THE INFLUENCE OF CATIONS ON ACTIVATED SLUDGE BEHAVIOR

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(ABSTRACT)

This study investigated the influence of cations on biopolymer characteristics and sludge properties. Settling and dewatering properties of sludges were measured and correlated to the biopolymer characteristics. In addition, effects of cations on sludge conditioning with polymer were studied.

Experiment mainly consisted of two parts, reactor study and batch study. In reactor study continuous-flow reactors were operated. Variables used included varing type of substrate, addition of magnesium or sodium, and changes in pH. The batch study included conditioning tests on the sludges with combinations of cationic polymer and salts.

Biopolymers were extracted using alkali extraction followed by gel filtration and subsequent carbohydrate and protein analysis. The sludge settling and dewatering were measured in terms of SVI and specific resistance, respectively. Sludge filtering rate (TTF) was used to measure the conditioning efficiency.

The results indicated that the influence of cations depended on the type and concentration of salt. An optimal concentration of Mg was found to improve biopolymer binding. The organic composition of feed also affected biopolymer characteristics. A higher pH combined with a high amount of sodium released biopolymer and resulted in sludge deflocculation. A relationship between unbound biopolymer and soluble effluent COD was observed but no discernible relation between biopolymer

binding and sludge settling and dewatering properties was found. Cations was found to reduce polymer requirement during sludge conditioning.

In addition, the amount of extactable biopolymers by alkali extraction was greatly influenced by salt. Magnesium inhibited the release of biopolymer, but sodium improved the efficiency of biopolymer extraction.

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I Introduction

The ability of microorganisms to form readily settleable sludge flocs is vital in obtaining a high quality of effluent in the activated sludge process. A well-flocculated biomass is required not only to control the degradation of organic waste but also to prevent the washout of biomass to the effluent. Furthermore, the extent of bioflocculation determines the efficiency of sludge handling processes such as sludge thickening and dewatering.

At the present time, it is widely accepted that self- flocculation of microorganisms results from the interparticle bridging of naturally occurring polymers, or so-called extracellular polymers (ECP) or biopolymers (1,2). The mechanism for interparticle bridging by biopolymers is believed to be through the adsorption of biopolymer segments on the colloid surfaces.

Although the theory of bioflocculation is well developed, the factors that influence biopolymer adsorption are not well understood. The adsorption characteristics of biopolymers are thought to be altered by variables such as the nature of the substrate, pH, cations (mixture and concentration) and mixing (period and intensity) (3,4).

Researchers have developed correlations among sludge settling and dewatering properties, the nature of substrates and biopolymers. In addition, many workers have proved that cations, especially divalent cations, are necessary for some species of dominant activated sludge microorganisms to agglomerate (5). According to their postulation, divalent cations reduce the repulsive forces and serve as a bridge to link the anionic biopolymers and negatively charged colloids. This enhances the attachment of the biopolymer to the surface of the colloidal particles.

However, little data exist investigating the relationships between cations and the binding characteristics of biopolymers. The research presented here attempts to provide information relevant to this topic. The primary objectives of this research were:

- to investigate the role of cations on the binding characteristics of biopolymers.
- to improve understanding of the influence of biopolymer binding on sludge properties.
- to study the effects of cations on sludge conditioning with polymers.

II LITERATURE REVIEW

The treatment efficiency of an activated sludge process is determined by two steps, the substrate oxidation in aeration basins and the sludge separation in the following clarifers. The developement of a stable floc structure is required for the adsorption of organic substances on the substrate utilization step (1), the capture of both biological and abiotic colloids (2), and the rapid separation of sludge flocs under quiescent settling.

The degree of bioflocculation affects sludge handling as well. Since activated sludge flocs contain a substantial amount of water, sludge dewatering by mechanical ways is usually performed to reduce sludge volume for final disposal. Therefore, a well flocculated sludge able to resist the high shear force during sludge pumping and processing and allow water to pass through quickly can eliminate many problems (7).

Theory of Bioflocculation

Although the agglomeration of bacterial cells in activated sludge process occurs spontaneously under usual operational conditions, this behavior is not well understood.

A bacterial cell can be viewed as a hydrophilic biocolloid, attached by macromolecules (called as biopolymers or ECPs), in the forms of capsules, flagellae, fibrils, filaments and limbriae of various kinds (4,8). These biopolymers contain functional groups which, within the physiological pH range, bear nonionic or anionic charges through acid-base interactions (4). As a result, bacterial cells usually carry a net negative charge on the cell surfaces. For cell to associate, the electrostatic repulsion between the cells having like charge must be overcome (4,8).

Numerous mechanisms have been proposed to describe the self- flocculation of bacterial cells. Past investigations (3,9-14) have rejected charge neutralization and the presence of a particular group of bacteria as the major mechanism of bioflocculation. At the present time, the bridging model of extracellular biopolymers is the most satisfactory interpretation.

In natural environments, microorganisms are capable of producing a matted mass of polysaccharide fibers (glycocalyx), extending outward from membranes. The glycocalyx allows cells to attach to surfaces and/or bind to others, and provides the advantages in protecting from enemies and competing for limited nutrient sources (15).

The production of polymeric substances by activated sludge microorganisms is a common phenomenon when substrate availability is limited. An accumulation of polysaccharide material on cell surfaces, as noted by Pavoni et al. (11), was first observed by Mckenney (14) and other researchers later on (16,17). Busch and Stumm (3) and Tenney and Stumm (5) proposed that bioflocculation is a result of interpartical bridging by the biopolymers, which are excreted or exposed at the cell surfaces. These biopolymers function similarly to synthetic anionic and nonionic polyelectrolytes (3,4). A general model of flocculation proposed by Eriksson and Hardin (18) suggested that biopolymers initiate the flocculation of biomass through bridging the distance between electrostatically stabilized cells and form weak and easily broken flocs. As more polymers are synthesized in a stable process, the flocs will be stronger by firm binding. Up to a certain level, further polymers are favorable, but an excess will have a dispersing effect.

The physical interactions leading the extended biopolymer segments to attach to others or adsorb on cell surfaces are thought to be hydrogen bonding, anion interchange with adsorbed anions(such as OH^-) or interchanges with cations on or in the

immediate vicinity of the cell surfaces (4,19). Because of mixing and short distance between cells, these interactions are believed to overwhelm the local electrostatic repulsion (20). These interactions are influenced by many factors such as the nature of biopolymers, the surface properties of colloids, the intensity and time of mixing, pH and the divalent cation concentration (4).

Self-flocculation is normal after reaching the declining or endogenous phase. The amount of biopolymer required for optimal aggregation is related to the total surface area (21). Busch and Stumm (3) pointed out that only under the declining or endogenous growth can an adequate amount of biopolymers accumulate on the cell surfaces allowing efficient flocculation to occur. During the logarithmic growth phase, the rapid increase in surface area leads to less biopolymers per unit surface of sludge floc. As a result, particles tend to be dispersed (3).

Nature and Composition of Biopolymers

Extracelluar polymers, as concluded by Clarke and Forster (2), probably originate from sewage itself, autolysis of cell walls and entrapped microorganisms. Hejzlar and Chudoba (20) found that both primary and secondary effluents contain sugars, uronic acids and amino acids, indicating a heterpolysaccharide character of biopolymer. The authors thought that biopolymers were microbial waste products; therefore, they might be found everywhere where bacteria exist. The biopolymers are excreted into the cultivation medium in the forms of either intermediates, or end products, which are resistant to further biodegradation (22). An increase in soluble effluent COD corresponding to the increase in unbound biopolymers has been proved by several researchers (23,24).

Vollam and Mcloughlin (23) studied a batch system fed with Brovine Serum Albumin (BSA) and found a release of biopolymers and a better flocculation, corre-

sponding to a decrease in cell dry weight. Therefore, they related the production of biopolymers to cell autolysis. Gulas et al. (24) indicated that the cell lysis is prompted by the presence of the enzymes capable of breaking cell walls. However, the finding by Gulas et al. directly conflict with others in respecting to the buildup of biopolymers during the high growth rate period. The authors explained that such a result is probably due to the autolytic enzymes that have a maximum activity during the logarithmic growth phase.

Since the characteristics of wastewater vary from time to time and from plant to plant, it is expected that the precise composition of extracellular polymers differs corresponding to the microbial predominance (11). However, all biopolymers contain polysaccharide, protein, DNA, and RNA but in various proportions. The variety in biopolymer composition is caused by the differences in substrate, operational condition and the extraction method for biopolymer determination (11).

It appears that carbon, nitrogen, phosphorus, and sulfur play critical roles on the characteristics of biopolymers. Nutrient alternation or imbalance will cause physicological changes in the surface components (26).

Harris and Michell (19) suggested that high carbohydrate wastes, for example, sucrose, will lead to an overproduction of polysaccharide and a stable microbial suspension. The excess polysaccharide might severely inhibit the conditioning efficiency of polymers.

Wilkinson (16) concluded that the deficiency in nutrients (N,P,S) will stimulate the production of polysaccharide. His statement is confirmed by the finding that a nitrogen-rich feed (25) yields a biopolymer composed mostly of protein. Whereas, the major constituent of the biopolymer from a carbohydrate feed (9) is carbohydrate. Kiff (25) found that an increase in loading rate and readily available carbohydrates could result in a production of capsular slime as a storage product. The biopolymer

produced is found to have a high C/N ratio. After a rebalancing of the feed C/N ratio, a 35% reduction in the carbohydrate fraction was found.

Kajornatiyudh (5) proposed that nitrogen can promote the production of protein biopolymer, and then improve the binding of carbohydrate biopolymer, resulting in a sludge with a better dewatering property.

Sludge Settling, Conditioning and Dewatering

The relationship between the nature of biopolymers and sludge settling and dewatering properties has been studied in depth by many researchers. Colloidal biopolymers are found to cause poor sludge filtration rate (27). High effluent turbidity is sometimes associated with a high concentration of carbohydrate biopolymer (22).

By comparing the difference in surface condition under a microscope, Chao and Keinath (28) concluded that surface roughness affects sludge settling and thickening. The rough surface of bulking sludge has a greater frictional force which can reduce the interface settling velocity and result a high SVI. The major role of metallic coagulant is to smooth the suface of flocs rather than change floc density.

Tenney and Stumm (4) suggested that anionic substances such as polysaccharide that control the magnitude of surface charge density are more important in determining sludge characteristics than nonionic substances such as protein.

The electrophoretic mobility measurement studied by Forster (29) showed that the average mobility is directly proportional to the SVI. Forster considered that the settling property of activated sludge is determined by some metabolic processes, altering the zeta potential of particles. The high value of SVI may result from an overgrowth of polyhydroxybutyrate induced by nitrogen-defficient feed solution. The sludge cells under such a condition are still agglomerated by biopolymers. However,

at the same time, the flocs stay in an expanded state by the increased electrostatic force, due to high surface charge density.

A "stability index" for sludge is proposed by Steiner et al. (21), based on that a linear relationship between SVI and surface charge. The authors suggested that such an index will help workers to predict the bulking potential of their sludge.

Goodwin and Forster (30), examining the chemical component of biopolymers extracted by heat extraction, found no recognizable relation between settlement property and protein biopolymer. On the other hand, they found that sludge settlement deteriorates as the lipid fraction increases. The surface interaction for lipids appears to be in part ionic and in part hydrophobic. Contradictory to the results from Steiner et al. (21), Goodwin and Forster (30) found that polysaccharide fraction increases as settlement improves. The authors explained that this could be due to either that a considerable amount of total polysaccharide is in a intra-cellular form or that polysaccharide becomes more difficult to be extracted as settlement deteriorates.

Heukelekian and Weisbury (31), cited by Forster and Choudhry (29), suggested that SVI is directly related to the bound water content of sludge. The polysaccharide material in activated sludge is found to be capable of adsorbing a large amount of water (27). Forster and Choudhry (29) found that the poor- settling sludge with a high negative charge contains more bound water than the sludge which settles well. Friedman (32) noted that the amount of bound water associated with the biopolymers is determined by the physical and chemical nature of biopolymers.

It appears that the extent of binding of biopolymers impacts the sludge settling and dewatering. Kajornatiyudh (5) concluded that biopolymers produced by activated sludge microorganisms contain bound and unbound fractions. When the amount of bound biopolymers is significant, sludge flocculation is improved and sludge settling and dewatering are better. There seems to be a relationship between the binding of

protein and carbohydrate biopolymer. Kajornatiyudh suggested that the binding of carbohydrate biopolymer is improved in the presence of protein biopolymer. Deflocculation corresponds to a low bound protein biopolymer and, at the same time, high unbound carbohydrate biopolymer concentration

Robert and Olsson (26) proposed that, similar to BET adsorption model, the floc matrix serves as the adsorbent and the natural anionic biopolymers as the adsorbate. The adsorbed biopolymers are easily desorbed or deslodged by mixing. The authors concluded that the major mechanism for cationic polymer sludge conditioning is through the neutralization of the charges on anionic colloidal biopolymers, resulting in a cross-linking between polyelectrolytes and biopolymers. Therefore, when the MLSS is low, the optimal polymer dose for conditioning is determined by colloidal particles rather than the solids content of sludge.

Studies by Novak et al. (33) and Novak and Haugan (34,35) further describe the mechanism of sludge conditioning. They found that the optimal dose occurs near the zero charge point; therefore, the conditioning of sludge by cationic polymers is proved to be through charge neutralization. The primary mechanism for metallic conditioners is through providing adsorption sites for colloidal biopolymers. Mixing intensity greatly affects the performance of conditioning. Since biopolymers are weekly bound to sludge flocs, it is expected that a large quantity of biopolymers will be desorbed by intensive mixing. Therefore, intensive mixing increases the requirement of conditioning dosage but yields a sludge more resistant to disruption and deterioration during sludge processing.

The Roles of Cations and pH on the Behavior of Biopolymers

In many mineral processing operations, the presence of a small amount of divalent cations, e.g., Ca, can significantly improve the flocculation of small mineral

particles by anionic polyelectrolytes (8). This is thought to be through the formation of complex between cations and polymers, resulting in changes in the adsorption characteristics of polymers. Pavoni et al. (11) postulated that the role of cations on enhancing the flocculation capacity of polymers is to change the structural configuration of polymers or to provide a coordination bridge between polymers and the activated sites on the surface of colloids.

Cations involve in the bioflocculation in two ways, either in helping biopolymer bridging or in reducing the surface charge. Busch and Stumm (3) and Stumm and Morgan (36) stated that the flocculation of a polymer system of like charges will only be observed in the presence of an polyelectrolytes, usually a salt. Even a trace amount of a third ions seems to be adequate (3).

For bacteria to adhere on surface or associate together, divalent cations such as magnesium can help the linkage between anionic sugar units at the end of glycocalyx (15). Dugan et al. (37) found that high molecular weight polymer can adsorb a large amount of water and monovalent cations (e.g., Na, K), and can exchange monovalent cations with polyvalent cations. Thereby, the polymers become more hydrophobic and the degree of flocculation is enhanced.

The calcium ion adsorption study by Steiner et al. (21) showed that the adsorption mechanism of cations on solid biopolymers, either in a solid form or adhered to a solid medium, is different to that on soluble biopolymers. This is attributed to the difference in the chemical nature of the adsorption sites and therefore in the strength of binding. As stated by the authors, cations are adsorbed on soluble biopolymers through salt formation with carboxyl groups. Such an interaction is irreversible at a normal pH range. On the other hand, adsorption of cations on the solid biopolymers occurs by attaching to hydroxyl units in the hexose and pentose molecules on the polysaccharide polymer by electric attraction.

Tezuka (6), working with a non-capsulated Flavobacterium dominant in activated sludge, reported that the culture was perfectly flocculated in the medium containing both calcium and magnesium ions, regardless of what point in the growth or station the culture was. When the flocs were suspended in deionized water and shaken for a few minutes, the flocs were deflocculated. Therefore, the author concluded that some mineral component(s) in the medium are required for floc formation.

In contrast, the response of other bacterial strains to the addition of cations is quite different to that of Flavbacterium . Tezuka found that the addition of magnesium salt inhibits the flocculation of strains Zoogloea and Pseudomonas. Angelbeck (38) proved that failure in the aggregation of Z. ramigera strains ZRC and I16M by the addition of calcium and magnesium occurs only during the logarithmic growth state.

The effects of cations on pure cultures have been studies by many researchers, who all found that cations affect bacterial growth.

The Hofmeister series, as described by Holm and Sherman (39), showed that natural salts affect the coagulation of biocolloids and the swelling and other physical properties of protein. The order of influence of varies cations are arranged as Ca > Sr > Mg > Cs > Rb > K > Na> Li.

Winslow and Dolloff (40), confirmed the finding of Hotchkiss (41), concluded that a wide variety of metallic cations (Na, K, Ca, Mg, Ba, Pb, and Hg) stimulates the growth of bacteria when present in a sufficient low concentration. But when in a sufficient high concentration, bacteria growth is inhibited. Such an effect is associated to the increase in permeability in diluted salt solution and the decrease in permeability or perhaps the rupture or some irreversible physical changes in the cell membrane when in high concentration of salt solution. The effects on bacterial growth vary corresponding to the concentration rather than the type of cations. The general conclusions by the authors are described below:

1. The divalent ions are generally eight to ten times as powerful as the monovalent ions. However, diluted monovalent ions tend to be more favorable in both viability and biological activity in the stimulating zone. For example, a 0.1 M solution of NaCl or KCl is generally stimulating while a similar concentration of CaCl(2) or MgCl(2) solution is general toxic.

2. Mixtures of a monovalent and a divalent salt or two monovalent salts are more favorable. The effects of the mixture of magnesium with either sodium or potassium in the proportion of ten parts of monovalent ion to one part of divalent ion in acid and neutral media is a direct function of the ionic activity of the cations presented. When the mixture is mixed in different proportion but with a constant molarity, in the stimulating zone, it shows additive effects.

3. Hydrogen ion is more powerful than monovalent cations with a coefficient of activity 30,000 times. The finding by Winslow and Falks (42), as cited by Winslow and Dolloff (40), found that the effects of sodium and calcium are additive in acidic solution but is antagonistic at a pH of 7.0 or more. This is due to the interference on the capacity of bacterial cells to neutralize the alkalinity of the menstruum.

III MATERIALS AND METHODS

3.1 System Operation

To investigate the role of monovalent and divalent cations on the binding of biopolymers in activated sludge system, and on the sludge settling and dewatering characteristics, continous-flow reactors were operated. The operational variables used included varying type of substrate, addition of magnesium or sodium in various concentrations, and changes in pH. The experimental procedure consisted of eight operational periods. The operational condition for each period is listed in Table 3.1.

Two continous-flow reactors, with a working volume of approximately 8.5 liters, were operated in parallel in a constant temperature room where the temperature was maintained at 20 $\pm$ 1°C. An adjustable baffle was placed into each reactor to create two interconnecting chambers for aeration and settling. An air stone diffuser was placed beside the baffle to provide an oxygen source and concurrently complete mixing. The schematic diagram of a continuous-flow reactor system is shown in Figure 3.1.

An activated sludge culture obtained from the aeration basin from the wastewater plant at Blacksburg, VA. was acclimated to the synthetic substrate that contained the chemicals listed in Table 3.2 and 3.3. To generate the sludges with different characteristics, two different kinds of organic substrate were prepared. Bactopeptone and a mixture of bactopeptone/dextrose in a ratio of 2:1 based on COD were used as organic carbon source and yielded a feed having an average COD of approximate 350 mg/l. Ammonium chloride was added to compensate the deficiency of nitrogen source when bactopeptone/dextrose was used. A phosphate buffer was utilized for both nutrient requirement and pH control. Sodium chloride and magnesium chloride were added in the feed solution to analyze the roles of cations on the bioflocculation

Table 3.1 Operational conditions during each period

Periods	Conditions
A	Bactopeptone only, no salt added, unadjusted pH
B	Bactopeptone only, 250 mg/l Mg, unadjusted pH
C	Bactopeptone only, 500 mg/l Mg, unadjusted pH
D	Bactopeptone only, 2300 mg/l Na, unadjusted pH
E	Bactopeptone only, 2300 mg/l Na, pH=8
F	Bactopeptone only, 1200 mg/l Na, pH=8
G	Bactopeptone/dextrose, no salt added, unadjusted pH
H	Bactopeptone/Dextrose, 4600 mg/l Na, unadjusted pH

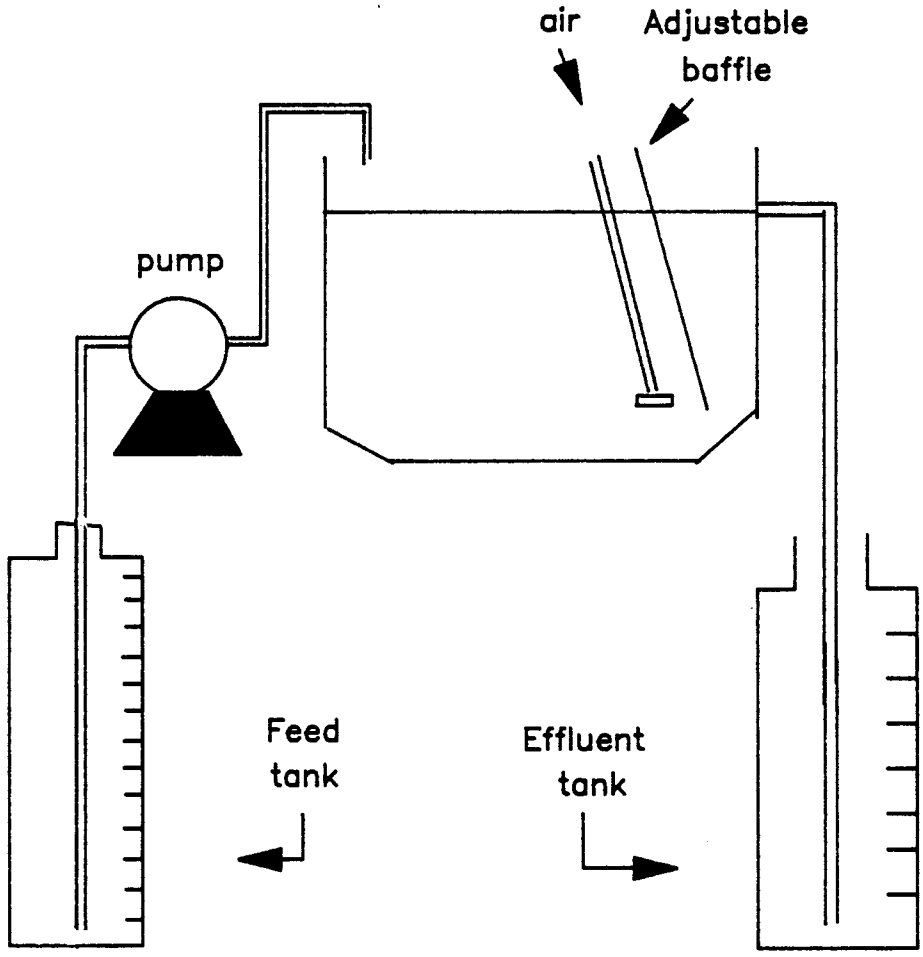

Figure 3.1 Schematic diagram of continous-flow reactor system

TABLE 3.2 Preparation of feed solution

Chemicals	Concentration, mg/l of tap water
Substrate I	
Bactopeptone	311
Substrate II	
Bactopeptone	250
Dextrose	150
NH_4Cl	64
Mineral nutrients	
K_2HPO_4	47.5
KH_2PO_4	292
$FeCl_3.6H_2O$	2.43*

*only in period A,B,C,E.

TABLE 3.3 Characteristics of feed solution

Parameters	Concentration
COD	350 mg/l
TKN	42 mg/l as N
Total P	80 mg/l as P
pH	about 7
Cations, mg/l	
Ca	11.5
Mg	4.3
Fe	0.2
Mn	0.01
Na	13.1
K	31.7

of activated sludge. An increase of one pH unit in the mixed liquor was achieved by addition of 50 ml of 5 N NaOH in the feed solution. The feed solution was pumped continuously by a Master-Flex pump (Cole-Parmer Co, Chicago, IL) at a flow rate of 6 ml per minute, providing a hydraulic retention time of one day. In addition, disinfection of feed lines and tanks by Chlorox solution was executed intermittently (every 3 to 4 days).

The solids retention time (sludge age) was maintained at 8 days by wasting a certain amount of sludge, directly from reactors after lifting the baffles and mixing completely. The exact volume of sludge wasted was obtained according to the following equation:

$$\Theta = \frac{XV}{XVw + Xe\,Q}$$

therefore

$$Vw = \frac{XV - Q\Theta cXe}{\Theta c\,X} = \frac{XV - 8QXe}{8X}$$

where

Θc: sludge age, 8 days

Xe: effluent suspended solids, mg/l

V : reactor volume, liters

Vw: volume of sludge wasted, liters

Q: flow rate, l/day

The wasted sludge was used in the measurement of MLSS, SVI, specific resistance(r), conditioning tests and biopolymer determination.

3.2 General Analysis

COD, TKN, Total Phosphorus, cation(Na, K, Ca, Mg, Fe) were performed to determine the characteristics of feed solution. The MLVSS, MLSS and pH were performed to monitor the operation of reactors. Samples from effluent tanks were detected SS and soluble COD. All analyses followed the procedures described in Standard Methods for the Examination of Water and Wastewater (43). The COD test was performed according to the close reflex dichromate method. Total phosphorus was determined by using the ascorbic method with preliminary digestion. The TKN analysis used Macro-Kjeldahl digestion and titration method. Analysis of cations in feed solution utilized a model 703, Perkin-Elmer (Norwalk, CN) Atomic Adsorption Spectrophotometer.

3.3 SVI

The SVI test was used to determine the settling ability of sludge. One liter of sludge was poured into a one-liter graduated cylinder. The volume of sludge in the cylinder after 30-minute settling was read and the value of SVI was calculated according to the following equation :

$$\text{SVI, ml/g} = \frac{\text{settled sludge volume(ml)} \times 1000}{\text{MLSS, mg/l}}$$

3.4 Specific Resistance (r)

Specific resistance, a measurement of the sludge filtering rate, was used to compare the filtration characteristics of different sludges. The device for specific resistance, drawn in figure 3.2, includes a Buchner funnel, Whatman No. 40 ashless filter papers, a vacuum pump with a vacuum gauge and a 100 ml graduated cylinder.

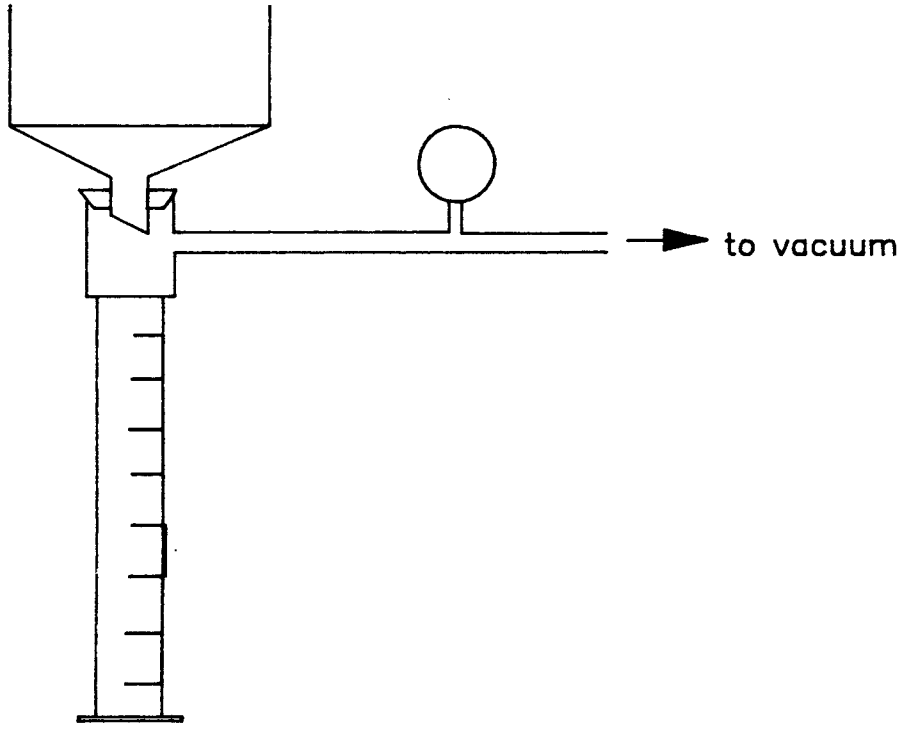

Figure 3.2 Schematic diagram of specific resistance device.

Sludge of 100 ml was filtered through the pre-sealed filter paper by applied a vacuum of 15 inches Hg. The volume of filtrate was recorded with time. The specific resistance was calculated as follows:

$$r = \frac{2PA^2b}{uC}$$

where

r = specific resistance, m/kg

A = area of filter paper, m^2

u = dynamic viscosity, $N*sec/m^2$

b = slope of t/v(sec/ml) versus V (ml) plot

C = solids per unit volume of filtrate, g/cm^3

determined by:

$c = 1/[(100-Ci)Ci]-[(100-Cf)Cf]$

where Ci = initial solids content of influent sludge, %

Cf = final solids content of sludge cake, %

3.5 Sludge Filtration Rate (TTF)

The sludge filtration rate is used to measure the time required for a specific volume of filtrate using the same device for determining the specific resistance (see 3.4 specific resistance). In this study, TTF was used as a parameter in evaluating the performance of conditioners. After conditioning, 100 ml samples was filtered through a Buchner funnel at a pressure of 15 psi and the time for the first 70 ml of filtrate to pass through the filter was measured.

3.6 Specific Oxygen Uptake Rate (SOUR)

The measurement of oxygen uptake rate was accomplished by using a dissolved oxygen probe with a stirrer. Before the measurement of oxygen depletion was begun, 400 ml of sludge was spiked with 10 ml of feed solution and aerated for 10 minutes. The value of SOUR (1/min) was obtained by dividing the slope (mg/l/min) of dissolved oxygen versus time by the MLSS of the sample (mg/l).

3.7 Biopolymer Analysis

The preliminary data determined by Kajornatiyudh (5) have shown that alkali extraction fo!'owed by centrifugation can obtain a maximum biopolymer yield without destroying bacterial cells. Therefore, this method was used to harvest biopolymers throughout this study.

When the reactor operation had reached steady state, 100 ml sludge samples were adjusted to pH 11 with 1 N NaOH solution and were slowly stirred on a Fisher Flex-Mix mixer for one hour. The samples were then centrifuged at 5000 rpm for 10 minutes by a Beckman Model J-21C centrifuge. Supernatants were carefully separated from the precipitated pellet and were stored at 4°C. Analyses of molecular weight distribution, protein, and carbohydrate were performed within 6 hours after biopolymer extraction.

The measurement of unbound biopolymer was achieved by centrifuging non-pH adjusted sludge samples and following the same procedures as the pH adjusted samples.

3.7.1 Gel Filtration

Gel chromatography (gel filtration) was used to separate organic compounds with different molecular weights. The Sephadex G-75 from Phamacia Fine Chemical

(Piscataway, NJ) was chosen in this study because of it suitable fraction range, as shown in Table 3.4.

The schematic diagram of a gel filtration column is shown in Figure 3.3. Dry gel beads were swollen in distilled water for at least 24 hours before used, and then poured into a 1 cm x 30 cm cylindrical glass column. After each trial, gel was taken out , washed with sufficient distilled water and repacted in column in order to prevent interference. The procedures for analyzing samples are listed below:

1. Surface water level was lowered to gel surface and, then, the outlet valve was closed,
2. A 2 ml sample was carefully injected to gel surface and the outlet valve was opened, allowing the sample to drain into gel column. Sample collection started immediately. Approximately 1 ml was collected in each test tube,
3. The outlet valve was closed again and gel column was connected to the eluant distilled water container,
4. Sample collection continued with a fraction collector, about 1 ml in each test tube,
5. The samples in test tubes were measured for absorbance at a wavelength of 280 nm with a Beckman Instruments Inc.(Irvine, CA) DU-6 Spectrophotometer.

The calibration of gel column was carried out by using 5 known molecular weight substances (see Appendix A-1 and A-2).

For determining protein and carbohydrate in fractionated samples, same procedure was repeated but about 4 ml was collected in each test tube.

Table 3.4 Sephadex gels and their properties

Gel	Dry Particle Diamater, um	Fractionation Range	
		Peptide & Globular Protein	Dextran
G-25	50-100	1,000-5,000	100-500
G-50	50-150	1,500-30,000	500-10,000
G-75	10-40	3,000-70,000	1,000-50,000

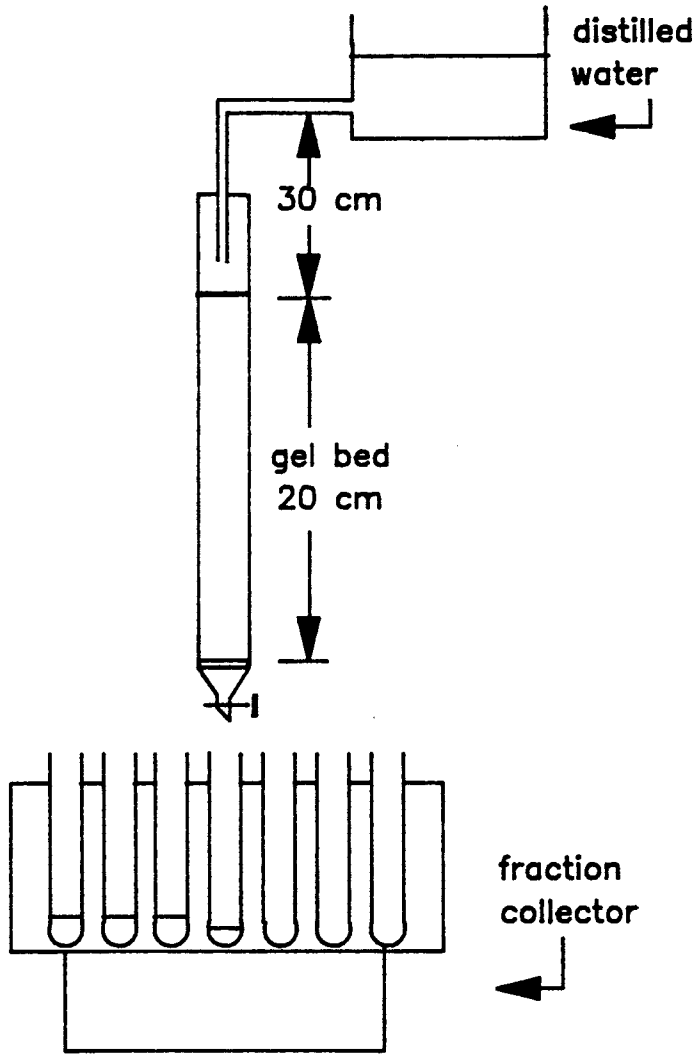

Figure 3.3 Schematic diagram of gel filtration device.

3.7.2 Chemical Analysis

The fractionated samples from gel filtration were performed protein and carbohydrate measurements. The protein content was measured as Bavine Serum Albumin (BSA) by the Lowry procedures described by Cooper (44). The carbohydrate concentration was determined as glucose by the phenol-sulfuric acid method described by Duois et al. (45). The detailed reagents and procedures are described below:

Protein

Reagents:

a. 100 g sodium carbonate in 100 ml (final volume) distilled water;

b. 1 g Copper sulfate in 100 ml (final volume) distilled water;

c. 2.177 g sodium potassium tartrate in 100 ml (final volume) distilled water;

d. 5 ml of 2 N Folin-Phenol reagent (available premixed) added to 50 ml distilled water.

Procedures:

1. A mixed reagent was prepared in a proportion of 20 ml of reagent A, 1 ml of reagent B and 1 ml of reagent C;
2. 1 ml of the mixed reagent was added to 2 ml sample and vortexed immediately before went to next sample;
3. The samples were incubated at room temperature for 15 minutes;
4. While the samples were incubating, reagent D was prepared;

5. After incubation, 3 ml of reagent D was added to the sample and vortexed immediately before went to next sample;
6. Samples were incubated at room temperature for additional 45 minutes;
7. The absorbance of each sample was measured against a reagent blank at 660 nm with a Bechman Instrument, Inc. DU-5 spectrophotometers;
8. Protein concentrations of samples were determined according to the standard curve prepared by BSA (see Appendix A-3).

Carbohydrate

Reagents:

A. Phenol, 5 % (weight/weight) solution in distilled water

B. concentrated sulfuric acid. reagent grade.

Procedures:

1. 1 ml of reagent A was added to 2 ml sample and vortexed immediately;
2. Before went to next sample, 5 ml of concentrated sulfuric acid was added and vortexed immediately;
3. After incubation for 30 minutes at room temperature, the absorbance of each sample was measured against a reagent blank at 490 nm using a Busch and Lomb(Rochester, NY) spectronic 20 spectrophotometer;
4. a standard curve for carbohydrate determination (see Appendix A-4) was obtained by a standard glucose solution.

IV Results and Discussion

In this chapter data for biopolymer characteristics, sludge settling and dewatering properties and sludge conditioning under various operating conditions are presented and discussed.

A comparison of biopolymer characteristics was based on three parameters: gel filtration profile to analyze the molecular weight distribution, normalized total biopolymer including the protein and carbohydrate content to quantify the amount of biopolymers produced by microorganisms in each period, and the ratio of unbound to total biopolymer to evaluate the extent of biopolymer binding. Table 4.1 summarizes the protein and carbohydrate content and their distribution (ratio of unbound to bound biopolymer) derived from the values tabulated in Table B-1 through B-8 in Appendix B.

In addition to the reactor study, conditioning tests on the sludges were conducted. In these tests, combinations of cationic polymer (Betz 1195) and salts (Mg and/or Na) were used to elucidate the influence of monovalent and divalent cations on sludge conditioning with polymers.

Biopolymer Characteristics

Bactopeptone Fed Sludge

In order to determine the influence of the operational variables, it was necessary to first characterize biopolymers produced during the bactopeptone feeding period. The bactopeptone feed period served as the control period to which other operational conditions are compared. As shown in Figure 4.1, the bactopeptone fed sludge generated biopolymer with three major molecular weight fractions. The relative elution time and molecular weight of each fraction are listed in Table 4.2. These fractions are classified, based on the molecular weight, as HMW fraction (MW greater

Table 4.1 Content and distribution of biopolymers

	Period		Total % MLVSS	Unbound mg/l	Unbound % Total	Bound % MLVSS
		HMW	18	44.4	37.8	11.2
	A	MMW	4.6	23.6	78.1	0.5
		LMW	1.0	9.4	100	0.6
		HMW	1.1	3.6	43	0.8
	B	MMW	2.4	2.5	13.4	2.0
		LMW	0.5	5.8	1.6	0.3
Portein		HMW	5.6	18.8	45.1	4.8
	C	MMW	10.1	30.6	40.8	6.0
		LMW	8.2	41.9	69.4	3.4
		HMW	12.8	20.4	22.8	10.1
	D	MMW	19.8	20.0	10.7	9.3
		LMW	2.5	3.1	6.5	1.2
		HMW	18.3	25	18.4	14.3
	E	MMW	20.2	11.2	48.7	18.6
		LMW	2.1	5.8	36.7	0.6
		HMW	13.8	20.8	23.1	10.6
	A	MMW	0	0	0	0
		LMW	14.4	0	0	14.4
		HMW	10.3	44.7	56	6.3
	B	MMW	6.1	27	57	2.8
		LMW	3	30.1	100	0.4
Carbohydrate						
		HMW	7.3	-	-	-
	C	MMW	3.2	-	-	-
		LMW	4.2	14	44.7	2.3
		HMW	14	0.9	0.9	13.7
	D	MMW	2.9	-	-	-
		LMW	1.3	2.6	3.3	2.9
		HMW	13.2	105	100	0
	E	MMW	3.1	27.4	100	0
		LMW	2.1	18.8	100	0

(continued)

Table 4.1 Content and distribution of biopolymers (cont.)

	Period		Total % MLVSS	Unbound mg/l	Unbound % Total	Bound % MLVSS
Protein	F	HMW	20.2	19.4	10.2	18.1
		MMW	19.8	20.0	10.7	17.6
		LMW	2.8	18.4	69.7	1.3
	G	HMW	30.1	7.8	3.9	29.1
		MMW	12.4	4.2	5.0	10.7
	H	HMW	30.3	18.6	9.2	20.9
		MMW	11.8	12.2	15.1	8.5
Carbohydrate	F	HMW	16.2	61	39	9.7
		MMW	1.7	12	75.9	0.4
		LMW	0.4	0	0	0.4
	G	HMW	6.0	30.2	75	0.9
		MMW	5.4	22.2	62	0.3
	H	HMW	5.3	2.8	7.8	4.7
		MMW	1.8	1.4	12.2	1.5

- : off-scale reading

* total , % MLVSS :

$$\frac{\text{(conc. in tube, mg/l)x(volume collected)}}{\text{volume of sample subjected to column,ml}} \times \frac{100}{\text{MLVSS}}$$

* bound, % MLVSS:

$$\frac{\text{(con. of total, mg/l)-(conc. of unbound, mg/l)(100)}}{\text{MLVSS, mg/l}}$$

* unbound, mg/l:

$$\frac{\text{(con. in tube,mg/l)x(volume collected)}}{\text{volume of sample subjected to column,ml}}$$

* unbound, % total:

$$\frac{\text{unbound, mg/l(100)}}{\text{total, mg/l}}$$

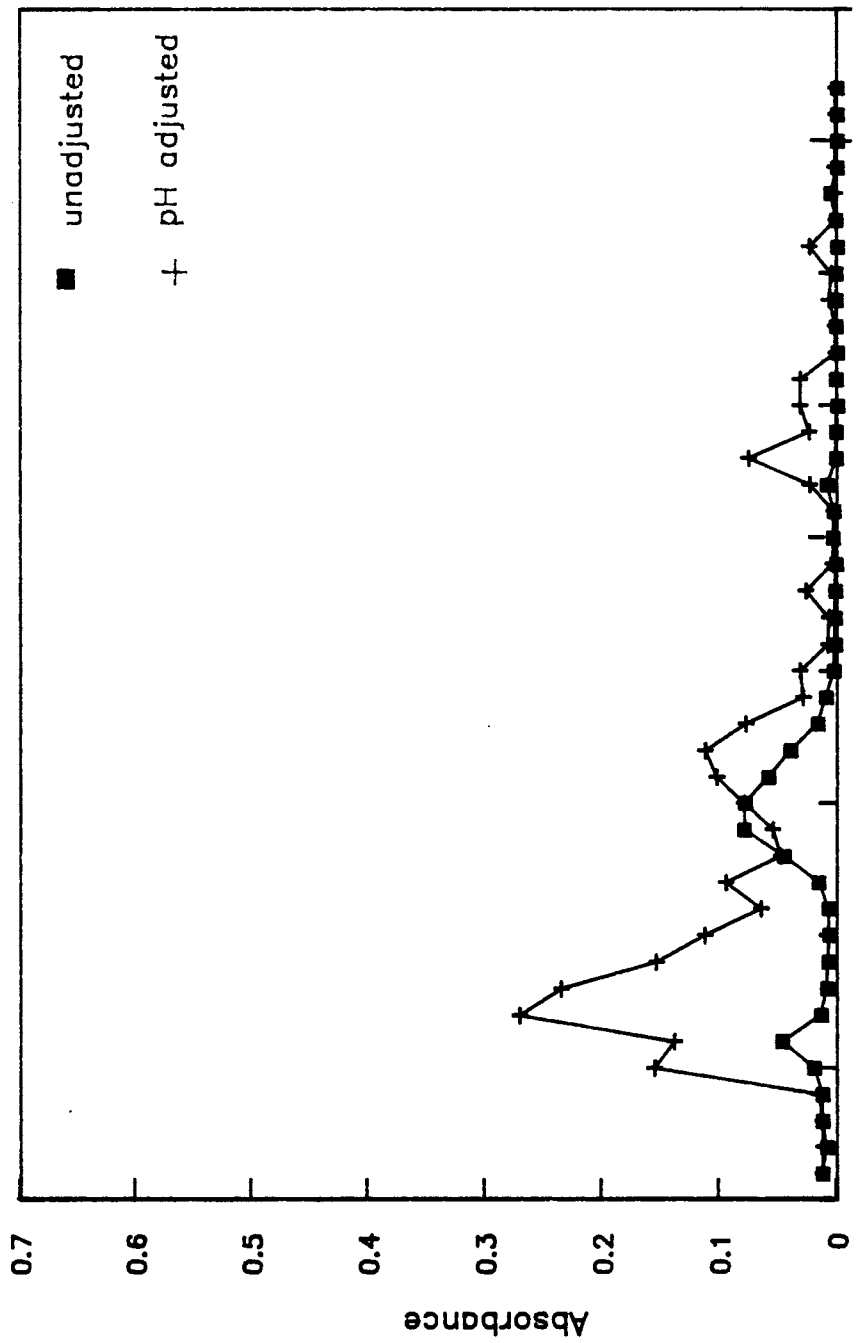

Figure 4.1 Gel filtration of biopolymer in period A (Bactopeptone, no salt, unadjusted pH)

Table 4.2 Molecular weight distribution of biopolymers

Group	Elution Time	Molecular Weight
HMW	before 12 ml	> 40,000
MMW	12- 24 ml	between 40,000 and 10,000
LMW	after 24 ml	< 10,000

than 40,000), MMW fraction (MW between 40,000 and 10,000) and LMW fraction (MW less than 10,000). The HMW and MMW fractions measured in the bactopeptone fed sludge agree with the finding from other studies (5,46,47) which show a similar molecular weight distribution. However, there is little information about the LMW fraction. This could be due to the various methods in extracting biopolymers and different gel used to separate organic compounds. In addition, the presence of LMW biopolymer may be the result of degradation (depolymerization) of some HMW or MMW biopolymer during alkali extraction.

The extent of binding can be estimated from the height between the unadjusted and pH adjusted gel filtration profile. In figure 4.1, it appears that almost all the HMW and LMW biopolymers were bound to sludge flocs, whereas, the majority of MMW biopolymer was in the unbound form.

Further analyses of the chemical composition in each fraction show a large bound fraction of protein and carbohydrate. The data in Table 4-1 show that these fractions were composed of protein and carbohydrate in quite different amounts and degrees of binding. The HMW fraction contained more protein than carbohydrate, but the bound fraction of protein was less than that of carbohydrate. The MMW fraction was almost entirely protein and was mostly in free form. The LMW fraction contained a small amount of free protein and a large quantity of bound carbohydrate. These data indicate that sludge fed with bactopeptone produced biopolymers which contained a slightly higher amount of carbohydrate than protein. Most of the carbohydrate was bound to sludge flocs, whereas, more than a half of protein was free in solution.

Some of the protein characterized as biopolymer may be undegraded bactopeptone. However, bactopeptone is a readily degradable substrate and the sludge age of 8 days in the system provided a substrates-limiting situation; therefore,

the portion of biopolymer attributed to undegraded bactopeptone is considered to be minor.

Influence of Mg on Biopolymer Characteristics

In this section, results from period B (250 mg/l addition of Mg) and Period C (500 mg/l addition of Mg) are presented and compared to those from period A (no Mg added). Figure 4.2 and Figure 4.3 show the gel filtration profiles of samples from period B and period C, respectively. For easier comparison the results in Table 4.1 are plotted as bar charts. The amounts of protein and carbohydrate generated in these periods are presented in Figure 4.4, and the extent binding of these biopolymers is shown in Figure 4.5.

As seen in Figure 4.2 and 4.3, the UV absorbance of total HMW biopolymer was significantly decreased for both 250 and 500 mg/l Mg(+2) addition. The MMW biopolymer did not change as drastically as the HMW fraction. One possibility for these changes is that during the extraction process coagulation of biopolymer occurred, resulting in low absorbance reading. It is known that magnesium precipitates as magnesium hydroxide at high pH. Novak and Haugan (35) postulated that metal hydroxide can coagulate biocolloids by supplying an adsorption surface. Therefore, it is possible that coagulation occurred during the alkali extraction process. The biopolymer-Mg(OH)(2) complex was then co-precipitated by centrifugation or was less sensitive to UV light due to the change in biopolymer nature and resulted in low absorbance readings.

The analyses of chemical composition, as shown in Figure 4.4, further confirm the possibility of the occurrence of coagulation during biopolymer extraction. Lower values of total biopolymers, particularly in HMW protein were observed at 250 mg/l of

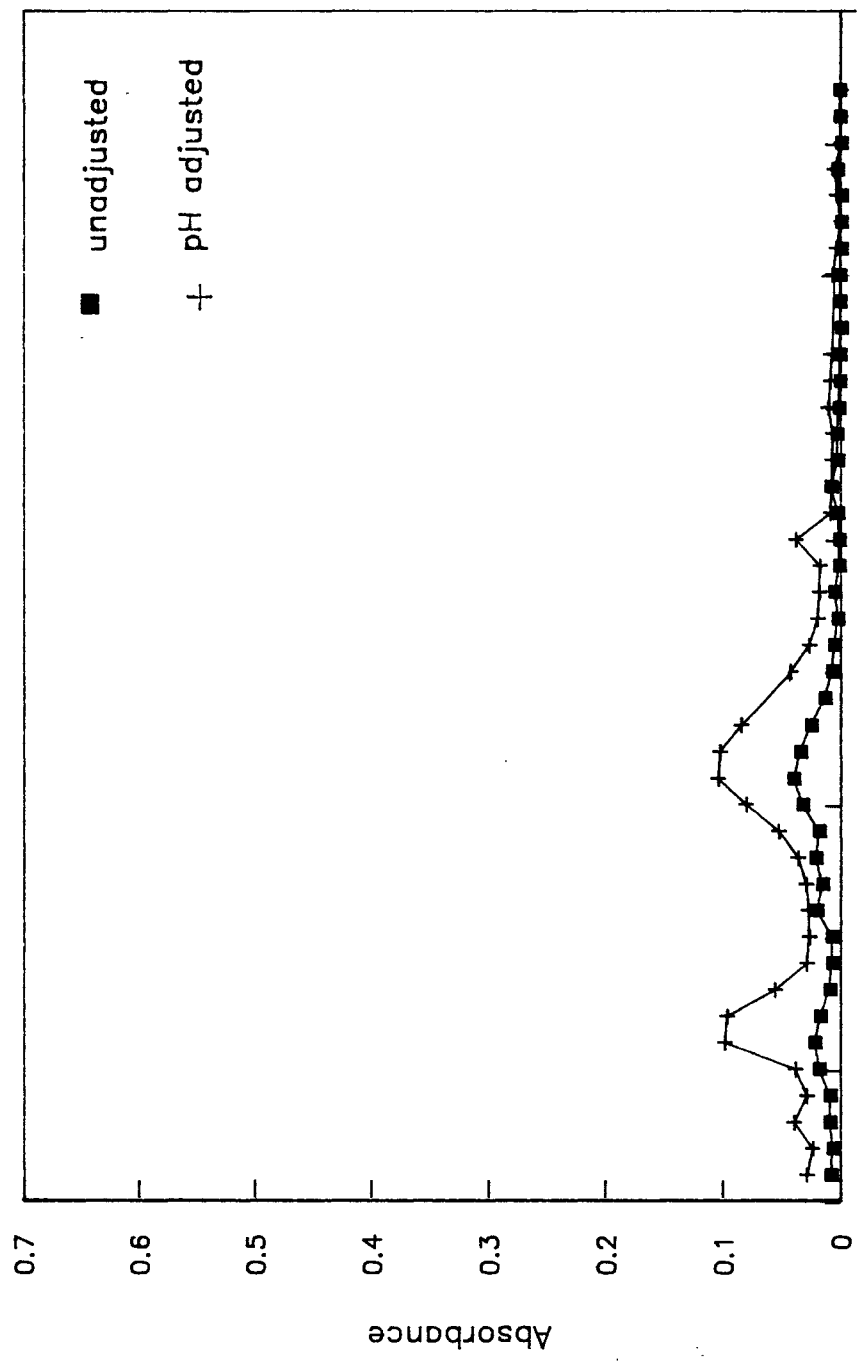

Figure 4.2 Gel filtration of biopolymer in period B
(Bactopeptone, 250 mg/l Mg, unadjusted pH)

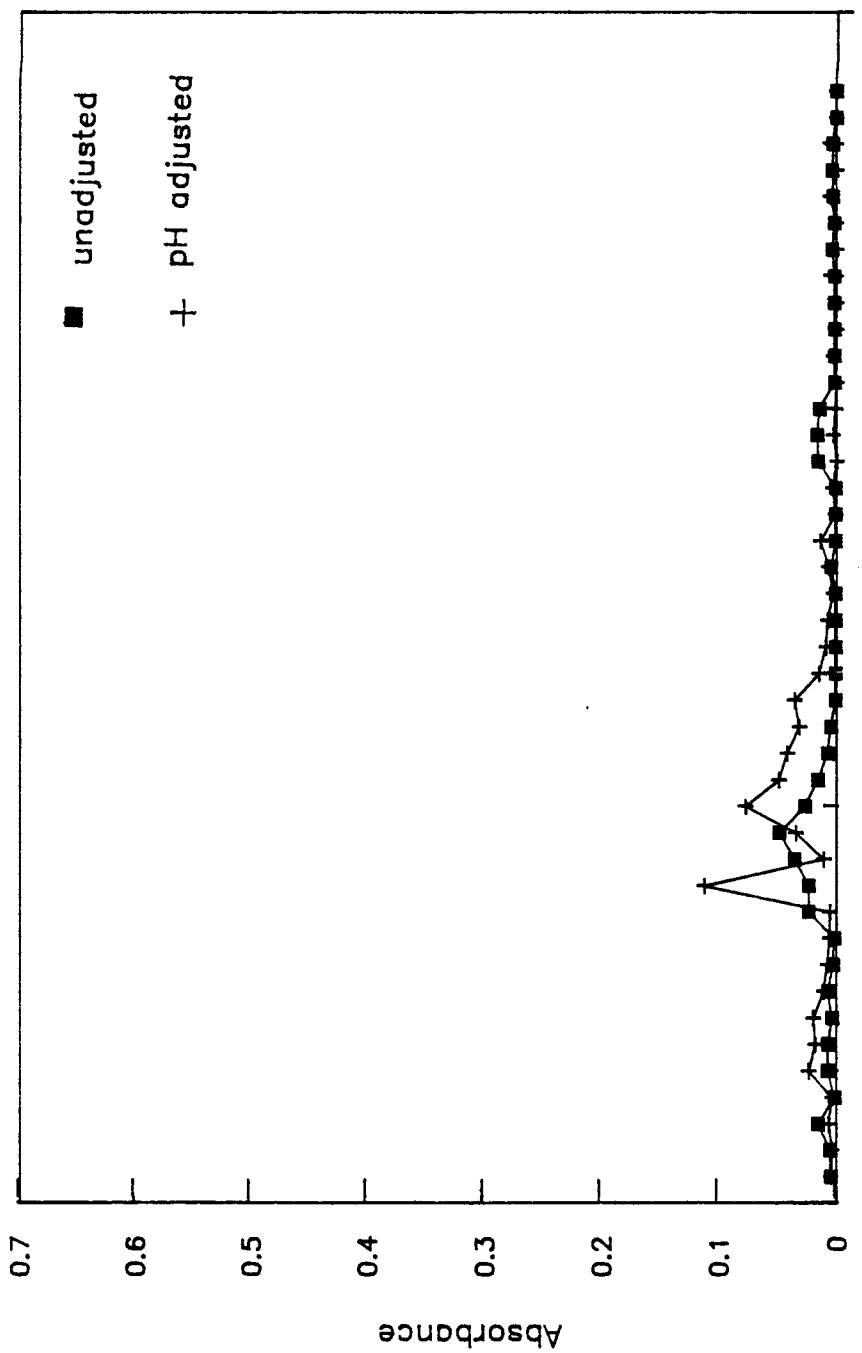

Figure4.3 Gel filtration of biopolymer in period C
(Bactopeptone, 500 mg/l Mg, unadjusted pH)

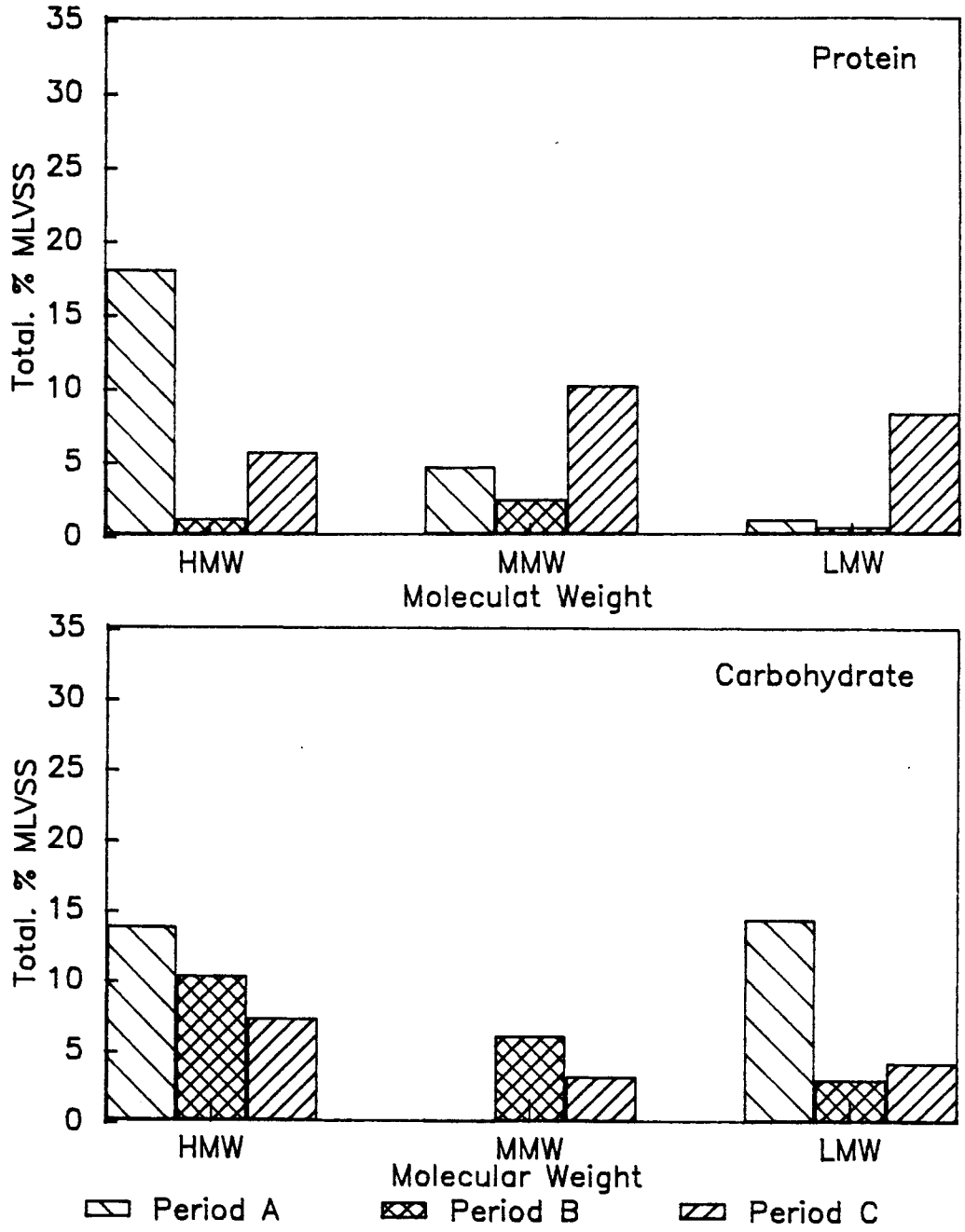

Figure 4.4 Effects of Mg addition on biopolymer composition, period A: no salt, period B: 250 mg/l Mg, period C: 500 mg/l Mg.

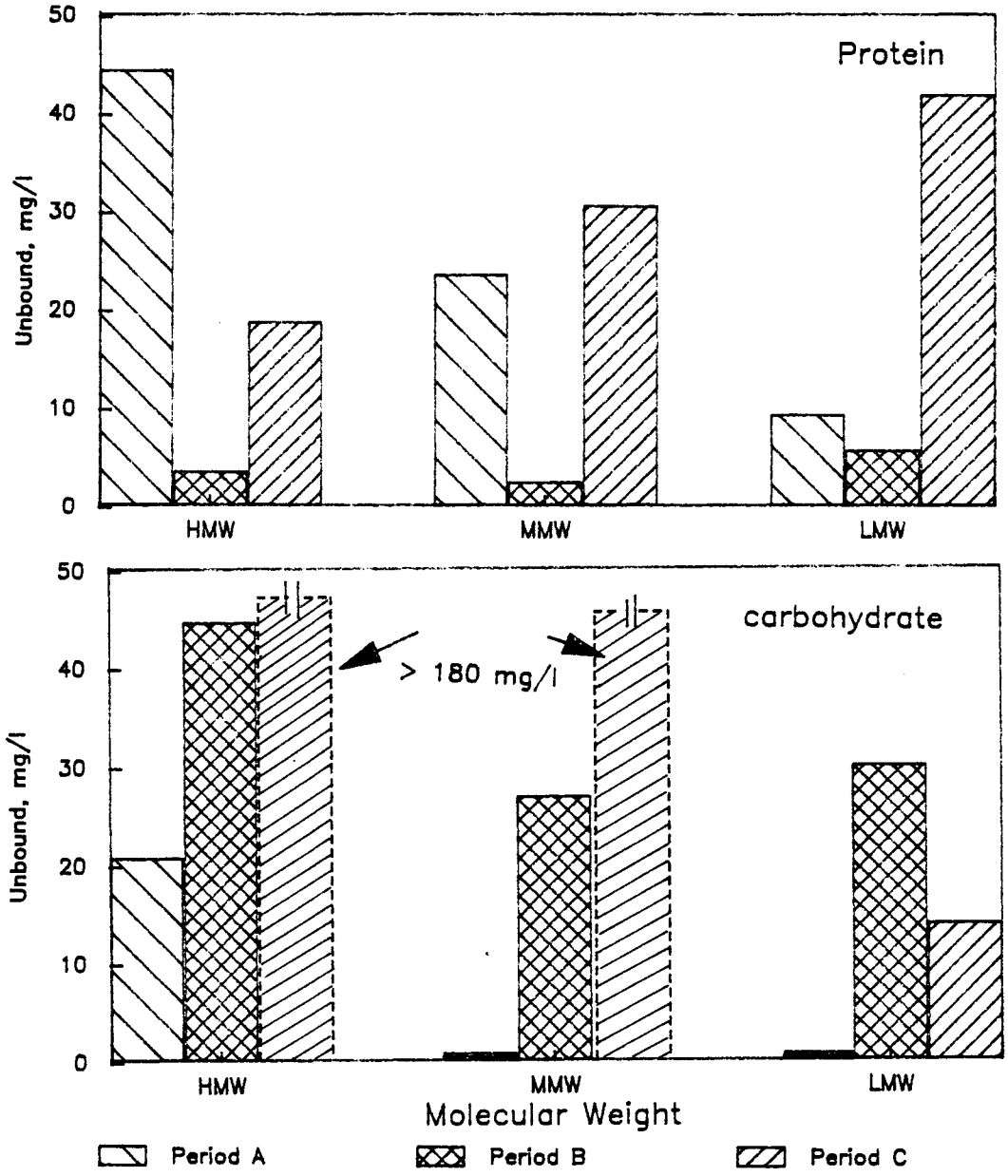

Figure 4.5 Variances in biopolymer binding by Mg addition, period A: no salt, period B: 250 mg/l Mg, period C: 500 mg/l Mg.

Mg. As more Mg was added, the carbohydrate decreased but protein increased, indicating the different adsorption character between protein and carbohydrate.

An additional gel filtration study was conducted to directly determine if biopolymers were coagulated by magnesium hydroxide during alkali extraction. The results of this experiment will be presented in the latter section but support the coagulation explanation.

It is likely that coagulation during biopolymer extraction interfered with the total biopolymer determination. Therefore, it is not possible to measure the binding extent of biopolymers by analyzing the ratio of unbound to total biopolymer. However, it is still possible to estimate the extent of binding by considering changes in the concentration of free biopolymer.

It appears that there is an optimal dose of Mg for improving binding of protein, and over that concentration, protein will be dislodged into bulk solution. As indicated in Figure 4.1 through 4.3, 250 mg/l of Mg mainly reacted with unbound MMW biopolymer and resulted in about 50 percent of reduction in UV absorbance. No significant changes in HMW and LMW fractions were observed. As the Mg concentration increased to 500 mg/l, unbound HMW biopolymer decreased but MMW biopolymer released.

The analyses of unbound protein and carbohydrate concentration give a more detailed understanding. As shown in Figure 4.5, the addition of 250 mg/l of Mg decreased all unbound protein but increased all unbound carbohydrate. As more Mg was added, protein was released. This indicates that the binding of free protein is influenced by salt concentration. The optimum concentration for Mg to have the best binding of free protein is in the range of around 250 to 500 mg/l, and over 500 mg/l, Mg will have an adverse effect on protein binding.

During carbohydrate determination, a dark brown color was observed in the samples after reagent addition and resulted in off-scale reading in UV absorbance and uncertainty in analyzing the change of carbohydrate. Such an off-scale reading may be due to contaminations or because there actually was an extremely high concentration of carbohydrate in the samples.

Influence of Na and pH on Biopolymer Characteristics

In this section, data in period A (no salt, unadjusted pH), period D (2300 mg/l Na, unadjusted pH), period E (2300 mg/l Na, pH = 8) and period F (1200 mg/l Na, pH = 8) are discussed and correlated to biopolymer characteristics. Figure 4.6 through 4.8 present the gel filtration profiles of samples from each period. The curves of pH-adjusted samples show that total biopolymer changed significantly, whereas unbound biopolymer did not change a lot. Differences presented in Figure 4.1 and 4.6 indicate that addition of 2300 mg/l of Na increased the yield of HMW biopolymer. This increase in biopolymer yield may be caused from the stimulation of biopolymer generation, or from a greater release of bound biopolymer at the presence of Na during alkali extraction. Such an increase in biopolymer was not significantly influenced by a raise in pH. As shown in Figure 4.6 and 4.7, while the pH in system was raised from 7 to 8 but the Na concentration was same, the peaks remained similar. As Na concentration was reduced to a half (1200 mg/l) but pH was maintained at 8, the HMW fraction decreased. No significant change in total MMW biopolymer was observed except the peak shape, as shown in these figures, indicating that the effect on the MMW fraction is system specific. Therefore, these suggest that the production of HMW biopolymer is influenced by the concentration of Na rather than increase in pH. On the other hand, both pH and Na have influence on the MMW fraction.

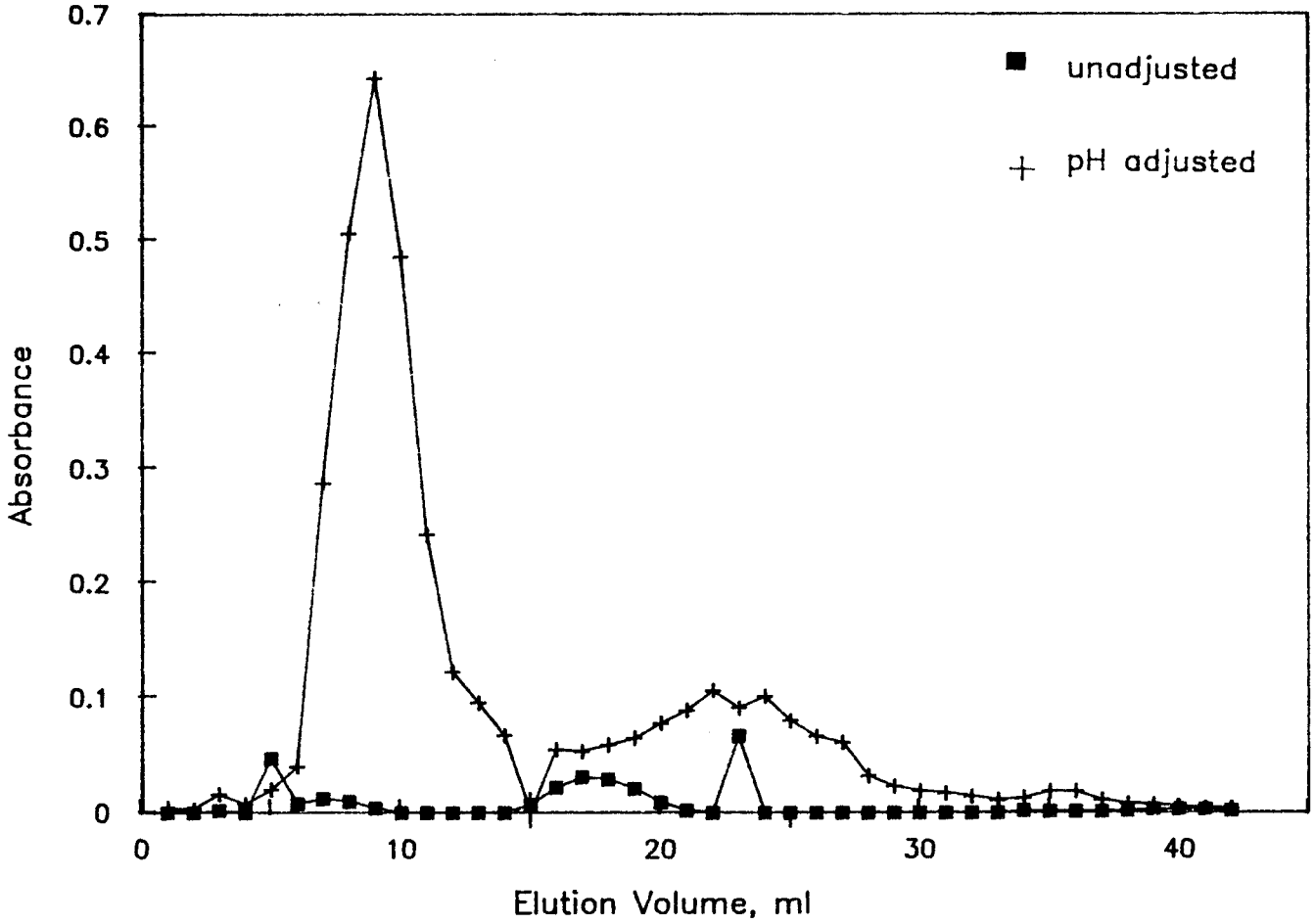

Figure 4.6 Gel filtration of biopolymer in period D
(Bactopeptone, 2300 mg/l Na, unadjusted pH)

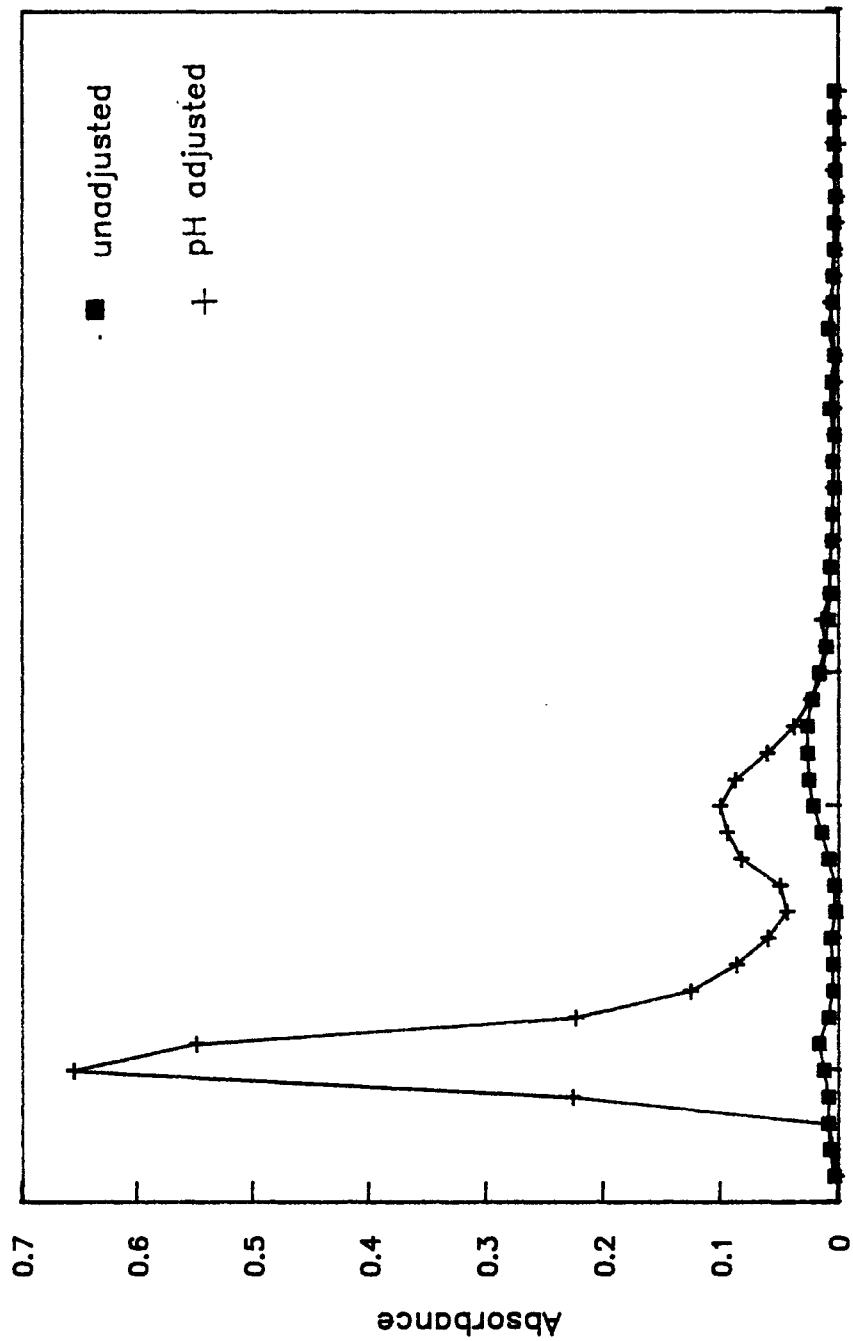

Figure 4.7 Gel filtration of biopolymer in period E (Bactopeptone, 2300 mg/l Na, pH=8)

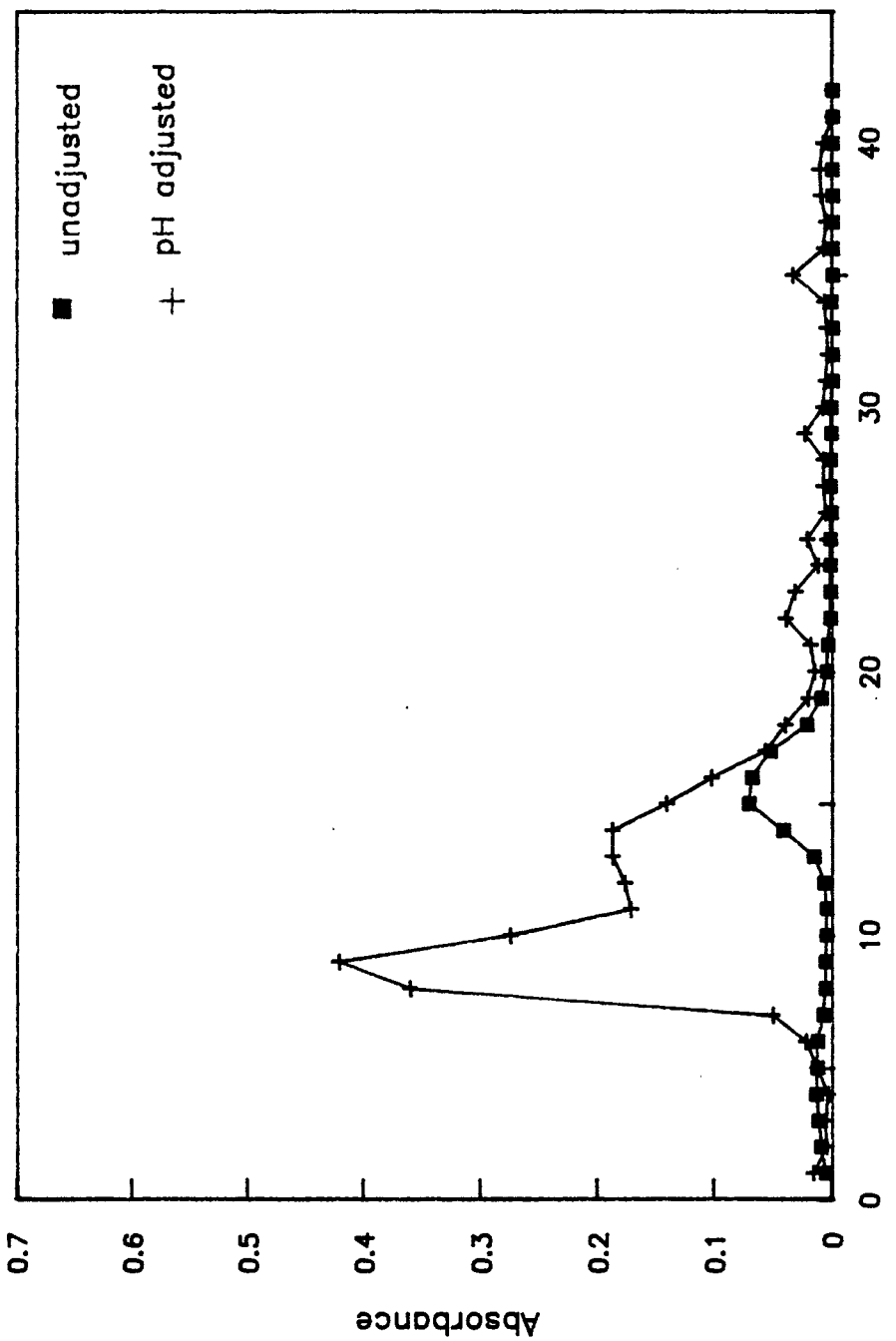

Figure 4.8 Gel filtration of biopolymer in period F
(Bactopeptone, 1200 mg/l Na, pH=8)

The results from protein and carbohydrate analysis show some inconsistencies as compared to those from gel filtration. Figure 4.9 shows the total biopolymer produced in each period. Unlike the data shown in the gel filtration profiles, change in Na or pH did not vary the yield of HMW biopolymer. On the contrary, an increase in total MMW protein at period D, E and F is observed. It appears that both Na and pH have influence on the binding degree of biopolymer. As seen in Figure 4.10, operation at 2300 mg/l of Na and unadjusted pH (period D) improved the binding of biopolymers, particularly those in the HMW fraction, but as the pH was raised to 8, the binding of carbohydrate was worsened. Almost all the carbohydrate was in free form during period E. When the Na concentration was reduced, carbohydrate release was less, but it was still very significant. According to Pavoni et al (11), as pH increases, more functional groups on biopolymers ionize with correspondingly more reaction sites been created. Also, as mentioned by Weber (48), to be effective in coagulation, a polymer molecule must interact with sites on the surface of the colloidal particles. Therefore, if additional particles are not available, this increased surface negativity will create a large repulsive force between biopolymers or between biopolymers and negatively charged particles. As the repulsive force becomes large enough to overcome the original physical interactions which allow biopolymers to adsorb on particles, biopolymers are bound less tightly to sludge flocs. When pH was increased to high level, such as the pH for biopolymer extraction, deflocculation occurred and biopolymers were released from sludge flocs. This probably can explain the reason that carbohydrate released to solution in period E and also the mechanism for biopolymer stripping by increase of pH.

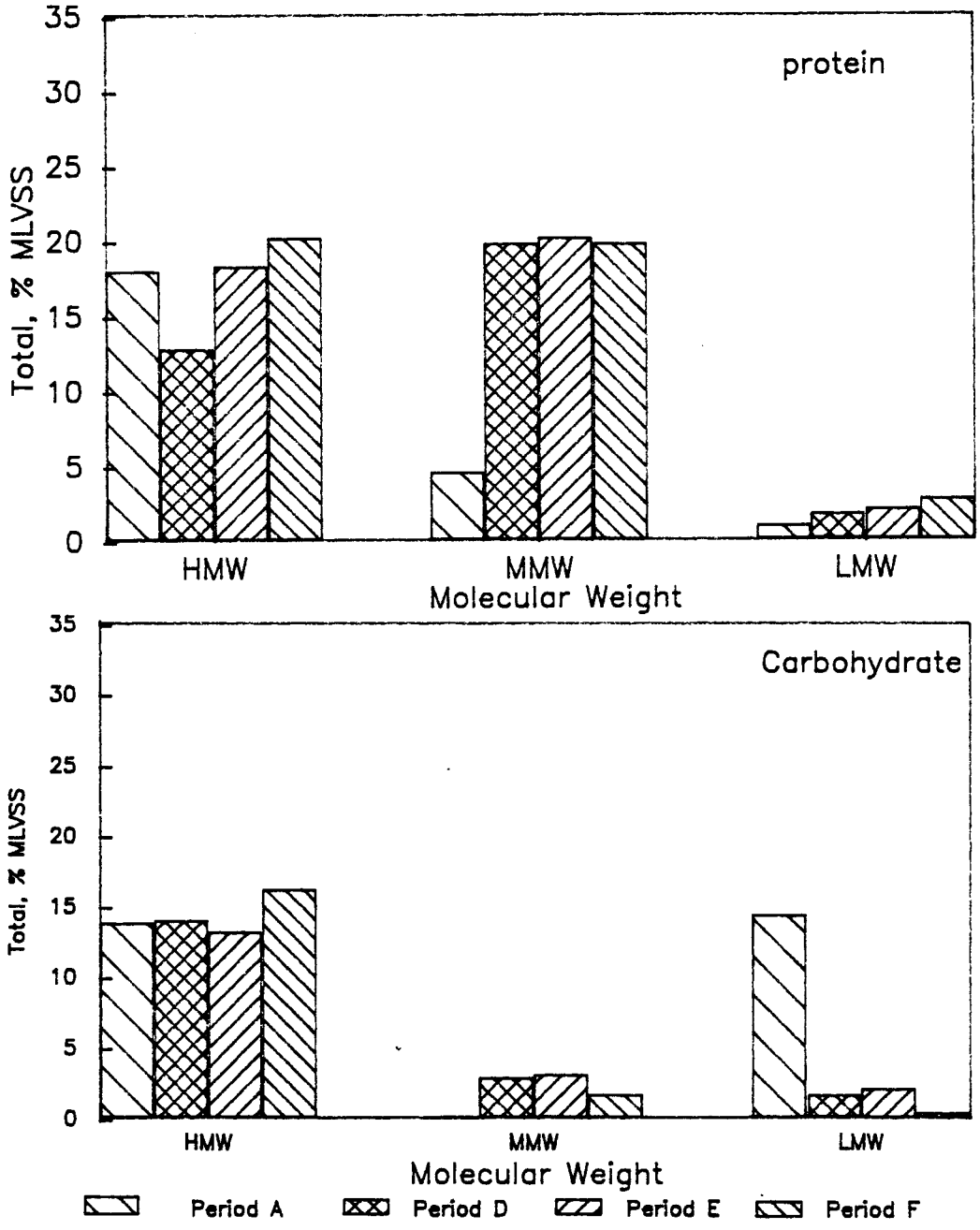

Figure 4.9 Variances in biopolymer production by Na addition and change of pH, period A: no salt, unadjusted pH, period: 2300 mg/l Na unadjusted pH, period E: 2300 mg/l Na, pH=8 period F: 1200 mg/l Na, pH=8.

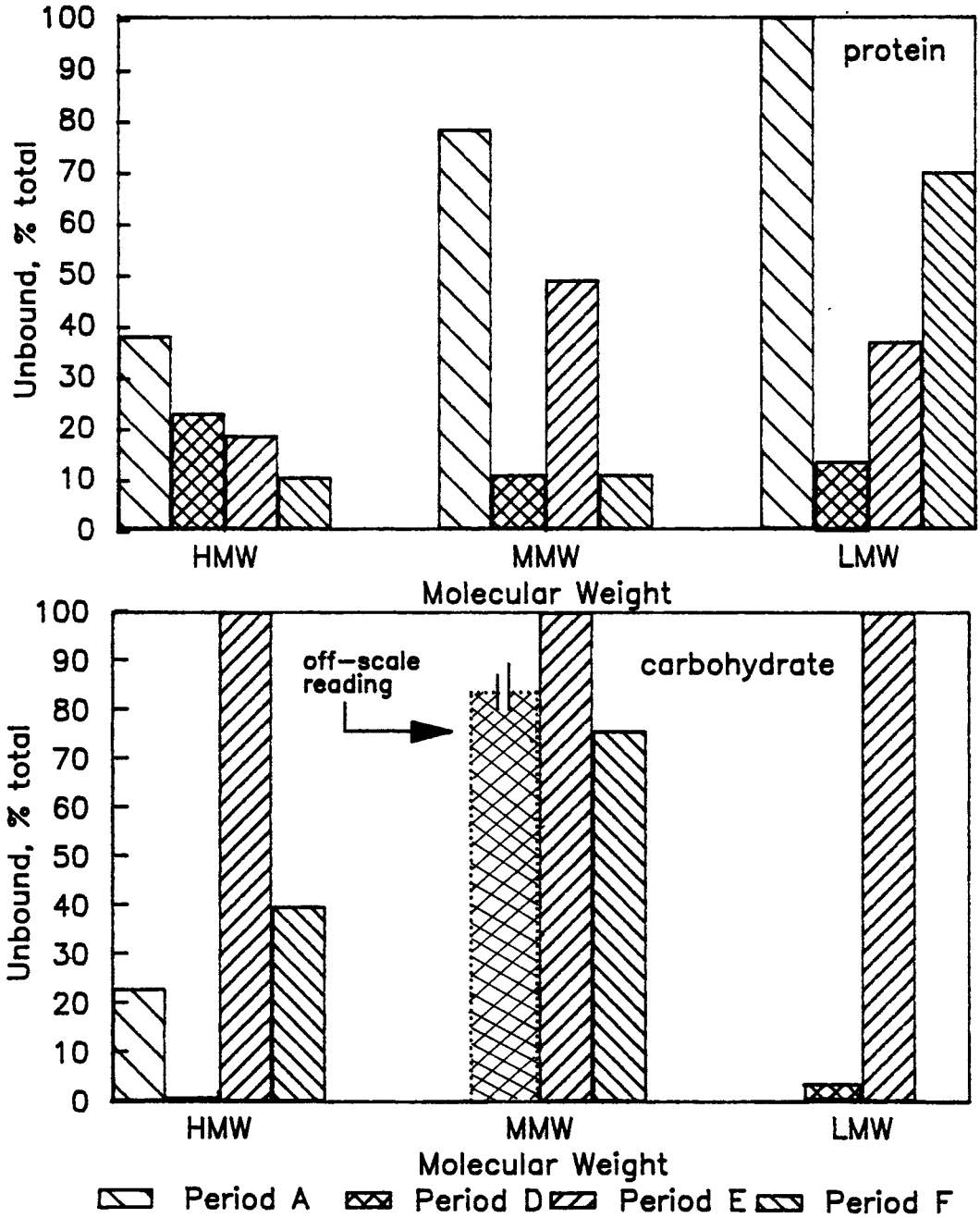

Figure 4.10 Variances in biopolymer binding by Na addition and change of pH, period A: no salt, unadjusted pH, period D: 2300 mg/l Na, unadjusted pH, period E: 2300 mg/l Na, pH=8, period F: 1200 mg/l Na, pH=8.

Influence of Dextrose and 4600 mg/l Na on Biopolymer Characteristics

Several studies have shown that differences in organic substrate can generated an activated sludge with different biopolymer characteristics. In addition to organic substrate, nutrients such as nitrogen determine the behavior of biomass. In this study, the system was operated on a bactopeptone/dextrose feed under nitrogen-rich conditions.

As seen in figure 4.11, the bactopeptone/dextrose fed sludge generated biopolymers in only HMW and MMW fractions. With the addition of nitrogen, as indicated in Figure 4.12 and 4.13, protein generation was stimulated and bound tightly to flocs. Carbohydrate production, on the other hand, was reduced and only a minor portion was attached to the floc surface.

The biopolymer characteristics of bactopeptone/dextrose fed sludge agree reasonable well with those found by Kajornatiyudh (5), who indicated that a lower amount of extractable carbohydrate biopolymer is generated and only about one-half of it is bound to flocs for the glucose fed sludge supplied with nitrogen. Moreover, nitrogen supplement improved the binding of the protein biopolymer.

When 4600 mg/l of Na was applied in system, biopolymer production did not change but the binding of carbohydrate was improved; correspondingly, protein was released.

It appears that, from Figure 4.5, 4.10, and 4.13, there is a competition between protein and carbohydrate for binding on floc surfaces. The changes in the amount of unbound protein biopolymer generally corresponded to the inversely changes in that of unbound carbohydrate biopolymer. Since only limited adsorption sites on particle surface are available, competition may occur between the increased biopolymer.

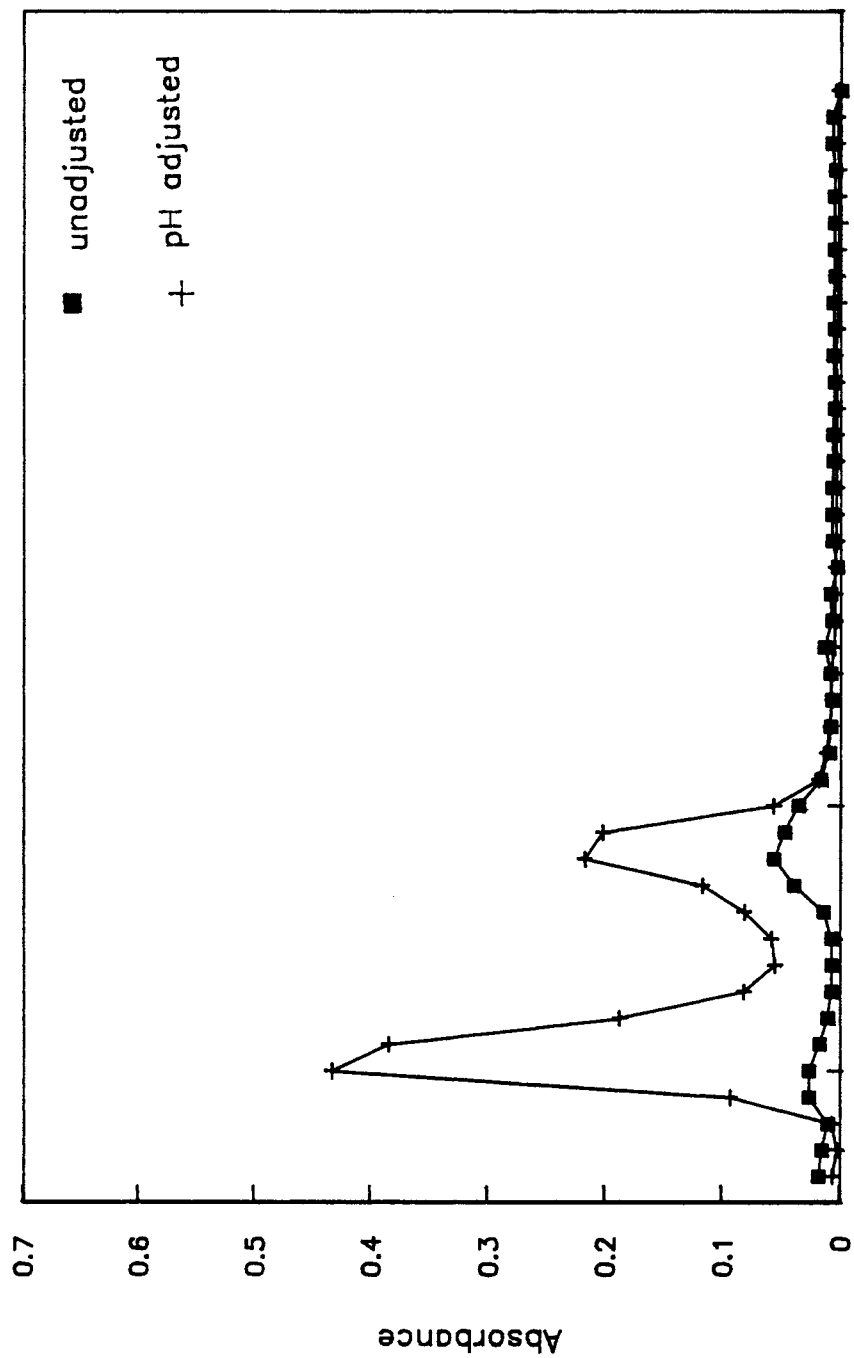

Figure 4.11 Gel filtration of biopolymer in period G
(Bactopeptone/Dextrose, no salt, unadjusted pH)

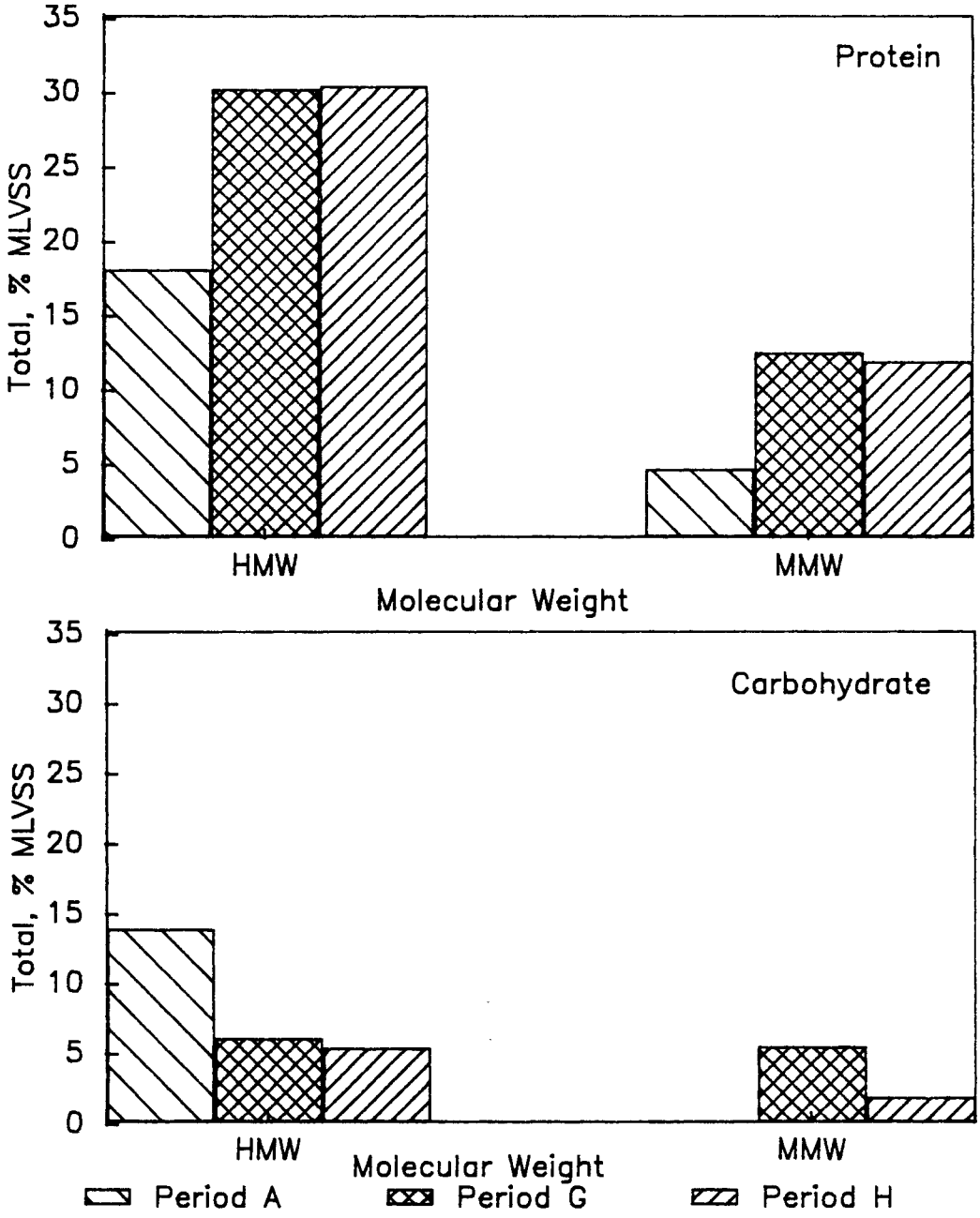

Figure 4.12 Variances in biopolymer production by change of substrate and Na addition, period A: Bactopeptone, no salt, period G: Bactopeptone/Dextrose, no salt, Period H: Bactopeptone/Dextrose, 4600 mg/l Na.

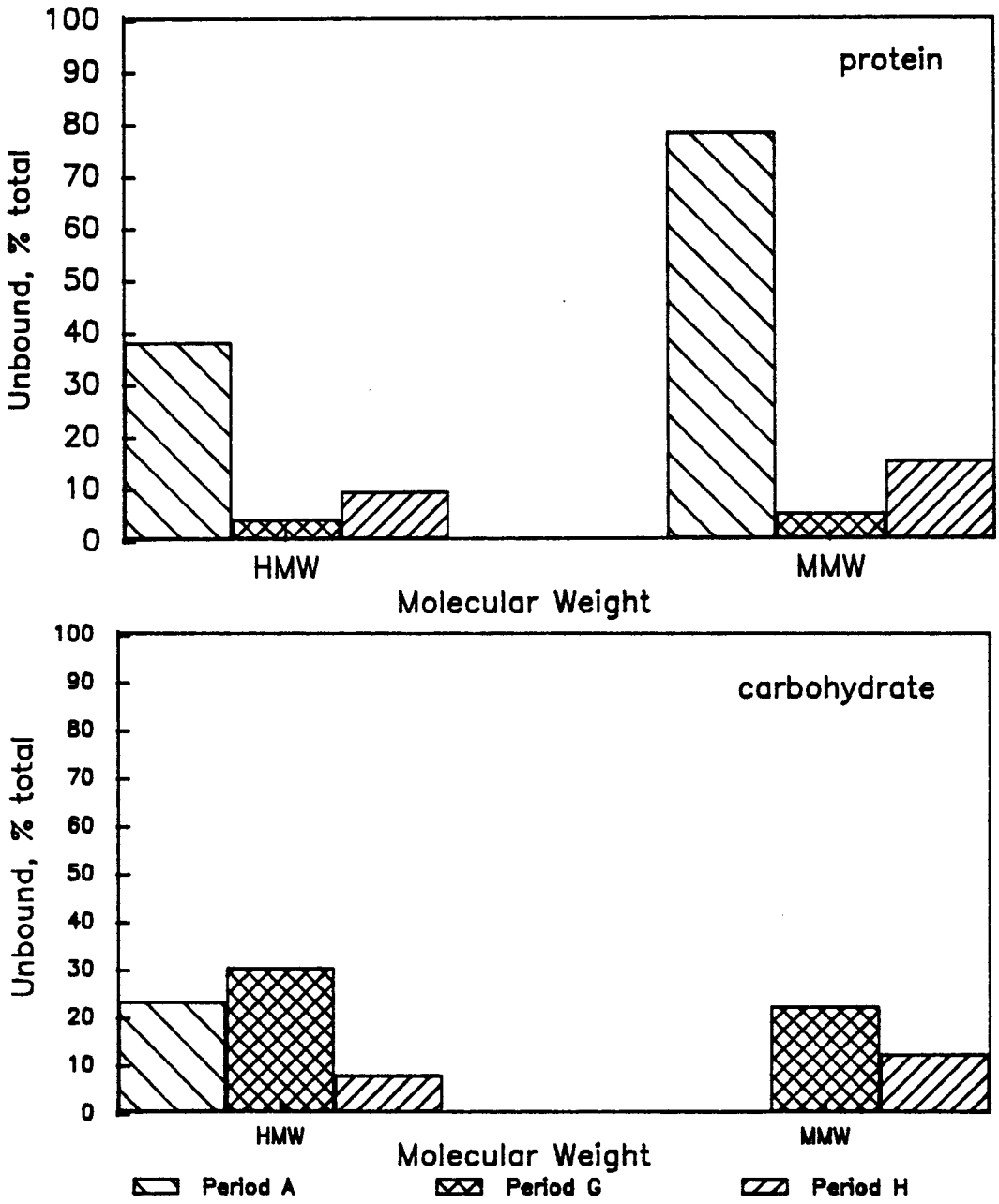

Figure 4.13 Variances in biopolymer binding by change of substrate and Na addition, period A: Bactopeptone, no salt, period G: Bactopeptone/Dextrose, no salt period H: Bactopeptone/Dextrose, 4600 mg/l Na.

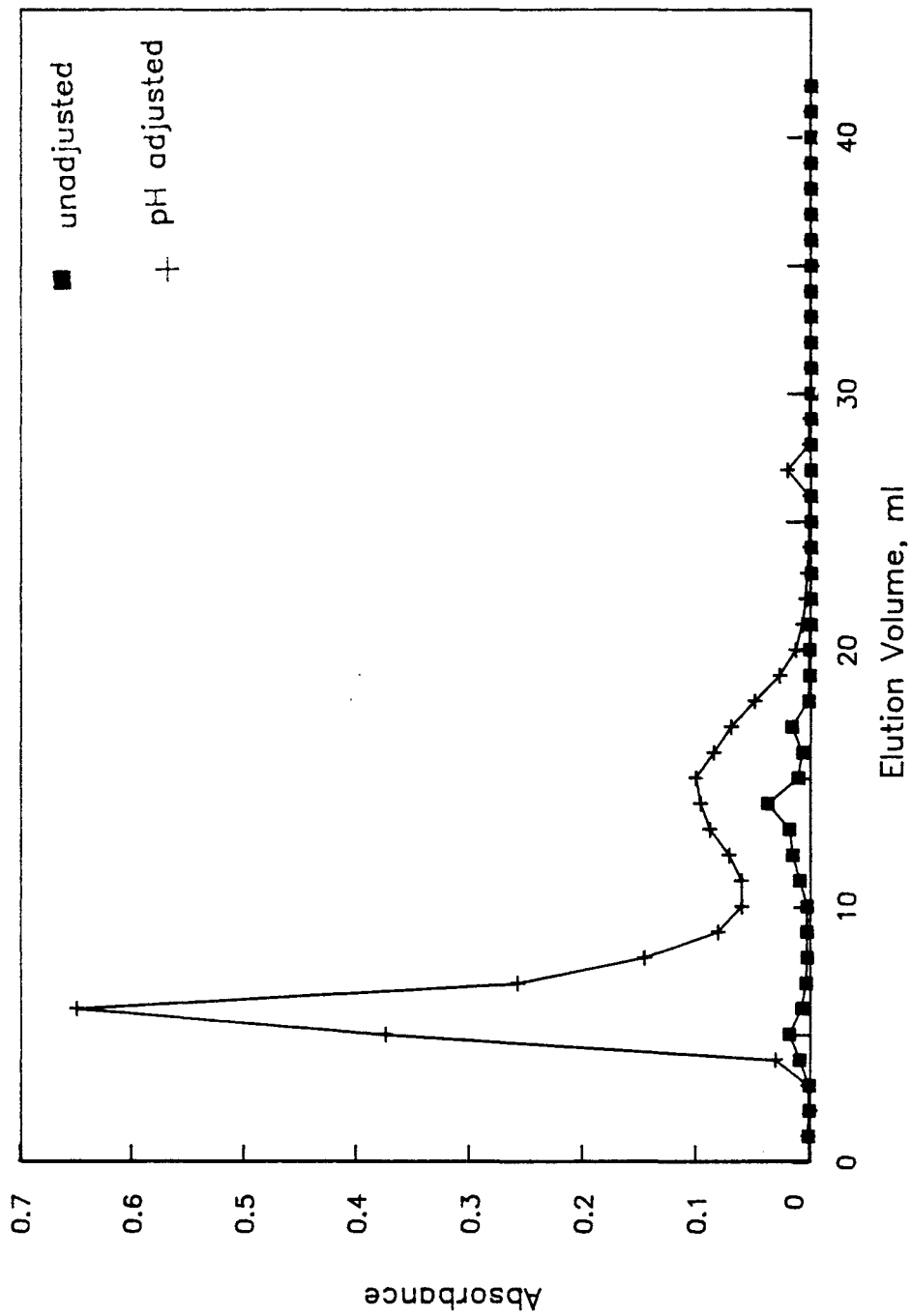

Figure 4.14 Gel filtration of biopolymer in period H
(Bactopeptone/Dextrose, 4600 mg/l Na, unadjusted pH)

Operational Differences

Visual examination on sludge flocs and general parameters including MLSS, MLVSS/MLSS, effluent SS, SOUR, pH and soluble effluent COD were performed to differentiate the reactor operation in each period. Sludge Volume Index and specific resistance were measured to compare the difference in sludge settling and dewatering properties. Diurnal raw data of reactor performance during each period are presented in Table B-9 through B-16 in Appendix B. When the values of MLSS and effluent SS attained stability, the system was considered to be at steady state and data during the steady state period were averaged and are presented in Table 4.3.

Bactopeptone fed sludge was light-brown, irregularly shaped and dominant in stalked and free swimming types of higher microbial forms. Throughout this bactopeptone feed period, a distinct interface between sludge blanket and supernatant liquor was present in the settling chamber. According to the low values of SVI (70 ml/g) and effluent SS (25 mg/l), this sludge could be judged as a well-flocculated sludge. However the specific resistance was somehow high, probably resulting form the rough surface of sludge flocs, according the conclusion from Chao and Keinath (28).

Salt addition, either Mg or Na, increased the inorganic fraction of sludge flocs. In addition, as salt was added into system, it appears that the biological population was influenced, based on the microscopic observation which found that higher forms of microorganisms did not appear as significant as before salt addition.

As Mg was applied, the pH of mixed liquor dropped from 7 to 6. As more Mg was added, the pH dropped more. This indicates that some unknown solid species or ions existed in the system and consumed alkalinity. This may be localized Mg(OH)(2) precipitation.

Table 4.3 Summary of average sludge performances in each period

Parameters	A	B	C	D	E	F	G	H
MLSS,mg/l	684	761	910	817	805	817	743	865
MLVSS/MLSS, %	97.3	92.1	91.3	92.0	93.3	92.0	90.8	84.7
Effluent SS, mg/l	25	17	14	31	21	31	79	70
SVI,ml/g	70	81	86	115	65	115	235	194
r^*,m/kg ($*10^{12}$)	10.6	4.9	2.6	18	17	18	126	89
SOUR,1/min ($*10^{-4}$)	10.1	7.9	11.3	11.1	9.4	11.1	3.6	9.8
pH	7.0	6.0	5.5	7.0	8.0	7.0	6.0	7.0
Effluent COD mg/l	41	67	55	30	93	30	57	45

Salt addition lowered the volatile ratio of sludge flocs. As indicated in Table 4.3, non-volatile fraction increased about 6 % in both the bactopeptone and bactopeptone/dextrose fed sludges when salt was added. This confirms the finding by Randall (46), who stated that some cations (K, Na, Mg and Ca) are taken up by microorganisms.

No consistent improvement or deterioration in SVI or specific resistance is found by addition of Mg or Na or increase in pH for the bactopeptone fed sludge. The reduction of specific resistance in period B and period C probably is the result of the formation of some unknown solid species, as described previously.

However, there are some unique phenomena in period D and period E. The floc during period D (2300 mg/l Na, unadjusted pH) was big and irregularly shaped with clear finger-like branches. This type of floc structure was only observed in this period and disappeared when the pH of mix liquor was increased (period E). The increase in SVI and r and decrease in effluent COD in period D corresponding to the big sludge flocs were probably the direct result of a better binding of HMW biopolymer as discussed in the previous section. These agree with the finding by Randall (46) and Kajornatiyuth (5), who concluded that increases in SVI and r * and a decrease in soluble effluent COD correspond to an increase in bound biopolymer in sludge flocs.

As the pH was raised in period E, the SVI dropped and the soluble effluent COD increased significantly, indicating the occurrence of a slight deflocculation and corresponding to the release of carbohydrate biopolymer discussed previously.

When dextrose was added to the feed, excessive growth of filamentous microorganisms caused deterioration of sludge settling and dewatering even though nitrogen was supplied in the system. The growth of filamentous microorganisms

makes it impossible to correlate the change of sludge properties to biopolymer characteristics.

When 4600 mg/l of Na was added in the bulking sludge, the SVI was significantly reduced and the settling ability was recovered within a few days. However, the specific resistance still remained high. Microscopic observation indicated that long filaments were broken and spread through the bulk solution. It appears that filamentous microorganisms are more sensitive to Na than regular microorganisms. Short filaments lessened the repulsion between flocs and resulted in a lower SVI, but the broken filaments in solution may interfere with sludge dewatering as indicated by a high specific resistance value.

Relationships Between Biopolymers and Operational Characteristics

SVI data show a linear relationship with effluent SS, as indicated in Figure 4.15. This confirms the finding by Randall (46), who concluded that an increase in bound biopolymer in flocs corresponds with high SVI but allows less solids to washout to the effluent.

The COD was found to correspond to unbound biopolymer. As seen in figure 4.16, when unbound total biopolymer increased, soluble effluent COD increased. Figure 4.17 shows a correspondence with an identical relationship between soluble effluent COD and unbound HMW biopolymer, indicating that the majority of effluent COD was contributed from the HMW fraction.

Fitzgerald (47) stated that r was related to the amount of HMW biopolymer. Kajornatiyudh (5) concluded that an increase in specific resistance corresponded to a low amount of bound protein. Randall (46), in contrast, suggested that HMW biopolymer is capable of incorporating considerable amount of water, resulting in aggregates which are not closely packed and hard to be dewatered. In this study the

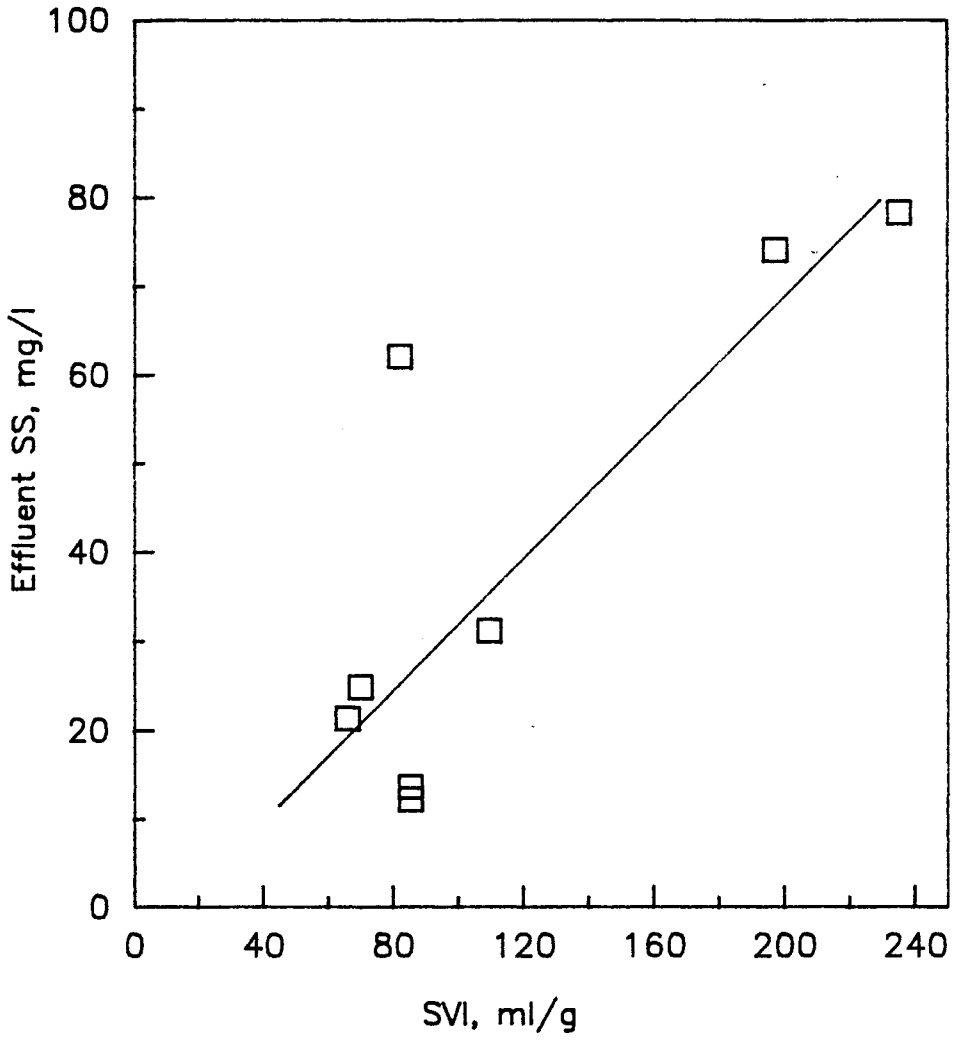

Figure 4.15 SVI vs. effluent SS.

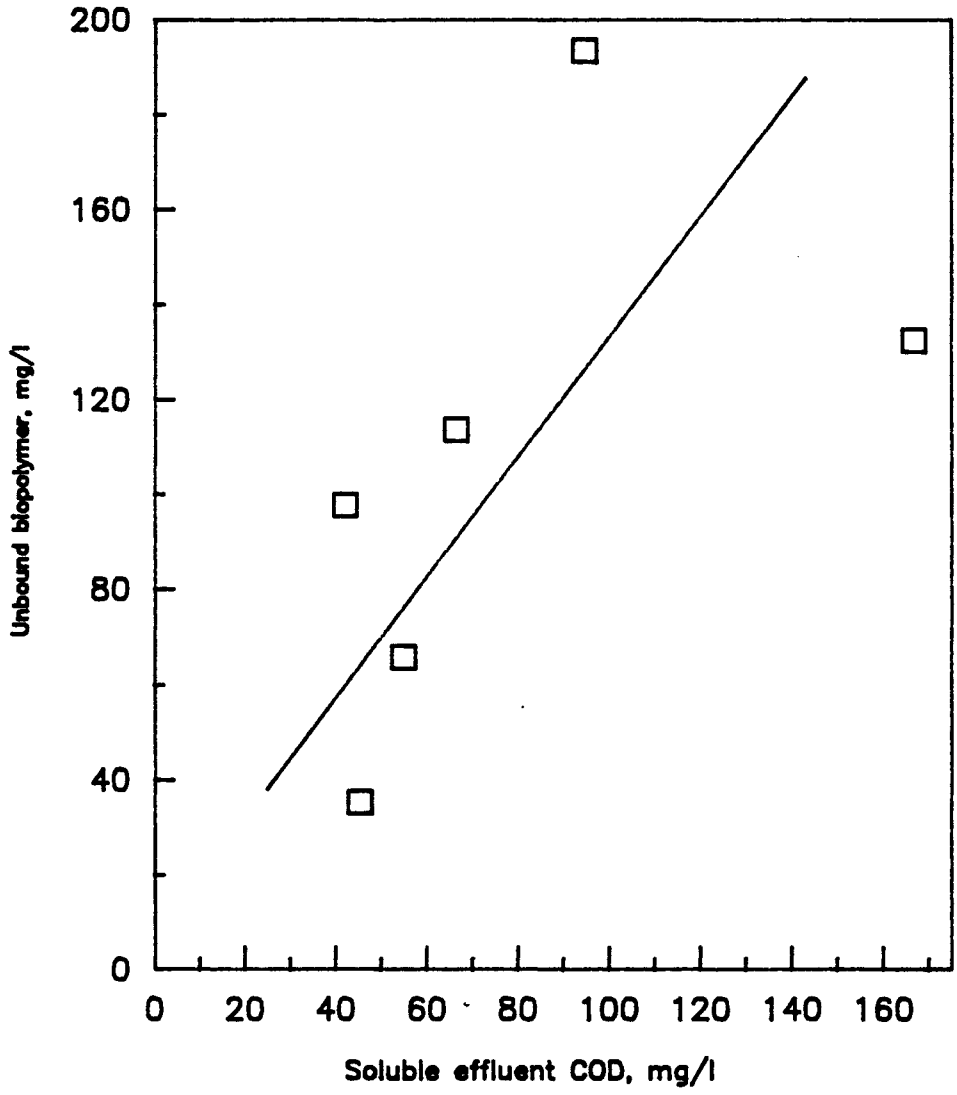

Figure 4.16 Soluble effluent COD vs. total unbound biopolymer

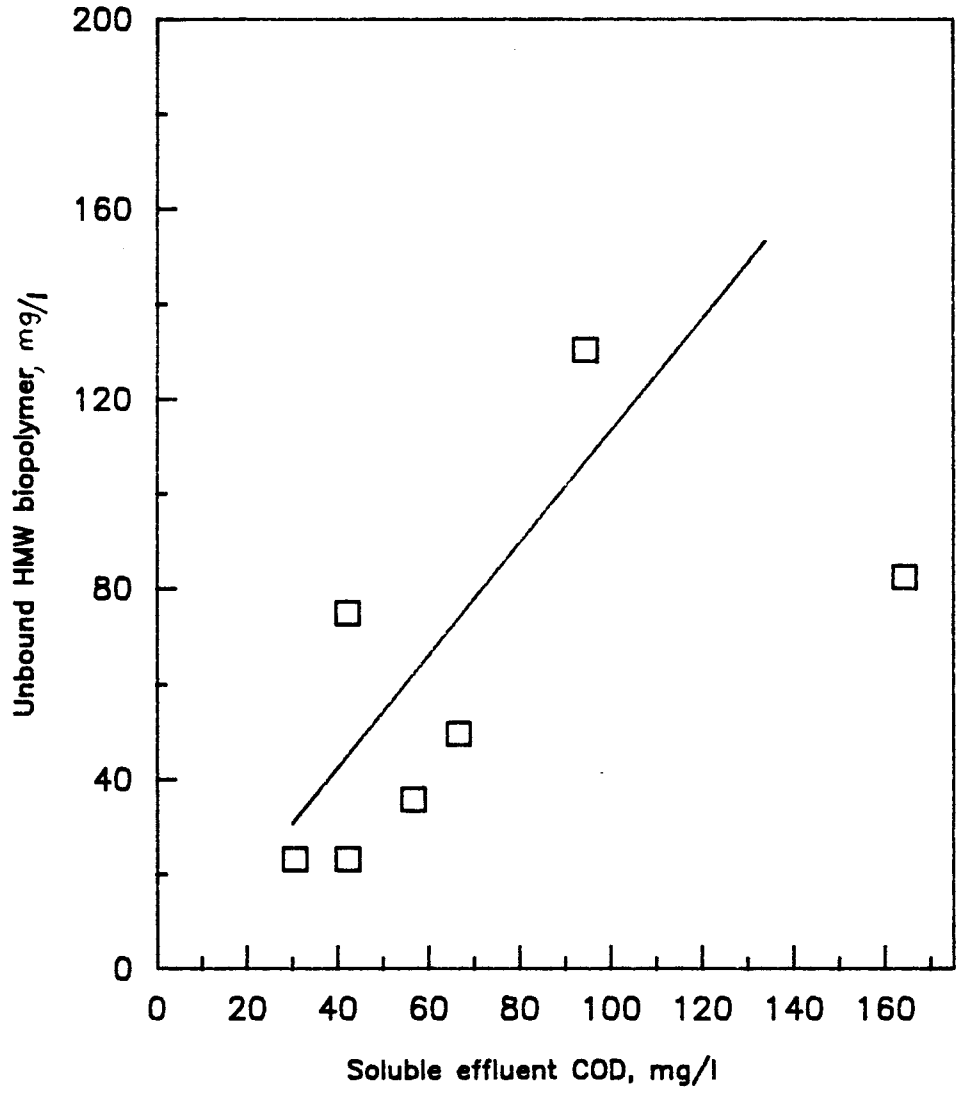

Figure 4.17 Soluble effluent COD vs. unbound HMW biopolymer.

expected variances in sludge settling and dewatering corresponding to the changes in biopolymer characteristics did not clearly emerge. No correlation is observed among biopolymer, SVI and r * , as shown in Figure 4.18 and 4.19.

Batch study

Binding of Biopolymers with Na and Mg

The biopolymer data in period B and period C strongly indicate that coagulation may occur in the extraction process. Therefore, in order to have a clear picture on the binding between Mg and biopolymers and to determine if Na is involved in the binding process, batch tests were conducted. In this study, 500 mg/l of Mg and three different concentrations of Na were added to the sludge from period E (2300 mg/l Na, pH =8). After salt addition, samples were rapidly mixed for 1 minute and slowly mixed for 30 minutes. An additional 1-hour mixing was performed for the pH adjusted samples. Gel filtration was run to identify the changes of biopolymers.

It is found that the flow rate for the eluent of samples added with either Mg or Na was faster than that of the original, corresponding to an early appearance of peaks in gel filtration. This indicates the occurrence of coagulation of fine particles.

As seen in Figure 4.20, addition of 500 mg/l of Mg did not change the UV absorbance of raw samples. The addition of Na at the same time with Mg did not increase or decrease the UV reading until Na concentration reached as high as 17700 mg/l. At this concentration the readings in both HMW and MMW fractions increased, indicating the possibility of cell lysis.

Similarly to the results in period C, alkali extraction did not harvest any bound biopolymers from the samples contained Mg. As seen in Figure 4.20, UV readings of HMW and LMW fractions of pH adjusted samples were reduced significantly, particularly in the HMW fraction. The UV reading of pH adjusted samples were even

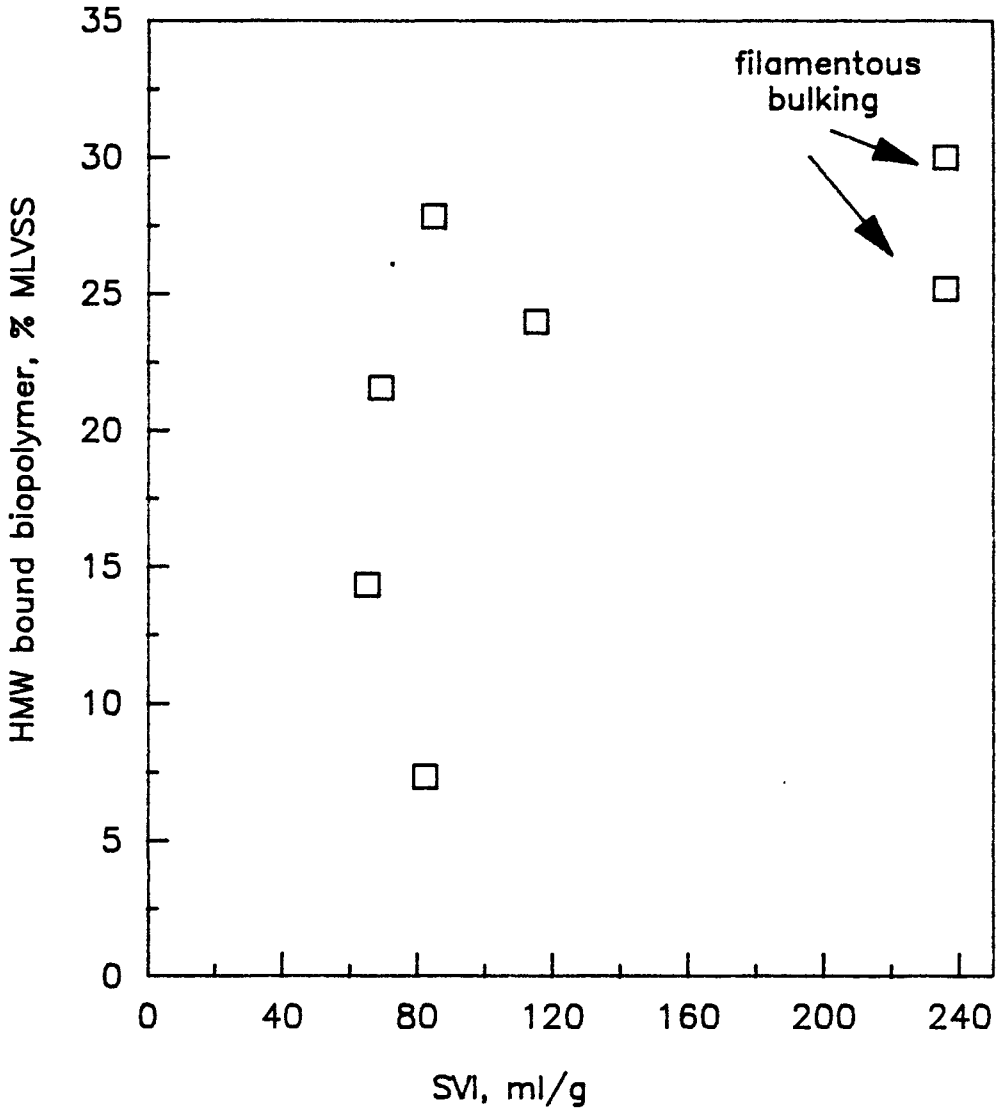

Figure 4.18 SVI vs. bound HMW biopolymer

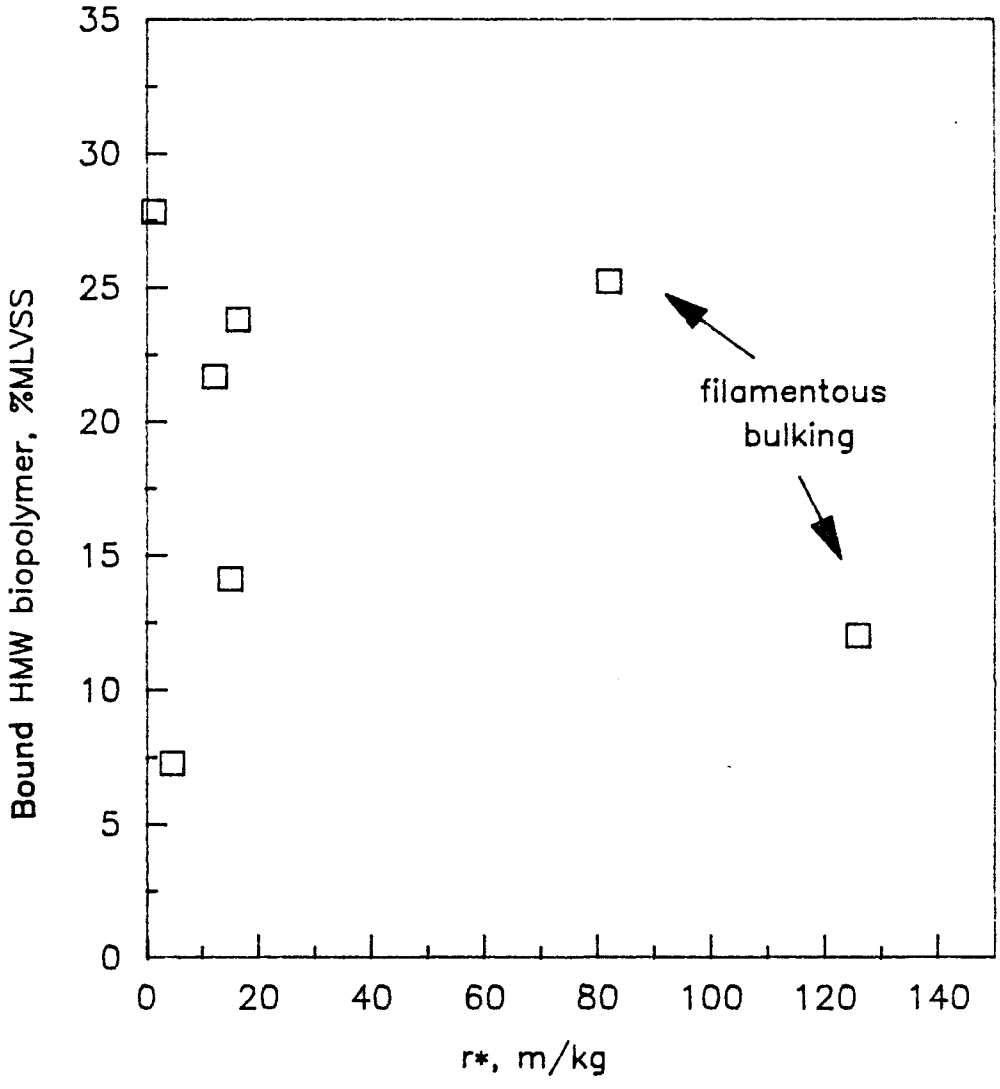

Figure 4.19 Specific resistance vs. bound HMW biopolymer

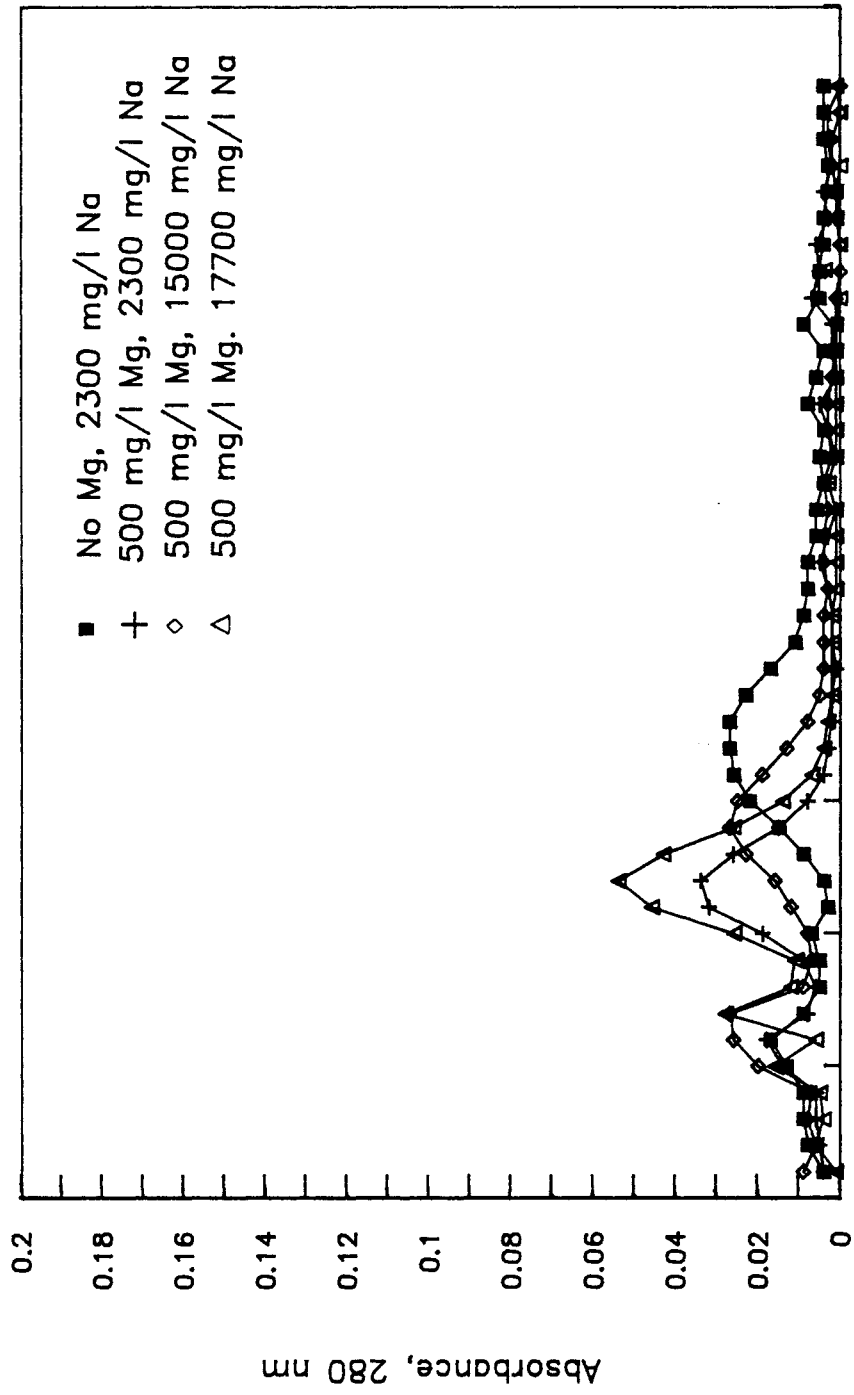

Figure 4.20 Binding of free biopolymer by Mg and Na, analyzed by gel filtration

lower than those of unadjusted samples, indicating that no bound biopolymer was extracted.

It appears that there was interaction or probably competition between Mg and Na, which lead to a different response. As seen in Figure 4.21, Addition of Na at the same time with 500 mg/l of Mg inhibited the adsorption of biopolymer on Mg(OH)(2) flocs. The MMW biopolymer was recovered as Na concentration was high. However, only a part of HMW fraction was recovered. Therefore, it appears that the adsorption of HMW biopolymer on Mg(OH)(2) floc was much tighter than that of MMW biopolymer.

According to these data, it is clear that coagulation occurred and a significant amount of HMW biopolymer was adsorbed on Mg(OH)(2) flocs. However, it is believed by the author that the binding extent is dependent on the nature of the biopolymer. In order to have a better understanding, further chemical analyses are required.

Conditioning Tests

Cation addition can reduce the dose requirement of cationic polymer. As reported by Novak and Haugan (35), cationic polymer coagulates sludge through neutralizing the charge of free biopolymers. The role of cations in polymer conditioning is to enhance the adsorption of biocolloids to the sludge surface; therefore, increasing the filtering rate. Three different sludges, sewage sludge from Blacksburg wastewater treatment plant, sludge from period A (bactopeptone only), and sludge from period E (bactopetone, 2300 mg/l Na, pH =8) were conditioned with cationic polymer, Betz 1195. Polymer combined with Mg or Na was added to sludge samples right before mixing. After 30-second rapid mixing (100rpm), samples were slowly mixed (30 rpm) for additional 1 minute and then the sludge filtering rate (TTF) was measured.

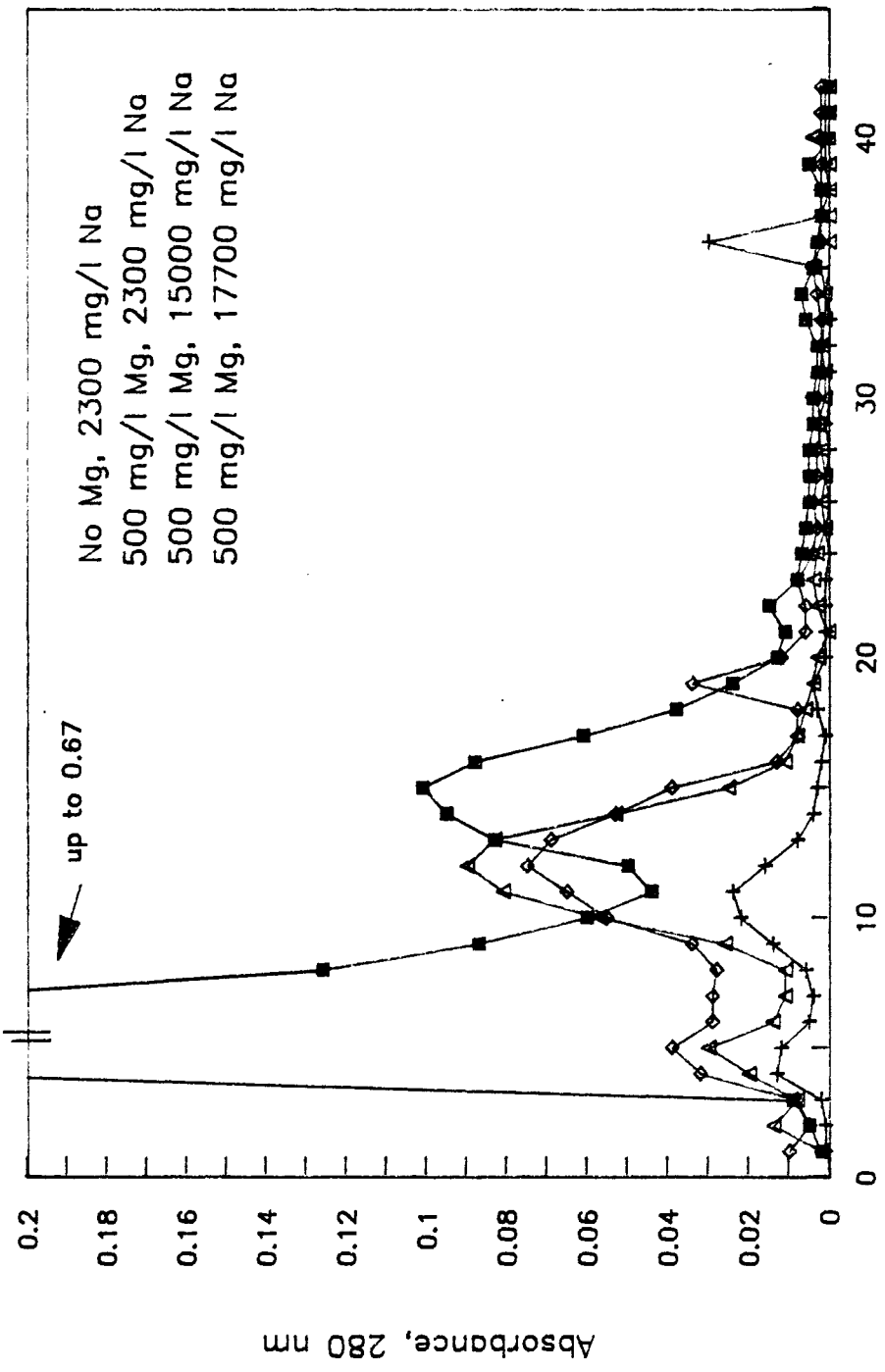

Figure 4.21 Binding of biopolymer at pH 11 by Mg and Na measured by gel filtration

The representative conditioning curves describing the sludge conditioning with Na, Mg and polymer are presented in Figure 4.22. As expected, Mg had a better conditioning ability than Na did, both in dosage and increasing the filtering rate. Metal ion was less efficient than HMW cationic polymer.

An additional test was performed to gain a further insight into the sludge conditioning with polymer. The sludge was taken from a batch-type reactor fed with bactopeptone substrate with COD value of about 300 mg/l and had a sludge age of 8 days. The MLSS value in the system was approximately 700 mg/l and the SVI value was about 100 ml/g. The conditioning test of this sludge with Betz 1195 found that the optimal dose was 15 mg/l and the sludge filtering time was reduced from 32 to 10 seconds by the optimal dose. The supernatants of this sludges without conditioning and conditioned by the optimal dose of polymer were run gel filtration. Figure 4.23 shows that cationic polymer mainly neutralized and than coagulated the HMW biopolymer colloids. A 50 percent reduction in UV absorbance in the HMW fraction corresponding with a significant improvement in sludge filtering rate reveals that HMW biopolymer is the major factor in determining the sludge dewatering.

When polymer was added with Mg and Na, unlike work alone, the conditioning efficiency of polymer is much different. For the sewage sludge, as shown in Figure 4.24, both Na and Mg reduced polymer requirement but did not improve the sludge filtering rate. The concentrations of Na or Mg required for the best performance in reducing polymer requirement were the same, indicating that Na had the same conditioning ability as Mg when it was added with polymer. It also implies that charge neutralization by salt addition is not the main mechanism in reducing polymer requirement, based on that the optimum concentration (also the molarity) of Na and Mg for reducing polymer requirement was similar. The performance curve of Mg in figure 4.24 (b) shows an identical trend to the conditioning curve in Figure 4.22 (b),

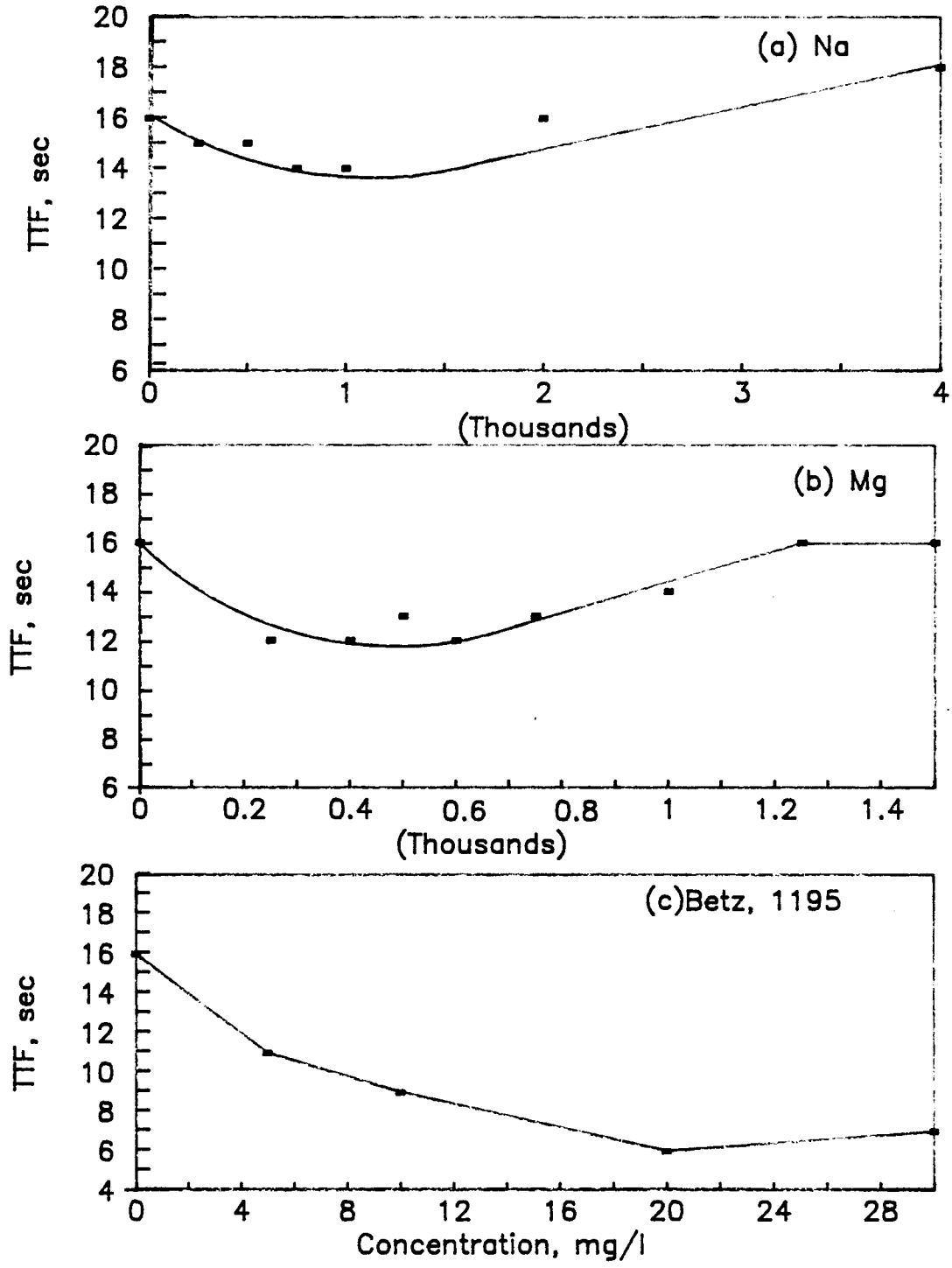

Figure 4.22 Conditioning curves of Na, Mg, and polymer Betz 1195

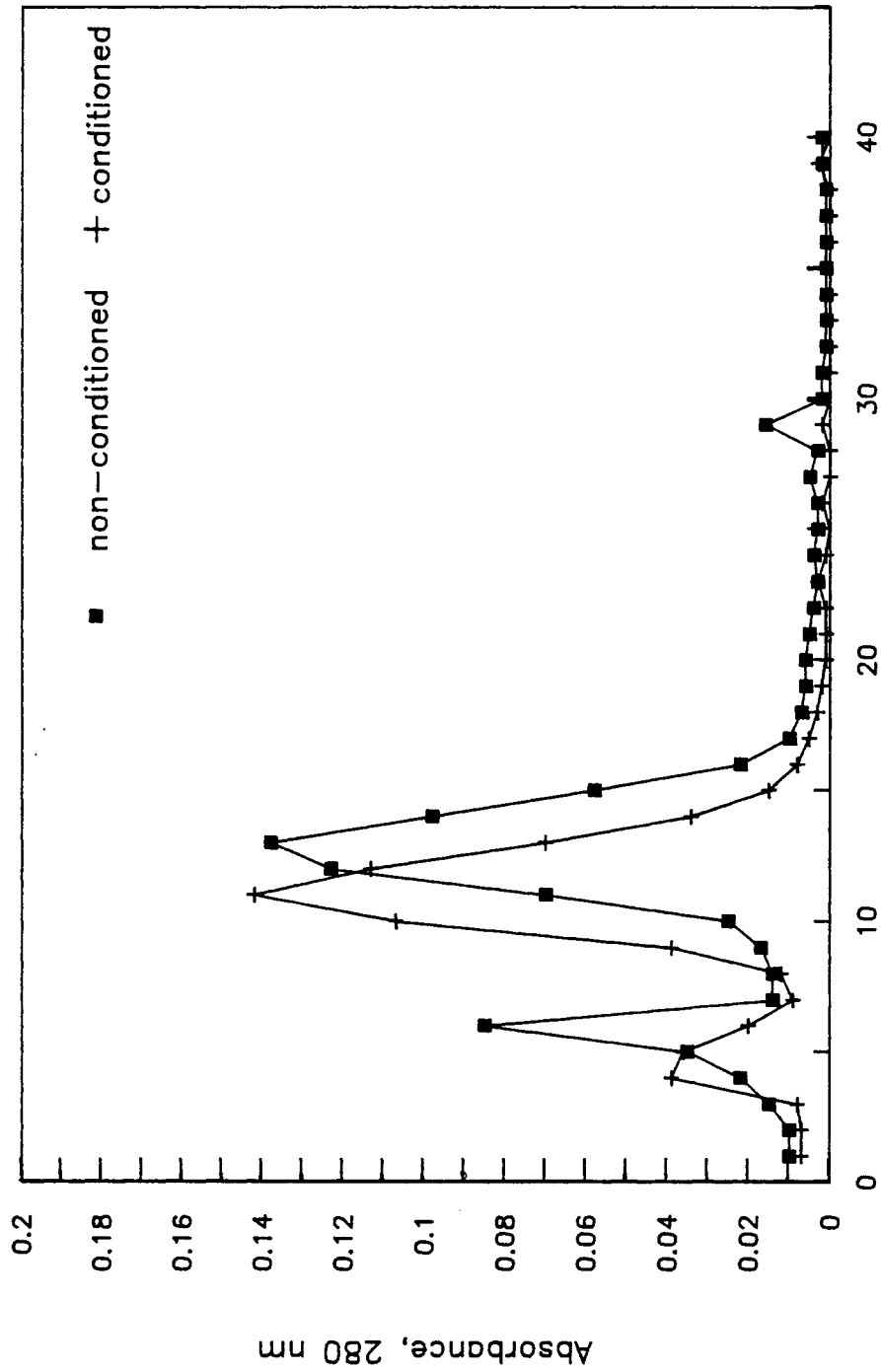

Figure 4.23 Gel filtration of biopolymer conditioned by polymer

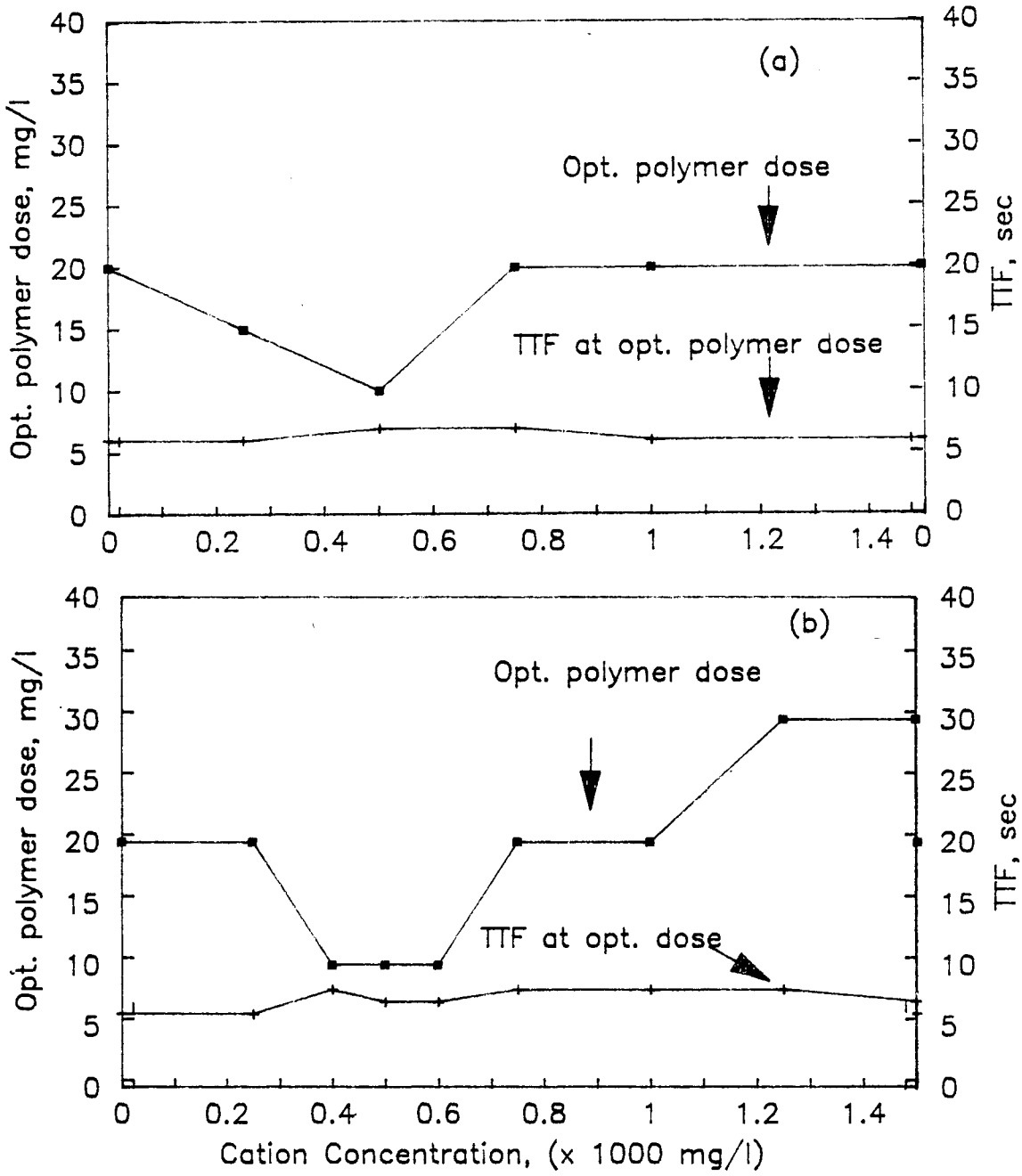

Figure 4.24 Influence of cation on sewage sludge conditioning.
(a) Na (b) Mg

whereas, the curves for Na in Figure 4.22 and 4,24 are quite different. This indicates that the role of Na in polymer conditioning may be different to that of conditioning alone.

The conditioning tests for the bactopeptone fed sludges performed differently. Figure 4.25 shows that Mg did not improve the conditioning at all. In contrast, the addition of 500 mg/l Mg, the concentration that performed the best at sewage sludge, even deteriorated the sludge conditioning. The conditioning of the sludge from period E (2300 mg/l Na, pH =8) was much worse by 500 mg/l of Mg. As discussed previously, an optimal dose of Mg was observed. The addition of 500 mg/l Mg in reactor system caused the release of protein (see Influence of Mg on biopolymer characteristics); therefore, the increase in polymer requirement at 500 mg/l Mg confirms that Mg over the optimal dose will deteriorate sludge dewatering property.

Many factors may involve in causing the different responses in sewage sludge and bactopeptone fed sludge, for example, pH, mixing time and intensity during conditioning and variances in biopolymer characteristics. As been discussed previously, sludge from period A (bactopeptone, no salt) had more free protein, whereas deflocculation occurred in the sludge from period E (2300 mg/l Na, pH=8) and a lot of carbohydrate was released to solution. Also the sewage sludge would be believed to contain the biopolymer with different characteristics. Since different compound has its special adsorption, under limited data, it is difficult to analyze.

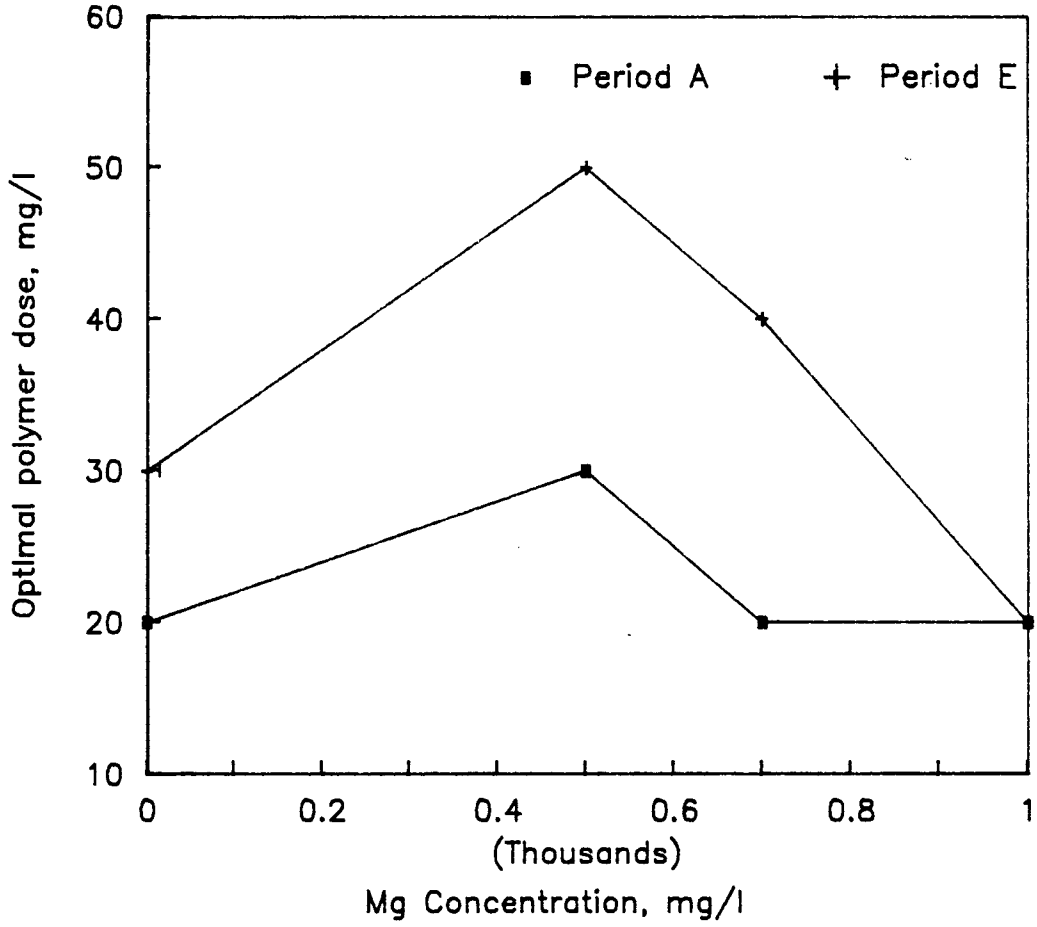

Figure 4.25 Influence of Mg on the polymer requirement of bactopeptone fed sludge conditioning, period A: no salt, unadjusted pH, period E: 2300 mg/l Na, pH=8.

V Conclusions

Although biopolymer binding was influenced by by salt addition the expected responses in sludge settling and dewatering properties corresponding to changes in biopolymer characteristics were not seen in this study.

The conclusions based on the data in this study are:

1. Salt addition influences the binding of biopolymers. The influence is dependent on the type and concentration of of salt. In this study, the optimal dose of magnesium for producing a better biopolymer binding was at about 250 mg/l. When magnesium concentration was increased to 500 mg/l, release of biopolymers occurred. No optimal dose was found by Na addition.

2. The organic composition in feed solution affects biopolymer characteristics. Bactopeptone feed generated biopolymer composed of more bound carbohydrate than bound protein. On the other hand, dextrose feed with nitrogen supplement produced biopolymer which contained more bound protein but less bound carbohydrate.

3. An increase in pH combined with a high amount of sodium can release biopolymer, particularly carbohydrate, and result in deflocculation of sludge flocs. A lower SVI value and a higher specific resistance were found to correspond to the release of biopolymer.

4. An increase in unbound biopolymer corresponds to an increase in soluble effluent COD, suggesting that in certain cases the effluent COD may be largely comprised of unbound biopolymer.

5. Both Na and Mg can reduce the dose requirement of cationic polymer.

6. Cations can influence the amount of extractable biopolymer by alkali extraction. Magnesium will reduce the yield of extractable biopolymer. Gel filtration and chem-

ical analysis both found that the HMW biopolymer was coagulated by magnesium hydroxide during alkali extraction. High concentrations of sodium, on the contrary, can improve the efficiency of biopolymer extraction.

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APPENDICE

Appendix A : Standard curves

Appendix B : Raw data of reactor study

Appendix C : Raw data of batch study

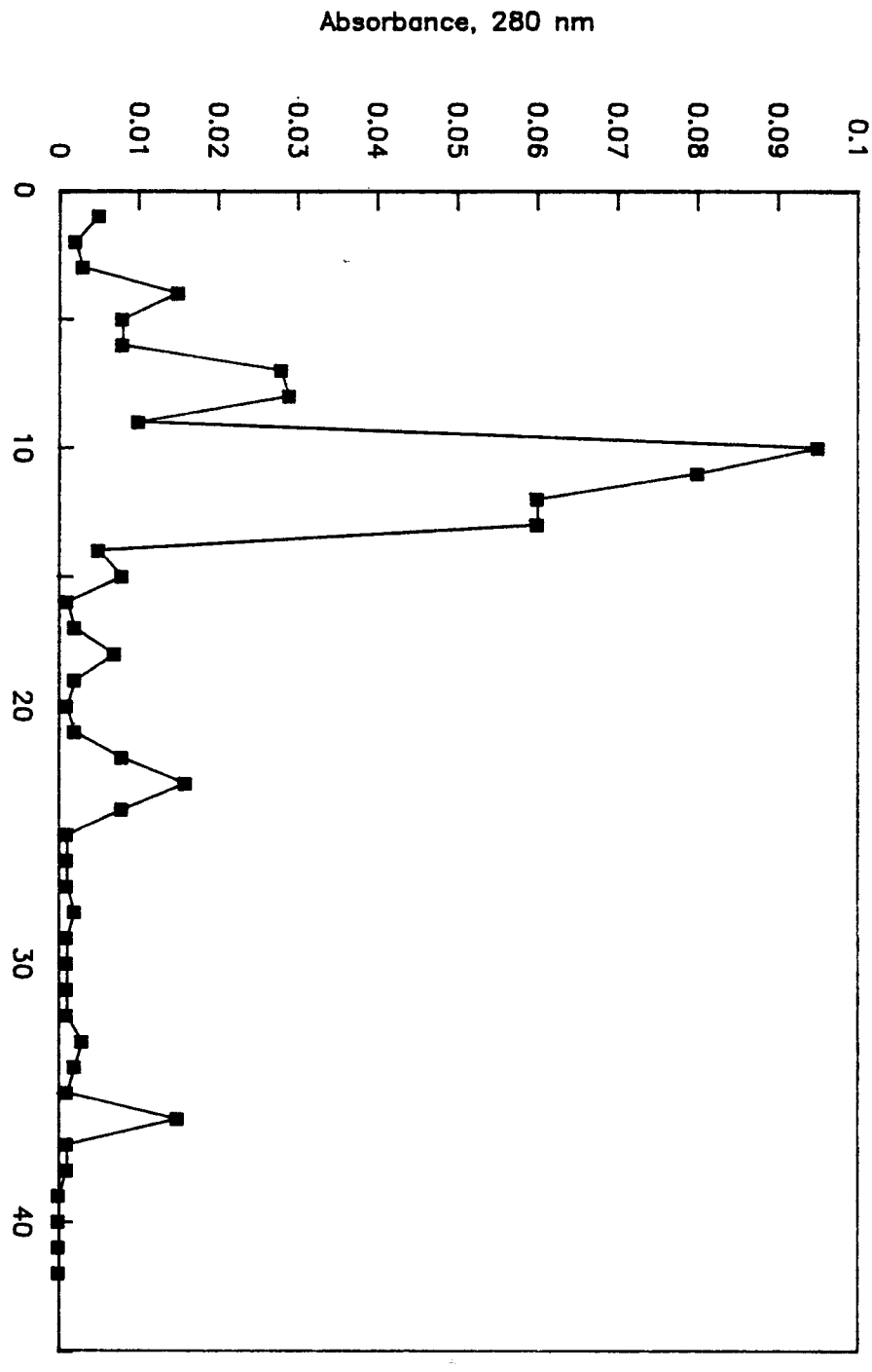

Figure A—1 Gel filtration of 5 known molecular weight substances

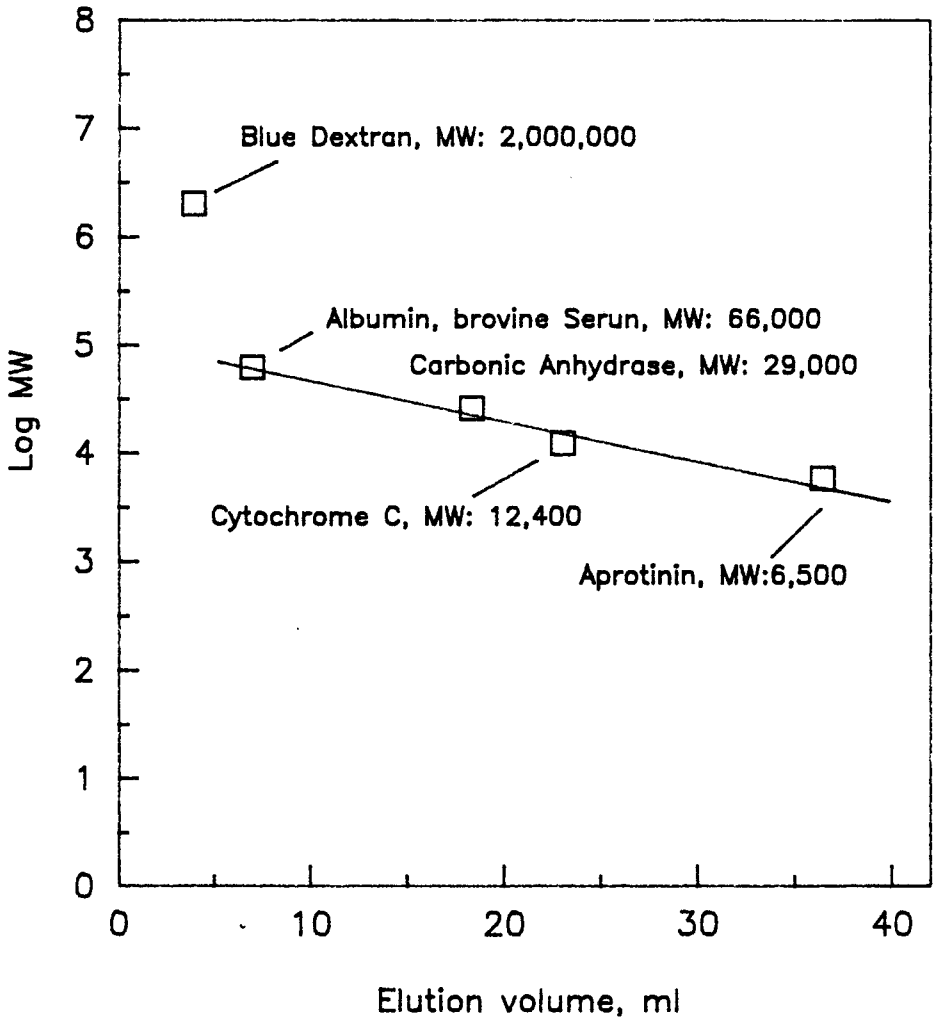

Figure A—2 Calibration curve for Sephadex G—75 column.

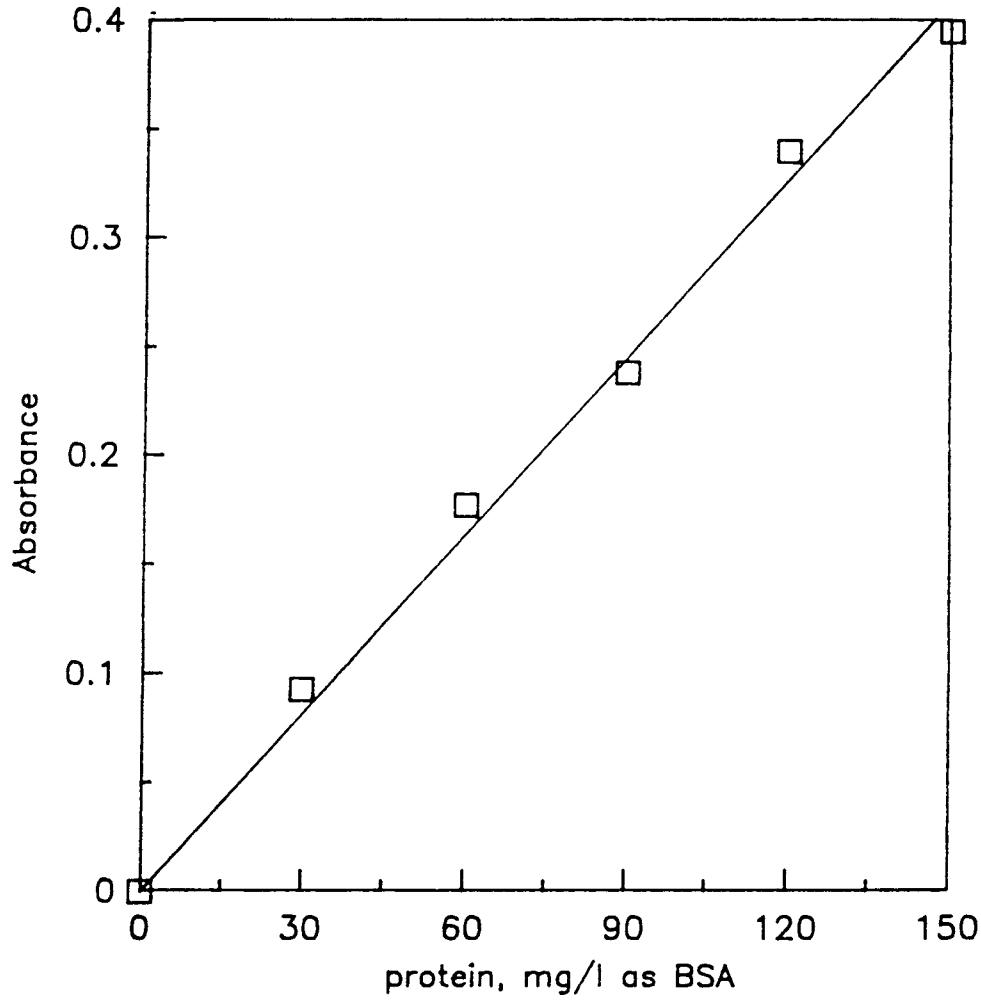

Figure A–3 Calibration curve for protein determination.

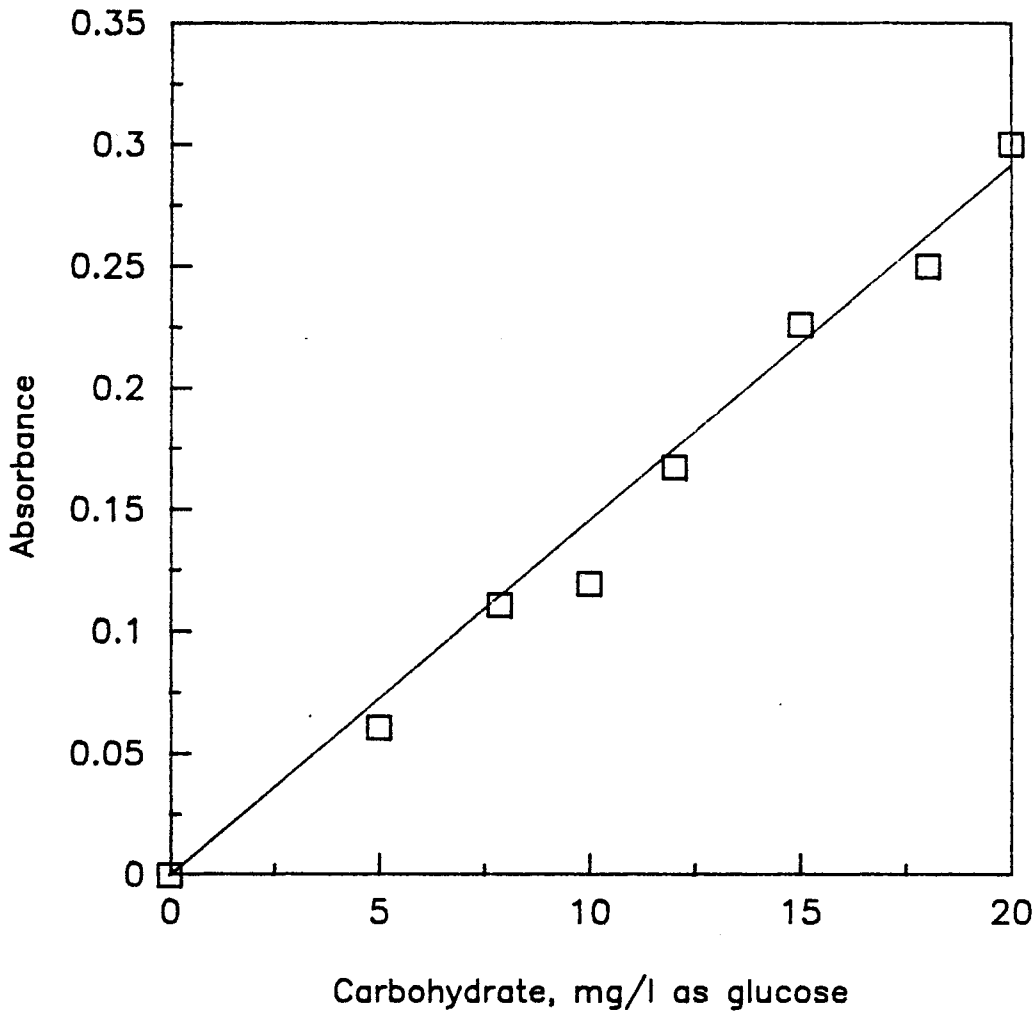

Figure A—4 Calibration curve for carbohydrate determination.

Table B-1 Protein and carbohydrate concentrations in period A

	Elution volume, ml	Total mg/l	Unbound mg/l	Bound mg/l	Bound/MLVSS %
Protein	4	9.6	2.9	6.7	1.0
	8	20.3	14.3	6.0	0.9
	12	28.9	5.0	23.9	3.57
	16	6.1	8.2	0.0	0.0
	20	7.9	1.8	6.1	0.91
	24	1.1	1.8	0.0	0.0
	28	0.0	0.7	0.0	0.0
	32	0.4	3.2	0.0	0.0
	36	1.4	0.4	1.0	0.15
	40	1.3	0.4	0.9	0.13
Carbohydrate	4	33.8	9.0	24.8	3.70
	8	7.9	1.4	6.5	0.97
	12	3.2	0.0	3.2	0.48
	16	0.0	0.0	0.0	0.00
	20	0.0	22.7	0.0	0.00
	24	0.0	0.0	0.0	0.00
	28	6.5	0.0	6.5	0.97
	32	29.5	0.0	29.5	4.40
	36	0.7	0.0	0.7	0.10
	40	10.1	0.0	10.1	1.51

Table B-2 Protein and carbohydrate concentrations in period B

	Elution volume, ml	Total mg/l	Unbound mg/l	Bound mg/l	Bound/MLVSS %
Protein	3	0.0	0.2	0.0	0.00
	6	0.3	1.3	0.0	0.00
	9	1.0	0.4	0.6	0.07
	12.5	3.6	0.4	3.2	0.38
	16	2.9	0.9	2.0	0.24
	19.5	5.7	0.2	5.5	0.66
	23	0.0	0.2	0.0	0.00
	26.5	0.0	0.2	0.0	0.00
	30	1.1	0.0	1.1	0.13
	33.5	0.6	2.2	0.0	0.00
	37	0.4	0.9	0.0	0.00
Carbohydrate	3	36.0	4.7	31.3	3.75
	6	5.7	7.2	0.0	0.00
	9	6.0	13.7	0.0	0.00
	12.5	4.7	3.6	1.1	0.13
	16	2.7	3.2	0.0	0.00
	19.5	19.2	10.4	8.8	1.05
	23	5.1	1.8	3.3	0.40
	26.5	2.4	4.3	0.0	0.00
	30	5.3	3.6	1.7	0.20
	33.5	4.1	7.9	0.0	0.00
	37	1.4	1.4	0.0	0.00

Table B-3 Protein and carbohydrate concentrations in period C

	Elution volume, ml	Total mg/l	Unbound mg/l	Bound mg/l	Bound/MLVSS %
Protein	3	8.6	2.9	5.7	0.70
	6	6.6	2.7	3.9	0.48
	9	8.3	3.5	4.8	0.59
	12	12.5	3.4	9.1	1.12
	15	13.4	3.7	9.7	1.20
	18	14.1	7.8	6.3	0.78
	21	13.9	3.1	10.8	1.33
	24	8.6	5.8	2.8	0.35
	27	8.1	6.2	1.9	0.23
	30	7.2	11.6	0.0	0.00
	33	8.0	3.6	4.4	0.54
	36	10.8	3.6	7.2	0.89
	39	6.1	2.9	3.2	0.40
Carbohydrate	3	9.3	-	-	-
	6	10.2	-	-	-
	9	2.2	-	-	-
	12	14.4	19.4	0.0	0.00
	15	3.2	7.9	0.0	0.00
	18	0.7	-	-	-
	21	1.8	5.0	0.0	0.00
	24	10.1	1.4	8.7	1.07
	27	1.4	1.4	0.0	0.00
	30	3.6	2.9	0.7	0.09
	33	6.5	0.7	5.8	0.72
	36	4.3	0.0	4.3	0.53
	39	5.0	4.3	0.0	0.00

- : off-sacle reading

Table B-4 Protein and carbohydrate concentrations in period D

	Elution volume, ml	Total mg/l	Unbound mg/l	Bound mg/l	Bound/MLVSS %
Protein	4	0.0	0.8	0.0	0.00
	8	31.6	7.4	24.2	3.18
	12	13.0	2.0	11.0	1.45
	16	32.5	1.1	31.4	4.13
	20	3.4	2.1	1.3	0.17
	24	2.3	1.8	0.5	0.04
	28	2.1	1.7	0.4	0.04
	32	1.5	0.3	1.2	0.17
	36	0.9	0.7	0.2	0.01
Carbohydrate	4	25.9	1.8	24.1	3.17
	8	23.0	0.0	23.0	3.03
	12	0.7	0.0	0.7	0.09
	16	8.6	108	0.0	0.00
	20	1.4	1.4	0.0	0.00
	24	0.9	1.0	0.0	0.00
	28	0.4	0.0	0.4	0.01
	32	1.0	0.8	0.2	0.01
	36	0.0	0.0	0.0	0.00

Table B-5 Protein and carbohydrate concentrations in period E

	Elution volume, ml	Total mg/l	Unbound mg/l	Bound mg/l	Bound/MLVSS %
Protein	4	2.1	0.0	2.1	0.26
	8	51.4	2.1	49.3	6.20
	12	14.2	10.4	3.8	0.48
	16	32.5	3.2	29.2	3.69
	20	24.6	2.4	22.2	2.79
	24	18.0	0.7	17.3	2.18
	28	0.0	0.0	0.0	0.00
	32	0.0	0.0	0.0	0.00
	36	0.0	0.0	0.0	0.00
	40	5.0	2.9	2.1	0.26
Carbohydrate	4	26.6	36.0	0.0	0.00
	8	11.5	11.5	0.0	0.00
	12	10.8	5.0	5.8	0.73
	16	3.6	3.6	0.0	0.00
	20	2.9	4.7	0.0	0.00
	24	2.2	2.5	0.0	0.00
	28	2.8	2.9	0.0	0.00
	32	1.4	2.9	0.0	0.00
	36	4.3	3.6	0.7	0.09
	40	2.2	2.9	0.0	0.00

Table B-6 Protein and carbohydrate concentrations in period F

	Elution volume, ml	Total mg/l	Unbound mg/l	Bound mg/l	Bound/MLVSS %
Protein	4	4.3	1.8	2.5	0.25
	8	58.2	2.9	55.3	5.45
	12	32.8	5.0	27.8	2.74
	16	68.9	6.1	62.8	6.19
	20	18.6	0.7	17.9	1.76
	24	5.7	3.2	2.5	0.25
	28	0.0	2.1	0.0	0.00
	32	4.6	2.1	0.0	0.00
	36	5.0	3.6	1.4	0.14
	40	3.6	1.4	2.2	0.22
Carbohydrate	4	45.3	19.4	25.9	2.55
	8	17.3	9.0	8.3	0.82
	12	13.8	2.1	11.7	1.15
	16	5.1	4.2	0.9	0.09
	20	2.8	1.8	1.0	0.10
	24	0.0	0.0	0.0	0.00
	28	0.0	0.0	0.0	0.00
	32	0.0	0.0	0.0	0.00
	36	0.9	0.0	0.9	0.09
	40	0.8	0.0	0.8	0.08

Table B-7 Protein and carbohydrate concentrations in period G

	Elution volume, ml	Total mg/l	Unbound mg/l	Bound mg/l	Bound/MLVSS %
Protein	4	0.0	0.7	0.0	0.00
	8	74.2	2.5	71.1	9.56
	12	26.8	0.7	26.1	3.48
	16	31.8	2.1	29.7	3.96
	20	5.7	0.0	5.7	0.76
	24	4.3	0.0	4.3	0.57
Carbohydrate	4	5.8	3.6	2.2	0.24
	8	10.1	7.9	2.2	0.29
	12	4.3	3.6	0.7	0.09
	16	13.0	5.4	7.6	1.01
	20	4.3	3.6	0.7	0.09
	24	0.7	2.1	0.0	0.00

Table B-8 Protein and carbohydrate concentrations in period H

	Elution volume, ml	Total mg/l	Unbound mg/l	Bound mg/l	Bound/MLVSS %
protein	4	1.4	2.1	0.0	0.00
	8	75.3	4.3	71.0	11.09
	12	24.3	2.9	21.4	3.34
	16	31.4	4.3	27.1	4.23
	20	8.9	1.8	7.1	1.11
	24	0.0	0.0	0.0	0.00
Carbohydrate	4	1.4	0.7	0.7	0.11
	8	12.2	0.7	11.5	1.80
	12	4.3	0.0	4.3	0.67
	16	4.3	0.7	3.6	0.56
	20	1.4	0.0	1.4	0.22
	24	0.0	0.0	0.0	0.00

Table B-9 Operating parameters in period A

Date	Day	MLSS mg/l	ESS mg/l	SVI ml/g	r* m/kg	pH	SOUR 1/min
8/29	73	885	16	73	-	-	-
8/30	74	850	14	82	-	-	-
8/31	75	815	9	75	-	-	-
9/2	77	745	8	107	-	-	-
9/3	78	790	12	99	-	5.6	-
9/4	79	790	13	82	-	6.2	-
9/6	81	760	-	66	-	-	-
9/8	83	680	21	74	-	5.7	-
9/9	84	740	36	54	-	6.8	-
9/10	85	725	38	44	-	7.2	-
9/11	86	715	26	49	-	7.2	-
9/13	88	700	29	43	-	7.2	-
9/14	89	670	11	60	-	6.8	-
9/16	91	695	19	58	-	6.4	-
9/18	93	610	17	66	1.6×10^{13}	6.7	6.6×10^{-4}
9/19	94	530	30	94	1.0×10^{13}	7.4	14.7×10^{-4}
9/20	95	590	33	76	5.8×10^{12}	7.5	8.9×10^{-4}
9/21	96	610	36	77	-	7.4	-
9/22	97	670	18	75	-	7.4	-
Ave.(day 79-95)		684	25	70	10.6×10^{12}	7.0	10.1×10^{-4}

Table B-9 Operating Parameters in period A (cont.)

Date	Day	MLVSS/MLSS	COD, mg/l	
		%	feed	effluent
9/10	85	91.7	300	56
9/11	86	91.0	-	-
9/18	93	99.2	-	-
9/19	94	95.3	310	44
9/20	95	97.5	319	22

Table B-10 Operating parameters in period B

Date	Day	MLSS mg/l	ESS mg/l	SVI ml/g	r* m/kg	pH	SOUR 1/min
8/8	52	510	28	59	-	-	-
8/11	55	550	33	64	-	-	-
8/12	56	684	36	44	-	-	-
8/14	58	680	15	59	-	-	-
8/16	60	844	11	47	-	-	-
8/17	61	816	18	49	-	-	-
8/20	64	495	9	81	-	-	-
8/21	65	705	12	71	3.6×10^{12}	6.1	$8.2\times^{10-4}$
8/22	66	820	22	73	8.9×10^{12}	5.6	6.1×10^{-4}
8/23	67	680	27	88	-	5.7	9.3×10^{-4}
8/24	68	755	18	86	2.2×10^{12}	5.9	-
8/26	70	870	11	75	-	-	-
8/27	71	735	13	95	-	-	-
Ave.(day 65-71)		761	17	81	4.9×10^{12}	6.0	7.9×10^{-4}

Table B-10 Operating Parameters in period B (cont.)

Date	Day	MLVSS/MLSS	COD, mg/l	
		%	feed	effluent
8/21	65	90.6	342	67
8/23	67	93.6	334	67

Table B-11 Operating parameters in period C

Date	Day	MLSS mg/l	ESS mg/l	SVI ml/g	r* m/kg	pH	SOUR 1/min
7/29	42	675	12	192	-	5.2	-
7/31	44	750	34	133	-	-	-
8/2	46	620	27	129	-	-	-
8/4	48	810	21	123	2.0×10^{13}	5.4	11.4×10^{-4}
8/5	49	775	27	84	4.4×10^{12}	5.5	-
8/8	52	745	28	81	-	-	-
8/11	55	750	26	87	-	-	-
8/17	61	788	37	125	-	-	-
8/21	65	790	15	114	-	5.7	-
8/22	66	1085	17	74	1.9×10^{10}	-	9.3×10^{-4}
8/23	67	835	18	84	-	5.5	-
8/26	70	995	18	75	-	5.4	-
8/27	71	845	20	83	1.4×10^{12}	5.6	13.3×10^{-4}
Ave.(day 65-71)		910	14	86	2.6×10^{12}	5.5	11.5×10^{-4}

Table B-11 Operating Parameters in period C (cont.)

Date	Day	MLVSS/MLSS	COD, mg/l	
		%	feed	effluent
7/29	42	90.3	332	30
8/21	65	92.1	334	94
8/23	66	-	334	77
8/27	71	91.7	-	-

Table B-12 Operating parameters in period D

Date	Day	MLSS mg/l	ESS mg/l	SVI ml/g	r* m/kg	pH	SOUR 1/min
7/20	33	760	62	119	6.5×10^{12}	-	-
7/21	34	775	19	103	12×10^{12}	-	-
7/22	35	715	72	105	-	-	-
7/23	36	725	42	103	12×10^{12}	6.8	-
7/24	37	775	31	97	11×10^{12}	-	-
7/26	39	795	12	101	-	-	-
7/27	40	880	38	114	-	6.7	9.1×10^{-4}
7/28	41	985	27	105	-	-	-
7/29	42	830	20	108	-	-	-
7/30	43	890	34	106	-	-	-
7/31	44	815	62	123	-	6.8	8.1×10^{-4}
8/2	46	620	28	153	-	-	-
8/4	48	760	28	132	21×10^{12}	6.8	8.9×10^{-4}
8/5	49	780	27	90	28×10^{12}	6.9	9.3×10^{-4}
Ave.(day 39-49)		817	31	115	18×10^{12}	7.0	11.1×10^{-4}

Table B-12 Operating Parameters in period D (cont.)

Date	Day	MLVSS/MLSS	COD, mg/l	
		%	feed	effluent
7/20	33	92.0	330	39
7/24	37	92.0	330	21
8/5	49	91.3	332	33

Table B-13 Operating parameters in period E

Date	Day	MLSS mg/l	ESS mg/l	SVI ml/g	r* m/kg	pH	SOUR 1/min
9/21	96	945	20	67	-	8.2	-
9/23	98	800	21	56	-	8.2	-
9/26	101	820	17	62	-	8.0	-
9/29	104	795	14	69	14×10^{12}	7.9	9.3×10^{-4}
10/2	107	895	24	62	18×10^{12}	8.4	8.6×10^{-4}
10/4	109	845	14	65	14×10^{12}	8.3	-
10/9	114	670	36	67	20×10^{12}	8.4	10.4×10^{-4}
Ave. (day 101-114)		805	21	65	17×10^{12}	8.0	9.4×10^{-4}

Table B-13 Operating Parameters in period E (cont.)

Date	Day	MLVSS/MLSS	COD, mg/l	
		%	feed	effluent
9/26	101	92.6	420	121.9
10/2	107	93.7	400	68.6
10/4	109	92.8	390	83.7
10/9	114	94.2	396	99.4

Table B-14 Operating parameters in period F

Date	Day	MLSS mg/l	ESS mg/l	SVI ml/g	r* m/kg	pH	SOUR 1/min
9/4	79	675	28	96	-	7.6	-
9/6	81	585	29	120	-	8.0	-
9/8	83	665	16	105	-	7.8	-
9/9	84	715	15	98	-	7.4	-
9/10	85	685	17	117	-	7.4	-
9/11	86	735	39	116	-	7.1	-
9/13	87	820	7	98	-	7.3	-
9/14	88	800	10	100	-	8.6	-
9/16	89	785	13	89	-	8.2	-
9/18	90	895	14	84	2.3×10^{12}	8.1	13.4×10^{-4}
9/19	91	1015	12	74	0.9×10^{12}	8.3	14.7×10^{-4}
9/20	92	980	19	71	1.3×10^{12}	8.3	-
Ave. (day 87-92)		883	13	86	1.5×10^{12}	5.0	1.4×10^{-4}

Table B-14 Operating Parameters in period F (cont.)

Date	Day	MLVSS/MLSS	COD, mg/l	
		%	feed	effluent
9/10	85	94.9	340	39
9/11	86	92.5	-	-
9/18	90	92.3	336	157
9/19	91	93.6	350	170
9/20	92	91.3	-	-

Table B-15 Operating parameters in period G

Date	Day	MLSS mg/l	ESS mg/l	SVI ml/g	r^* m/kg	pH	SOUR 1/min
10/27	132	685	41	96	-	-	-
10/28	133	540	49	93	-	-	-
10/29	134	550	50	93	-	-	-
10/30	135	510	62	98	-	-	-
10/31	136	560	68	107	-	-	-
11/1	137	575	106	104	-	-	-
11/2	138	575	144	118	-	-	-
11/4	140	710	114	204	-	6.3	-
11/6	142	745	83	215	83×10^{12}	6.1	3.4×10^{-4}
11/7	143	740	48	250	185×10^{12}	6.1	4.5×10^{-4}
11/8	144	755	66	245	110×10^{12}	6.2	2.8×10^{-4}
11/10	146	765	84	261	-	6.1	-
Ave.(day 140-146		743	79	235	126×10^{12}	6.0	3.6×10^{-4}

Table B-15 Operating Parameters in period G (cont.)

Date	Day	MLVSS/MLSS	COD, mg/l	
		%	feed	effluent
11/4	140	95.1	390	60
11/6	142	86.4	-	-
11/7	143	91.7	360	53
11/8	144	89.8	405	57

Table B-16 Operating parameters in period H

Date	Day	MLSS mg/l	ESS mg/l	SVI ml/g	r* m/kg	pH	SOUR 1/min
10/26	131	510	56	78	-	6.7	-
10/27	132	525	57	76	-	-	-
10/28	133	615	47	83	-	6.7	-
10/29	134	600	49	89	-	-	-
10/30	135	505	40	119	-	-	-
11/1	137	650	94	100	-	6.5	-
11/2	138	620	116	137	-	-	-
11/4	140	600	120	242	-	-	-
11/6	142	795	100	1006	68×10^{12}	6.0	-
11/7	143	640	160	1536	74×10^{12}	6.1	-
11/8	144	805	156	398	64×10^{12}	6.5	-
11/10	146	850	119	206	-	6.7	-
11/13	149	930	99	285	-	-	7.1×10^{-4}
11/16	152	860	36	150	97×10^{12}	7.3	10.4×10^{-4}
11/20	156	820	24	134	81×10^{12}	8.0	11.9×10^{-4}
Ave.(day 140-146		865	70	194	98×10^{12}	7.0	9.8×10^{-4}

Table B-16 Operating Parameters in period H (cont.)

Date	Day	MLVSS/MLSS	COD, mg/l	
		%	feed	effluent
11/13	159	81.4	390	53
11/16	161	85.6	368	37
11/20	164	87.1	371	45

Table C-1 Raw data of conditioning tests

Sludge: Blacksburg Wastewater Tretment Plant

Mg Conc. mg/l	Polymer dose mg/l	TTF sec	Na Conc. mg/l	Polymer dose mg/l	TTF sec
	0	16		0	15
0	10	9	250	10	9
	15	6		15	6
	20	6		20	6
	30	7			
	0	12		0	15
	10	8	500	5	10
250	15	8		10	7
	20	6		20	7
	0	12		0	14
	10	8		10	9
400	15	8	750	15	8
	20	8		20	7
				30	7
	0	13		0	14
	5	11	1000	10	9
500	10	7		20	6
	20	7		30	7
	30	9			
	0	12		0	16
	5	11		10	11
600	10	7	2000	20	6
	20	8		30	7
	30	9			
	0	13		0	18
750	10	9		10	13
	20	8	4000	20	8
	30	8		30	7
				40	9
	0	14			
1000	10	9			
	20	8			
	0	16			
1250	20	9			
	30	8			

Table C-2 Raw data of conditioning tests

Sludge from period A			Sludge from period E	
Mg Conc. mg/l	Polymer dose mg/l	TTF sec	Polymer dose mg/l	TTF sec
	0	26	0	23
	5	18	5	19
0	10	12	10	13
	20	6	20	9
	30	12	30	7
			40	9
	0	31	0	19
	10	16	5	18
500	20	8	20	12
	30	7	40	9
	40	7	50	7
			60	9
	0	16	0	20
	10	11	10	13
700	20	7	20	11
	30	11	40	9
	40	11	50	7
			60	9
	0	22	0	17
	5	25	5	16
1000	10	13	10	13
	20	11	20	10
	30	13	30	10

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