer from activated sludge flocs is based on the minimization of bacterial cell disruption. Of all the extraction methods conducted by the aforementioned researchers, only one of them checked the possibility of cell disruption in their extraction method. If cell lysis occurred during extraction, the cell materials would have been released and these materials might have been measured as extracellular polymer, which would have led to a wrong estimation of the actual quantity of polymers present in the

activated sludge sample. Nishikawa and Kuriyama (46) claimed that the EDTA extraction method could be used to obtain mucilage material without breaking down the living cells. Their method was later tested by Brown and Lester(39).

Brown and Lester (39) compared five different extraction methods on cultures of activated sludge, synthetic activated sludge obtained from a laboratory reactor, and a pure culture of Klebsiella aerogenes. In their comparison, hexose sugar and hexuronic acid concentrations were used as measures of extracellular capsular or slime polymers, and protein and deoxyribonucleic acid (DNA) concentrations were used as measures of the degree of cellular disruption. Those five extraction methods were,

1. high-speed centrifugation (modified Pavoni et al.'s method),
2. ultrasonication,
3. ultrasonication and high-speed centrifugation,
4. steaming extraction,
5. sodium hydroxide extraction (modified Tezuka's method), and
6. EDTA extraction (Nishikawa and Kuriyama's method).

They concluded that steaming treatment was the most effective extraction method for activated sludge. This conclusion was based on the fact that this method removed a

significant quantity of extracellular polymer from the flocs with minimal cellular disruption. Ultrasonication was found to released low concentrations of extracellular polymers from all cultures.

In 1982, Rudd et al. (49) used a combination of ultrasonication and centrifugation to quantify extracellular polymer produced by Klebsiella aerogenes under varying cultural conditions. They claimed that this method achieved the highest recovery of extracellular polymer. However, Gehr and Henry (25) proposed a different removal technique. Their method was composed of five steps to be used to extract the capsular part of the polymer. The method includes blending the activated sludge precipitate at high speed followed by centrifugation. Forster and Clarke (50) studied the relationship between the polymer production from activated sludge and the treatment operation by using centrifugation as a sole method of polymer extraction.

2.6 CHEMICAL COMPOSITION

At the present time it is widely accepted that the major component of BEP is polysaccharide. Wilkinson (23) provided an extensive literature review about the extracellular polysaccharides of bacteria. He mentioned that these polysaccharides can be divided into three main groups according to their morphological localization; intracellular

polysaccharides, cell wall polysaccharides, and extracellular polysaccharides located outside the cell wall.

Dudman and Wilkinson (34) extracted both the slime and capsular extracellular polymers from Klebsiella aerogenes and found polysaccharides which contained approximately 50% glucose, 29% uronic acid, 10% fucose, and 1% galactose. Wilkinson (23) worked on strains of K. aerogenes type 54; A3 which produces slime and capsular polysaccharide, and A3 "S1" that produces only slime polysaccharide. He found that all the polysaccharides studied contained glucose, fucose, and a uronic acid as the three component sugars in essentially the same molecular proportions. Also the slime and capsular polysaccharides showed identical chemical composition. These polysaccharides contained about 48% glucose, 10% fucose, and 29% uronic acid. Further studies on the slime polysaccharides of strain A3 (S1) showed that the uronic acid was glucuronic acid and that the molecule was highly branched.

Churms and Stephen (51) also studied the capsular polysaccharide of Klebsiella aerogenes type 54, type 4 and type 64. They found that the polysaccharides of K-type 4 and type 54 were similar. They appeared to have a relatively simple structure. The constituent sugars were glucose, mannose, and glucuronic acid. For K-type 64, the sugars were rhamnose, glucose, and mannose. The molar ratio of those neutral sugars were 1. 0:2. 0:1. 5 with acetate, pyruvate and

uronic acid residues. They concluded that relatively simple sequences of sugars were repeated throughout the entire molecular structure of these polysaccharides.

Another type of bacteria that has been studied extensively is Zoogloea ramigera. These bacteria are commonly found in activated sludge and are among many other bacteria that are responsible for bioflocculation. Friedman et al.(52) found that these bacteria produce a capsular matrix which cuases them to adhere together as a packets of cells with globular shape and fingerlike projections, which have been considered to be characteristic of the genus Zoogloea. The individual packets appear to adhere one to another by intermeshed fibrils that measured 2 to 5 nm in diameter. The fibril polymer appears to be polyglucose and is susceptible to cellulase. Friedman and Dugan (28) studied the Z. ramigera strain 115 and reported that their extracelular polymers were composed of glucose and galactose. Wallen and Davis (41) also found glucose and galactose with the addition of mannose in the biopolymer of the strain designated NRRL B-3669M. Besides these neutral sugars, Tezuka (48) found two amino sugars in the extracellular polymer of Z. ramigera and identified the compounds as glucosamine and possibly fucosamine. Farrah and Unz (47) conducted studies on the isolated extracellular polymer from different strains of Zoogloea, MP6 and 106. The analytical report of hydrolyzed extracellular polymer on strain MP6 showed the composition

of the polymer as reducing substances, amino sugar, hexose, uronic acid, and ether-soluble material with the per cent wt/wt ratio of 20:17:2:1:0 respectively. After acid hydrolysis of the biopolymer they found that amino sugars were the principal components. They further commented that the different descriptions of extracellular polymer associated with organisms described as Z. aerogenes were likely a result of the taxonomic confusion surrounding the genus Zoogloea rather than a result of different isolation and characterization procedures being used by different investigators.

The same investigators also extracted extracellular polymer from activated sludge and found that the ratio of reducing substances: amino sugar: hexose: uronic acid: ether-soluble material was 25:15:7:2:0. Steiner et al. (53) extracted the slime portion of the BEP and reported that the monosaccharides present varied between different sludge samples. However hexose, pentose, and glucuronic acid were present in all the sludges investigated. This finding was consistent with Sutherland's (54) which described that the most common monosaccharides found in BEPs are hexoses: D-glucose, D-galactose, and D-mannose.

Many other bacteria have been investigated in the past. Gaudy and Wolfe (55) extracted the capsular polysaccharides from Sphaerotilus natans, bacteria that cause sludge bulking problems, and found that the major components were fucose,

galactose, glucose, and glucuronic acid in approximately equimolar amounts. These polysaccharides were associated with variable content of protein. Nagahama et al. (37) isolated bacteria from polluted water and sludges in the factories manufacturing sweet potato starch and they identified these bacteria as slime-producing strain Aerobacterium radiobacter. They reported that the polysaccharides produced by these bacteria were composed of galactose, glucose, succinic acid, and pyruvic acid. El-Sayed et al (56) investigated the extracellular polysaccharides of Agrobacterium tumefaciens and found that they consisted of hexasaccharide repeating-units having D-glucose as the sole sugar component, and pyruvic acid, O-succinyl, and O-acetyl in the molar ratios of approximately 5. 8:1. 03:0. 91:0. 87. They also studied Agrobacterium radiobacter polysaccharide and found glucose, galactose, succinic acid, and pyruvic acid which was similar to what had been reported by Nagahama et al.(37) . They expressed the molar ratios of D-glucose, D-galactose, pyruvic acid, O-succinyl, and O-acetyl as approximately 3. 1:2. 2:1. 2:1. 1:1. 02.

Forster (57) mentioned in his article that BEP's contain lipids, nucleic acids, proteins and carbohydrates. However, it is not known whether these compounds are present as individual compound or complexed such as glycoproteins, lipoproteins or glycolipids.

The inclusion of protein as one of the constituents of extracellular polymer is still a subject of debate. As described above, some of the workers did report the presence of protein in the polymer. However, Brown and Lester (39) considered protein as a cellular constituent. They even used protein and DNA contents to measure the extent of cellular disruption in their extraction method.

In conclusion, most investigators contend that the major component of BEP is carbohydrate. The most common monosaccharides are hexoses: D-glucose, D-galactose, and D-mannose. These sugars may be in the form of relatively simple sequences and repeat themselves throughout the entire molecular structure of the polysaccharide. Novak and Haugan (8) concluded in their article that;

> "the specific indentification of the biopolymers remains the subject of some debate, but these polymers definitely contain polysaccharides, and perhaps protein and nucleic acids."

2.7 SEPHADEX GEL FILTRATION OF BACTERIAL EXTRACELLULAR POLYMER

Gel filtration or gel chromatography has recently been used to classify polymers in activated sludge. Novak et al. (8) reported that gel filtration is a promising method for determining molecular weight distributions in the supernatant of activated sludge without causing cell lysis or altering

extracellular polymer properties. Churms and Stephen (51) used this chromatographic method to determine the molecular weight distribution of some capsular polysaccharides from Klebsiella bacteria at various stages of acid hydrolysis. Dewalle and Chain (54) studied the kinetics of formation of humic substances in activated sludge systems and their effect on flocculation by using the combination of reverse osmosis, ultrafiltration, and gel filtration to isolate humic substances from. the sludge samples. Minear and Christman (59), and Obiaga and Ganczarczyk (60) analyzed the activated sludge effluent by gel filtration and found two molecular weight fractions.

Novak and Haugan (8) proved that high speed centrifugation does not strip extracellular polymers from activated sludge flocs and one of their approaches was by using gel filtration. They compared the molecular weight distribution of the polymeric materials from the settled supernatant and extracted supernatant by centrifugation and found no distinct differences. The result indicated two major fractions in the supernatants; one with the molecular weight approximately 800-1,500, and the other one between 3,000 and 5,000.

Later Rudd et al. (49) used gel filtration with Sephadex G-50 to recover biopolymer from their extracted samples. They claimed that the recovery was 95 percent and the relative molecular weight exceeded 10^4.

About the history of gel filtration , this method became an established laboratory technique with the introduction of Sephadex in 1959 (61). Sephadex is a registered trademark of Pharmacia Fine Chemicals. The basic process of gel filtration is described by Pharmacia Fine Chemicals (61) as:

> Molecules larger than the largest pores of the swollen Sephadex, i. e. above the exclusion limit, cannot penetrate the gel particles and therefore they pass through the bed in the liquid phase outside the particles. They are thus eluted first. Smaller molecules, however, penetrate the gel particles to a varying extent depending on their size and shape. Molecules are therefore eluted from a Sephadex bed in order of decreasing molecular size. . . . .
> As the molucules pass through the bed at different rates, they emerge from the outlet of the column separated from each other. . . . . When all the molecules have been eluted from the Sephadex bed, the column is ready for another experiment.

Pharmacia Fine Chemicals also describes Sephadex as:

> Sephadex is a bead-formed, dextran gel. Sephadex is prepared by cross-linking selected dextran fractions with epicholrohydrin.

The G-types of Sephadex differ in their degree of cross-linking and hence in their fractionation ranges. Table 2 shows the G-types of Sephadex and their fractionation ranges that are usually used in the area of activated sludge research.

Table 2

Sephadex gels and their properties (8).

Type	Dry particle diameter, μm	Fractionation range	
		Peptides and Globular Proteins (MW)	Dextrans (MW)
G-25fine	50-100	1,000-5,000	100-5,000
G-50	50-150	1,500-30,000	500-10,000
G-75	10-40	3,000-70,000	1,000-50,000

III. ANALYTICAL METHODS AND TECHNIQUES

3.1 SYSTEM OPERATION

Two identical completely mixed, continuous flow laboratory-scale activated sludge systems were operated in parallel in a controlled temperature room where the temperature was maintained at 20±1 degree C.

Each of these activated sludge operating units had a working volume of approximately 8.5 l and was constructed of transparent plexiglass. The detailed dimensions of a unit are shown in Figure 2. The unit has two interconnected chambers, aeration and clarification, which were separated by an adjustable baffle. A diffuser stone was placed inside the aeration chamber to supply air and to induce a mixing effect in the chamber.

The influent substrate solution was pumped by a Calgon chemical feed pump Model P-8 at a constant rate to the aeration chamber from an 20 liter plastic carboy through standard medical intravenous (I.V.) tubes. These influent tubes and container were disinfected with Chlorox solution intermittently (every 2 or 3 days) and were rinsed with tap water to remove traces of disinfectant. A schematic diagram of the experimental reactor set up is shown in Figure 3.

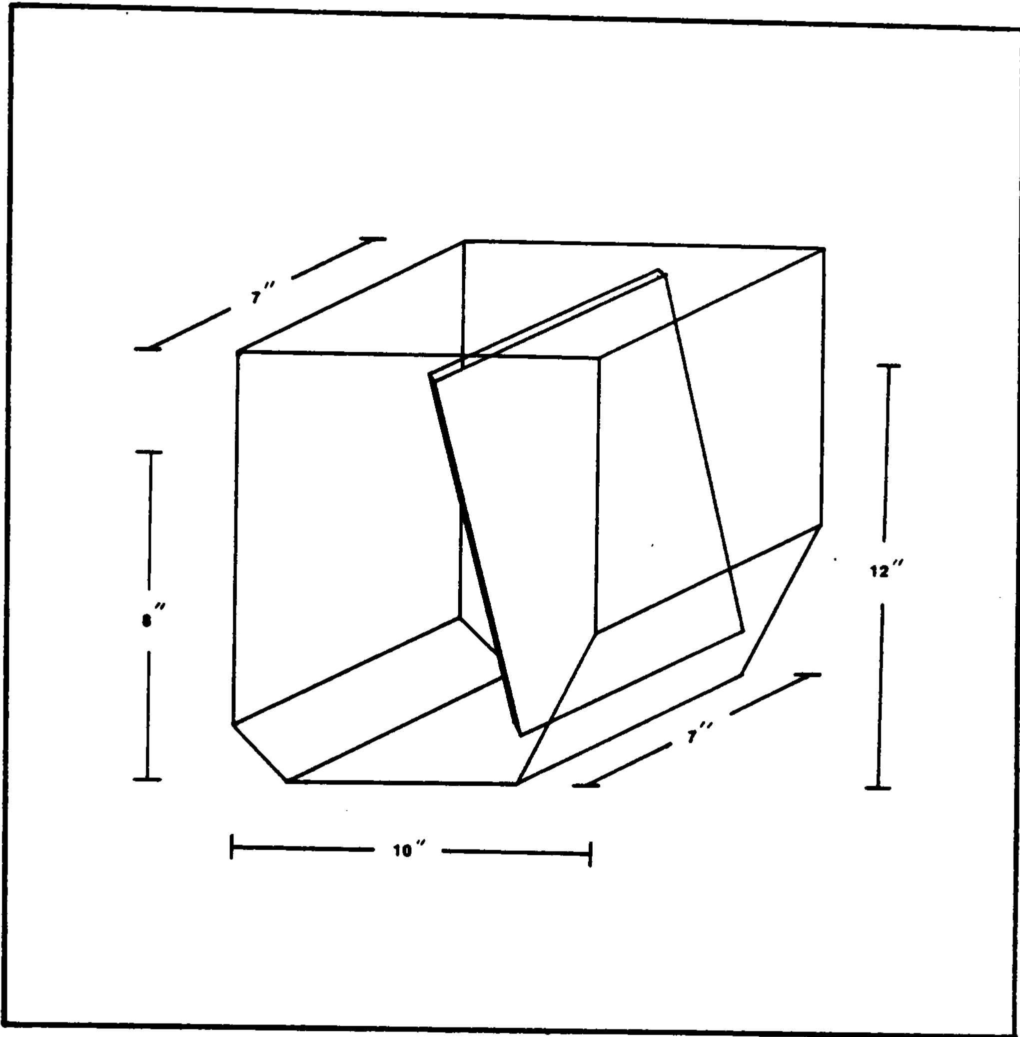

Figure 2. Detailed dimensions of the operating unit.

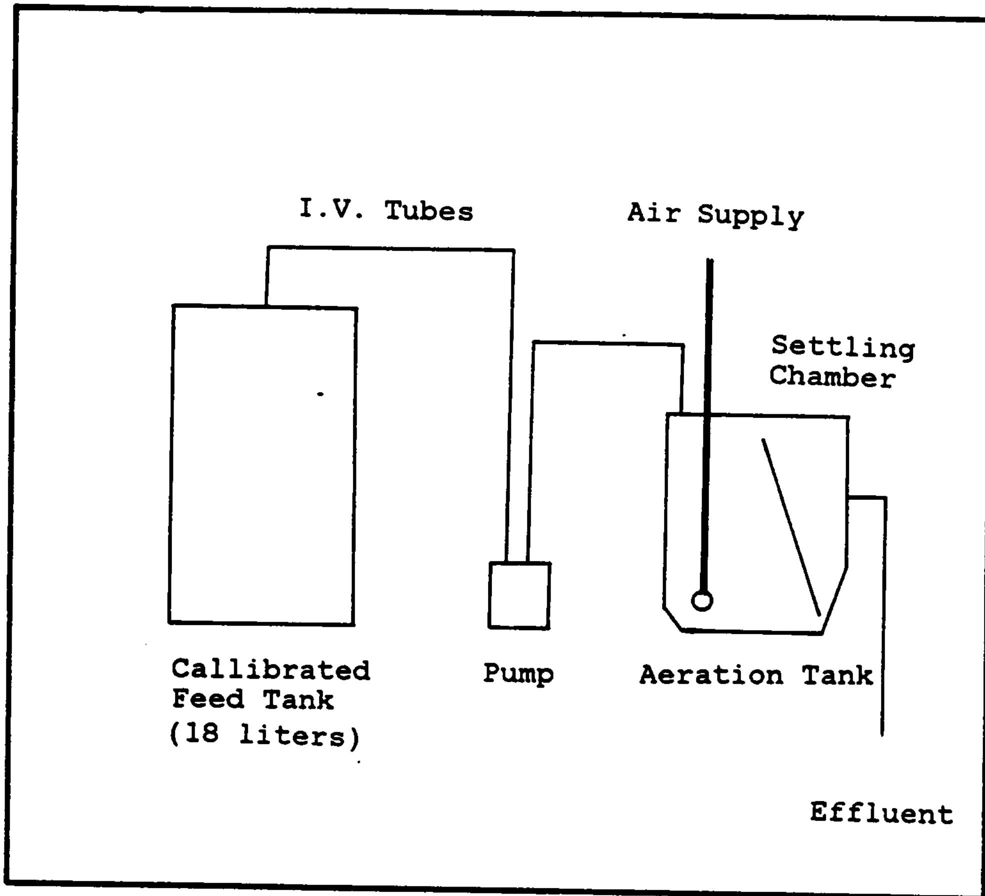

Figure 3. A schematic diagram of the operating unit.

An activated sludge was obtained from the aeration basin of a wastewater treatment plant. In the early phase of the research, the sludge used was from the Blacksburg, Virginia Municipal Wastewater Treatment Plant, and later, from the industrial wastewater treatment system of the Celanese Company located in Pearisburg, Virginia.

To start the system, the activated sludge was poured into the reactor to a total volume of 8.5 l. Aeration was begun immediately after the reactor had been filled. After 15 minutes of aeration and mixing, an adjustable baffle was placed in the slot. When the solids in the settling chamber had settled, feeding through the influent line was started at a pre-determined rate. The analysis of sludge characteristics and the extraction of BEPs were started on the next day. Fresh sludges obtained from either the Blacksburg or Celanese plant were also analyzed in order to compare changes in sludge and biopolymer properties .

3.2 FEED SOLUTION

In order to better understand the production of BEP and their chemical and physical characteristics, various types of substrates were supplied to the micro-organisms in the activated sludge being examined. These substrates included milk, bacto-peptone, glucose, nitrogen, and Celanese raw wastewater. Nitrogen in the form of NH_4^+ and NO_3^- were added

to the influent to verify the influence of nitrogen and its limitation on BEP production and, consequently, activated sludge flocculation and dewatering characteristics. The entire research was separated into 6 periods as shown in Table 3.

The composition of the substrate used in the research are categorized in Tables A1 to A8 (Appendix A). Those tables include the type of sludge being studied at that time, the substrate and its concentration, feed rate, hydraulic retention time, and sludge age (θ_c).

3.3 SAMPLING PROCEDURE

Daily sludge wastage in the form of mixed liquor suspended solids (MLSS) was performed from each reactor by stopping the influent line and removing the baffle to allow mixing of the solids between the aeration basin and the clarifier. The volume of sludge to be wasted was measured to attain the desired θ_c.

Gulas and Bond (38) defined sludge age as the average time a unit of biomass remains in the treatment system. The mathematical expression of sludge age for a completely mixed process is shown in equation 1 by Randall and Benefield (62):

$$\theta_c = \frac{V_a X}{Q_w X + (Q - Q_w) X_e} \quad (1)$$

where

θ_c	=	sludge age (days)
V_a	=	volume of aeration tank (liters)
X	=	average mixed liquor volatile suspended solids in the completely mixed aeration basin (mg/l)
Q_w	=	sludge wasting rate (l/d)
Q_e	=	influent flow rate (l/d)
X_e	=	average volatile suspended solids in effluent (mg/l)

To simplify the operation of the laboratory unit, it was assumed that solids lost in the effluent could be neglected. Therefore, equation 2 can be reduced to

$$\theta_c = \frac{V_a}{Q_w} \quad \text{or} \qquad (2)$$

$$Q_w = \frac{V_a}{\theta_c} \qquad (3)$$

In this study, θ_c values of 10 and 17 days were used. These θ_c's corresponded to a wastage volume of 850 ml and 500 ml, respectively, based on the reactor volume of 8.5 l.

The daily sludge wasted was then used to determine the suspended solids, SVI, and other settling characteristics , sludge physical characteristics, and BEP. The extracted biopolymer was further analyzed for chemical composition.

Table 3

Research schedule

Periods	Date	Description of Period
A	April 13-30	Two reactors, fed with glucose and bacto-peptone.
B	May 19-June 12	One reactor, initially fed with glucose and then changed to bacto-peptone
C	June 22-July 10	Two reactors, fed with glucose and glucose+NH_4Cl, both in buffer solution
D	July 11-Aug. 7	Two reactors, fed with glucose+NH_4Cl and glucose+$Ca(NO_3)_2 \cdot 4H_2O$ both in Celanese raw wastewater.
E	Oct. 30-Nov. 27	Two reactors, fed with Celanese raw wastewater and Celanese raw wastewater +NH_4Cl

3.4 ANALYTICAL METHODS AND TECHNIQUES

3.4.1 SOLIDS DETERMINATION

Both suspended solids and total solids were determined in accordance with Standard Methods for the Examination of Water and Wastewater (63). Sample volume of 20 ml was drawn from the well mixed reactor for suspended solids analysis. The sample was then filtered through 5.5 cm Reeve Angel 934 AH glass filters and a 5.5 cm Nalgene Buchner funnel. The same volume of sample was also analyzed for total solids. A Mettler Ac 100 Balance was used to weigh filters and aluminum pans for all samples.

3.4.2 SVI

Sludge settling characteristics were examined using the Sludge Volume Index (SVI) test which has a formula:

$$SVI = \frac{\text{ml of sludge} \times 1000}{\text{suspended solids conc., mg/l}} \qquad (4)$$

The SVI test was performed by filling a one-liter graduated cylinder with MLSS. After 30 minutes, the settled sludge volume was measured and the SVI calculated in accordance with equation 4.

3.4.3 SPECIFIC RESISTANCE

A wastewater sludge volume of 100 ml from the daily waste was filtered through a 9 cm Buchner funnel, using Whatman No. 40 ashless filter paper (Figure 4), and the volume of filtrate (v) was measured as a function of time (t).

The specific resistance of sludge was then calculated as follows:

$$r^* = \frac{2PA^2b}{\mu W} \tag{5}$$

where

r^* = specific resistance, m/kg

A = area of filter paper, m^2

μ = dynamic viscosity, $Nsec/m^2$

b = slope of t/v versus v plot

p = pressure, N/m^2

W = dry solids deposited per unit volume of filtrate, kg/m^3

The pressure used in this analysis was maintained at 15 inches Hg or approximately 25 psi. This specific resistance was determined in accordance with Standard Methods for the Examination of Water and Wastewater (63).

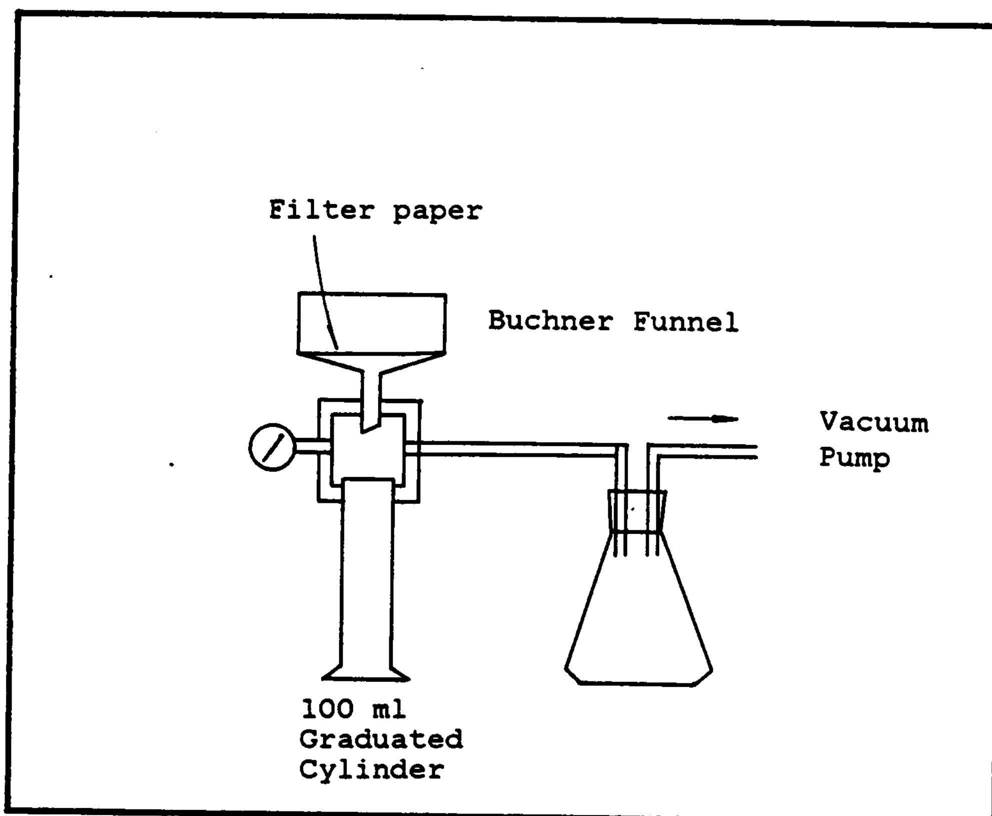

Figure 4. Buchner funnel apparatus for determining sludge dewatering rates.

3.5 CHEMICAL ANALYSIS OF BIOPOLYMER

All the biopolymer samples to be studied were prepared by adjusting the pH to 11 using sodium hydroxide. This biopolymer extraction procedure is described in section 3.5.5. The supernatant of the sludge was then used for chemical analysis.

3.5.1 CELLULAR DEOXYRIBONUCLEIC ACID

DNA was measured by the Burton Diphenylamine test as described by Webb and Levy (64). The procedure for this method is:

APPARATUS

- Spectrophotometer

REAGENTS

a. Modified diphenylamine reagent: This is prepared by dissolving 1.5 g of steam-distilled diphenylamine in 100 ml of redistilled acetic acid and adding 1.5 ml of concentrated sulfuric acid. The reagent must be stored in the dark.

At the time of use, add 0.10 ml of aqueous acetaldehyde (16 mg/ml) to each 20 ml of reagent required.

b. DNA Standard: Calf thymus DNA is dissolved in 5 nM NaOH to give 400 ug/ml: this is stable at 4 degree C for 6 months. Working standards are prepared by mixing measured

volumes of stock with 0.5 N $HClO_4$ and heating 15 mintues at 70 degree C.

PROCEDURE

a. one or two ml of test sample is mixed by a Vortex-Genie model K-550-G with 2 volumes of the modified diphenylamine reagent (containing acetaldehyde) in test tubes.

b. Standards containing known amounts of DNA (10-50 ug/ml) in 0.5 N $HClO_4$, and a reagent blank containing 0.5 N $HClO_4$ but no DNA are prepared in a similar manner.

c. The tubes of mixture are incubated at 30 degree C for 16-20 h.

d. Absorbances are measured at 600 nm by a Spectronic 20 spectrophotometer against the blank and compared with the standard curve.

3.5.2 PROTEIN ANALYSIS

The protein determination method used involves the binding of Coomassie Brilliant Blue G-250 to protein. Bradford (65) described this method as rapid, sensitive, and reproducible for the quantitation of protein, and as superior to the standard Lowry Method.

The principle involved in this method is the conversion of the red form of Coomassie Brilliant Blue G-250 to the blue form upon binding of the dye to protein.

APPARATUS

- Spectrophotometer
- Test tubes

REAGENTS

a. Protein Reagent: Coomassie Brilliant Blue G-250 (100 mg) is dissolved in 50 ml 95 % ethanol. To this solution 100 ml 85 % (w/v) phosphoric acid is added. The resulting solution is diluted to a final volume of 1 liter before use.

b. Protein Standard: Stock solution of 1 mg/ml albumin is prepared. Set of protein standards (e.g. 0, 10, 20, 40, 80, and 100 ug/0.1ml) are prepared from this stock solution.

PROCEDURE

a. 0.1 ml of test sample, set of protein standards, and distilled water (blank) are pipetted into separated test tubes.

b. 5 ml of protein reagent is added into the tube and immediately vortexed.

c. Repeat step b for all tubes.

d. The tubes are allowed to stand at room temperature for at least 2 min.

e. Absorbance are measured at 595 nm against the blank. The developed color is stable for about 1 h.

3.5.3 CARBOHYDRATE ANALYSIS

The carbohydrate content of the biopolymer was measured by a method developed by Dubois et al. (66). The detailed procedure is presented below.

REAGENT

a. Concentrated sulfuric acid

b. Reagent grade phenol, 80 % by weight

c. Carbohydrate standards

Glucose stock solution of 50 mg/l was prepared. A set of standards (0, 4, 12, 20, 25, 40, 50 mg/l) was prepared from this stock solution and the final volume of each standard and sample was 2 ml.

PROCEDURE

a. To each tube, add 0.05 ml phenol reagent followed by rapid addition of 5.0 ml concentrated H_2SO_4

b. Let the sample and standard stand at room temperature for 30 minutes.

c. Measure absorbance at 488 nm against the blank.

3.5.4 GEL FILTRATION

Gel filtration analysis was also performed on the supernatant of the raw and extracted sludges. The detailed theory behind gel filtration can be found in reference 61. Figure 5 shows the schematic set up of this experiment.

Sephadex G-75 was used in the chromatography because its size gave two distinguished peaks without overlapping and also it has a suitable fractionation range, as shown in Table 2 (page 30).

In this procedure, dry gel was allowed to swell in distilled water for at least 24 h. The swollen gel beads were then poured into a 1 cm x 36 cm cylindrical glass tube. The gel column was allowed to settle and more gel beads were filled until there was a space height of 4 cm left at the top of the glass tube. An intravenous tube from the eluant distilled water vessel was connected to the top of the glass column and the flow rate was controlled by a stopcock at the exit line of the tube. A supernatant sample of volume 2 ml was added at the top of the column.

The exit supernatant sample was continuously monitored by a Buchner Fracto Scan ultra-violet light source set at a wave length of 280 nm. The Fracto-Scan was connected to a recorder. A typical output of organics concentration recorded is shown in Figure 6. The eluant from the column was collected in a Eldex Universal fraction collector, calibrated to collect exactly 5 ml in each test tube.

Those samples collected in the fraction collector were analyzed for protein and carbohydrate content. Later in the research, the output from the recorder had so much noise that it became difficult to evaluate the peak height. Therefore, the collected samples in the fraction collector were analyzed

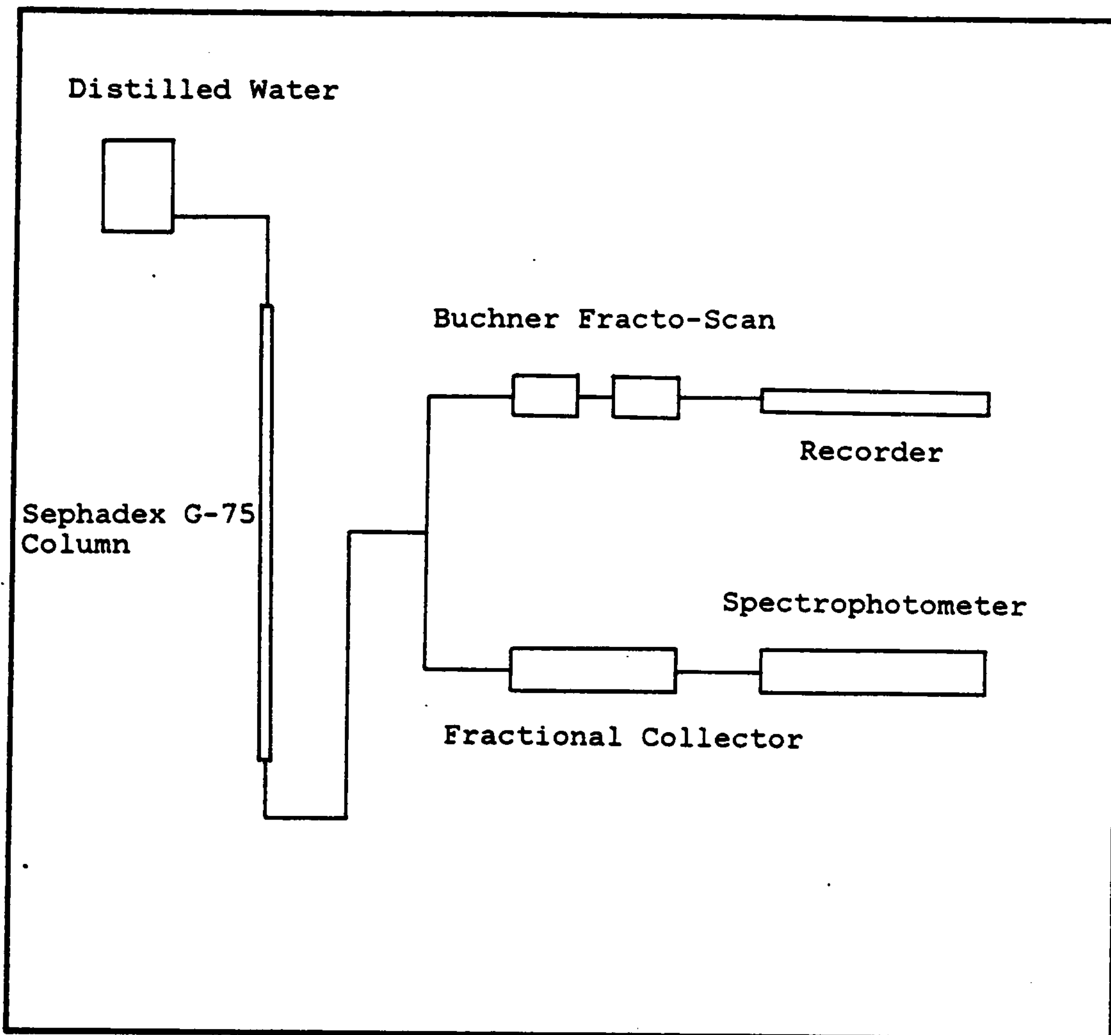

Figure 5. Experimental set up of gel filtration chromatography.

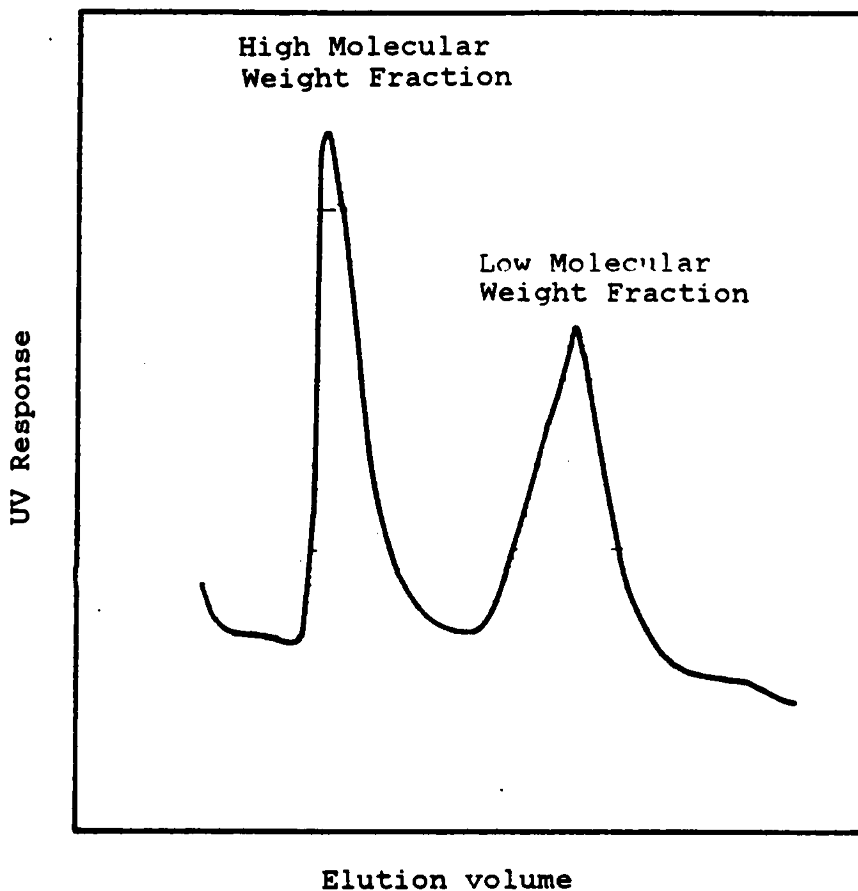

Figure 6. Typical chromatogram for organics in the sludge supernatant liguor.

by the Bausch & Lomb Spectronic 710 spectrophotometer. The light source equipped with this machine was a deuterium lamp and the wave length was set at 280 nm. The transmittance of the samples were recorded for each tube and plotted as elution volume VS (100-% transmittance).

3.5.5 EXTRACTION METHODS

The selection of a method for extracting BEP should be based on the maximization of the polymer yields, and the minimization of bacterial cell disruption. In a preliminary experiment, an attempt was made to find a suitable extraction method for activated sludge. Two extraction methods: pH adjustment and steaming, were compared. Brown and Lester (39) found these to be the best methods for extraction.

The extracted organic substance in the supernatants obtained by each method and the controls were separated from each other and tested for relative quantity using gel filtration (Sephadex G-75). For analysis of the extent of bacterial cell disruption, DNA was selected as the indicator of cell lysis and was measured by the modified diphenylamine test. The extracted supernatants which contained biopolymers were further tested for physical and chemical properties which were adsorption at different pHs, and carbohydrate and protein contents, respectively.

The extraction was begun on a sludge sample as soon as it was wasted from the laboratory reactor. The pH of the sludge sample was adjusted to 11 with 0.5 N NaOH and was slow stirred on a Fisher Flex-mix mixer for 1 h. The sample was then centrifuged at 5,000 rpm by a Beckman Model J-21C Centrifuge for 10 min. and the supernatant filtered through a milipore filter paper before analysis by gel filtration. The precipitate pellet from centrifugation was discarded.

The steaming extraction was conducted in a similar manner to the pH-adjustment method except that fresh activated sludge was steamed in a steaming chamber for 10 min. The steamed sample was then centrifuged at 5,000 rpm for 10 min. and the supernatant was analyzed by gel filtration.

A control, in which fresh activated sludge was centrifuged without raising the pH or steaming, was also analyzed for comparison.

3.6 ADSORPTION TEST

One goal of this study was to determine the extent of adsorption of extracted biopolymers. In this test, 100 ml of supernatant from the pH extraction was mixed with 100 ml of pulverized carbon solution at a concentration of 20 mg/l. The mixed solution was then separated into 6 jars. The pH of each solution was lowered to 11, 8, 7, 6, and 3 with 0.5

N HCl. Another set of experiments was performed in the same manner but with the raw sludge supernatant at its natural pH.

Those two sets of solutions were mixed at 70 rpm by a jar test apparatus or a 6-paddle stirrer for 1 h and then centrifuged at 5,000 rpm for 10 min. The supernatants were analyzed for biopolymers by gel filtration.

IV. RESULTS

4.1 EXTRACTION METHOD

A comparison of two extraction methods, steaming and pH-extraction is provided in this section. Figure 7 shows representative gel filtration chromatograms of the processed supernatants. The chromatograms show two major molecular weight fractions of bacterial extracellular polymers in the extracted and the raw sludge (control) supernatants. Both pH 11-extracted and steaming extracted supernatants contained higher quantity of polymers than the control for both the high and low molecular weight fractions. Extraction by adjusting the pH to 11 provided a substantially higher yield of biopolymer than the steaming method.

The analysis of DNA in those supernatants indicated that no significant cell lysis occurred when the sample pH was raised to 11 whereas extraction by steaming resulted in a considerable DNA generation, suggesting significant cell lysis had occurred.

Table 4 lists absorbances of the raw sludge, pH-adjusted, and steamed-treated supernatants as 0.04, 0.10, and 0.18 units, respectively. According to the developed DNA standard curve in Figure 8, the DNA concentrations were 50 mg/l, 120 mg/l, and 220 mg/l. These results indicated that extraction

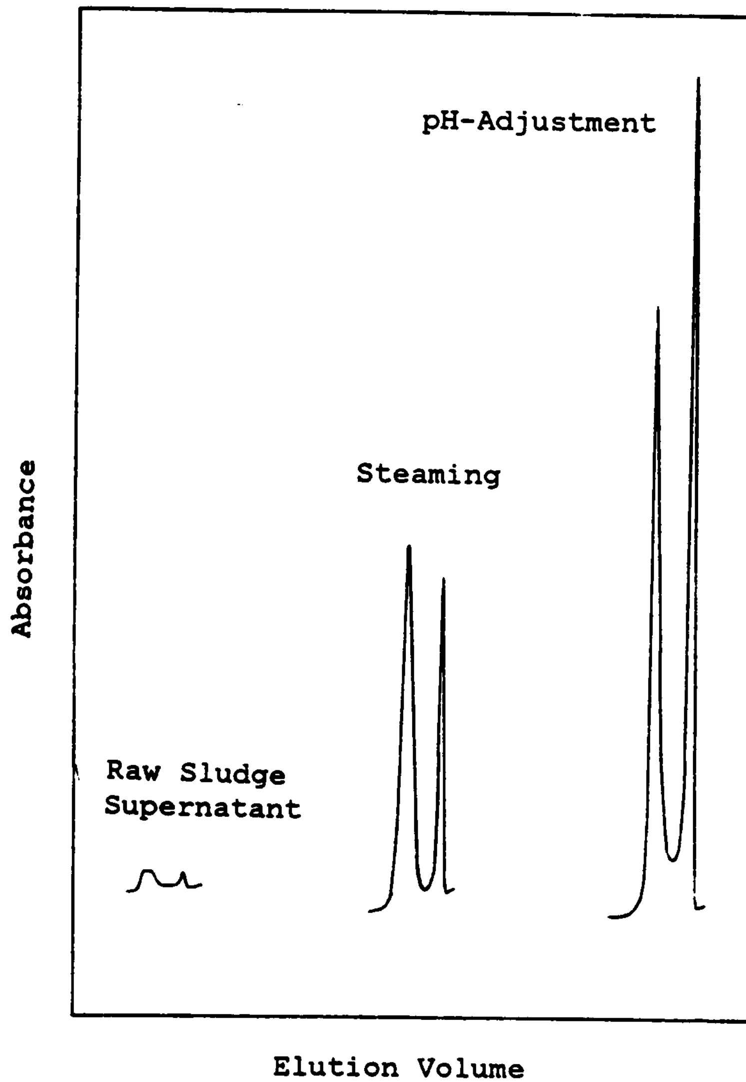

Figure 7. Comparison of extracted biopolymer yields using different extraction methods.

Table 4

DNA absorbance (experiment 1)

Samples	Absorbance (UV units)	Conc., mg/l
raw sludge supernatant	0.040	50
pH 11 extracted supernatant	0.100	120
steamed treated supernatant	0.180	220

See absorbance readings for standard curve in Appendix B.

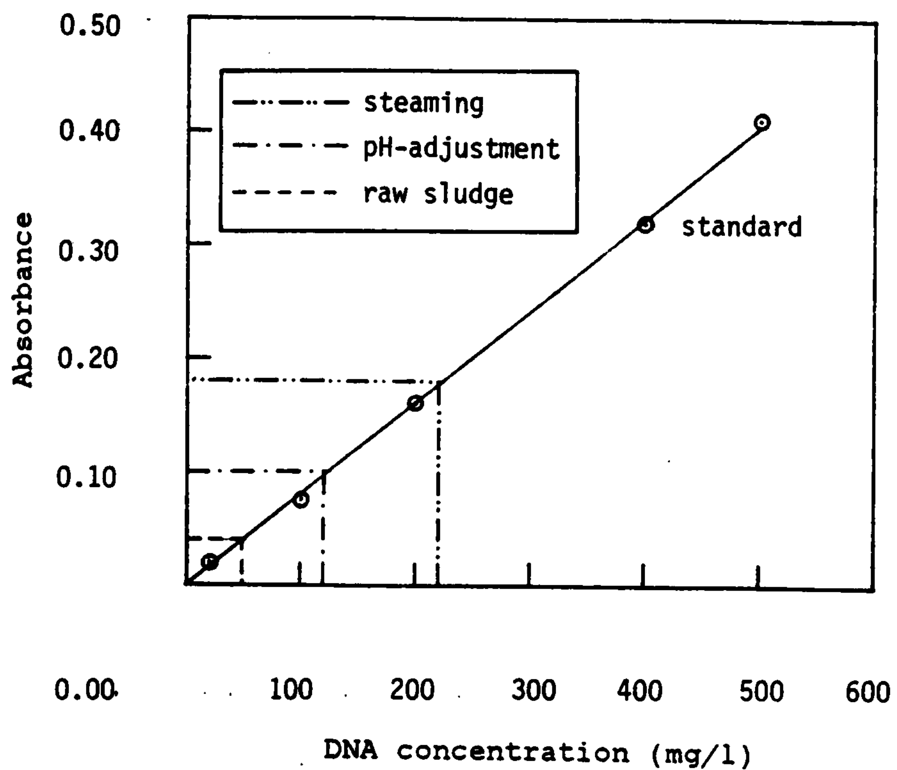

Figure 8. DNA standard curve and comparison of methods.

of biopolymer by steaming causes more cell lysis than the pH-extraction method.

In order to confirm the above results another experiment was conducted and the results are shown in Figure 9 and in Table 5. The concentration of the DNA in the supernatant after extraction by pH-adjustment was only three percent higher than the control or the raw sludge supernatant at its natural pH. This small difference indicates very minor cell disruptive effects occurred when extraction by adjusting the pH to 11 was used.

Figures 7 and 8 also indicate that less material is extracted by steaming but the extracted matter is higher in DNA. This suggests cell lysis occurs when steaming is used.

From the above discussion, the pH-extraction method was chosen as the routine method for extraction of biopolymers. However, before this method could be used routinely, optimization of the procedure was necessary to reduce experimental time and maximize recovery. Therefore, further studies were conducted to better define the procedures for this test

The steps involved in the routine pH-extraction method were adjustment to pH 11, 1 h stirring, and centrifugation at 5,000 rpm for 10 min. Using this combination, a high yield of biopolymer was obtained without using excessive time during extraction. This combination did not extract all the polymers in the supernatant sludge sample but did appear to

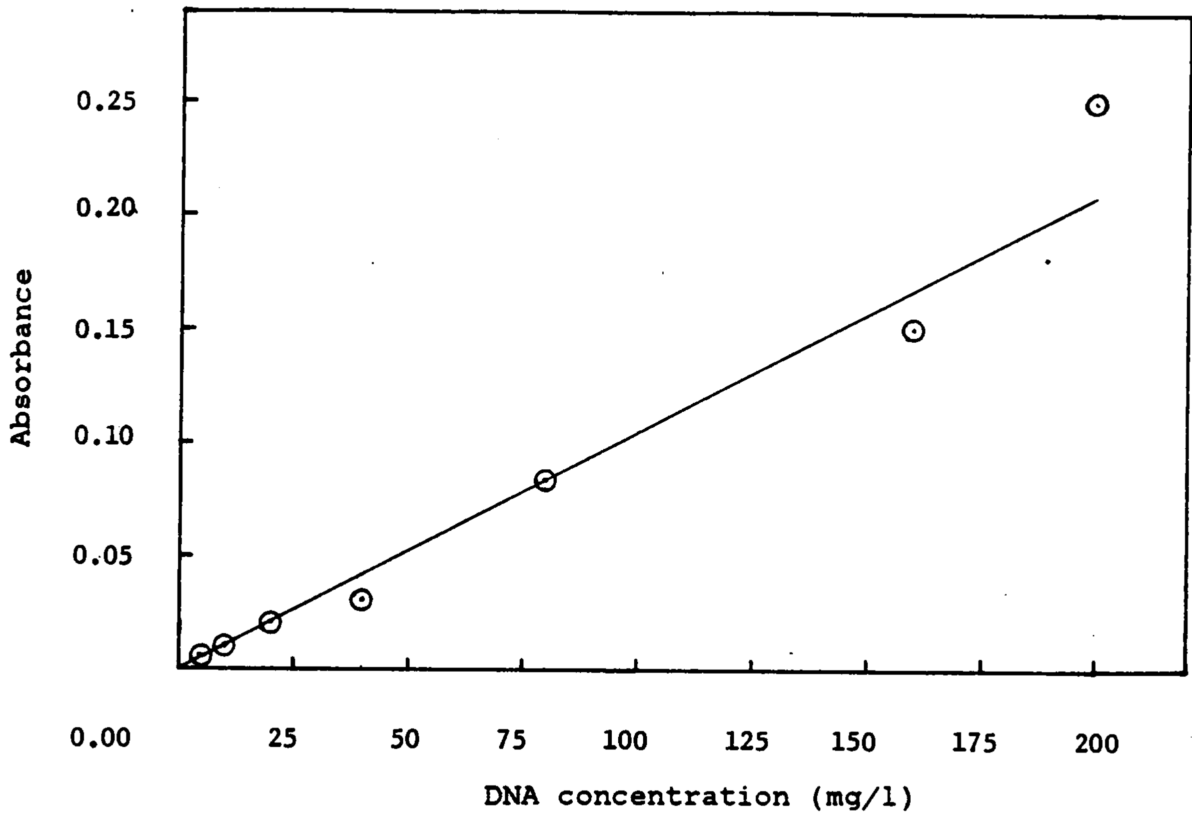

Figure 9. DNA standard curve (experiment 2).

Table 5

DNA absorbance (experiment 2)

Samples	Absorbance (UV units)	Conc., mg/l
raw sludge supernatant	0.228	220
pH 11 extracted supernatant	0.235	227

See absorbance readings for standard courve in Appendix B.

provide a quantitative distribution of molecular weights similar to longer extraction times. Figure 10 and 11 show the results that led to this selection. A stirring period of 2 h at pH 11 resulted in the highest extraction for both high and low molecular fractions but there was no significant difference when it was compared to extraction for 1 h at the same pH. To avoid spending too much time in the extraction procedure, a contact time of 1 h was chosen.

Figure 11 shows extraction at various pHs at a 1 h stirring period. By increasing the pH of the activated sludge sample, more biopolymers were released from the sludge flocs. At pH 12 the highest quantity of polymers were released but polymerization of low to high molecular weight compounds, which was undesirable seemed to occur. This is evidenced by a decrease in the lower molecular weight fraction while the higher fraction increased. This polymerization phenomenon was also observed by Ghassemi and Christman (68). They studied a variety of naturally colored water and reported that under highly alkaline conditions, the color molecules appeared much larger, presumably due to polymerization. The comparison of organic materials presented in pH 11 and raw sludge supernatants indicates that very little polymerization had occurred.

In Figure 12, it can be seen that the highest quantity of extracted polymers was obtained at low centrifugal force. However, with a difficult-to-settle sludge, some small flocs

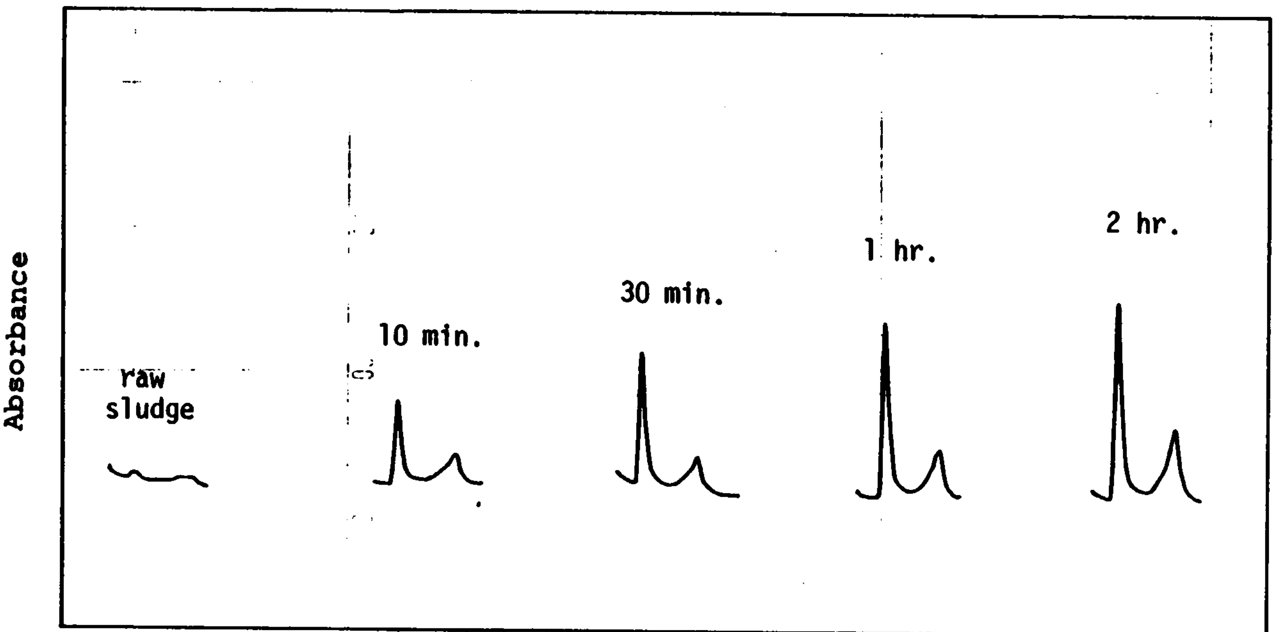

Figure 10. Extraction of biopolymer at pH 11 using different contact times.

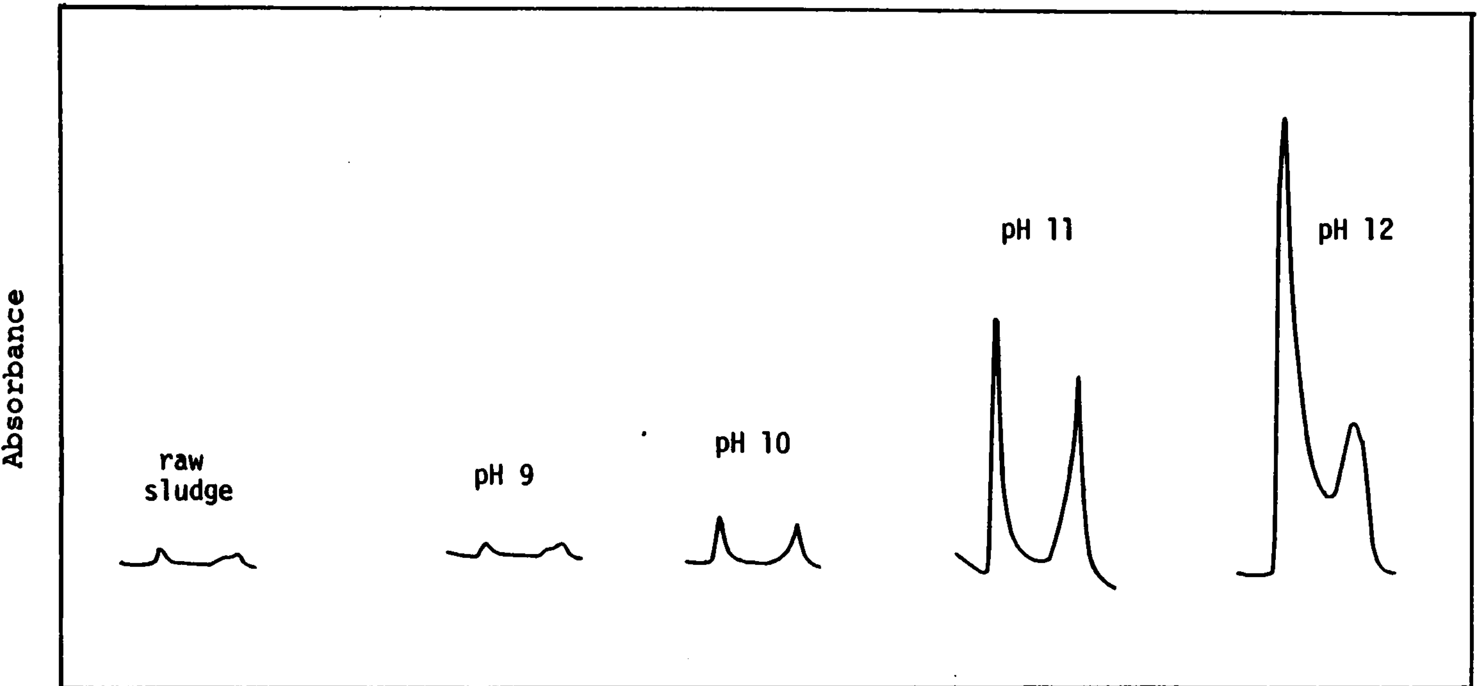

Figure 11. Extraction at different pHs using a one hour contact time.

were still suspended in the supernatant. Therefore, a centrifugal force of 5,000 rpm was chosen because it was sufficient to remove all the bacterial cells and small flocs in the supernatant, and yet provided a high yield of biopolymer.

4.2 COMPOSITION

Chemical analysis of the extracted polymer was performed directly on the processed supernatant. The results show that the extracted polymers contained large amounts of both protein and carbohydrate. At the early stage of this research, protein was quantified by the Lowry method and carbohydrate by the Phenol-Sulfuric Acid Colorimetric method. Later the Lowry method was replaced by a superior protein-dye (Coumassie Brilliant Blue G-250) binding method. Prior to this chemical analysis, the high and low molecular weight fractions were separated from each other using gel filtration. The results of the protein and carbohydrate analyses are summarized in Table 6, 7, and Figure 13.

In Table 6, the total carbohydrate content of the extracted biopolymer was 245 mg/l which was more than seven times the unextracted sample (34 mg/l). In contrast, the protein ratio of the extracted to unextracted samples was only 2.5 (Table 7). This indicates that more polysaccharide

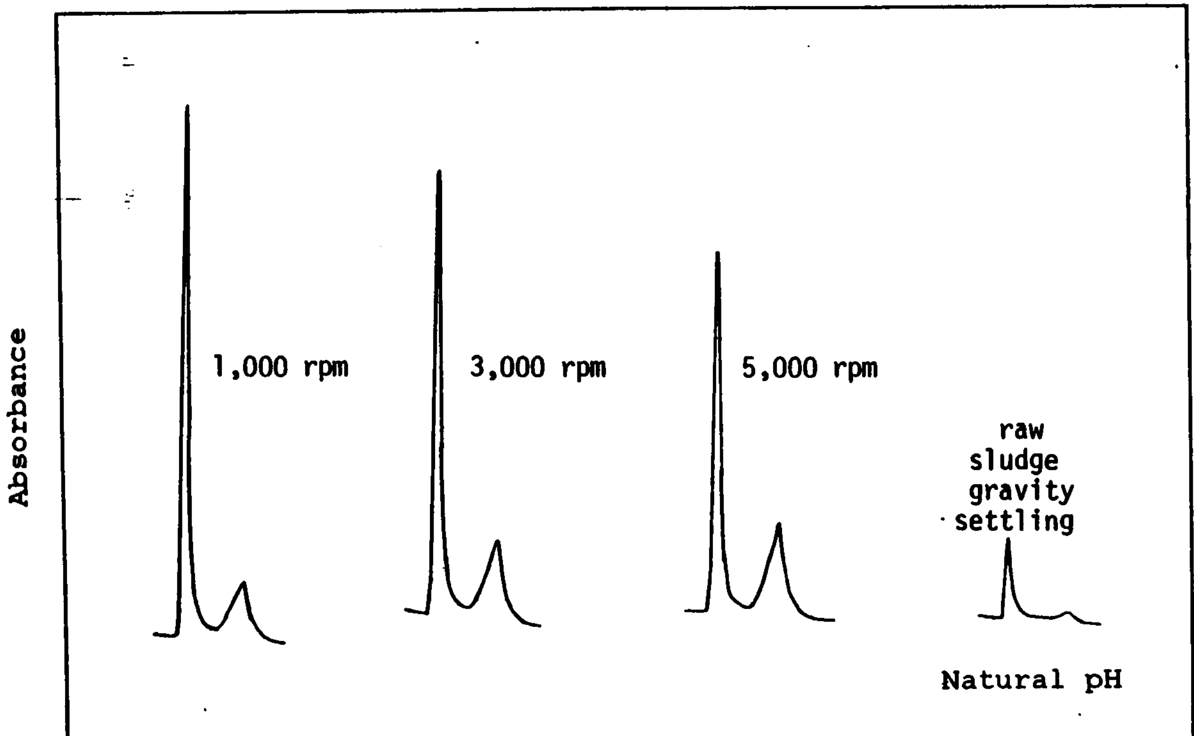

Figure 12. pH 11 extraction with different centrifugal forces.

Table 6

Carbohydrate absorbances and their concentrations

Samples	Absorbance (UV units)	Carbohydrate Conc., mg/l
pH 11 (1:1)	0.450	245 (total)
raw sludge (1:1)	0.062	34 (total)
<u>elution volume (ml)</u>		
<u>pH 11</u>		
0 - 5	0.013	4
5 - 10	0.100	27
10 - 15	0.145	40
15 - 20	0.047	13
20 - 25	0.040	11
25 - 30	0.100	27
30 - 35	0.082	22
35 - 40	0.030	8
40 - 45	0.010	3
<u>raw sludge</u>		
0 - 5	0.019	5
5 - 10	0.000	0
10 - 15	0.020	6
15 - 20	0.005	1
20 - 25	0.002	0.5
25 - 30	0.025	7
30 - 35	0.020	6
35 - 40	0.005	1

Table 7

Protein absorbances and their concentrations

Samples	Absorbance (UV units)	Protein Conc., mg/l
pH 11 (1:1)	0.800	86 (total)
raw sludge (1:1)	0.325	35 (total)
<u>elution volume (ml)</u>		
<u>pH 11</u>		
0 - 5	1.700	92
5 - 10	0.700	38
10 - 15	0.750	41
15 - 20	0.250	14
20 - 25	0.115	6
25 - 30	0.040	2
30 - 35	0.050	3
35 - 40	0.045	2
<u>raw sludge</u>		
0 - 5	0.048	3
5 - 10	0.000	0
10 - 15	0.190	10
15 - 20	0.100	5
20 - 25	0.000	0
25 - 30	0.050	3
30 - 35	0.000	0
35 - 40	0.000	0

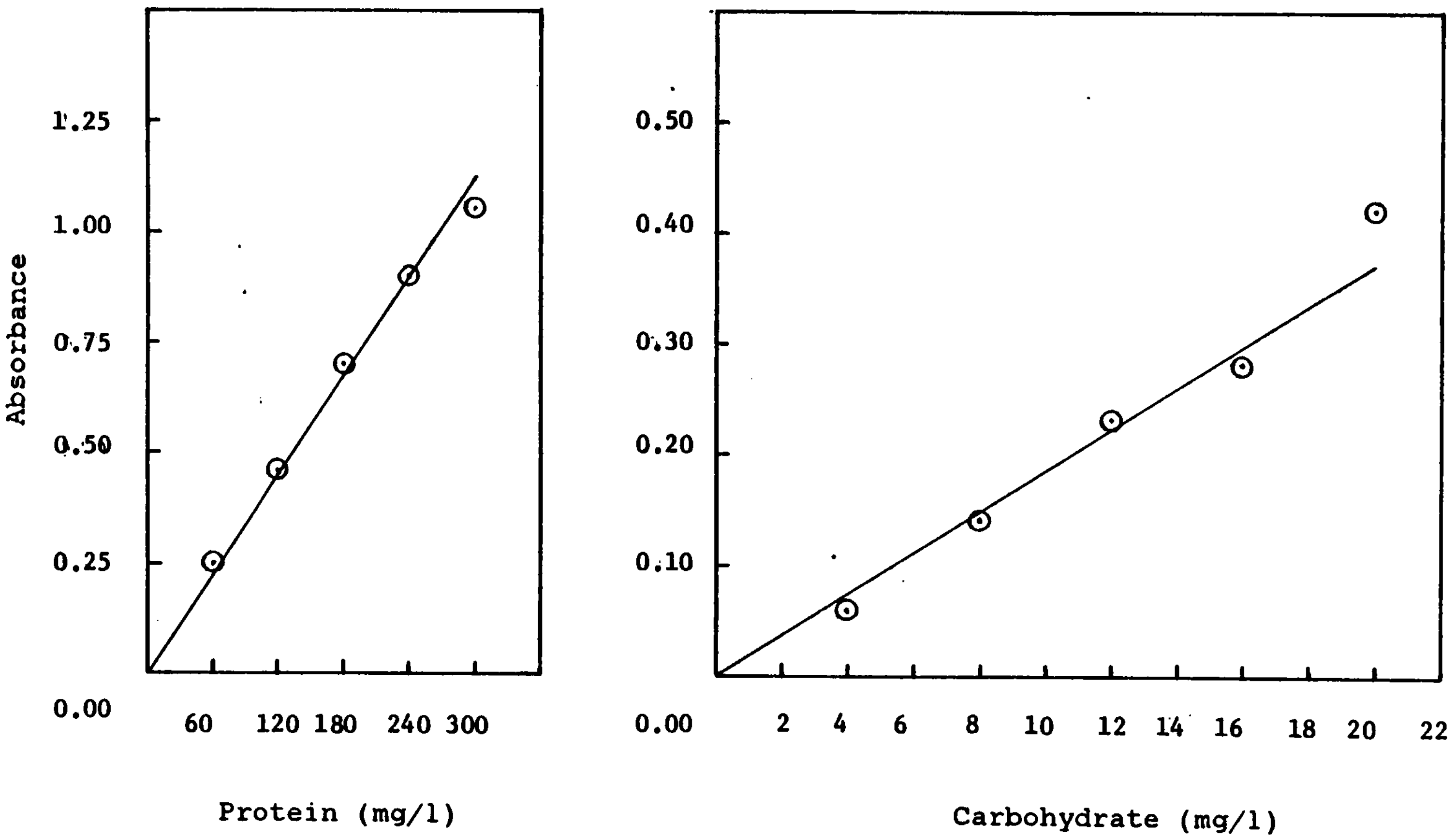

Figure 13. Protein and carbohydrate standard curves.

than protein was extracted. This conclusion is consistent with the finding of Carr and Ganczarczyk (31).

The protein and carbohydrate of separated biopolymers obtained from a fractional collector in Tables 6 and 7 are plotted against elution volume and are shown in Figures 14 and 15. The similarity is clearly seen when comparing the gel filtration chromatogram in Figure 7 with Figure 14 and 15. The data show that high protein concentrations are associated with the high quantities of biopolymer. The carbohydrate data suggest that the extracted sample had a low amount of low molecular weight biopolymers.

Therefore the determination of changes in protein and carbohydrate contents of extracted biopolymers from an activated sludge sample under various conditions may be useful in the study of flocculation and dewatering characteristics of that sludge.

All the above results were obtained by performing analyses on the activated sludge wasted from a simulated batch reactor fed with bacto-peptone with a 5 days sludge age (Table A1).

4.3 ADSORPTION TEST

As mentioned earlier it appears that bacterial extracellular polymers may play an important role in activated sludge bioflocculation. One of the most important physical characteristics that may determine why the activated

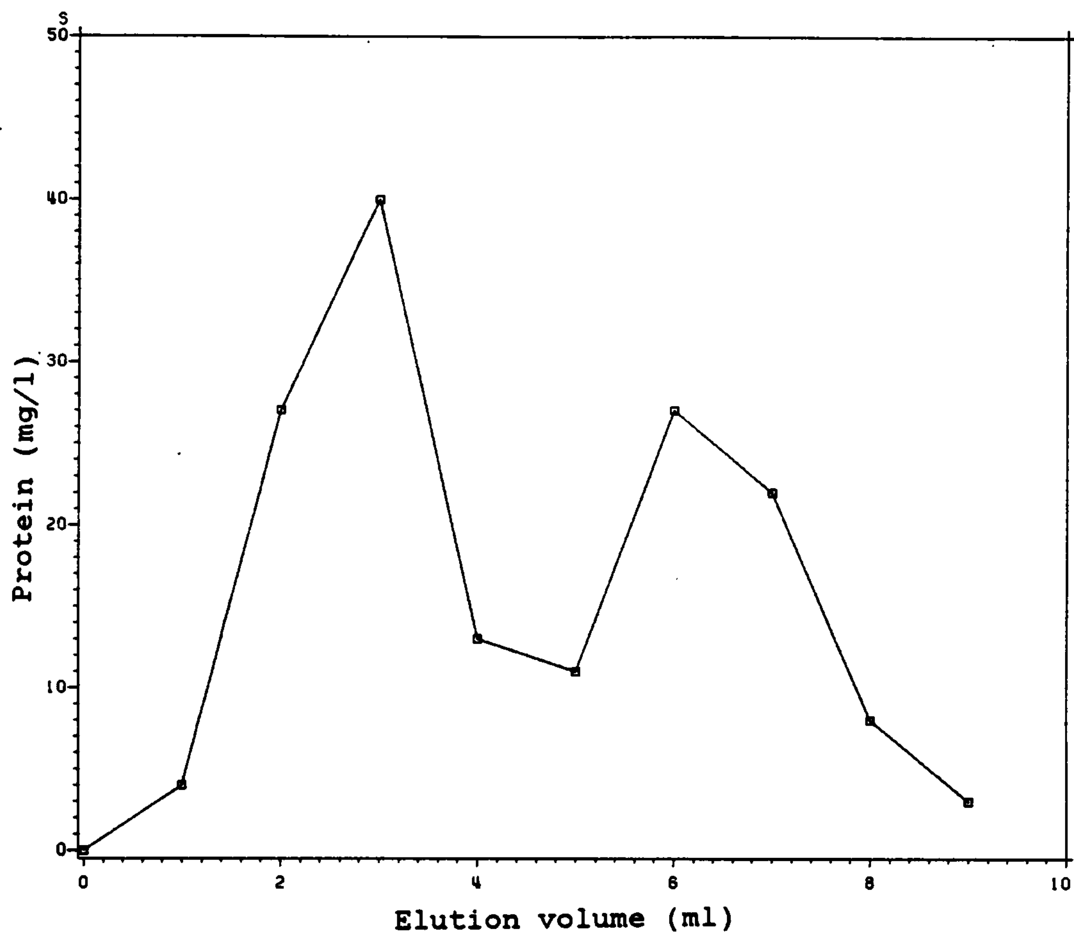

Figure 14. Protein content of biopolymer in the sample eluant.

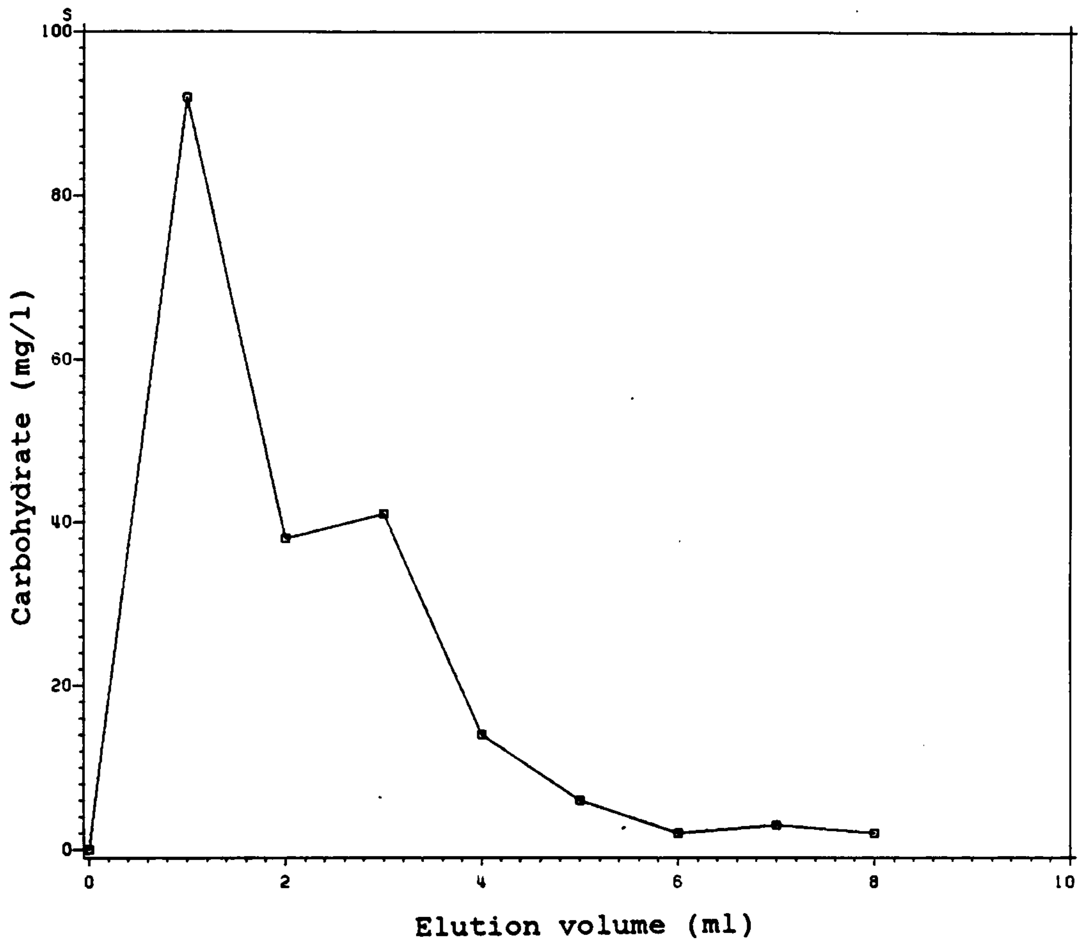

Figure 15. Carbohydrate content of biopolymer in the sample eluant.

sludge flocs are loose (poorly adsorbed) or dense (tightly adsorbed) is adsorption. Therefore, adsorption properties of BEP were studied.

Figure 16 shows gel filtration data following contact with activated carbon at various pH values. A carbon solution of 20 mg/l, when mixed with the extracted biopolymer solution at a volume ratio of one to one had different degrees of adsorptions when the pH of the solution was varied. Figure 16 (a) shows that raw sludge supernatant contained a small amount of biopolymer; however, adsorption of the high molecular weight fraction (greater than 5,000 based on Sephadex G-75 exclusion) was obvious. At pH 11, the pH of extraction, adsorption of the high molecular weight fraction of biopolymers was significant. The data indicate adsorption decreased as the pH was reduced to pH 4; however, adsorption on carbon increased again at pH 3 (Figure 16 (d)).

The chromatogram peaks of the high molecular weight fraction observed in Figure 16 were measured and plotted. The peak heights before and after adsorption are shown in Figure 17 and the degree of adsorption is presented in Figure 18.

Figure 18 clearly illustrates that high adsorption occurred at pH 11 and pH 3. The high degree of adsorption of BEP on carbon particles observed at pH 11 is unexpected. It was expected that very low adsorption would occur at pH 11 because the method of biopolymer extraction relys on

desorption at pH 11. At pH 4 and 6 no adsorption was observed. At pH 3, it was assumed from literature reports that the BEP approached a point of zero charge and charge neutralization of negatively charged biopolymers followed by bridging might be responsible for high adsorption. Also note that the high molecular weight fraction was more strongly adsorbed because their polymeric chains were longer.

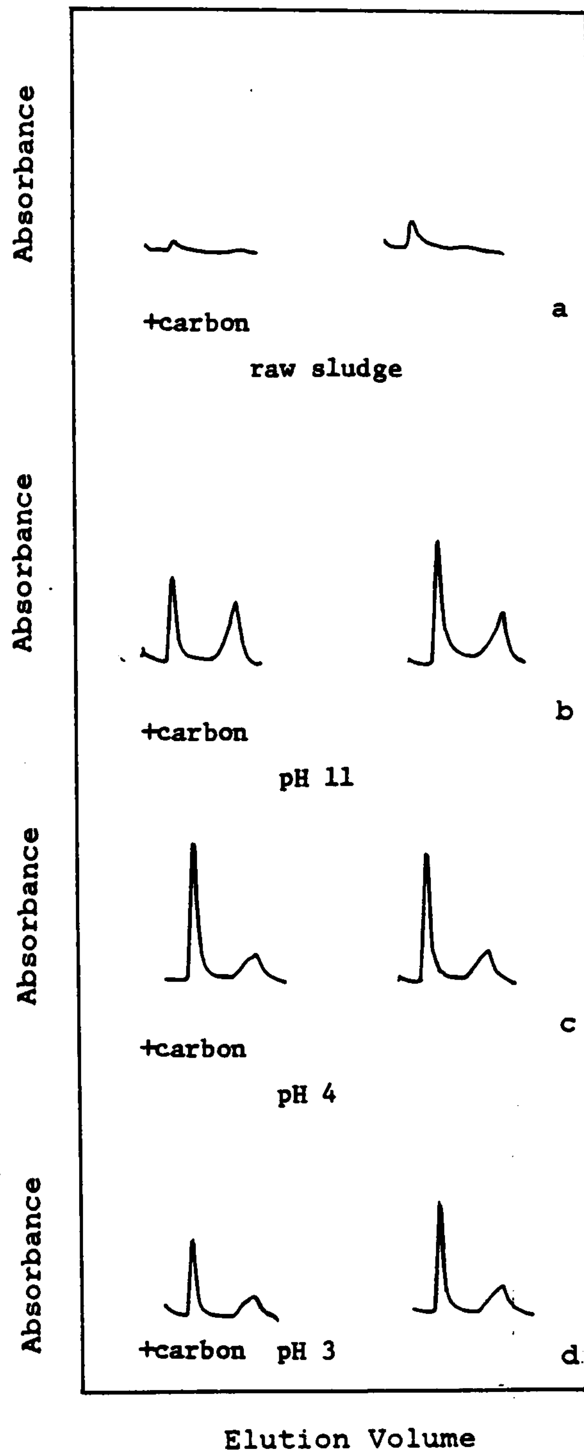

Figure 16. Measurement of adsorption of biopolymers on activated carbon at different pHs as indicated by gel chromatography.

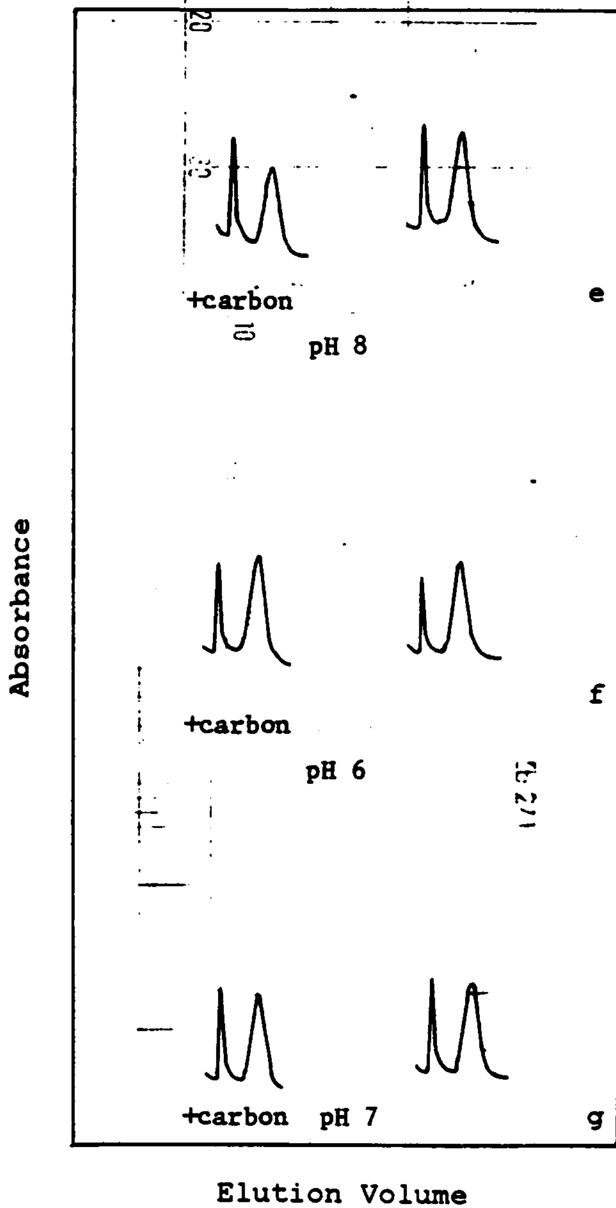

Figure 16 (continued)

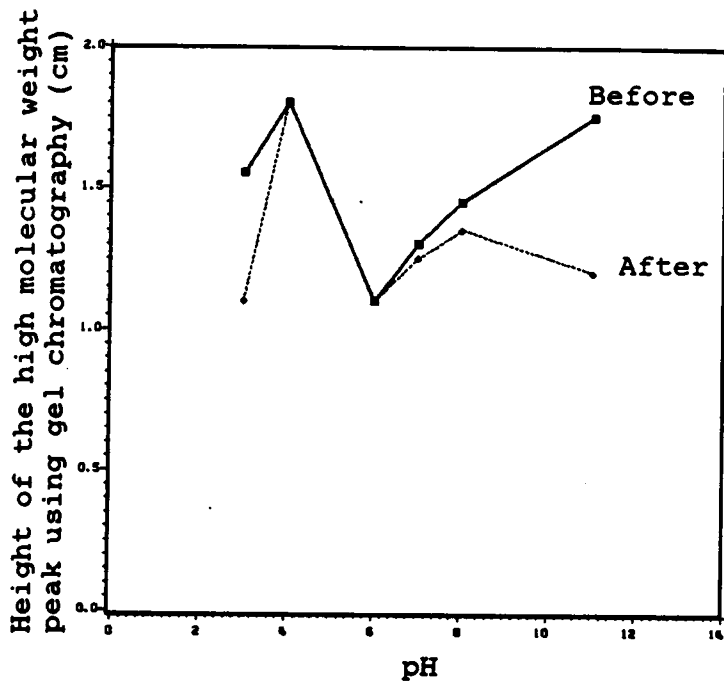

Figure 17. Peak heights of biopolymers remaining in solution before and after adsorption by activated carbon.

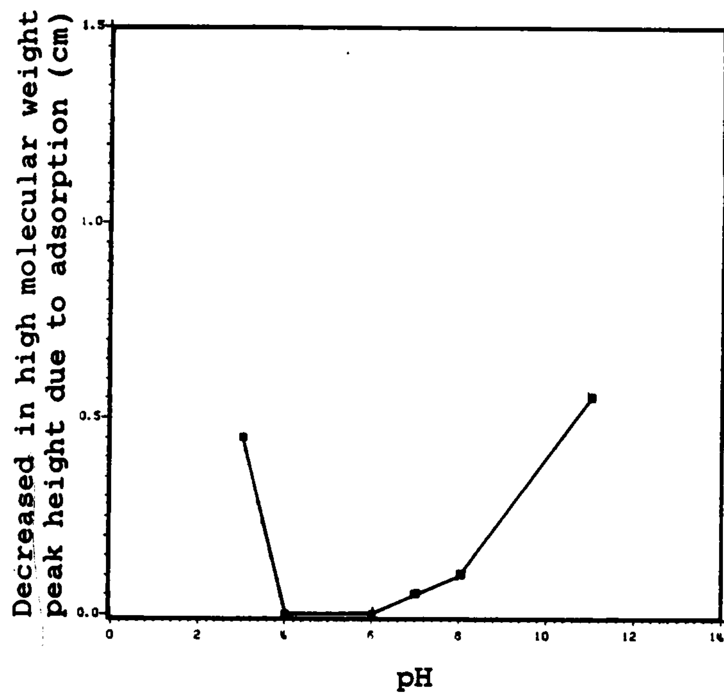

Figure 18. Removal of high molecular weight organics at different pHs by activated carbon.

4.4 CONTINUOUS FLOW REACTOR STUDIES

To investigate the role of bacterial extracellular polymers and their chemical characteristics in sludge settling and dewatering, continuous flow reactors were operated. These completely mixed, continuous reactors were operated to observe changes in sludge properties and biopolymer characteristics which might help to understand the nature of the flocculation and dewatering properties of activated sludge.

The operational conditions for each period for the reactor studies are presented in Table A1 through A8 (Appendix A). The data obtained during those periods were total suspended solids, sludge volume index, sludge specific resistances, and protein and carbohydrate contents of biopolymers in the extracted and the gravity settled supernatants. The relationship between dewaterability of sample activated sludge and the physical characteristics of the reactor and the chemical properties of biopolymers are discussed.

4.4.1 PERIOD A

Two reactors were operated during this period. One of the reactors was initially fed with milk and the feed was changed to glucose on the fourth day of the experiment. Bacto-peptone was fed to the other reactor. These substrates

were selected because glucose is known to produce bulking sludge while bacto-peptone generally produces sludge with good settling properties. By selecting those substrates, a major goal of this study which was to determine the relationship between BEP and sludge settling characteristics could be attained. The reason glucose was substituted for milk in one of the reactors was because milk caused such a poorly flocculated sludge that a significant loss of solids from the reactor occurred, making operation impossible.

Tables C1 through C3 in Appendix C summarize the data obtained during this period for each reactor. The data from those tables were plotted and are shown in Figures 19 and 20.

It appears that glucose fed to the reactor caused a deterioration in sludge properties. The deflocculation of the sludge is indicated by SVI values as high as 550 ml/g (Figure 19 (b)). This deflocculation caused an increase in sludge specific resistance (Figure 19 (c)) and a loss of solids from the reactor (Figure 19 (a)). As solids were lost, the SVI declined presumably due to the small amount of solids present and the absence of biopolymer for the formation of bridges, leaving only discrete particles (bacteria) for settling.

The carbohydrate and protein data obtained during this period may be subject to some interferences. The high protein content detected in the biopolymers of sludge from a bacto-peptone fed reactor may be the result of a protein

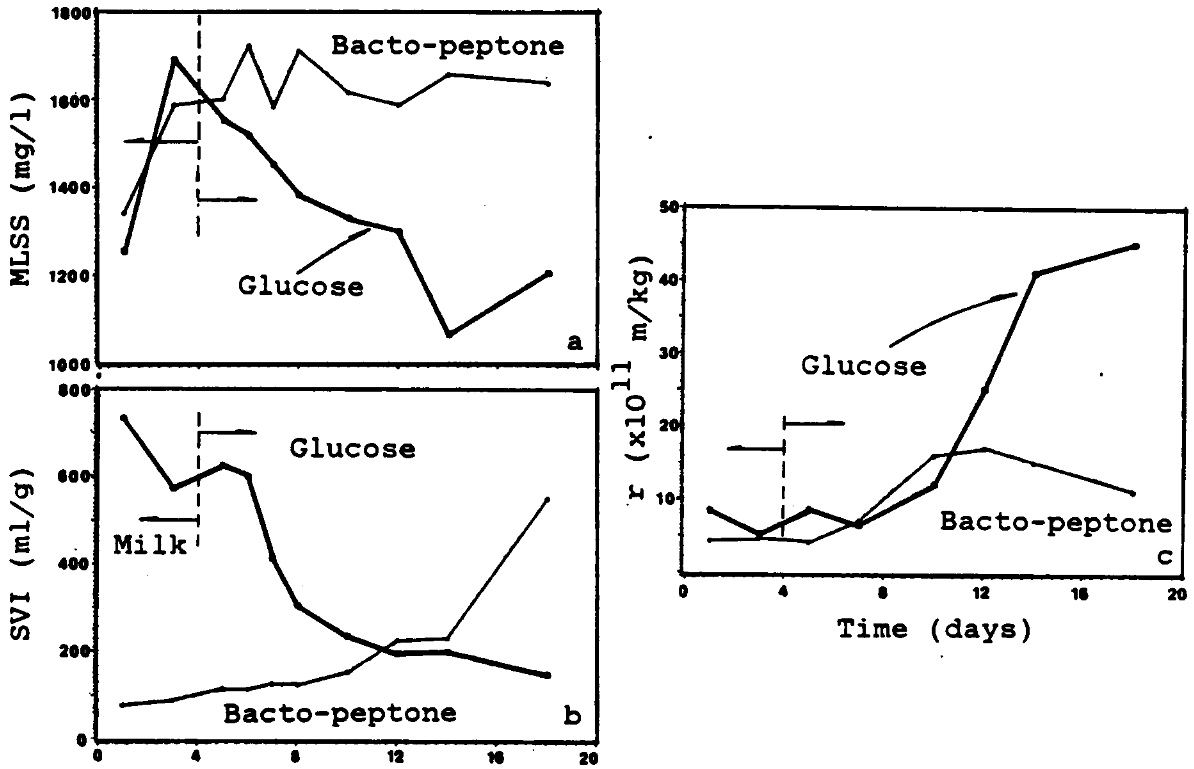

Figure 19. Reactor study data, period A: Physical characteristics of sludge.

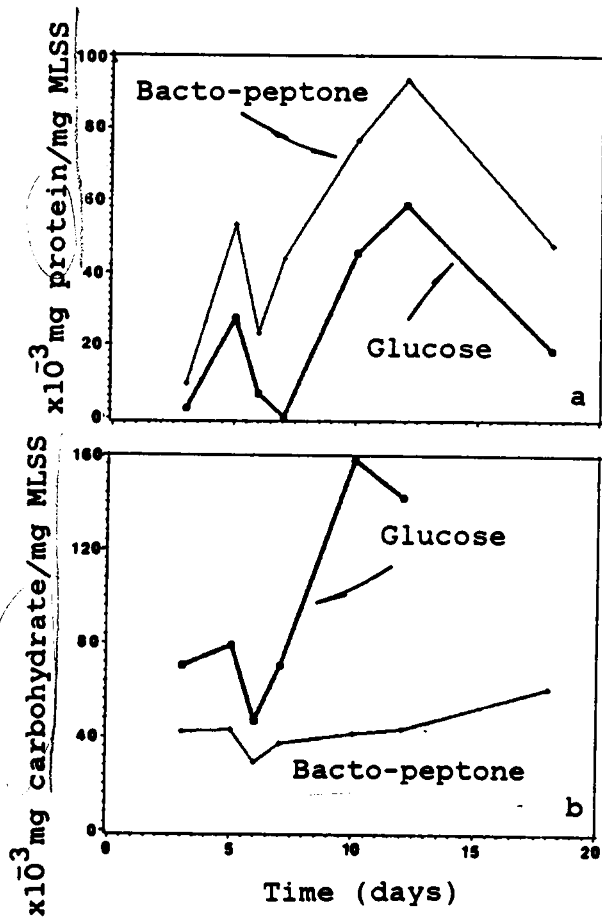

Figure 20. Reactor study data, period A: Chemical characteristics of BEP.

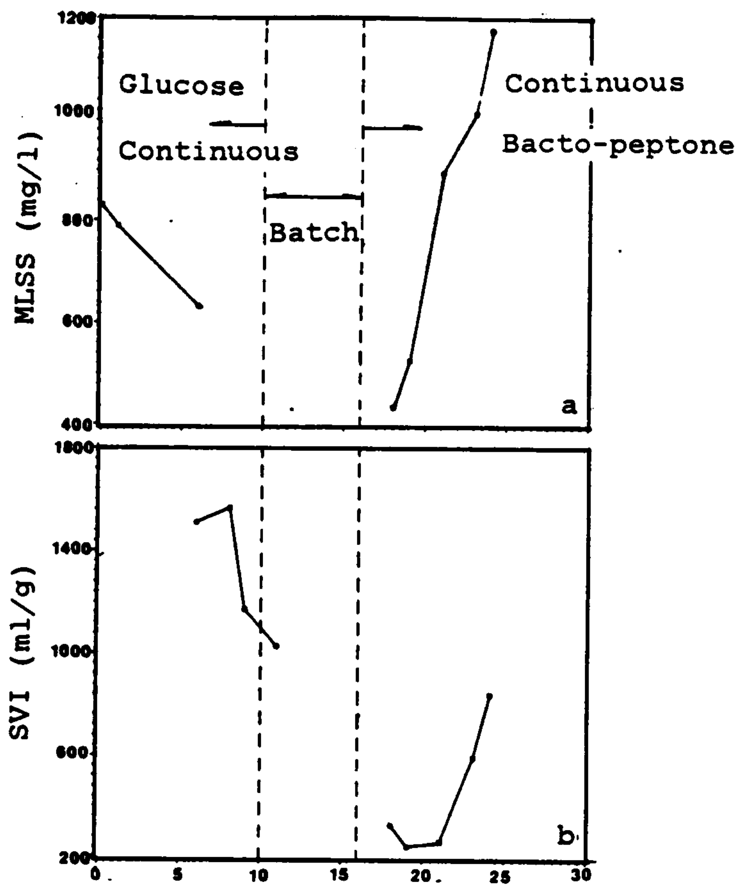

Figure 21. Reactor study data, period B: Physical characteristics of sludge.

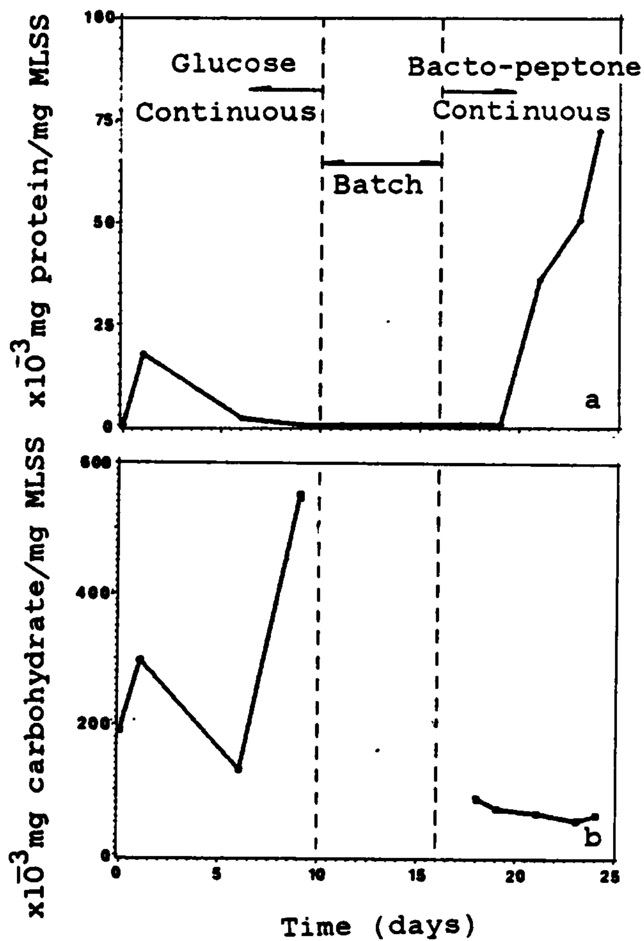

Figure 22. Reactor study data, period B: Chemical characteristics of BEP.

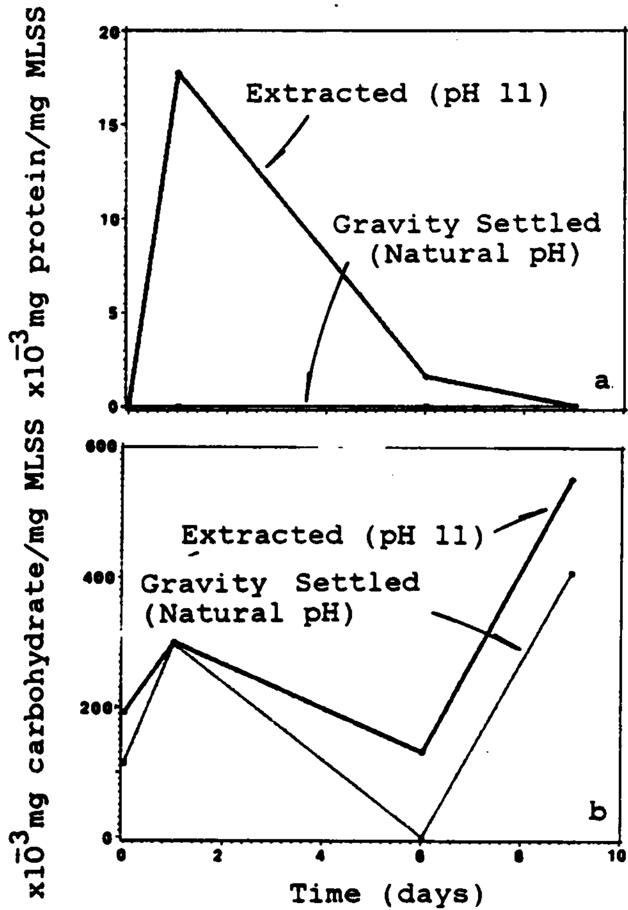

Figure 23. Reactor study data, period B: Carbohydrate and protein of biopolymer obtained from extracted and gravity settled sludge samples.

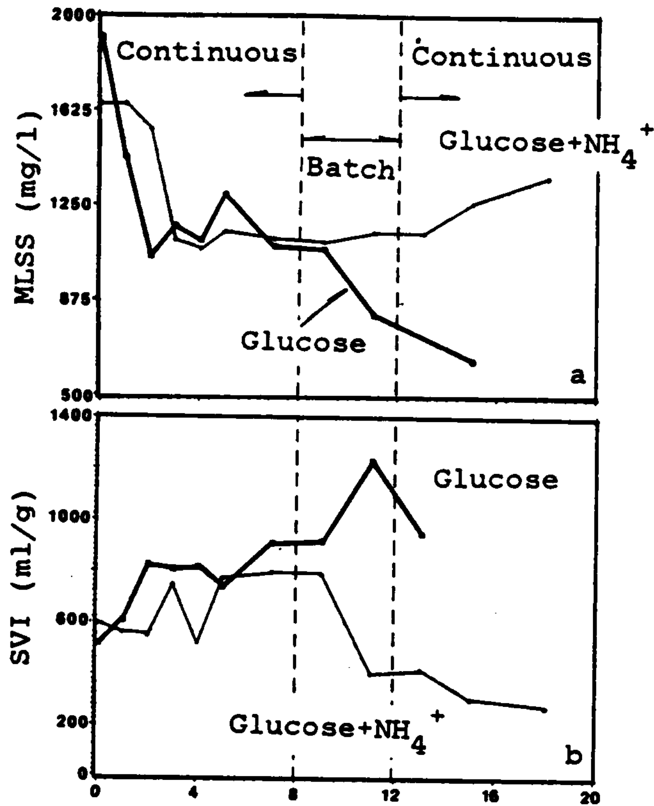

Figure 24. Reactor study data, period C: Physical characteristics of sludge.

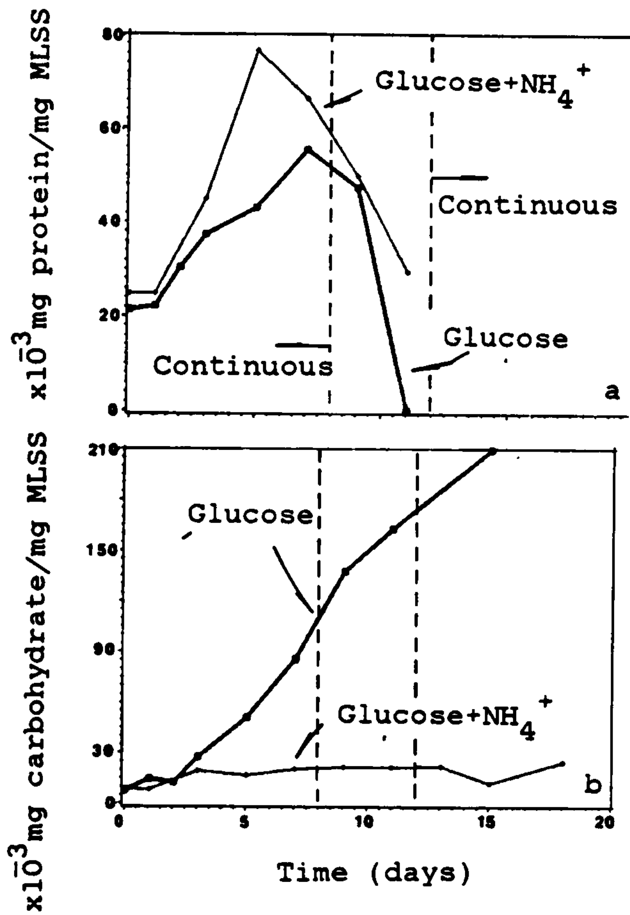

Figure 25. Reactor study data, period C: Chemical characteristics of BEP.

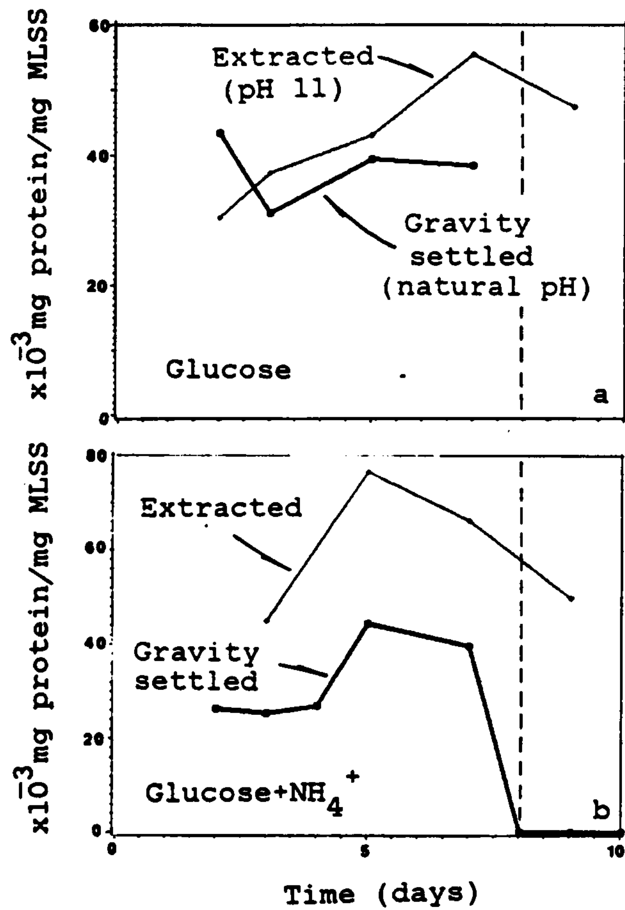

Figure 26. Reactor study data, period C: Protein of biopolymer obtained from exracted and gravity settled sludge samples.

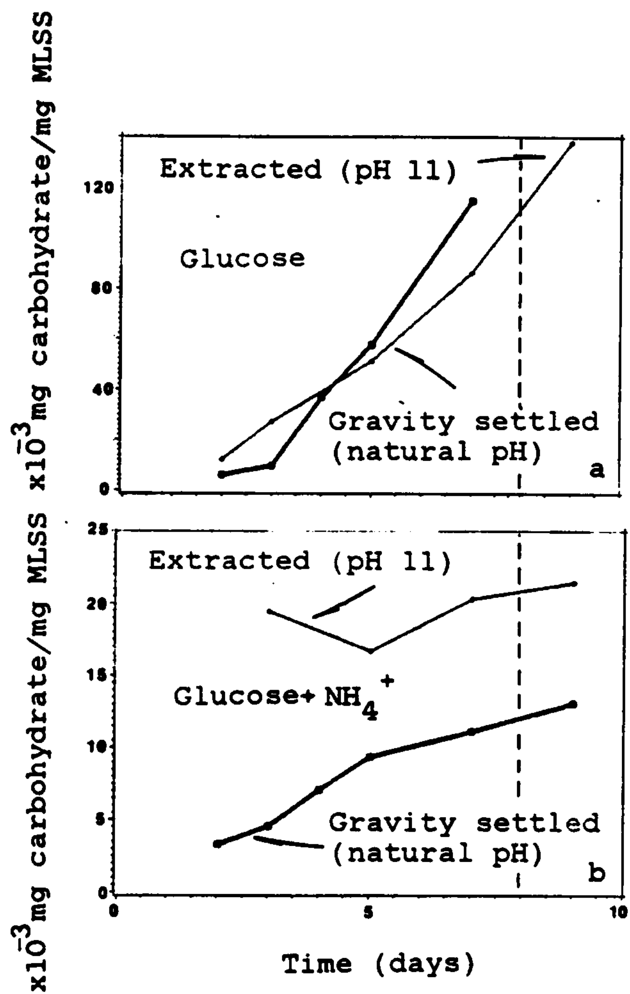

Figure 27. Reactor study data, period C: Carbohydrate of biopolymer obtained from exracted and gravity settled sludge samples.

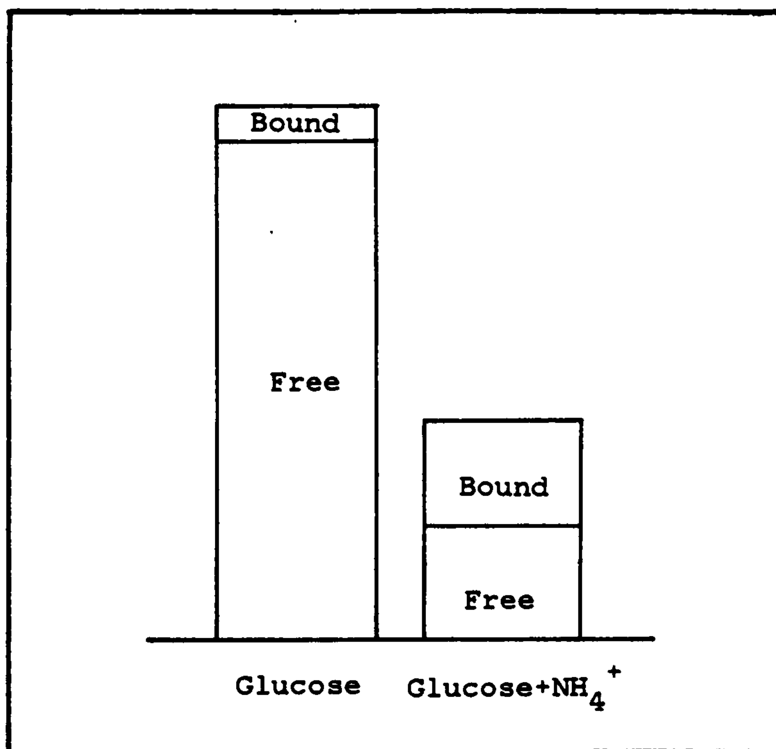

Figure 28. Bound and free carbohydrate biopolymers: Relative quantity of bound and free carbohydrate biopolymers for reactor studies in period C.

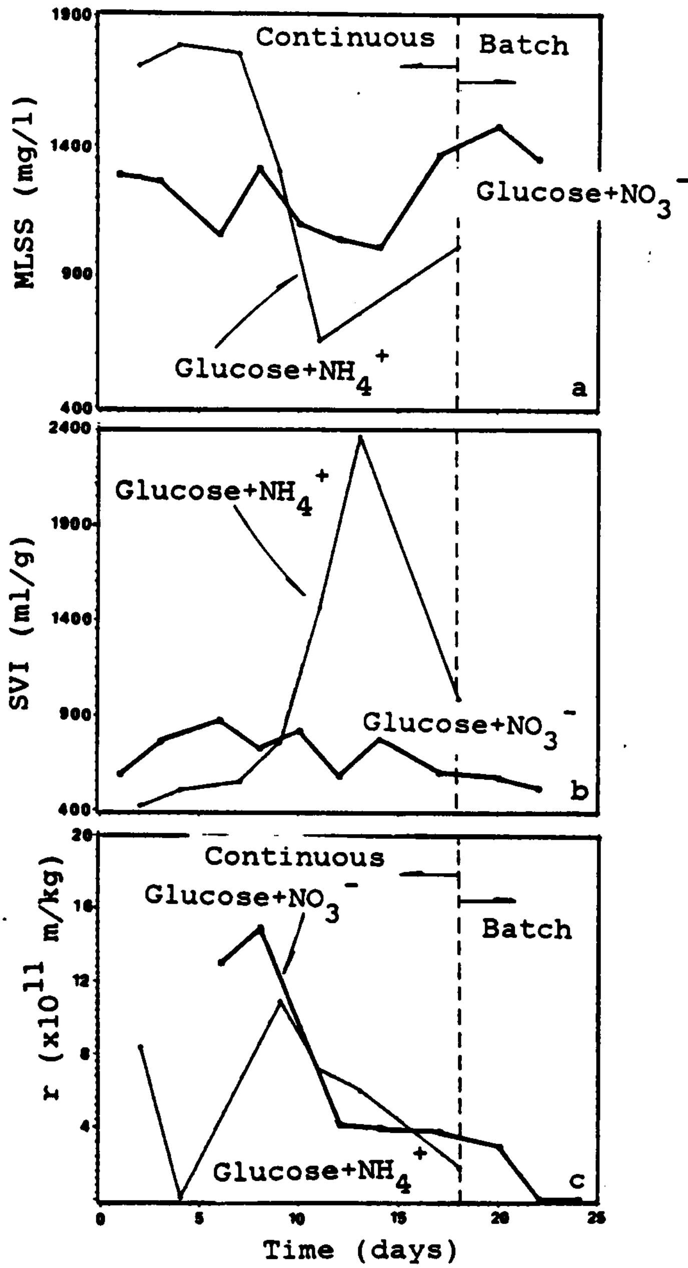

Figure 29. Reactor study data, period D: Physical characteristics of sludge.

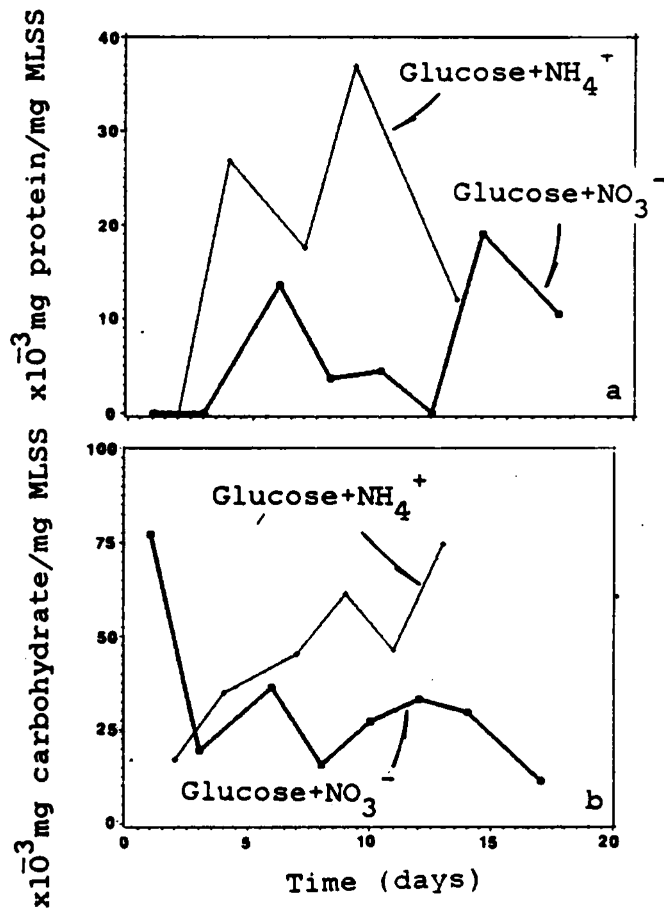

Figure 30. Reactor study data, period D: Chemical characteristics of BEP.

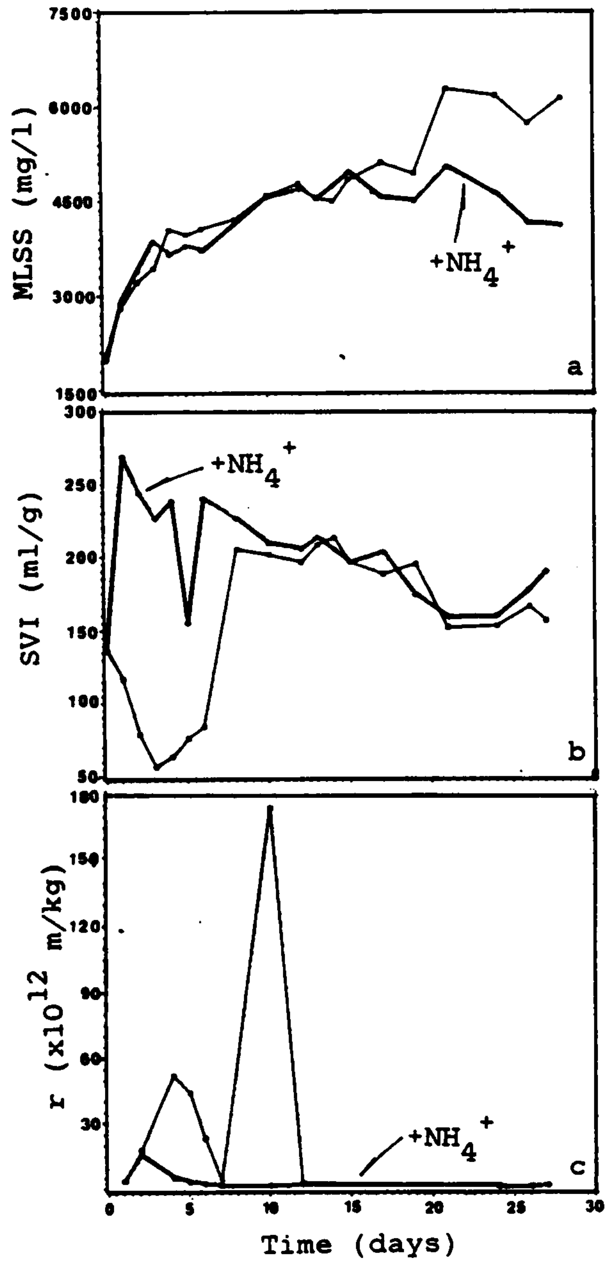

Figure 31. Reactor study data, period E: Physical characteristics of sludge.

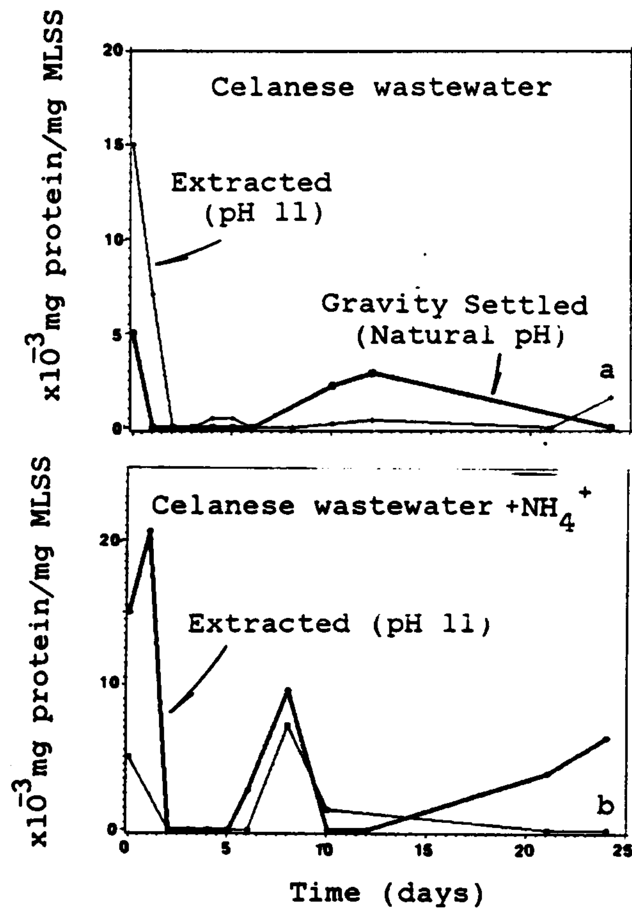

Figure 32. Reactor study data, period E: Protein of biopolymer from extracted and gravity settled supernatants.

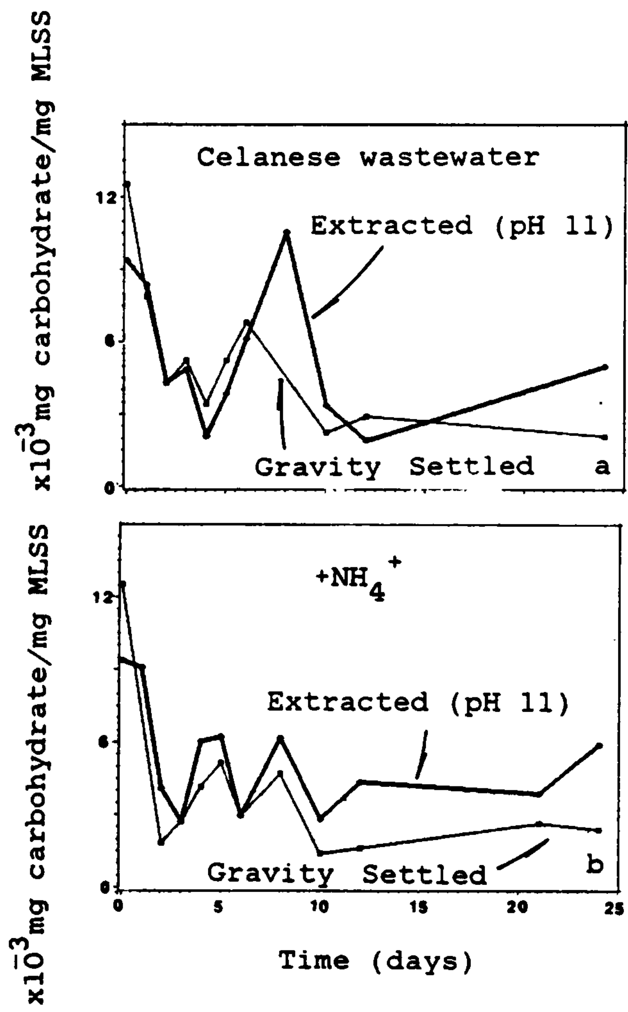

Figure 33. Reactor study data, period E: Carbohydrate of biopolymer from extracted and gravity settled supernatants.

interference from undegraded bacto-peptone. However, the effect of undegraded bacto-peptone is expected to be slight because bacto-peptone is readily degradable.

The high carbohydrate content detected in the glucose fed reactor may have also been due in part to interference by glucose in the sample. However, it is more likely that glucose caused the activated sludge to produce more extracellular polysaccharides. Figure 20 (a) and (b) also suggest that low protein to carbohydrate ratio led to poor dewatering. This occurred to the sludge fed with glucose. With bacto-peptone, this ratio was higher and the sludge dewatered well. Therefore it seems that the ratio of protein to carbohydrate and the binding properties of BEP to sludge flocs might be an important factor in determining the sludge dewaterability.

Several important observations resulted from operation of the continuous flow reactors during this period. Those observations are listed below.

1. Glucose as a feed substrate has a deleterious effect on sludge dewatering characteristics.

2. The presence of extracellular polysaccharides appears to influence the sludge dewatering rate.

3. The loss of solids from the glucose fed reactor but not the bacto-peptone fed reactor suggests a different binding capacity.

4.4.2 PERIOD B

The data collected during period A was instructive but did not lead to a clear picture of the relationship between the BEP and sludge physical properties. Therefore another reactor was operated during period B. Glucose was the main organic nutrient supplied to the system. However, on the tenth day of operation the system was changed to batch operation because the MLSS was low. Fitzgerald (68) successfully used this technique to reclaim solids and to improve sludge settling properties. The solids in the system accumulated to an acceptable level on the sixteenth day so continuous feeding was resumed and bacto-peptone was substituted for glucose. This switch in substrate was carried out in order to further study the role of BEP in flocculation under the high-nitrogen (bacto-peptone fed) and low-nitrogen (glucose fed) environments. Tables C4 through C8 contain the data obtained during this period and they are summarized in Figures 21, 22 and 23.

Figure 21 (b) clearly indicates an improvement in SVI when the reactor was operated under batch conditions. A significant increase in protein was detected in the supernatant liquor (Figure 22 (a)) beginning after 18 days of operation and appeared to be a direct result of switching the substrate from glucose to bacto-peptone. After this substitution was made, the extractable carbohydrate

concentration stopped decreasing (Figure 22 (b) and the SVI increased. The accumulation of solids after the beginning of bacto-peptone feed suggests that protein in the biopolymer is essential for proper floc formation.

Figure 23 (a) and (b) show a difference in settled and extracted biopolymers. Those data indicate that most protein was not present in the supernatant but was stripped from the floc surface (by raising pH to 11). With regard to carbohydrate, large portions were associated with the supernatant fraction. This suggests that pH 11 extraction either may extract more protein than carbohydrate, presumably because carbohydrate is poorly bound and not associated tightly with the floc, or pH 11 extraction is more specificed for protein.

Although all measurements were not conducted over the full operational period, two observations were made with regard to the sludge properties. First, as solids were lost, presumably because of deflocculation of the biofloc, the protein content was declining. Recovery of flocculation as evidenced by an increase in MLSS appeared to correspond to an increase in protein. Second, during the period when biomass was lost, the carbohydrate biopolymer appeared to be "free" or unbound as evidenced by the large supernatant biopolymer fraction. It should be mentioned that the carbohydrate in the supernatant liquor could still be considered biopolymer because the molecular weight was in the

range of 2,000-5,000 as estimated by gel filtration. This value is much greater than the molecular weight of glucose.

4.4.3 PERIOD C

After reviewing the data obtained during period B it appeared that protein biopolymer is not only critical to a proper floc formation but also is an important factor that determines the sludge flocculation properties. In order to clarify these assumption, another set of experiments was conducted.

Two reactors were operated side by side during this period. One of the reactors was fed with glucose and the other one with glucose and NH_4^+ as nitrogen suppliment. During the operation a problem of solids loss from both reactors (Figure 24 (a)) was solved by changing the systems to batch feed on the 8th day of operation. After 5 days of batch operation, continuous feed was resumed. The data obtained during this period are summarized in Table C9 through C13 and shown in Figures 24, 25, 26, and 27.

Figure 25 shows that sludge fed with glucose, again, produced high carbohydrate biopolymers and the results were poor settling and loss of solids. However, the presence of ammonia-nitrogen in the substrate seems to change those characteristics. The analysis of the sludge fed with glucose + NH_4^+-N showed an improvement of SVI and an increase in

MLSS. When the carbohydrate concentrations of biopolymers in gravity settled and extracted samples are compared (Figures 27 (a) and (b)), it was observed that more polysaccharide was extracted from sludge fed with glucose + NH_4^+ than the one without a nitrogen suppliment. The carbohydrate data (Table C11 and C13) in Figure 27 (c) and (b) can be plotted by a bar chart and is shown in Figure 24 using as a definition of bound biopolymer;

Bound biopolymer concentration	=	pH 11 Extracted biopolymer concentration	-	Gravity settled biopolymer concentration

Figure 28 shows that the sludge without ammonia-nitrogen produced a significant amount of carbohydrate biopolymer but almost all of it was not attached to the floc surface. However, when the sludge was supplied with ammonia-nitrogen, a lower amount of extractable carbohydrate biopolymer was present and about one-half was bound. This suggests that without ammonia-nitrogen, the glucose was used to produce carbohydrate biopolymer, most of which was contained in the supernatant liquor phase. These poorly bound biopolymers were responsibled for the poor sludge settling indicated in Figure 24 (b). Moreover, when the bound fraction of carbohydrate biopolymer was significant, good settling was observed.

Extracellular protein also has a pronounced effect on the settling characteristic of the activated sludge. Figure 26 (a) and (b) clearly suggest that the sludge fed with glucose + NH_4^+ had more extracellular protein bound to its floc than the sludge fed with pure glucose. This apparently led to good floc formation and consequently good settling. Therefore it is expected that the more bound protein present in the activated sludge, the better that sludge will settle.

It can be summarized that glucose fed sludge had only small quantities of bound protein and carbohydrate biopolymers. A large portion of those biopolymers were bound when ammonia-nitrogen was available to the sludge. These bound biopolymers appear to play an important role in improving sludge settling by producing better floc formation through improved binding.

4.4.4 PERIOD D

The purpose of study for this period was to compare different forms of nitrogen feed, ammonia-N and nitrate-N on the flocculation and dewatering of activated sludge and their influence on biopolymer production.

Two reactors were operated during this period, one was supplied with glucose + NH_4^+-N and the other one with glucose + NO_3^--N. The problem of solids loss during the operation was also encountered during this period and it was more

severe for the reactor with ammonia-nitrogen (Figure 29 (a)). After eighteen days of operation, both reactors were changed from continuous to batch feed until the end of the period. Table C14 through C16 summarize the data obtained during this period. Those data were plotted and are shown in Figures 29 and 30.

Figure 29 (b) and (c) indicate that sludge from both reactors settled and dewatered similarly. The improvement of specific resistance of sludge from both corresponded to an increase in MLSS, indicating flocculation had improved. when total carbohydrate concentration decreased.

Figure 30 (a) and (b) indicate that activated sludge fed with glucose + NH_4^+-N produced more overall biopolymers than the other one and the result was a slightly better sludge dewaterability. However, a sharp decrease in total protein concentration occurred on the 13th day of operation was observed and the result was an extremely high SVI, which indicates that deflocculation had occurred.

It is suggested that more experimental research will have to be conducted to derive any conclusions about the effect of different forms of nitrogen (ammonia and nitrate) on sludge flocculation and dewatering characteristics.

4.4.5 PERIOD E

The data collected during period D reflected wide variations in sludge and BEP properties when the reactors were started up with a sludge from the Celanese Plant. It is known that Celanese sludge often settles poorly and is badly deflocculated. There is also a low nitrogen level in the wastewater. Therefore, it is logical to investigate the effect of nitrogen on sludge flocculation and dewatering. During this period two reactors were operated. Both of them were fed with Celanese raw wastewater but ammonia-N was added to one of the reactor. The data monitored during this period are summarized in Tables C17 through C 21 and Figures 31, 32, and 33.

The results clearly reveal the relationship between extracellular protein and carbohydrate and the sludge specific resistance. The analysis of a sludge from the reactor fed with ammonia-nitrogen shows that when the bound extracellular protein was low, the sludge dewatered poorly. This is shown in Figure 32 (a) and 31(c) on the fourth and tenth days of operation. This relationship was also observed in the other reactor during the first two days of operation.

The relationship between the extracellular polysaccharide and sludge specific resistance was also observed in both reactors. The addition of ammonia-nitrogen seems to improve the sludge dewatering rate (Figure 31 (c)). The improvement

in sludge dewaterability occurred when a significant amount of carbohydrate biopolymer was bound (Figure 33 (b)). Those biopolymers apppear to bind bacterial colloids together to form well-flocculated flocs which dewatered easily.

The sludge from the reactor fed with Celanese raw wastewater without ammonia-nitrogen did not possess a significant amount of bound carbohydrate biopolymer. Nevertheless when a relatively high quantity of bound fraction was detected, the sludge specific resistance was low. This is clearly shown in Figure 31 (c) and Figure 33 (a) on the eighth day and after ten days of running the reactor.

Figures 31 (b), 32, and 33 show that the improvement of SVI in both reactors was the result of an increase in bound extracellular protein and carbohydrate. This finding is consistent with the previous experiments.

One of the major findings during this period of study was the relationship between protein and bound carbohydrate biopolymers. A high fraction of bound carbohydrate was associated with the presence of protein biopolymer. This result was detected in the sludge with NH_4^+ suppliment. It is assumed that nitrogen is the major factor that improves the sludge properties through the production of protein biopolymer.

V. DISCUSSION

The results of this research include extraction method, adsorption test, and continuous flow reactor studies. To evaluate methods for biopolymer analysis and establish relationships to sludge properties, the results were carefully analyzed.

5.1 EXTRACTION METHOD

It has been shown in the Results section that raising the pH to 11 is the most appropriate method for the extraction of bacterial extracellular polymer from the activated sludge. The method includes adjustment to pH 11, 1 h stirring, and centrifugation at 5,000 rpm for 10 min. This method is convenient for any laboratory studies. The method appeared to extract more protein than carbohydrate during period B. However, during period D and E the reverse was observed. The results suggest that pH 11 extraction is able to extract both protein and carbohydrate biopolymers from the floc surface.

The pH 11 extraction method has also been proved to cause minor cellular disruption (see Chapter IV). DNA concentrations in the extracted supernatant were used as a measure of the degree of the disruption. When this extraction method is combined with gel filtration

chromatography, it appears that the extracted biopolymer of different sludge samples can be compared and the binding of activated sludge floc can be estimated.

5.2 ADSORPTION TEST

The variation of adsorption of the BEP with pH was studied and as mentioned previously, maximum adsorption occurred at pH 3 and pH 11. The high adsorption detected at pH 11 was unexpected and may have been due to filtration by the glass wool at the gel surface or localized pH adjustment. At some point, the glass wool layer could have become loose, permitting particles to get through and cause an uneven flow of eluant or clogging of the voids between the gel beads.

The purpose of the adsorption experiment was to attempt to evaluate biopolymer binding properties. An expected result would have been as shown in Figure 34 (69).

Instead, the actual data varied from the expected pattern above pH 6. This result is believed to be an artifact of the procedure used, and several approaches could be used to better evaluate it. Comparison of a variety of sludges, better control over eluent pH, and control over eluent salt content may all improve the response. However, since the bound polymer estimation using the difference between pH 11 extracted and supernatant levels appeared to give reasonable

results, improvement in the adsorption method was not pursued further in this study.

5.3 CONTINUOUS FLOW REACTOR STUDIES

The continuous flow reactor study results suggested that the binding properties of BEP is an important factor in determining the dewaterability of an activated sludge. The major result of what is considered as good binding properties is proper floc formation. Good flocs are normally associated with low SVI and low sludge specific resistance and retention of biomass in the reactor. It was found that the presence of bound protein and carbohydrate biopolymers usually led to proper floc formation. The significance of those biopolymers are considered in detail as follows:

5.3.1 PROTEIN BIOPOLYMER

It was consistently observed during this investigation that protein biopolymer, especially the bound fraction, is critical to the formation of activated sludge flocs. A substantial production of these biopolymers occurred when nitrogen was available to the bacteria. The nitrogen could be in the form of NH_4^+-N, NO_3^--N, or as organic-N such as is found in bacto-peptone. Those sources of nitrogen are believed to be equally available for incorporation into the

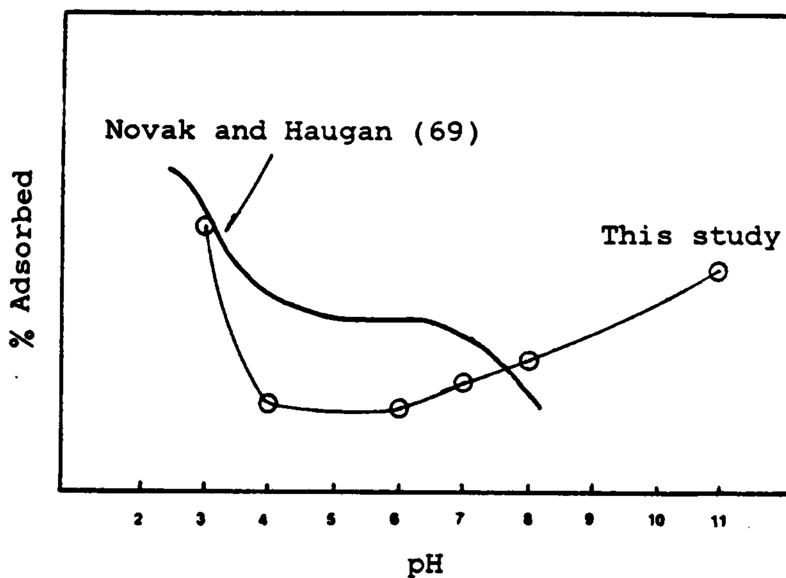

Figure 34. Effect of pH on biopolymer adsorption Comparison of the data of Novak and Haugan (69) with data from this study

protein biopolymer. This phenomenon was observed from the reactors fed with glucose and bacto-peptone (period A), glucose and glucose+NH_4^+-N (period C), glucose+NH_4^+-N and glucose+NO_3^--N (period D), and Celanese raw wastewater and Celanese raw wastewater+NH_4^+-N (period E). The sludge from those reactors with nitrogen supplement showed a high amount of protein biopolymers. Figures 35 and 36 show that substantial levels of bound protein were produced when nitrogen was available to the bacteria.

The effect of bound protein on sludge dewatering was clearly observed. Figure 37 shows that the specific resistance decreases when the amount of bound protein biopolymer increases. In addition, an improvement of specific resistance was observed when ammonia-N was present. It is suggested that when low amounts of biopolymer are bound to the floc, deflocculation or floc break-up occurs and the sludge dewaters poorly. A low specific resistance was observed when large amounts of protein biopolymer, which holds flocs tightly together, were present.

5.3.2 CARBOHYDRATE BIOPOLYMER

The relationship between extracellular polysaccharide and sludge dewatering was also observed during most of the experiments. It seems that free or loose biopolymer has a role in determining the sludge specific resistance.

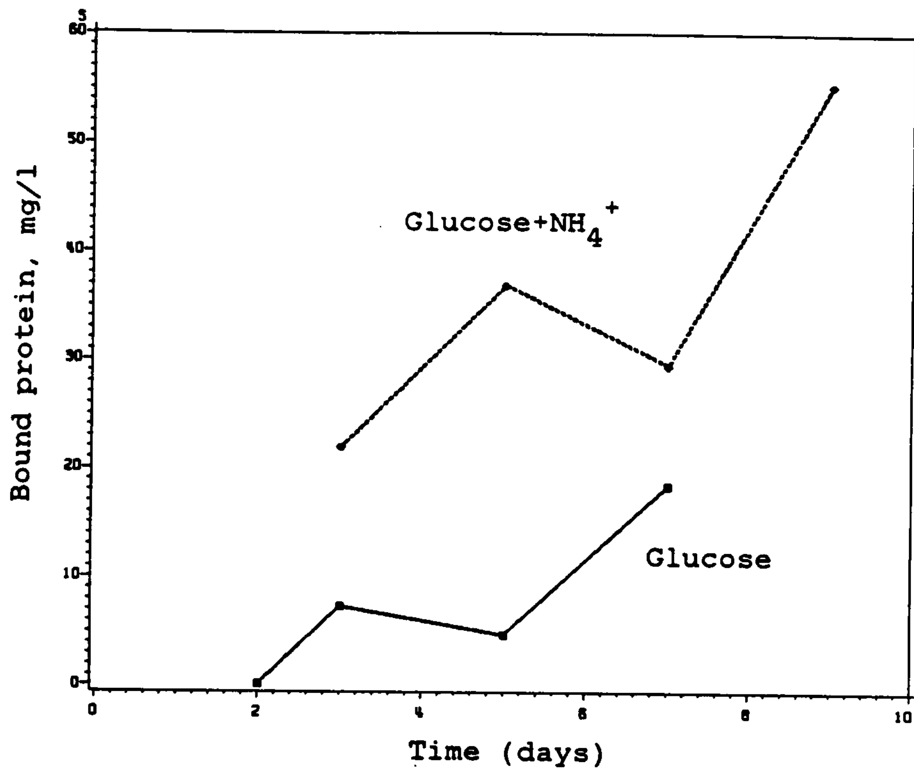

Figure 35. Effect of ammonia feed on bound protein biopolymer production, period C.

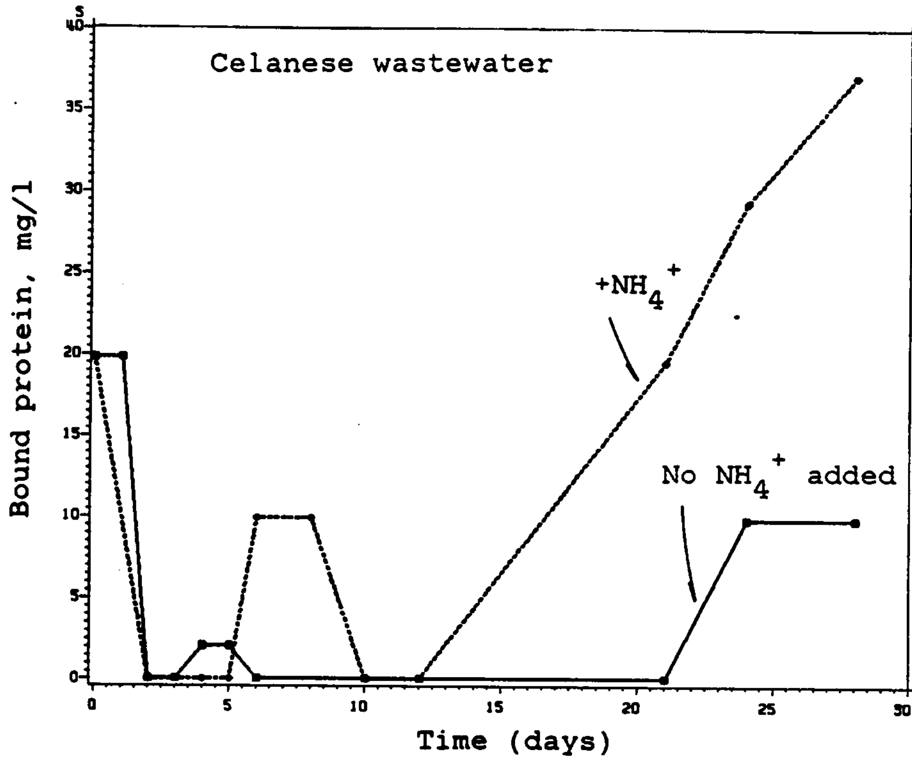

Figure 36. Effect of ammonia feed on bound protein biopolymer production, period E.

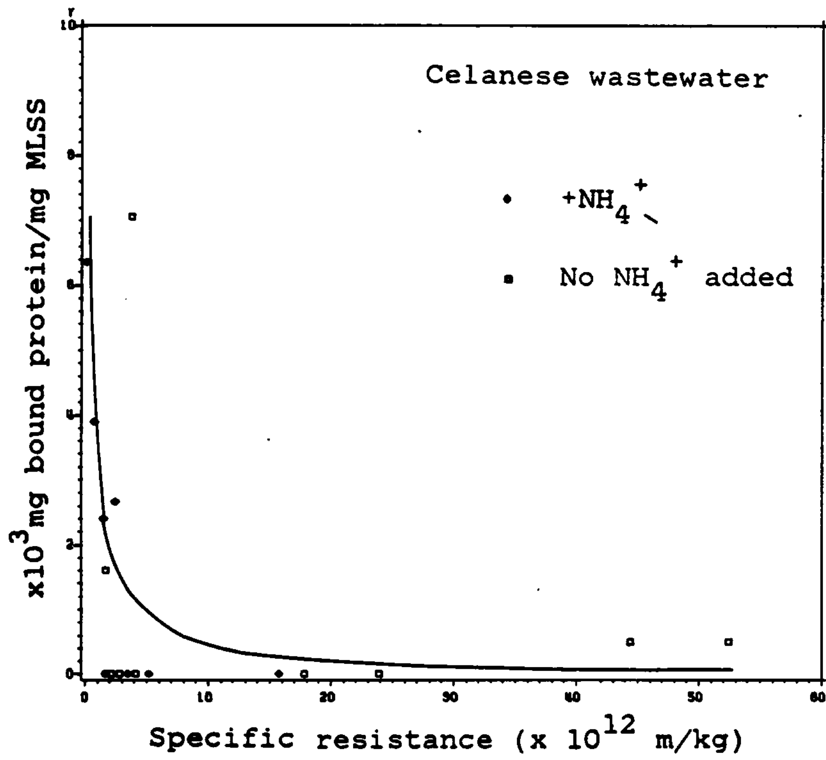

Figure 37. The effect of bound protein biopolymer on sludge specific resistance, period E.

The data obtained during period B indicate that most of the biopolymers in the reactor were loose or free and they were responsible for creating a sludge which settled poorly. During period E more data were collected and it was observed that for this period, nitrogen did not appear to affect the relationship between the bound extracellular polysaccharide and sludge specific resistance (Figure 38). This may be because nitrogen had already been added into the Celanese raw wastewater. [1] Other data confirmed that nitrogen plays a role in the production of protein biopolymer and consequently the improvement of sludge specific resistance.

The carbohydrate biopolymer also has an ability to bind to the floc surface. Its binding capacity is dependent of the presence of protein. Figure 39 is sketched based on the data obtained during period E to demonstrate this relationship. The relationship between total biopolymer (protein and carbohydrate) and specific resistance is shown in Figure 40. The Figure clearly demonstrates the importance of BEP on sludge dewatering.

Another condition that favored the production of extracellular carbohydrate was the use of glucose as feed to the reactors. This simple sugar is a known constituent of

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[1] The plant operators were not artifically adding nitrogen to the wastewater. It was only being added to the recycle sludge. However, the amount of nitrogen in the wastewater varies from time to time.

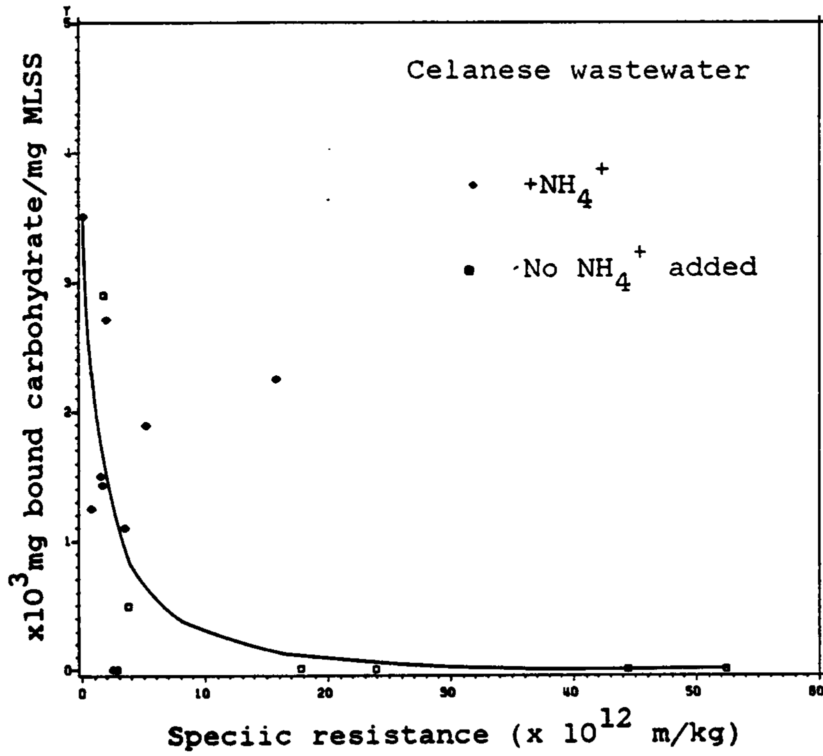

Figure 38. The effect of bound carbohydrate biopolymer on sludge specific resistance, period E.

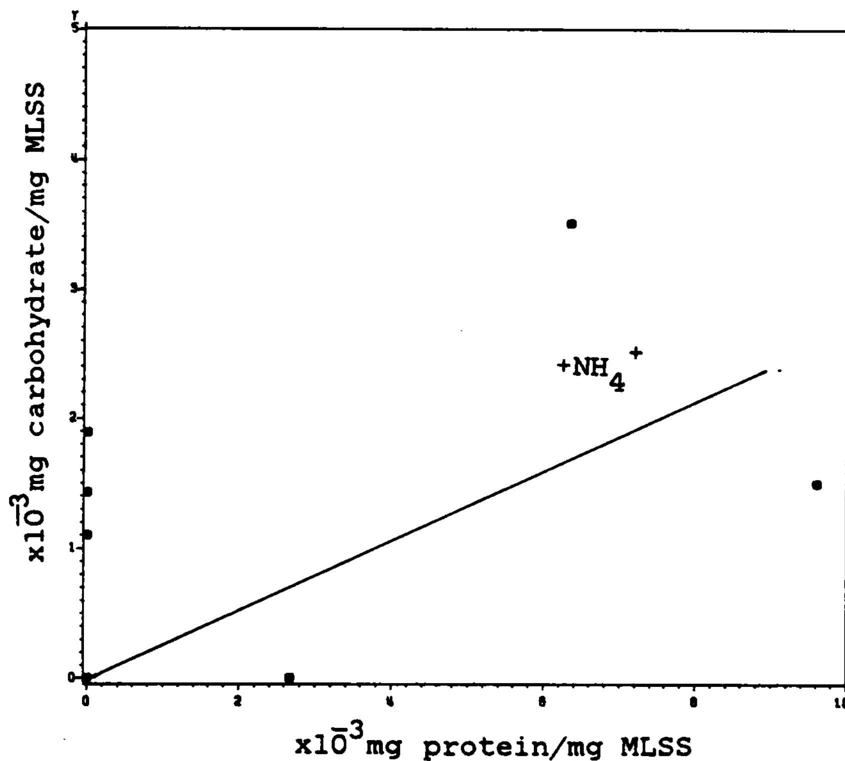

Figure 39. Relationship between protein and bound carbohydrate biopolymers (from sludge fed with Celanese wastewater).

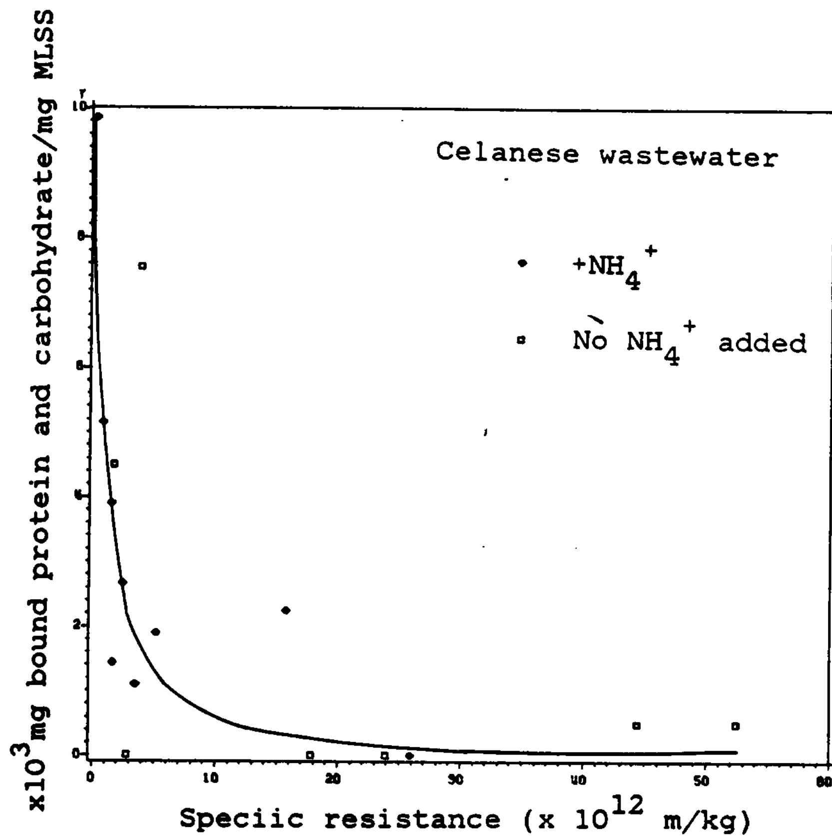

Figure 40. The effect of bound protein and carbohydrate biopolymers on sludge specific resistance, period E.

carbohydrate. When excess glucose was available and low nitrogen was present, the result was over production of carbohydrate biopolymer and these became loose and appearred in the sludge supernatant, resulting in poor dewaterability.

It is likely that protein biopolymers were better bound to the floc than carbohydrate biopolymers. This conclusion is derived from the finding that when protein biopolymer was detected, a good portion of them were bound. Therefore, it appears protein biopolymer plays a more important role in sludge binding and consequently sludge dewatering, than carbohydragte biopolymers.

5.3.3 SVI AND BIOPOLYMER

One of the parameters monitored throughout the study periods was sludge volume index (SVI). Figures 41 and 42 are typical plots of biopolymer VS SVI. Those figures show that good settling (low SVI) was not always associated with high concentrations of protein and carbohydrate biopolymers.

Figure 43 is proposed as an illustration of the effects of biopolymers on the settling and dewatering behaviors of sludges, based on the results observed throughout this study. Three different types of floc formation occur in activated sludge. Tight flocs usually settle well (low SVI) while flocs with loosely bound biopolymers have large amounts of water incorporated into the floc matrix and settle poorly

(high SVI). Badly deflocculated flocs are represented by discrete particles with little or no biopolymer and possess a relatively poor setting characteristic (high SVI).

Figure 43 also shows that the ease of sludge dewatering decreases when flocs are transformed from tight to loose flocs and finally to discrete particles.

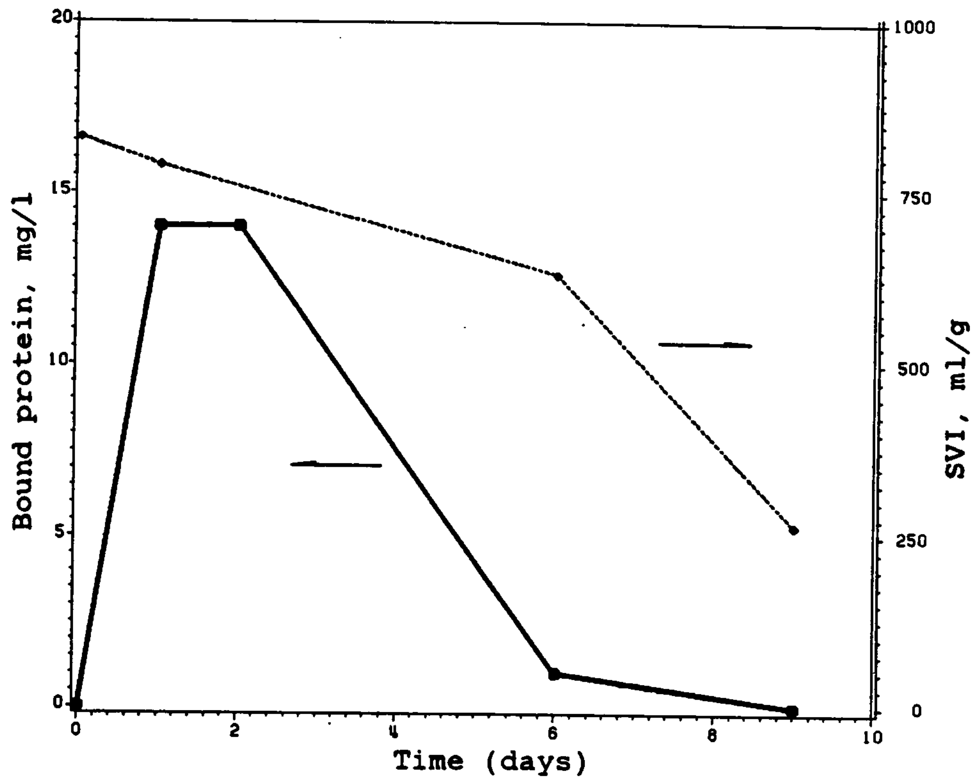

Figure 41. A comparison of bound protein biopolymer and SVI, period B (Bacto-peptone fed reactor)

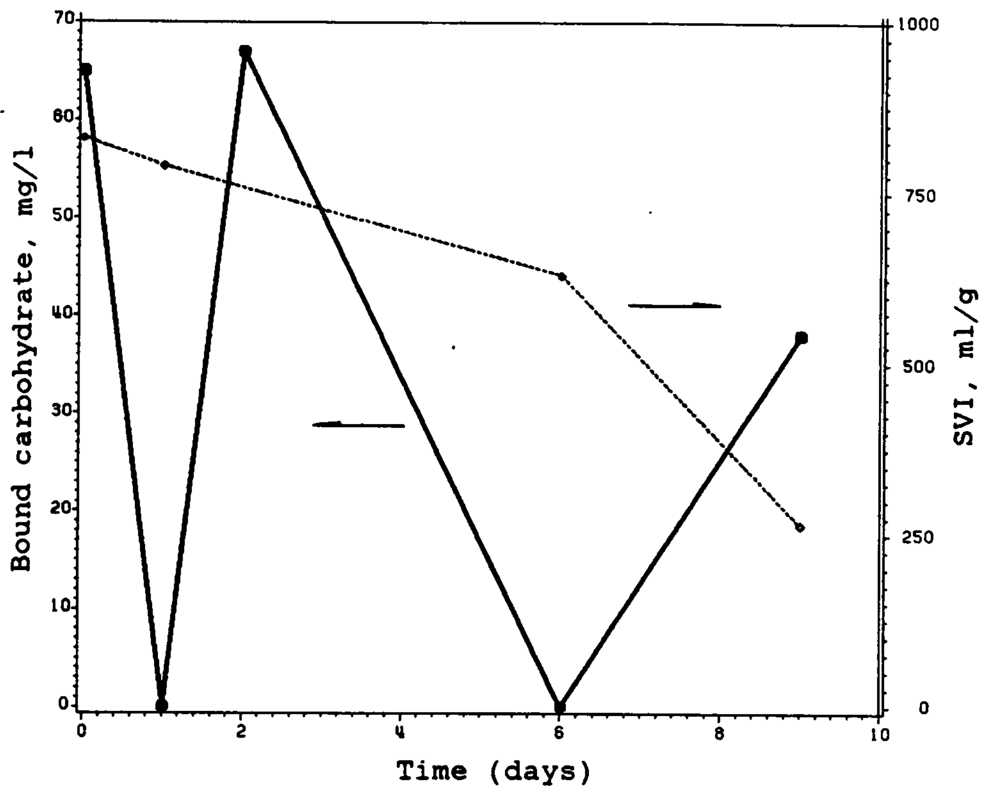

Figure 42. A comparison of bound carbohydrate biopolymer and SVI, period B (Bacto-peptone fed reactor)

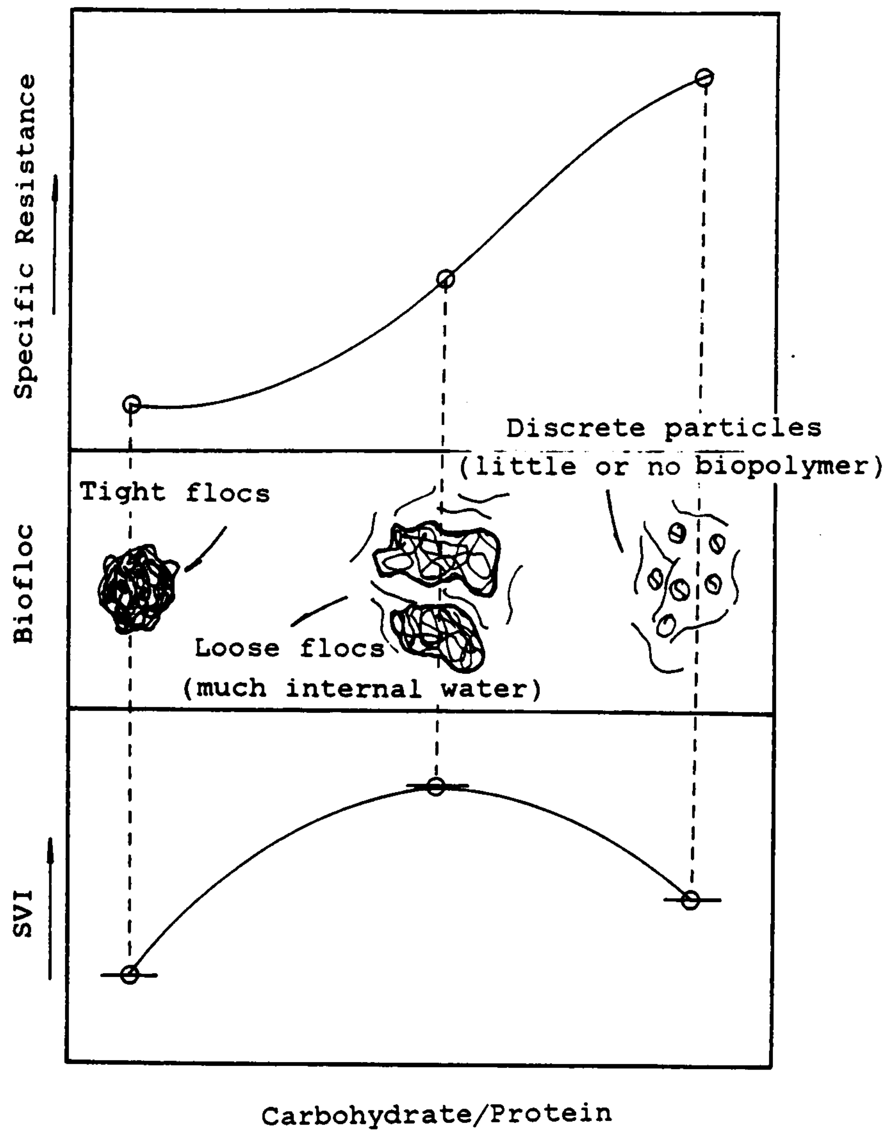

Figure 43. Proposed model for floc formation in relation to SVI and specific resistance.

V. Conclusions

The analysis of the experimental data obtained from the continuous flow reactor and adsorption studies suggests that BEP plays an important role in the flocculation of activated sludge. The conclusions derived from this study are summarized as follows:

1. The protein and carbohydrate biopolymers present in the activated sludge system are composed of bound and unbound fractions. Both fractions influence the sludge settling and dewatering properties. The major mechanism that is responsible for changes in those sludge properties is the extent of biopolymer binding.

2. When the amount of bound protein and carbohydrate is significant, the clumping of sludge flocs is improved and the results are better floc formation and settling.

3. The binding of carbohydrate biopolymer is dependent on the presence of protein biopolymer.

4. Sludge specific resistance is a function of the bound biopolymer, especially the bound fraction of protein biopolymer. The dewatering rate increases when the bound biopolymer increases.

5. Deflocculation of sludge flocs occurs when the bound protein biopolymer is low and/or the free carbohydrate biopolymer is high.

6. The nature of the feed substrate, such as its glucose and nitrogen content, has been found to affect the production

of specific types of biopolymers. When nitrogen is available, more protein biopolymers are produced and they are better bound to the sludge floc than the carbohydrate biopolymers. A condition with excess glucose and low nitrogen favors the over production of carbohydrate biopolymer. With low amounts of protein biopolymer, carbohydrate biopolymers are loosely bound and poor dewatering results.

7. Extraction of biopolymers from the activated sludge floc by adjusting the pH to 11 is superior to the steaming method. Very minor cell disruptive effects occur when this qualitative method is used.

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APPENDIX A. SUBSTRATE COMPOSITION

The following Tables summarize the substrate composition that was fed to the reactor during period A through E. The Tables also include the operating conditions on each period.

Table A1

Substrate composition during preliminary experiment period.

Component	Stock solution	ml/5.4 l
<u>Every other day</u>		
NH_4Cl		800 mg
$MgSO_4.7H_2O$	80 g/l	7
$MnSO_4.7H_2O$	8 g/l	7
$FeCl_3.6H_2O$	1 g/l	3.5
$CaCl_2$	3 g/l	7
KH_2PO_4	70 g/l	10
K_2HPO_4	48 g/l	10
<u>Every day</u>		
Bacto-peptone	64.5 g/l	98.5 ml
Glucose	112.5 g/l	229.0 ml
Nathiosulfate	12.4 g/l	5 ml

- Batch feed
- θ_c = 5 days

Table A2

Substrate composition and operating conditions during period A (April 13 - April 30)

Reactor	Component	Concentration/day
#1	Bacto-peptone in tap water	5.1 g/18 l/d
#2	Glucose in tap tap water	5.1 g/18 l/d

- Seeded sludge obtained from Blacksburg Municipal WWT Plant
- Batch feed with retention time = 12 h.
- θ_c = 10 day, daily sludge waste rate = 850 ml/d

Table A3

Substrate composition and operation conditions during period B (May 19 - June 12)

Component	Concentration/day
Glucose in tap water	5.1 g/18 l/d
Switched to bacto-peptone in tap water	5.1 g/18 l/d

- Seeded sludge obtained from Blacksburg Municipal WWT Plant
- Continuous feed
- May 29: Changed from continuous to batch feed (5.1 g/d) and θ_c = 10 day
- June 3: Resumed continuous feed, feed rate = 6.8 g/d, retention time = 12 h.
- June 4: Switched from glucose to bacto-peptone at the same feed rate and retention time.

Table A4

Substrate composition and operating conditions during period C (June 22 - July 10).

Reactor	Component	Concentration/day
#1	Glucose + substrate solution in Table A5	4.22 g/18 l/d
#2	Glucose + NH_4Cl (COD:TKN = 10:1) + substrate solution in Table A5	glucose = 4.22 g/18 l/d NH_4Cl = 1.72 g/18 l/d

- Seeded sludge obtained from Blacksburg Municipal WWT Plant
- θ_c = 10 days, however, from June 29 till the end of the period, θ_c was changed to 17 days.
- July 1: For reactor#1, changed from continuous to batch reactor and it was shut down on July 5.

Table A5

Substrate composition during period C.

Component	Stock solution	ml in 18 l	g in 18 l
$MgSO_4.7H_2O$	20 g/l	45	0.9
$MnSO_4.H_2O$	2 g/l	45	0.09
$FeCl_3.6H_2O$	45 g/l	45	4.5 mg
$CaCl_2$	1.5 g/l	45	0.07
KH_2PO_4	10.54 g/l	45	0.47
$NaHCO_3$	58.96 g/l	10	0.50
$NaCO_3$	63.6 g/l	5	0.32

Table A6

Substrate composition and operating conditions during period D (July 11 - August 7)

Reactor	Component	Concentration/day
#1	Celanese raw wastewater*	glucose = 4.22 g/18 l
	+ glucose + NH_4Cl	NH_4Cl = 1.72 g/18 l
	(COD:TKN = 10:1)	
#2	Celanese raw wastewater*	glucose = 4.22 g/18 l
	+ glucose	$Ca(NO_3)2.4H2O$ = 4.985 g/18 l
	+ $Ca(NO_3)_2.4H_2O$	
	(COD:TKN = 10:1)	

* see table A7

- Seeded sludge obtained from Celanese plant
- July 25: Changed from continuous to batch reactor

Table A7
Composition of
Celanese raw wastewater

Source	Component
Acetone recovery	- Water and condensate
	- Acetone
	- Mesityl oxide
	- Caustic
	- Finish oil*
CA	- Water and condensate
	- Acetic acid
	- Sulfuric acid*
	- Acetic anhydride*
	- Magnesium acetate
	- Caustic*
	- Soda ash*
	- Calcium chloride*
	- Methylene chloride*
	- Magnesium oxide*
	- Pulp fines
	- CA fines
	- Sludge*
AR/AM	- Water and condensate
	- Acetic acid
	- Acetic anhydride*
	- Ethyl acetate
	- Benzene
	- Methyl ethyl ketone
	- Caustic*
	- Ethyl alcohol
	- Methyl acetate
	- Acetone
	- Methyl cyanide
	- Methyl alcohol
	- Sodium acetate
	- Magnesium acetate
	- Calcium acetate
	- Calcium sulfates
	- CA fines
	- Hydrated lime*

* Typically enters by exception

Table A8

Substrate composition and operating conditions during period E (October 30 - November 27).

Reactor	Component	Concentration/day
#1	Celanese raw wastewater*	glucose = 4.22 g/18 l
#2	Celanese raw wastewater* + NH_4Cl	20 mg/l N then increased to 50 mg/l N

* see table A7

- Seeded sludge obtained from Celanese pland
- θ_c = 17 days
- Nov. 11: Increased NH_4Cl from 20 to 50 mg/l as N

APPENDIX B. STANDARD CURVES

This Appendix includes the standard curve data for the DNA, protein, and carbohydrate concentrations.

Table B1

DNA Standard curve (experiment 1)

Samples	Absorbance (UV units)	Conc., mg/l
std. 20 mg/l	0.020	20
std. 100 mg/l	0.075	100
std. 200 mg/l	0.160	200
std. 400 mg/l	0.320	400
std. 500 mg/l	0.410	500

Table B2

DNA Standard curve (experiment 2)

Samples	Absorbance (UV units)	Conc., mg/l
std. 5 mg/l	0.005	5
std. 10 mg/l	0.010	10
std. 20 mg/l	0.020	20
std. 40 mg/l	0.030	40
std. 80 mg/l	0.083	80
std. 160 mg/l	0.150	160
std. 200 mg/l	0.260	200

Table B3

Carbohydrate absorbances and their concentrations

Samples	Absorbance (UV units)	Conc., mg/l
std. 4 mg/l	0.250	60
std. 120 mg/l	0.460	120
std. 180 mg/l	0.700	180
std. 240 mg/l	0.890	240
std. 300 mg/l	1.050	300

Table B4

Protein absorbances and their concentrations

Samples	Absorbance (UV units)	Conc., mg/l
std. 4 mg/l	0.050	4
std. 8 mg/l	0.140	8
std. 12 mg/l	0.230	12
std. 18 mg/l	0.280	18
std. 20 mg/l	0.400	20

APPENDIX C. CONTINUOUS FLOW REACTOR STUDIES DATA

Table C1

Changes in the operating parameters of reactor#1 and reactor #2, period A

Date	Tss (mg/l)		SVI (ml/mg)		Spec. Resistance ($x10^{11}$ m/kg)	
	reactor #1	reactor #2	reactor #1	reactor #2	reactor #1	reactor #2
4/13	1253	1340	734	78	8.4	4.3
4/15	1687	1585	572	88	5.1	4.5
4/16	change feed from milk to glucose					
4/17	1550	1599	626	113	8.55	4.0
4/18	1515	1721	600	113	-	-
4/19	1451	1582	413	126	6.4	7.1
4/20	1381	1708	304	123	-	-
4/22	1327	1613	234	152	12.0	16.0
4/24	1298	1585	193	224	25.0	17.0
4/26	1062	1654	198	230	41.0	15.0
4/30	1205	1633	145	551	45.0	11.0

Reactor#1, feed substrate = milk and glucose
Reactor#2, feed substrate = bacto-peptone

Table C2

Changes in the total protein content of extracted biopolymers, period A.

Date	Day	Protein concentration (mg/ml)	
		Reactor#1	Reactor#2
4/13	1	-	-
4/15	3	4.2	14.8
4/16	Change feed from milk to glucose in reactor#1		
4/17	5	42.3	84.7
4/18	6	9.9	39.7
4/19	7	0.0	69.4
4/20	8	-	-
4/22	10	60.0	123.2
4/24	12	75.8	147.8
4/26	14	-	-
4/30	18	22.1	77.4

Table C3

Changes in the total carbohydrate content of extracted biopolymers, period A.

Date	Day	Carbohydrate concentration (mg/ml)	
		Reactor#1	Reactor#2
4/13	1	-	-
4/15	3	119	67
4/16	change feed from milk to glucose in reactor#1		
4/17	5	123	69
4/18	6	71	50
4/19	7	102	59
4/20	8	-	-
4/22	10	210	67
4/24	12	183	69
4/26	14	-	-
4/30	18	221	99

Table C4

Changes in the operating parameters of reactor#1, period B.

Date	Day	Tss (mg/l)	SVI (ml/mg)
5/19	0	830	-
5/20	1	790	-
5/25	6	630	1507
5/27	8	-	1566
5/28	9	265	1170
5/29	Changed from continuous to batch		
5/30	11	253	1027
6/2	14	-	-
6/3	15	-	-
	Resumed continuous feed with glucose (6.8g/d)		
6/4	Changed from glucose to bacto-peptone		
6/5	17	-	-
6/6	18	433	323
6/7	19	523	239
6/9	21	893	258
6/11	23	1010	594
6/12	24	1175	838
	Sludge got bad visually		

Feed substrate = bacto-peptone

Table C5

Changes in the total protein content of extracted biopolymers, period B.

Date	Day	Protein concentration (mg/l)
5/19	0	0
5/20	1	14
5/21	2	14
5/25	6	1
5/28	9	0
5/29	Changed from continuous to batch	
5/30	11	0
6/2	14	0
6/3	15	0
	Resumed continuous feed with glucose	
6/4	Changed from glucose to bacto-peptone	
6/5	17	0
6/6	18	0
6/7	19	0
6/9	21	32
6/11	23	51
6/12	24	85
	Sludge got bad visually	

Table C6

Changes in the total carbohydrate content of extracted biopolymers, period B.

Date	Day	Carbohydrate concentration (mg/l)
5/19	0	159
5/20	1	>235
5/21	2	170
5/25	6	82
5/27	8	-
5/28	9	146
5/29	Changed from continuous to batch	
5/30	11	>415
6/2	14	>>415
6/3	15	>415
	Resumed continuous feed with glucose	
6/4	Changed from glucose to bacto-peptone	
6/5	17	49.6
6/6	18	37
6/7	19	37
6/9	21	57
6/11	23	54
6/12	24	71
	Sludge got bad visually	

Table C7

Changes in the protein content of biopolymers in the supernatants obtained by gravity setting and pH adjustment, period B.

Date	Day	Protein concentration (mg/l)	
		Gravity	Extracted
5/19	0	0	0
5/20	1	0	14
5/21	2	0	14
5/25	6	0	1
5/27	8	0	-
5/28	9	0	0

Table C8

Changes in the carbohydrate content of biopolymers in the supernatants obtained by gravity setting and pH-adjustment, period B.

Date	Day	Carbohydrate concentration (mg/l)	
		Gravity	Extracted
5/19	0	94	159
5/20	1	>235	235
5/21	2	103	170
5/25	6	104	82
5/27	8	118	-
5/28	9	108	146

Table C9

Changes in the operation parameters of reactor#1
and reactor#2, period C.

Date	Tss (mg/l)		SVI (ml/mg)	
	reactor		reactor	
	#1	#2	#1	#2
6/22	1910	1645	513	596
6/23	1435	1645	606	559
6/24	1045	1545	823	550
6/25	1165	1110	806	748
6/26	1105	1075	815	521
6/27	1285	1143	739	770
6/29	1080	1113	907	795
	Change to batch			
7/1	1070	1110	916	791
7/3	810	1140	1230	404
	Resume continuous feed.			
7/5	450	1133	945	419
7/7	630	1258	-	302
7/10	-	1363		272

Reactor#1, feed substrate = glucose

Reactor#2, feed substrate = glucose + NH_4^+

Table C10

Changes in the total protein content of extracted biopolymers, period C.

Date	Day	Protein concentration (mg/ml)	
		Reactor#1	Reactor#2
6/22	0	40.8	40.8
6/23	1	31.8	40.8
6/24	2	31.8	-
6/25	3	43.5	50.0
6/26	4	-	-
6/27	5	55.3	87.5
6/29	7	59.9	73.7
	Changed to batch.		
7/1	9	50.7	55.3
7/3	11	0.0	33.5
	Resumed continuous feed.		
7/5	13	-	33.5
7/7	15	0.0	1.0
7/10	18	-	23.9

Table C11

Changes in the total carbohydrate content of extracted biopolymers, period C.

Date	Day	Carbohydrate concentration (mg/ml)	
		Reactor#1	Reactor#2
6/22	0	14.0	14.0
6/23	1	20.6	13.0
6/24	2	12.7	-
6/25	3	31.8	21.6
6/26	4	-	-
6/27	5	65.4	19.1
6/29	7	92.8	22.6
	Changed to batch.		
7/1	9	147.5	23.8
7/3	11	>132	24.5
	Resumed continuous feed.		
7/5	13	-	25.1
7/7	15	>132	14.5
7/10	18	-	33.7

Table C12

Changes in the protein content of biopolymers in the gravity settled supernatant, period C.

Date	Day	Protein concentration (mg/l)	
		Reactor#1	Reactor#2
6/24	2	45.4	40.8
6/25	3	36.3	28.1
6/26	4	29.0	29.0
6/27	5	50.7	50.7
6/29	6	-	47.9
7/1	7	41.5	44.2
7/3	8	-	0.0
7/7	9	-	0.0
7/10	10	-	0.0

Table C13

Changes in the carbohydrate content of biopolymers in the gravity settled supernatant, period C.

Date	Day	Carbohydrate concentration (mg/l)	
		Reactor#1	Reactor#2
6/24	2	6.4	5.1
6/25	3	11.4	5.1
6/26	4	40.7	7.6
6/27	5	73.8	10.7
6/29	6	-	8.3
7/1	7	123.7	12.4
7/3	8	-	9.3
7/7	9	-	14.5
7/10	10	-	8.6

Table C14
Changes in the operating parameters of reactor#1 and reactor#2, period D.

Date	Tss (mg/1)		SVI (ml/mg)		Spec. Resistance ($x10^{11}$ m/kg)	
	reactor		reactor		reactor	
	#1	#2	#1	#2	#1	#2
7/12	1285	-	584	-	-	-
7/13	-	1710	-	421	-	8.4
7/14	1260	-	754	-	-	-
7/15	-	1785	-	501	-	0.1
7/17	1055	-	867	-	13.0	-
7/18	-	1750	-	543	-	-
7/19	1305	-	717	-	14.9	-
7/20	-	1295	-	749	-	10.9
7/21	1095	-	808	-	-	-
7/22	-	645	-	1457	-	7.1
7/23	1035	-	570	-	4.1	-
7/24	-	398	-	2361	-	6.0
7/25	1005	-	766	-	3.9	-
7/28	1360	-	588	-	3.7	-
7/29	change from continuous to batch feed					
7/30	-	1010	-	980	-	1.7
7/31	1470	-	558	-	2.9	
8/2	1348	-	508	-	0.01	
8/4	-	-	-	-	0.005	-
8/6	1465	-	-	-	-	-

Reactor#1, feed substrate = Glucose+NH_4^+

Reactor#2, feed substrate = Glucose+NO_3^-

Table C15

Changes in the total protein content of extracted biopolymers, period D.

Date	Day	Protein concentration (mg/ml)	
		Reactor#1	Reactor#2
7/12	1	0.0	-
7/13	2	-	0.0
7/14	3	0.0	-
7/15	4	-	47.8
7/17	6	14.3	-
7/18	7	-	30.6
7/19	8	4.8	-
7/20	9	-	47.8
7/21	10	4.8	-
7/22	11	-	45.9
7/23	12	0.0	-
7/24	13	-	4.8
7/25	14	19.1	-
7/28	17	14.3	-
7/29	18	-	43.0

Table C16

Changes in the total carbohydrate content of extracted biopolymers, period D.

Date	Day	Carbohydrate concentration (mg/ml)	
		Reactor#1	Reactor#2
7/12	1	99.1	-
7/13	2	-	29.1
7/14	3	24.5	-
7/15	4	-	62.3
7/17	6	38.3	-
7/18	7	-	79.3
7/19	8	20.5	-
7/20	9	-	79.3
7/21	10	29.7	-
7/22	11	-	29.7
7/23	12	34.4	-
7/24	13	-	29.7
7/25	14	29.7	-
7/28	17	15.2	-
7/29	18	-	29.7

Table C17

Changes in the operating parameters of reactor#1 and reactor#2, period E.

Date	Tss (mg/l) reactor #1	Tss (mg/l) reactor #2	SVI (ml/mg) reactor #1	SVI (ml/mg) reactor #2	Spec. Resistance ($x10^{12}$ m/kg) reactor #1	Spec. Resistance ($x10^{12}$ m/kg) reactor #2
10/30	1980	1980	136	136	-	-
10/31	2810	2885	117	269	3.73	3.73
11/1	3215	3385	79	244	17.8	15.7
11/2	3435	3850	57	226	-	25.9
11/3	4040	3655	64	238	52.4	5.14
11/4	3965	3780	76	155	44.4	3.47
11/5	4060	3710	84	240	23.9	2.42
11/7	4190	4120	205	226	4.1	1.5
11/9	4575	4545	201	209	174	1.6
11/11	4755	4675	196	205	2.8	1.9
11/12	4520	4535	208	213	-	-
11/13	4470	4715	213	205	-	-
11/14	4820	4930	196	196	-	-
11/16	5095	4540	188	203	-	-
11/18	4920	4475	195	174	2.7	0.6
11/20	6265	5025	152	159	2.1	0.7
11/23	6165	4615	153	160	1.7	0.1
11/25	-	-	166	178	1.7	1.1
11/26	-	-	157	190	1.9	2.0

Reactor#1, feed substrate = Celanese raw wastewater

Reactor#2, feed substrate = Celanese raw wastewater+NH_4^+

Table C18

Changes in the total protein content of extracted biopolymers, period E.

Date	Day	Protein concentration (mg/ml)	
		Reactor#1	Reactor#2
10/30	0	29.7	29.7
10/31	1	19.8	59.5
11/1	2	0.0	0.0
11/2	3	0.0	0.0
11/3	4	2.0	0.0
11/4	5	2.0	0.0
11/5	6	0.0	9.9
11/7	8	0.0	39.6
11/9	10	0.9	0.0
11/11	12	1.8	0.0
11/20	21	0.0	19.5
11/23	24	9.8	29.3
11/27	28	9.8	39.1

Table C19

Changes in the total carbohydrate content of extracted biopolymers, period E.

Date	Day	Carbohydrate concentration (mg/ml)	
		Reactor#1	Reactor#2
10/30	0	18.6	18.6
10/31	1	23.5	26.2
11/1	2	13.8	13.8
11/2	3	16.6	10.4
11/3	4	8.3	22.1
11/4	5	15.2	23.5
11/5	6	24.8	11.0
11/7	8	44.2	25.5
11/9	10	15.2	12.8
11/11	12	8.9	20.3
11/20	21	-	19.5
11/23	24	30.6	27.2
11/27	28	27.2	23.8

Table C20

Changes in the total protein content of biopolymers in the gravity settled supernatant, period E.

Date	Day	Protein concentration (mg/ml)	
		Reactor#1	Reactor#2
10/30	0	9.9	9.9
10/31	1	0.0	-
11/1	2	0.0	0.0
11/2	3	0.0	0.0
11/3	4	0.0	0.0
11/4	5	0.0	0.0
11/5	6	0.0	0.0
11/7	8	-	29.7
11/9	10	10.1	6.3
11/11	12	13.7	7.6
11/20	21	-	0.0
11/23	24	0.0	0.0
11/27	28	0.0	2.0

Table C21

Changes in the total carbohydrate content of biopolymers in the gravity settled supernatant, period E.

Date	Day	Carbohydrate concentration (mg/ml)	
		Reactor#1	Reactor#2
10/30	0	24.8	24.8
10/31	1	22.1	-
11/1	2	13.8	6.2
11/2	3	17.9	10.4
11/3	4	13.8	15.2
11/4	5	20.7	19.3
11/5	6	27.6	11.0
11/7	8	-	19.3
11/9	10	10.1	6.3
11/11	12	13.7	7.6
11/20	21	-	13.2
11/23	24	12.7	11.0
11/27	28	11.9	15.3

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