THE ROLE OF BIOPOLYMERS IN THICKENING AND DEWATERING OF ACTIVATED SLUDGE

by

Jaidev Kunjur

Thesis Submitted to the faculty of the

Virginia Polytechnic Institute and State University

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

Sanitary Engineering

APPROVED:

William R. Knocke, Chairman

Clifford W. Randall

John T. Novak

December, 1982

Blacksburg, Virginia

ACKNOWLEDGMENTS

I wish to express my sincere appreciation to Dr. William R. Knocke for his excellent guidance and assistance during the course of my studies and in the completion of this research. I also wish to thank Dr. John T. Novak for his helpful advice and ideas. The inputs of Dr. Clifford W. Randall and Dr. Joseph Sherrard are greatly appreciated.

This thesis is dedicated to my parents (Neeradha and Ramadev) and my sisters (Shiela and Sandhya), all very special to me.

Finally, I would like to thank Ann Crate, for typing this thesis at such short notice.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF FIGURES (cont.)

LIST OF TABLES

I. INTRODUCTION

The activated sludge system is one of the most popular and versatile of the biological treatment methods available. The efficiency of the activated sludge system depends on the ability of microorganisms to degrade the different food sources present in the influent. In the initial phase of the process, organic matter is utilized and microbial mass is synthesized. The second phase, and ultimately the most significant in the development of high quality effluent, is the flocculation of microorganisms and other suspended or colloidal components into a readily settleable mass so that a clear, low organic content effluent may be obtained. It is also desirable that the settled waste biomass be easy to dewater for volume reduction considerations related to final disposal.

Prior research has shown that bacterial agglomeration may be accomplished according to accepted coagulation theory where naturally occurring anionic exocellular biopolymers coagulate bacteria via the interparticle bridging mechanism. The specific identification of these biopolymers remains the subject of some debate, but these polymers reportedly contain polysaccharides, proteins and nucleic acids. These high molecular weight biopolymers structure the individual cells into a lattice or three dimensional matrix with sufficient rigidity and porosity. So that the settled biomass may be dewatered using mechanical systems.

A better understanding of the fundamental nature of these polymeric materials and their role in biological sludge dewatering systems may lead to more economical designs and operation of treatment plants. In an attempt to provide research information relevant to this topic, the primary objectives of this study were formulated to:

1. investigate the qualitative and quantitative characteristics of exocellular materials produced in biological waste treatment systems, emphasizing such parameters as molecular weight distribution and chemical characterization in this effort.
2. examine the apparent relationship between biopolymer content and sludge dewatering rate to better establish whether or not a fundamental relationship exists between these two parameters.

II. LITERATURE REVIEW

2.1 The Activated Sludge Process

Activated sludge wastewater treatment is one of the most versatile methods for treating a wide variety of industrial and domestic wastewaters. In the first phase of this aerobic process, a consortium of microorganisms remove soluble organics from wastewater by conversion of the organic waste into new cells, energy and inert end products. The second phase involves the flocculation of microorganisms and other suspended or colloidal components into a readily settleable mass. The efficiency of this separation process determines effluent quality from the treatment plant. The settled waste biomass must be easy to dewater for volume reduction considerations. A filter cake of sufficient dryness must be obtained if a self sustaining incineration reaction is desired.

Tenney and Stumm (1) and Busch and Stumm (2) have postulated that bacterial agglomeration may occur according to accepted coagulation theory where naturally occurring anionic exocellular biopolymers coagulate bacteria via the interparticle bridging mechanism. The existence of biopolymers in activated sludge and their influence on sludge settling and dewatering has been well documented, although the specific identification of these biopolymers remains the subject of some debate.

2.2 Nature of Biopolymers in Activated Sludge

Bacteria in activated sludge produce a great variety of polymeric substances that are exuded as a slime or form a soft capsule which may be ten or more times the volume of the cell. In addition, cells which die during treatment rupture or lyse and release their contents. Most of the contents of lysed cells may be utilized as food by other bacteria, in contrast to capsular material and cell walls which are inherently more resistant to metabolic degradation. A larger number of hydrocolloids have been identified in capsular material and slimes.

A typical biological floc consists of a great variety of bacteria and occasionally other organisms such as protozoa, fungi and viruses, as well as abiotic suspended matter. A floc may thus comprise a broad spectrum of hydrophobic and hydrophillic interfaces (2). The cell walls, capsules, flagellae, fibrils, filaments and fimbrae of microorganisms are composed of proteins, lipids and polysaccharides combined in various proportions. The morphological appearance of the floc depends on the character of the microbial population and nutritional conditions in the medium. The degree of bioflocculation observed has been related to substrate availability. Conditions of declining or endogeneous growth have been found to yield better sludge settling characteristics in comparison to conditions of profilic growth (2,3). Busch and Stumm (2) hypothesized that an adequate concentration of polymer can accumulate per unit surface area only under conditions

of declining or endogeneous growth. Under prolific growth conditions, new microbial surfaces may be produced faster than surfaces can become covered with polymer. Cell binding polymeric materials conducive to flocculation are known to be synthesized by some strains of microorganisms especially under conditions where growth is limited by a nutrient such as carbon or nitrogen. The chemical transformations and condensations necessary for synthesis of polymers demands little energy and can be carried out by the cells even in the absence of growth (2).

Tenney and Stumm (1) described the naturally produced polymers responsible for the aggregation of bacterial cells as complex polysaccharides and polyamino acids that are either excreted or lysed at the surface of the cell during the endogeneous or declining phase of growth. Pavoni et al. (3) have shown that these polymers contain the major cellular components; DNA, RNA, proteins and polysaccharides. These polymeric materials are released in batch cultures by cell lysis after the period of log growth has ceased.

Forster (4,5) showed that the surfaces of activated sludge particles were of a polysaccharide nature. For good settling sludges (low sludge volume index (SVI) values) the main ionogenic material was glucouronic acid. By hydrolysis and thin layer chromatography, this polysaccharide was shown to be composed of galactose, fucose, mannose and glucouronic acid. It was also shown that the total carbohydrate content of the sludge increased

with the SVI. It was therefore suggested that nutritional imbalances cause these carbohydrate variations which result in alterations in the sludge surfaces as well as in flocculation and settling characteristics.

Wilkinson (6) examined bacterial polysaccharides and identified three major fractions which included intracellular polysaccharides, cell wall polysaccharides and extracellular polysaccharides. He noted that some bacteria form extracellular capsules, and others excrete chemically similar materials that are unattached to cell surfaces. Extracellular polysaccharides were therefore subdivided into slime polysaccharides (those external to the cell but not physically attached to it), and capsule polysaccharides (those attached directly to the exterior of the cell wall).

Gulas et al. (7) postulated that two regions appear to exist with regard to exocellular polymer content per unit of biomass and sludge age. At low sludge ages and high specific growth rates, the bacterial cells may undergo autolytic activity, releasing biopolymers into the medium. They showed that at a sludge age (θ_c) of two days, pinpoint flocs were present along with maximum polymer production. The existence of pin point floc at lower θ_c values was explained to be due to the autolysis of low molecular weight polymers whose agglutinative properties are deficient. At high sludge ages and low growth rates, autolysis of cells slows and polymer production again increased which agreed with the

classical theory of polymer production during endogeneous respiration of the cell. At high sludge ages, bacterial cells release high molecular weight polymers which are more amenable to efficient flocculation of microorganisms.

Saunders and Dick (8) studied the influence of θ_c on the chemical characteristics of activated sludge effluents. Slime organic matter contained low molecular weight (mol wt $\leq$ 500) and high molecular weight (mol wt > 100,000) subfractions, both of which decreased as θ_c increased from 0.8 days to 4.8 days. As θ_c was increased above 4.8 days, high molecular weight slime decreased to about five percent of the total effluent chemical oxygen demand (COD), and low molecular weight slime increased to about two-thirds of the total effluent COD. Filterable fractions of activated sludge effluents were shown to contain organic compounds varying in molecular size from that of low molecular weight volatile acids to cell wall fragments. The authors concluded that effluent organic compounds may be composed of microbial waste products, partially oxidized or hydrolysed influent organic compounds, or condensation products formed through the reaction of influent organic compounds and microbial waste products.

According to Dewalle and Chian (9) twenty to fifty percent of the effluent COD may be due to the presence of humus like substances. They postulated that the formation of refractory material possibly occurs after the removal of a readily available

carbon source. The amount of refractory material present in solution will depend on the adsorption properties of the humic materials towards bacterial cells. The carbohydrate content of the refractory material will influence the ability of humic materials to adsorb onto bacterial cells. The authors showed that if the amount of carbohydrate present in the humic and fulvic acid fraction is substantial, both fractions will adsorb onto bacterial cells. When carbohydrate content decreases, adsorption may not take place, resulting in poor flocculation and settleability. This decrease in adsorption would cause an increase in the concentration of refractory materials in the effluent. Humic acids have been reported to have a molecular weight between five thousand and a million, while the molecular weight of fulvic acids ranged from two thousand to ten thousand (9). Peter and Wuhrmann (10) stated that humic acids, isolated in substantial quantities in the effluent, may have a function in bridging cell particles and subsequent floc formation.

Brown and Lester (11) reported on previous research into the factors that affect concentrations of extracellular polymers in bacterial cultures and activated sludge. Some of these were: (1) the ratios in the growth medium of carbon to nitrogen, carbon to phosphorus and carbon to sulfur; (2) oxidation of extracellular polymers; (3) dissolved oxygen concentration; and (4) biochemical oxygen demand. They also found extracellular polymer concentrations in activated sludge increased as θ_c increased from three

days to nine days, then increased more gradually up to a θ_c of eighteen days, showing a direct relationship with mixed liquor suspended solids concentration. Polysaccharides and proteins were detected in samples extracted from pure cultures of Klebsiella aerogens and activated sludge. Deoxyribonucleic acid was also present in samples extracted from activated sludge. Brown an Lester concluded that increased polymer concentrations enhanced metal adsorption in the activated sludge process.

2.3 The Role of Biopolymers in Sludge Settling and Dewatering

Biopolymers can act as links between individual dispersed colloids, causing the particles to agglomerate into flocs and eventually settle out of suspension. Coagulation of bacteria in the activated sludge process may occur through the mechanism of interparticle bridging in which negatively charged bacteria are coagulated by naturally occurring biopolymers. Therefore, an activated sludge floc would consist partially of a matrix of bridging polymers to which bacteria may attach. These biopolymers may vary in molecular weight over a wide range. It is also likely that smaller polymers, not capable of interparticle bridging, may also be associated with the floc matrix (Novak and Hangan (12)).

Parker et al. (13) have shown that activated sludge flocs are composed of a system of semi-rigid floc matrices with a size range of one hundred to one thousand micrometers (μm) to which primary particles of 0.5 to 5.0 μm are weakly adsorbed. Roberts and

Olsson (14) described activated sludge flocs by an adsorption model where the floc matrix serves as the adsorbent and the adsorbate is comprised of natural anionic biopolymers. These colloidal biopolymers exist at equilibrium with additional biopolymers adsorbed to the floc surface. The adsorbed biocolloids may be dislodged from the floc surface by mixing. Their surface binding strength seems to be influenced by solution cation concentration and pH. Colloidal biopolymers were found to be responsible for poor sludge filtration rates and required the bulk of chemical conditioning agents. Based on the results of activated sludge conditioning studies using cationic polymers, Roberts and Olsson concluded that cationic polymers react primarily with unsorbed anionic biopolymers rather than with the activated sludge flocs. Therefore, the optimum polymer dose was found to depend on the equilibrium concentration of solution polymers.

Novak and Haugan (12) found that both high and low molecular weight cationic polymers perform best at or near the point of zero charge, as measured by electrophoretic mobility, indicating that the polymers function by charge neutralization of the solution colloids. When metal salts were used to condition activated sludge, a reduction in the concentration of anionic colloids was produced. This was explained to be due to coagulation by charge neutralization caused by positively charged kinetic intermediates or by providing positively charged metal hydroxide precipitants

to which the polymeric colloids adsorbed. The authors proposed an adsorption model for activated sludge flocs where total polymer may be comprised of three fractions: (i) polymer incorporated into the sludge floc; (ii) loosely adsorbed polymer; and (iii) free or supernatant anionic particles. It seems possible that changes in operating conditions for activated sludge such as pH, nutrients, growth conditions and mixing conditions would all influence the concentration of each fraction relative to the other two fractions.

Novak et al. (15) described activated sludge as a slurry containing an excess of anionic polymers. These anionic polymers were felt to interfere with sludge filtration rates. The thickening and dewatering properties of activated sludge were found to be influenced by the concentration of free colloidal anionic polymer in the supernatant liquor and not the anionic polymers associated with biological flocs. Colloidal solids, hydrolyzing metal ions, cationic polymers and acid were all successful coagulant aids. When activated sludge was conditioned with an anionic polymer, the authors found that sludge dewatering properties worsened even with small polymer doses. They concluded that the length of the polymer must be sufficient to bridge between sludge particles, and the ability of anionic polymer to bridge cells is adversely affected by the interaction of like charged particles.

The mechanism of attachment of anionic polymeric species to net negatively charged colloidal surfaces involves chemical forces such as hydrogen bonding, anion interchange with adsorbed anions (such as OH^-) or interaction with cations on the colloid surface (1,2,16). The length of the extended polymer segments attached to the sludge particle must be sufficient to bridge the minimum distance separating the particles (established by electrostatic repulsion of like particle charges) to form the floc matrix. This electrostatic interference due to like charges may be reduced by the addition of cations which could reduce the net negative charge on the sludge particles and, correspondingly, reduce the minimum distance separating the particles.

Wu et al. (17) examined biological sludge dewatering characteristics using continuous flow activated sludge treatment systems cultivated under different growth and nutrient conditions. They attributed poor sludge filtering properties to: (i) the excessive growth of long attached and free floating filamentous microorganisms; (ii) the existence of dispersed and pinpoint floc; and (iii) the overproduction of extracellular biopolymer that would produce a considerably higher surface charge around the sludge. Under severely limited amount of nitrogen in the wastewater, the growth of activated sludge was found to be subject to the undesirable properties mentioned above. Nitrogen deficient

filtration than nitrogen rich activated sludge when both the food to microorganism ratio and θ_c were identical.

Pitman (18) reported effluent suspended solids concentration as a good indicator of sludge dewatering characteristics. Effluent samples from activated sludge systems operated at a mean cell residence time of 7.5 days were cloudy, contained mobile microorganisms along with fine floc particles, and had poor settling and dewatering properties. Certain protozoa had a marked effect on bioflocculation, attached protozoa appearing to promote flocculation by removing finely divided particles from the system. Since mobile protozoa are dispersed, they have an adverse effect on flocculation. Pitman concluded that good bioflocculation occurs in the activated sludge plant when attached microorganisms are present in large numbers and mobile microorganisms are absent.

Friedman et al (19) showed that bacteria which were isolated on the basis of their characteristic flocculent growth habit all possess exocellular fibrillar polymers. Flocculation was felt to occur by entanglement of cells among fibrils or adsorption of cells to fibrils.

Becari et al. (20) studied bulking sludge and exocellular polymer production (ECP) under various operating conditions. In the first phase of their research, every decrease or increase in SVI was matched by a decrease or increase in ECP content. However, in the second phase, increased SVI corresponded to a decrease in ECP and vice versa. This apparently contradictory

data was interpreted by likening ECP behavior to that of a polyelectrolyte in the coagulation-flocculation process of a particle suspension. Only with optimum polyelectrolyte dosages does destabilization of the suspension and agglomeration of particles occur satisfactorily. When ECP production was higher than optimum, poor sludge flocculation caused an increase in SVI. Exocellular polymer production near the optimum resulted in a reduction in SVI due to an improvement in bioflocculation.

Erickson and Axberg (21) demonstrated that pollutants can have a significant influence on the aggregation and settling behavior of activated sludge even at rather low concentrations. The authors concluded that the amount of extracellular material critically determined the concentration where a pollutant or a flocculating agent gave its maximum positive or negative effect on aggregation and sedimentation.

2.4. Characterization of Biopolymer

Several methods have been used to extract extracellular polymers from activated sludge. Brown and Lester (22) compared five different bacterial extracellular polymer extraction methods on cultures of activated sludge, synthetic activated sludge and *Klebsiella aerogenes*. For the *Klebsiella aerogenes* culture, high speed centrifugation was the most effective extraction method. Steaming treatment was most effective for activated sludges, since it released a significant quantity of biopolymer and caused less

cellular disruption than ethylenediaminetetraacetic acid and sodium hydroxide treatments. Ultrasonication released low concentrations of extracellular polymers from all cultures.

Pavoni et al (3) used high speed centrifugation with a force of 32,000 x G for fifteen minutes in an effort to quantitatively extract polymers from activated sludge. In a recent publication, Novak and Haugan (23) demonstrated that high speed centrifugation does not strip polymer from activated sludge flocs. Further, the authors showed that no significant difference existed between centrifuged sludges and settled supernatant liquors concentration of high molecular and low molecular weight organic fractions (obtained following elution through Sephadex G-25 and G-75).

Prior research by Novak and Haugan (15) indicated that the most important biopolymer fraction interfering with sludge dewatering was in solution and not associated with the biological flocs. Gel filtration chromatography was found to be a promising method for determining molecular weight distributions in the supernatant of activated sludge without causing cell lysis or altering biopolymer properties.

Separation of molecules on Sephadex is obtained by a kind of molecular sieving. Small molecules move with the elutant both within and outside the Sephadex particles. Molecules larger than the pore size of the gel particles cannot penetrate the particles and, therefore, move rapidly down the column with the elutant.

The smaller particles penetrate the gel particles to a varying extent, depending on their shape and size. Therefore, the first molecules that exit the column will be molecules larger than the rated pore size of the gel.

The fractionation ranges give the general range of molecular sizes by globular proteins, peptides and dextrans that will be separated by the gels.

Several researchers have characterized secondary effluents using gel filtration chromatography. Sachdev (24) used Sephadex G-10, G-15 and G-25 to partially characterize the organics in treated effluents from trickling filter and contact stabilization plants. The organic carbon recovery from the Sephadex gel column was 85.1 to 97.8 percent. There was neither sorption of organics on the gels nor dissolution of dextran from the gels. Rebhun and Manka (25) showed that 40 to 50 percent of the organics in secondary effluents were humic substances (humic, fulvic and hymathomelanic acids), the fulvic acid being the major fraction of this class. The remainder of the organic matter consisted of ether extractables, anionic detergents, carbohydrates, proteins and tannins.

Manka et al. (26,27) compared the molecular weight distributions and the soluble organic groups in secondary effluents. The authors concluded that the main components of high molecular weight compounds in secondary effluents may be humic substances, proteins and carbohydrates (polysaccharides).

III. METHODS AND MATERIALS

The research objectives of this study were fulfilled by operating two continuous flow activated sludge reactors in a controlled temperature chamber. Sludge samples from the Christiansburg and Blacksburg wastewater treatment plants were also studied. A detailed description of the experimental techniques and associated analysis procedures follows.

3.1 System Operation

Two completely mixed, continuous flow activated sludge reactors were operated in a controlled temperature chamber where the temperature was maintained at 20 ± 1°C. From the twenty liter feed container shown in Figure 1, 18 liters/day of feed solution was introduced into both reactors through intravenous lines at a constant flow rate of 12.5 mL/minute. This provided a hydraulic retention time of twelve hours. The composition of feed solution is given in Table I. Sludge from each nine liter reactor was wasted every day by lifting the baffles, allowing complete mixing, and pumping 900 mL of biomass from each reactor into one liter measuring cylinders. The average mean cell residence time was therefore set at ten days. This sludge sample was then analyzed for dewatering and settling properties. The supernatant from settled sludge was characterized for biopolymer, protein, carbohydrate and humic acid concentration. To insure steady state conditions, pH, total suspended solids, effluent

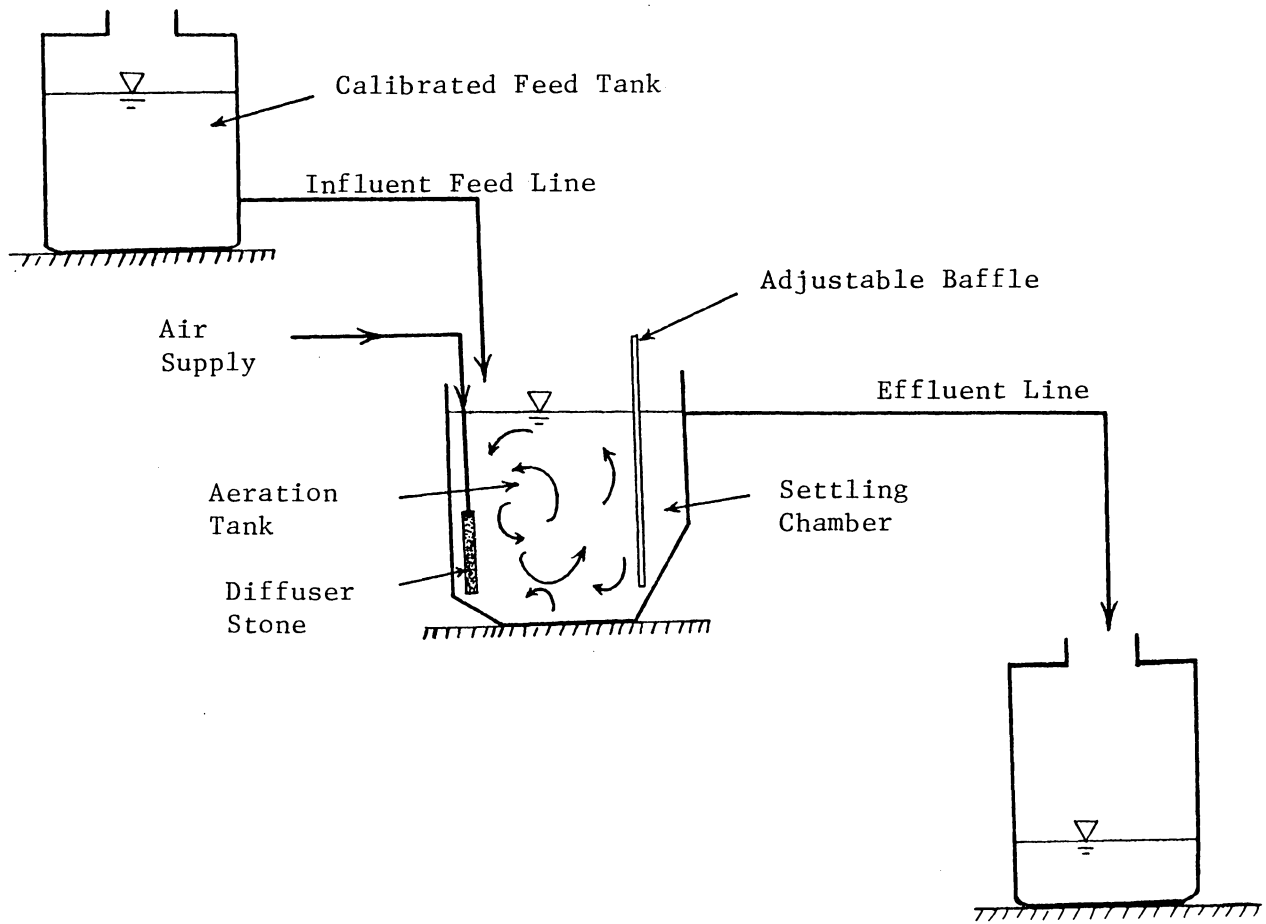

Figure 1. Experimental continuous flow reactor set up.

TABLE I. COMPOSITION OF INFLUENT SUBSTRATE SOLUTION

Nutrient	Influent Concentration (mg/L)
Bacto-peptone	491
$MgSO_4$ $7H_2O$	53
$MnSO_4$ $7H_2O$	53
$FeCl_3$ $6H_2O$	0.67
$CaCl_2$	4.0
KH_2PO_4	8.9 (mg/L as P)
K_2HPO_4	7.1 (mg/L as P)
Na_2CO_3	114 (mg/L as $CaCO_3$)

suspended solids were constantly monitored by techniques described in Standard Methods (28).

3.2 Sludge Dewatering and Settling Characteristics

a. Dewatering

Dewatering tests were conducted using the Buchner funnel apparatus shown in Figure 2. Equipment utilized included a 9 cm Buchner funnel, Whatman No. 40 ashless filter paper, a 100 mL graduated cylinder and a vacuum pump. One hundred milliliter waste sludge samples were used throughout all of the filtration studies. The dewatering procedure utilized has been described in greater detail by Vesilind (29). In general, once the vacuum was initiated, filtrate volumes were collected in the graduated cylinder and recorded as a function of time. The vacuum level was maintained at 15 inches Hg (approximately 7.5 psi).

The specific resistance of sludge was then calculated as follows:

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

where

r^* = specific resistance, m/Kg

P = pressure, N/m^2

A = area of filter paper, m^2

b = slope of θ/V versus V plot

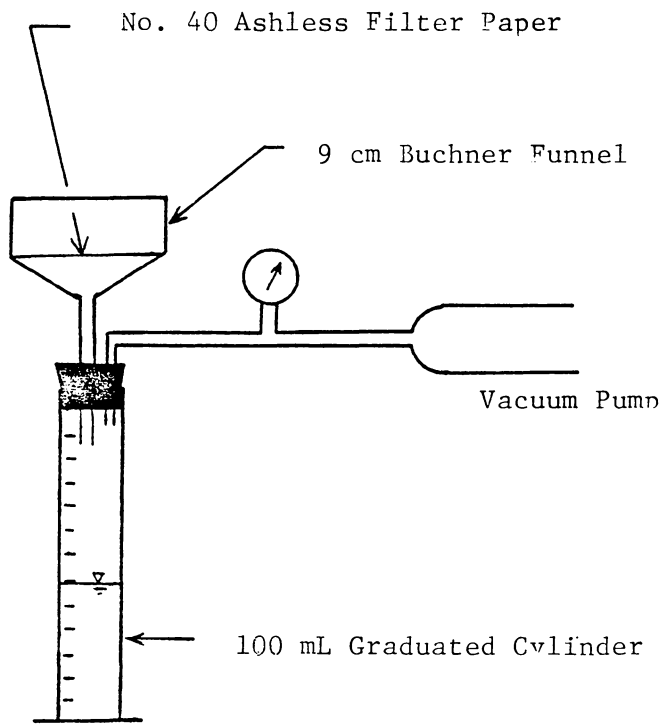

Figure 2. Buchner funnel apparatus for determining sludge dewatering rates.

μ = dynamic viscosity, $Nsec/m^2$

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

b) Settling characteristics:

Sludge settling characteristics were examined using the Sludge Volume Index (SVI) test. The test was conducted in a one-liter graduated cylinder, and the settled sludge volume measured after 30 minutes. The SVI is then calculated as follows (29):

$$SVI = \frac{\text{mL of sludge x 1000}}{\text{suspended solids concentration, mg/L}}$$

3.3 Supernatant Characterization

The supernatant from settled sludge samples was analyzed for biopolymer content and particle size distribution. The biopolymer was separated by gel chromatography and examined for protein, carbohydrate and humic acid concentrations.

a. Gel Filtration Chromatography

The experimental set up is shown in Figure 3. Sephadex G-50 was chosen because of its suitable fractionation range, as shown in Table II. Sufficient quantities of dry gel were placed in distilled water and allowed to swell for 24 hours. The swollen gel beads were then poured into a 2 cm x 90 cm cylindrical glass column up to a height of 80 cm. The eluant distilled water was stored in a glass vessel 30 cm above the column to insure

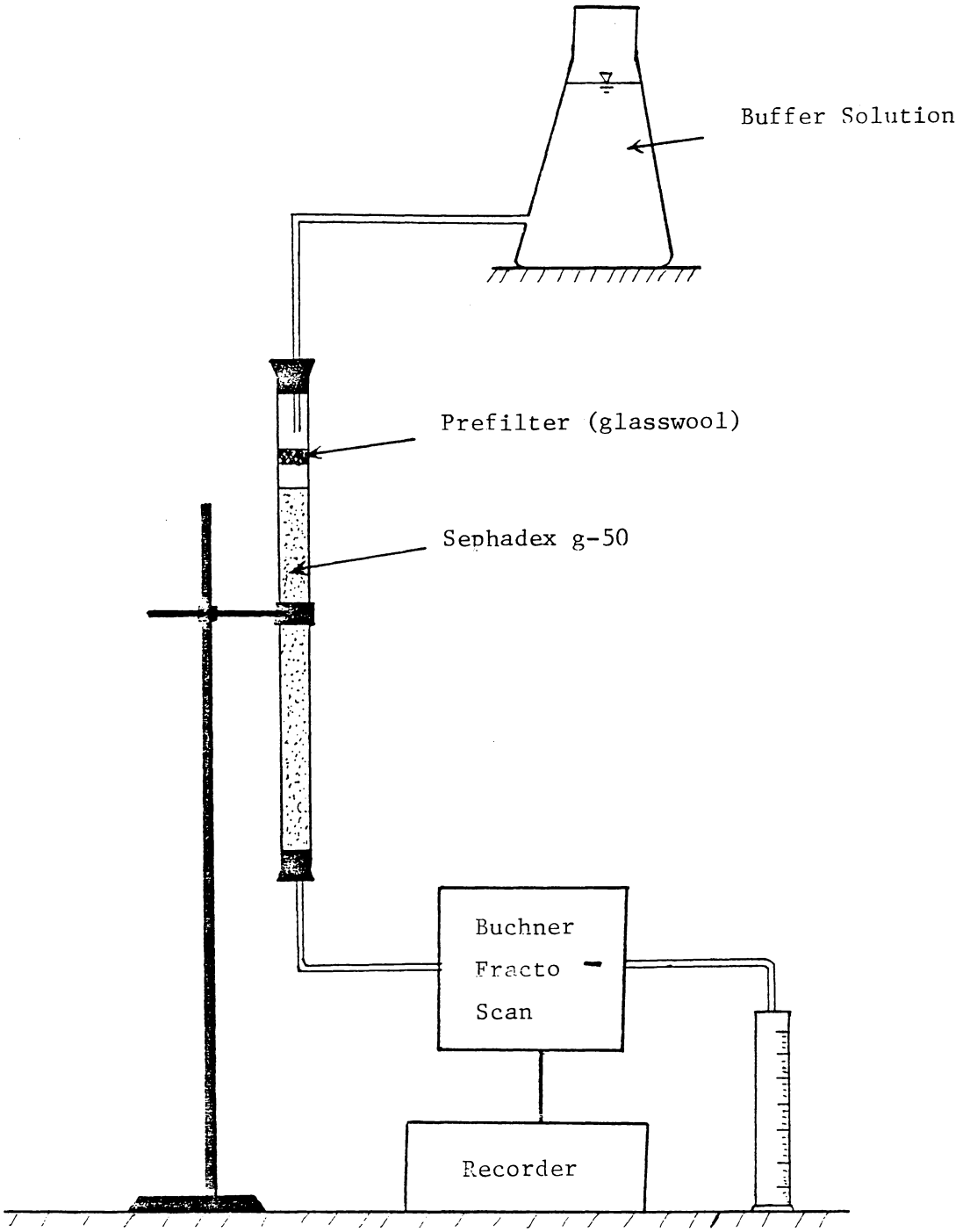

Figure 3. Experimental setup for gel filtration chromatography.

TABLE II. SEPHADEX GELS AND THEIR PROPERTIES

Type	Dry Particle Diameter, μm	Fractionation Range	
		Peptides and Globular Proteins (molecular weight)	Dextrans (molecular weight)
G-25	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

sufficient head such that the desired flow rate of 2 mL/min could be attained. The flow rate was controlled by the stopcock at the exit line of the tube. Sample volumes of 5 mL were added at the top of the column and eluted with 100 mL of distilled water.

The organics concentration in the eluant was continuously monitored by a Buchler Fracto Scan ultra-violet light source set at a wave length of 280 nm. This was connected to a recorder which monitored changes in output. The eluant from the column was collected in a fraction collector, calibrated to collect exactly 10 mL in each tube. From the calibration plot the molecular weight of high (HMV) and low (LMW) molecular weight fractions could be determined.

The peak heights are related to the concentrations of organics in the HMW and LMW fractions. The samples collected in the fraction collector were analyzed for Total Organic Carbon (TOC), proteins and carbohydrate content.

Prior to gel filtration, all supernatant samples were concentrated by a factor of 3:1 in a vacuum evaporator set at 40°C.

b. Total organic carbon:

The Dohrmann DC-50A/52A TOC analyzer and the DC-54 ultra-low level TOC analyzer systems were used to measure TOC concentration in the sludge supernatant and in the fraction samples collected during the gel chromatography tests.

c. Proteins:

Protein concentrations in the individual fractions were determined by the Lowry method (30). The detailed procedure is listed in Appendix D4.

d. Carbohydrates:

Submicro amounts of carbohydrates were detected by the phenol sulfuric acid reaction, developed by Dubios et al. (31). The detailed procedure is presented in Appendix D4.

3.4 Humic Acids

Insoluble humic acid like substances were isolated by a procedure developed by Rebhun et al. (25). Supernatant was filtered through a 0.45 m Millipore membrane. The filtrate was then mixed with an equal volume of diethyl ether in a separatory funnel. The water layer was then separated from the ether layer. The pH of the water layer was then lowered to pH 1.0 with hydrochloric acid, at which time a precipitate appeared. This precipitate was collected using a 0.45 m Millipore membrane filtration system and was subsequently washed with 50 mL ethyl alcohol. The precipitate was then allowed to dry in a vacuum dessicator. After one day, the weight of precipitable humic acids was measured by gravimetric determination.

3.5 Particle Size Analysis

A HIAC Mocel PC320 , 12-channel automatic particle size analyzer was utilized to obtain the characteristic size distribution of particles in various sludge supernatant samples. Analysis during this study utilized a HIAC-60 particle size sensor with a detection limit of 1 to 60 µm.

IV. RESULTS AND DISCUSSION

4.1 System Operation

Operating conditions such as pH, total suspended solids and effluent suspended solids concentrations for Reactor #1 and #2 are shown in Table III. Daily variations of each parameter are given in Appendix B.

4.2 Gel Filtration Chromatography

A typical chromatogram for a supernatant sample is shown in Figure 4. This type of separation of high molecular weight (biopolymer) organics and low molecular weight organics (LMW) was observed in all sample analyses after the supernatant had been concentrated by a factor of 3:1 (Chapter III). The elution volume V_e, is the quantity of solvent passing through the gel bed up to the appearance of the separated substance. This depends on the volumetric distribution of the gel bed, the quality of the gel and the properties of the separated substance. The elution volume for HMW biopolymer, V_e, was at 20 mL; V_e for LMW material was at 60 mL. Calibration of the column with organics of known molecular weight (Table IV) gave a linear relationship between the logarithm of molecular weight and elution volume as shown in Figure 5. The biopolymer or HMW fraction contained organics with a molecular weight near $>10^5$ and the LMW fraction consisted of organics with molecular weight near 1500. Total organic carbon (TOC) measurements, in milligrams per liter (mg/L) correlated

TABLE III. AVERAGE OPERATING PARAMETERS FOR REACTORS #1 and #2

Parameter	Reactor #1	Reactor #2
Mean Cell Residence Time, θ_c (in days)	10	10
Total Suspended Solids, mg/L	2,550	2,500
Effluent Total Suspended Solids, mg/L	25	29
Soluble Influent COD, mg/L	350	350
Soluble Effluent COD, mg/L	21	25
Influent pH	8.1	8.1
Tank pH	7.4	7.5
Influent TKN, mg/L	40	40

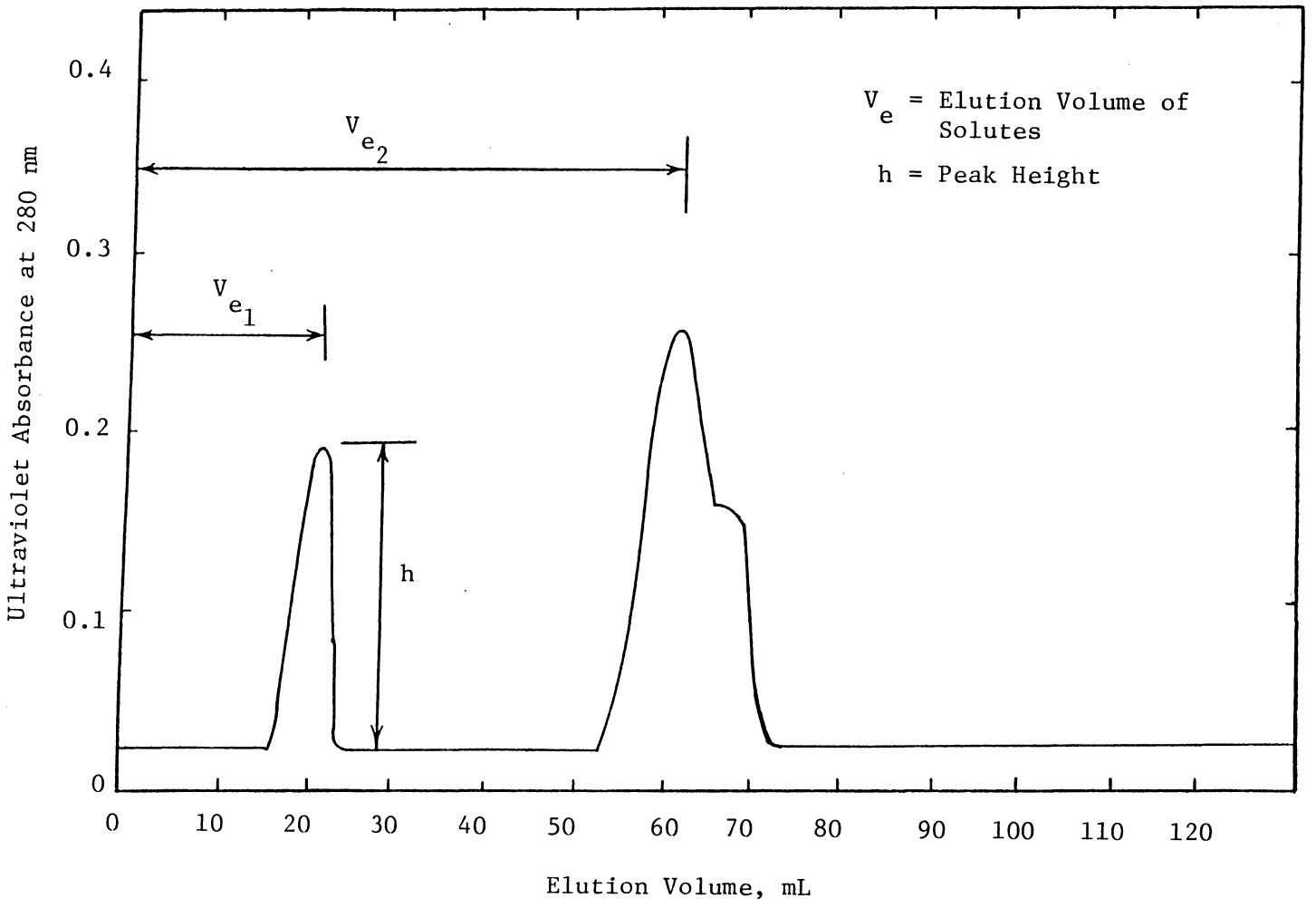

Figure 4. Typical chromatogram for size exclusion chromatography of sample.

TABLE IV. ORGANIC COMPOUNDS OF KNOWN MOLECULAR WEIGHT USED FOR CALIBRATION OF GEL FILTRATION COLUMN

Compound	Molecular Weight	Elution Volume, mL	log MW
Blue Dextran	2×10^6	19	6.3
Bovine Serum Albumin	6×10^4	27	4.8
Chymotrypsin A	2.5×10^4	30	4.4
Ribonuclease A	1.4×10^4	38	4.1
Acetyl Choline Tetra Hydrazine	3500	54	3.5

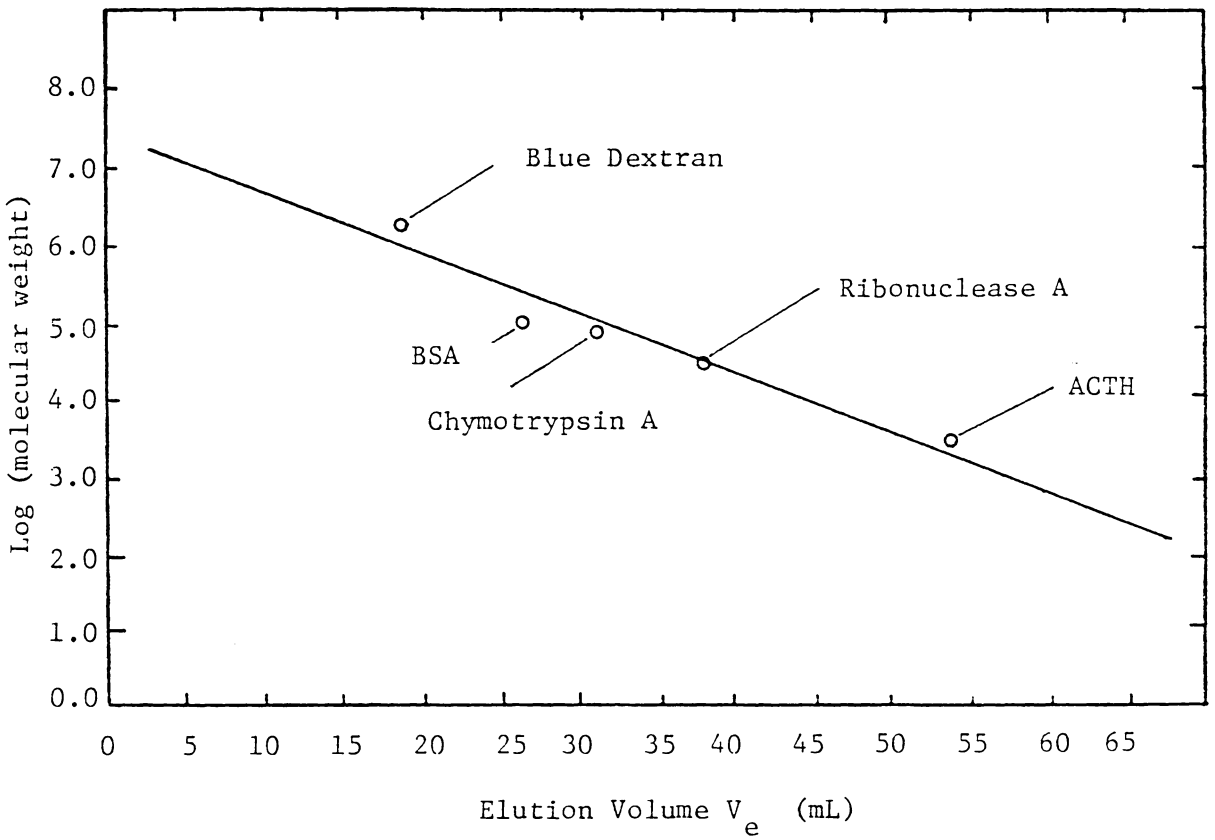

Figure 5. Calibration of Gel Filtration Column using organics of known molecular weight.

well with ultraviolet (u.v.) absorbance values for each sample analysis, an example of which is presented in Figure 6. Thus, TOC and uv analyses were used to quantify biopolymer concentrations.

Poor peak resolution was a problem during the initial stages of this research. The effect of vacuum evaporation and concentration on peak height and resolution is shown in Figure 7. The shape of the peaks were drastically improved by this technique, making the chromatogram data more reliable and accurate.

One concern about this technique was that vacuum evaporation might cause polymerization of low molecular weight organics, thus giving misleadingly high values for biopolymer concentration. By measuring TOC concentrations in the eluant fractions (Figure 8), a mass balance of influent and effluent organics could be made (Appendix A1). Compared to the samples concentrated twice (2:1), a 36 percent increase in TOC for 10:1 sample was observed. Although total TOC concentration has increased due to concentration of sample, the relative areas occupied by front peak (HMW) in relation to the back peak (LMW) remained almost constant. This implies that both the front and back peaks increased proportionally. A sample concentration factor of 3:1 was therefore used throughout this research. To ascertain how much of the sample organics was lost during gel filtration, the percentage of effluent TOC accounted for by the peaks is shown in Figure 8. An average of 71 percent of effluent TOC could be accounted for.

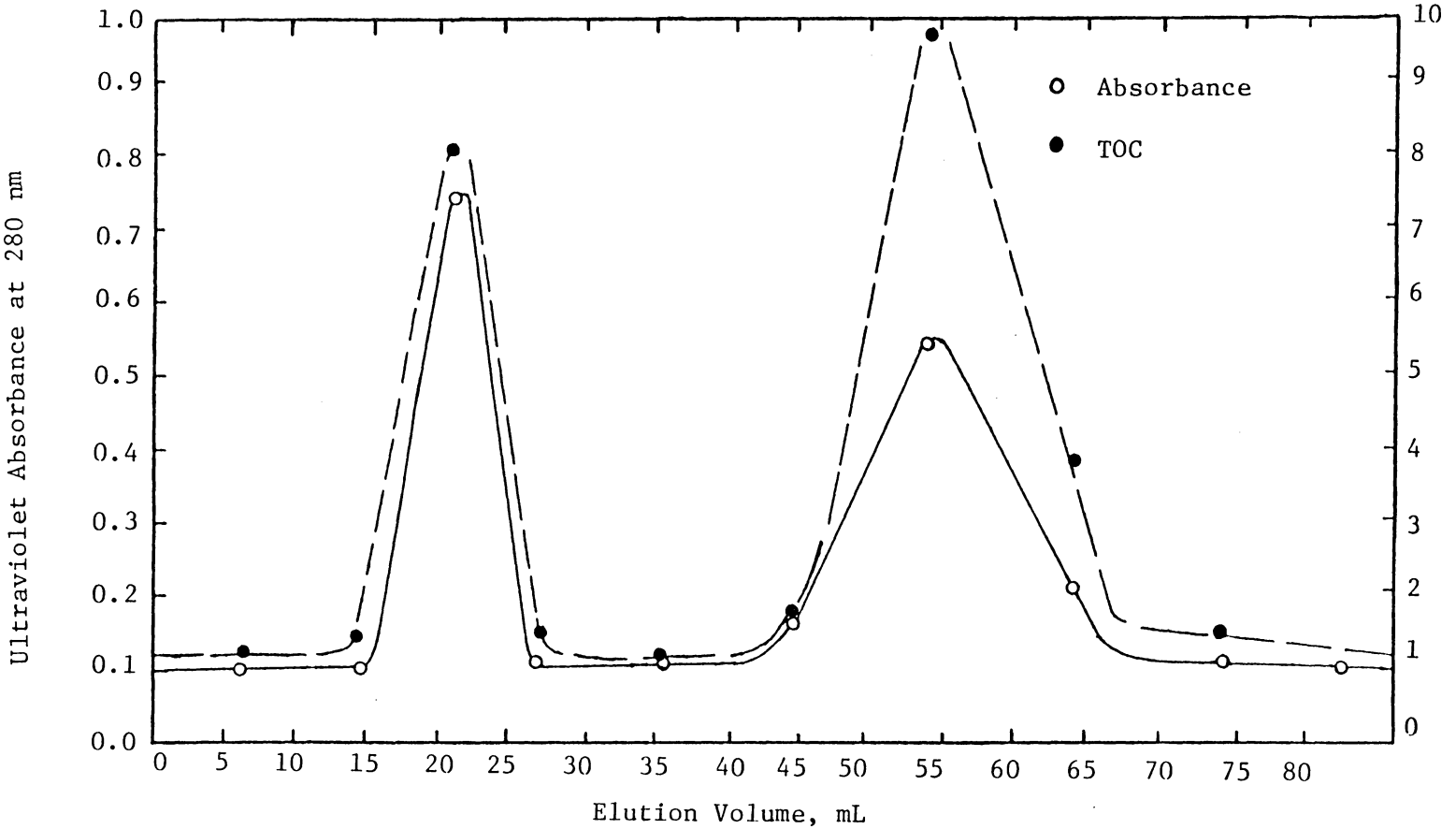

Figure 6. Absorbance versus TOC for fractions collected from the eluant.

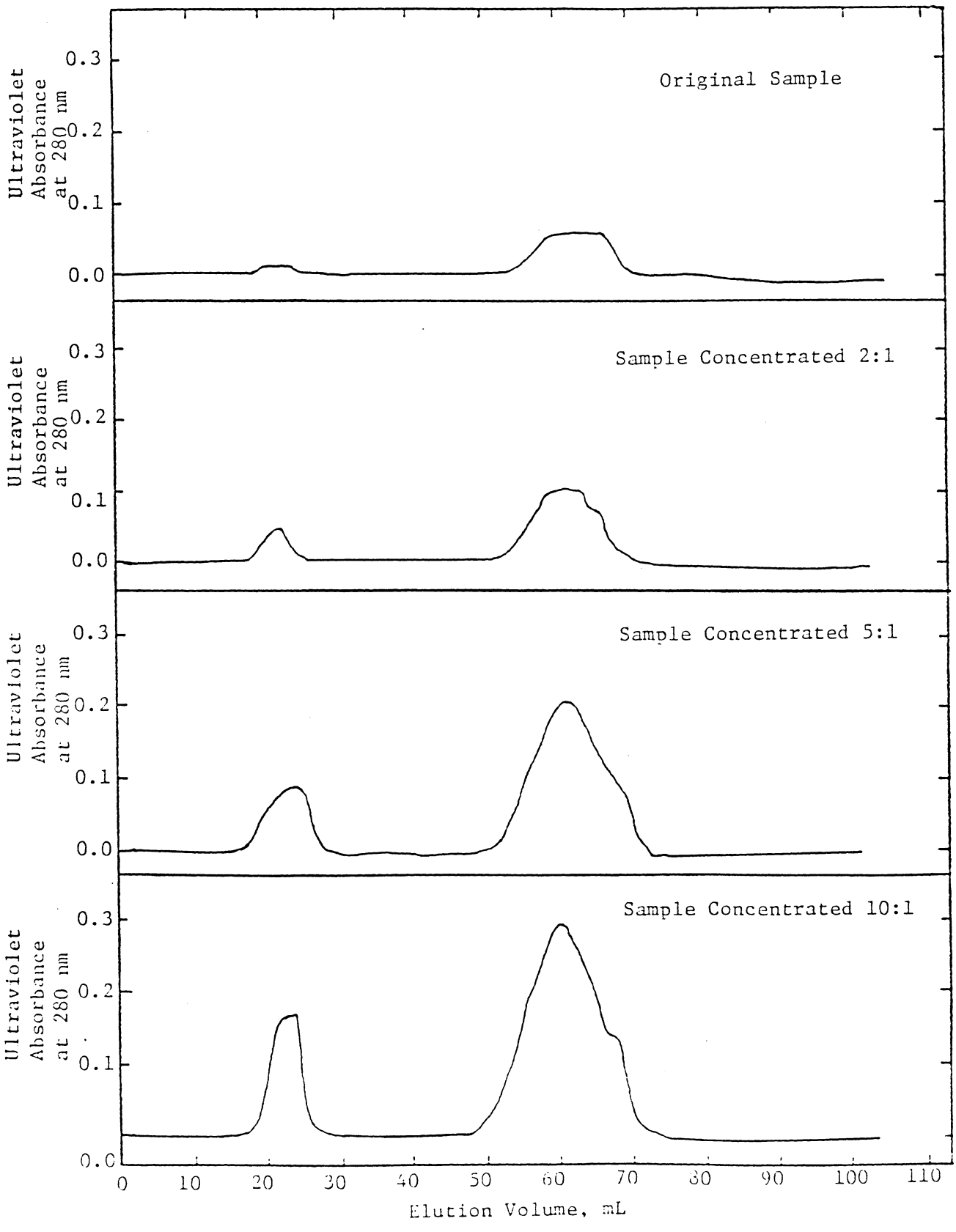

Figure 7. The effect of concentration of sample (at 40°C) on biopolymer peak heights.

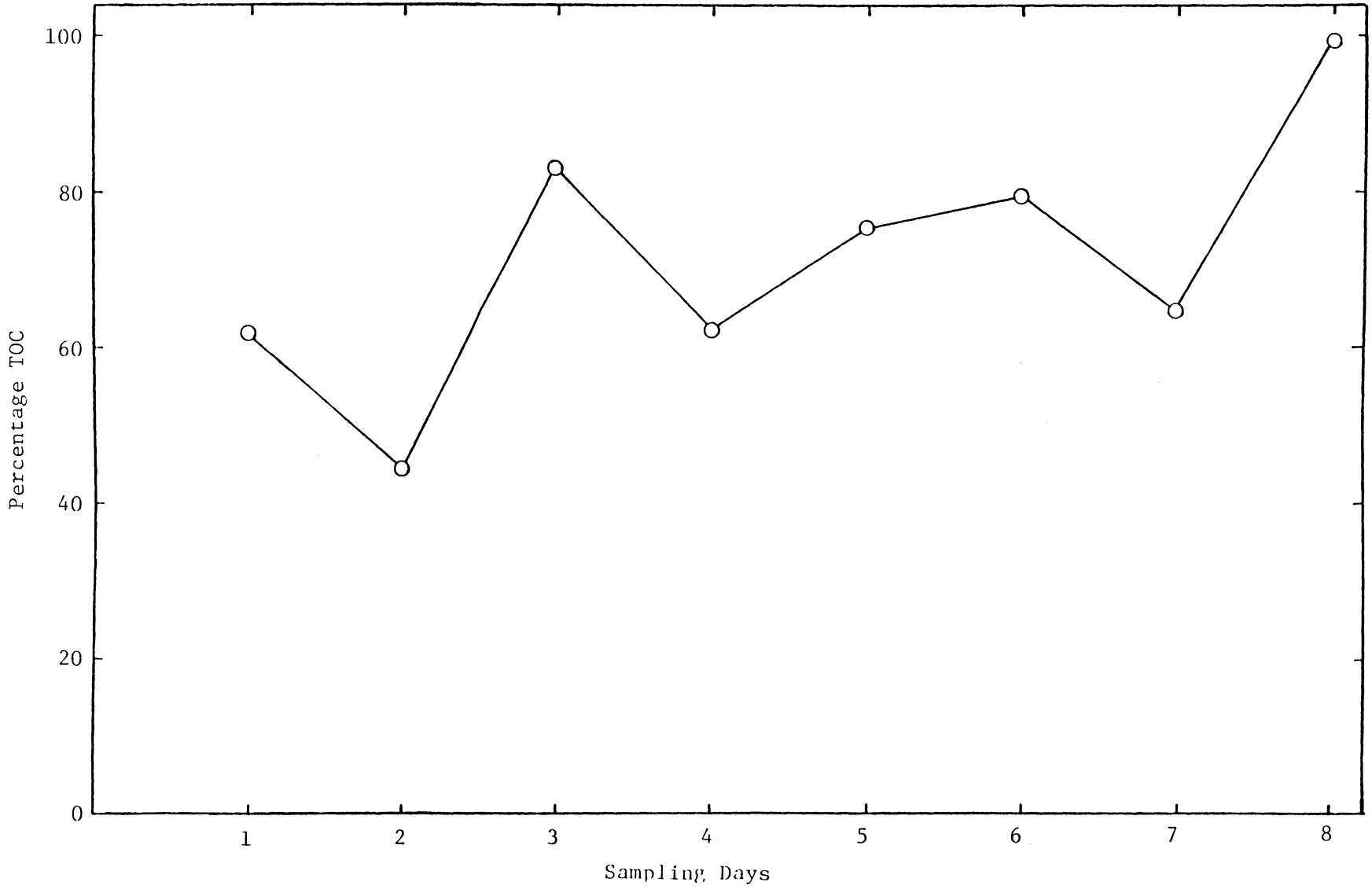

Figure 8. Percentage of effluent Total Organic Carbon accounted for by Chromatogram peaks.

The remaining 29 percent may have been lost due to sorption of organics on the gels or volatilization of low molecular weight organics. This value is much lower than the 85.1 to 97.8 percent recovery of organic carbon from Sephadex gel column as reported by Sachdev (24).

The relationship between effluent biopolymer concentration (in terms of front peak height, in millimetres) and effluent TOC for Reactor #1 is shown in Figure 9. Every increase or decrease in effluent TOC is matched by an increase or decrease in effluent biopolymer concentration. Investigation of filtrable fractions of activated sludge effluents by Saunders and Dick (8) has shown the effluent to contain organic compounds varying in molecular size from that of low molecular weight volatile acids to cell wall fragments, including numerous polymeric compounds such as proteins, polysaccharides and nucleic acids excreted by microorganisms. Biopolymer fractions collected may correspond to solution colloids, lysed cellular products and other microbial products, all of which will be measured as TOC.

4.3 Settling and Dewatering Properties

Waste sludge samples from Reactors #1 and #2 were analyzed at regular intervals for sludge dewatering and biopolymer concentration in the supernatant. Typical results are shown in Figures 10 and 11. It is interesting to note that even though reactor feed solution, temperature and pH for both reactors

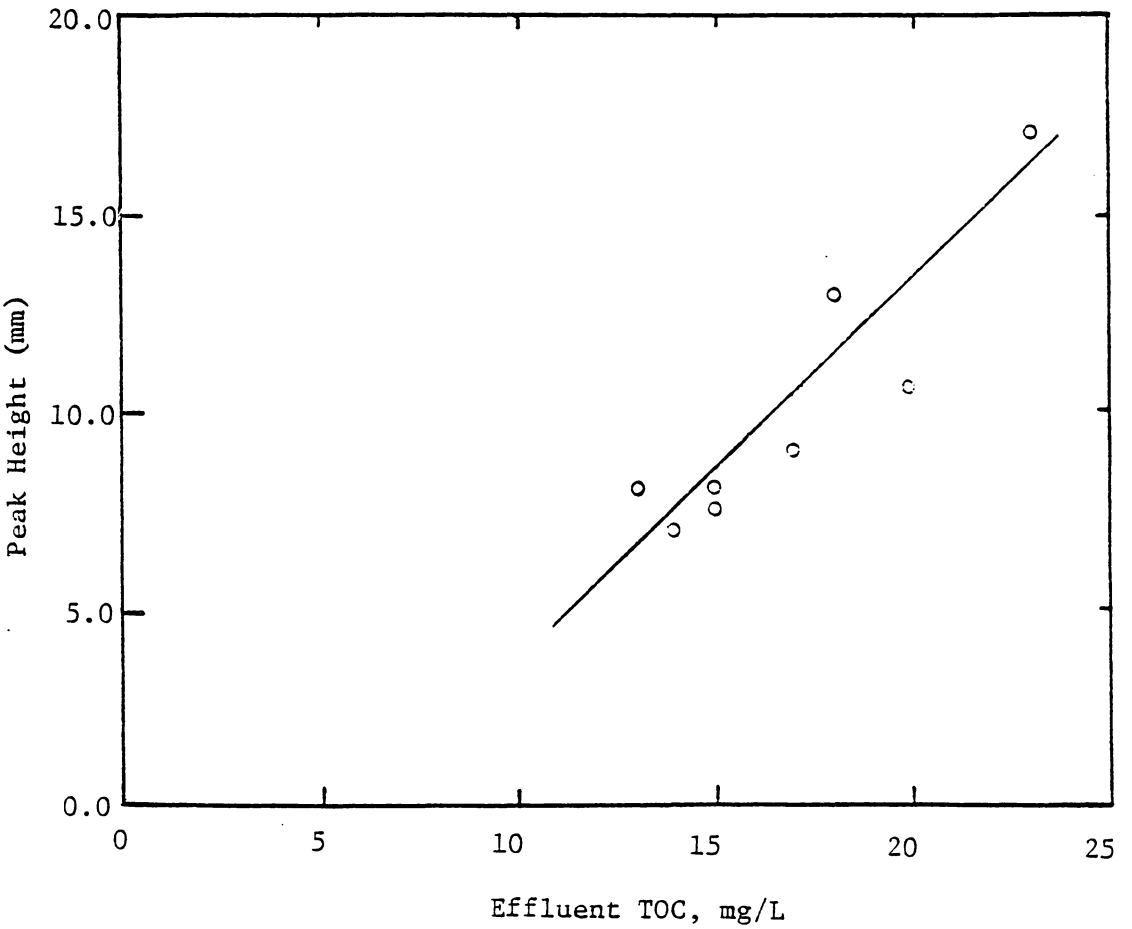

Figure 9. Relationship between effluent biopolymer content and TOC for Reactor 1.

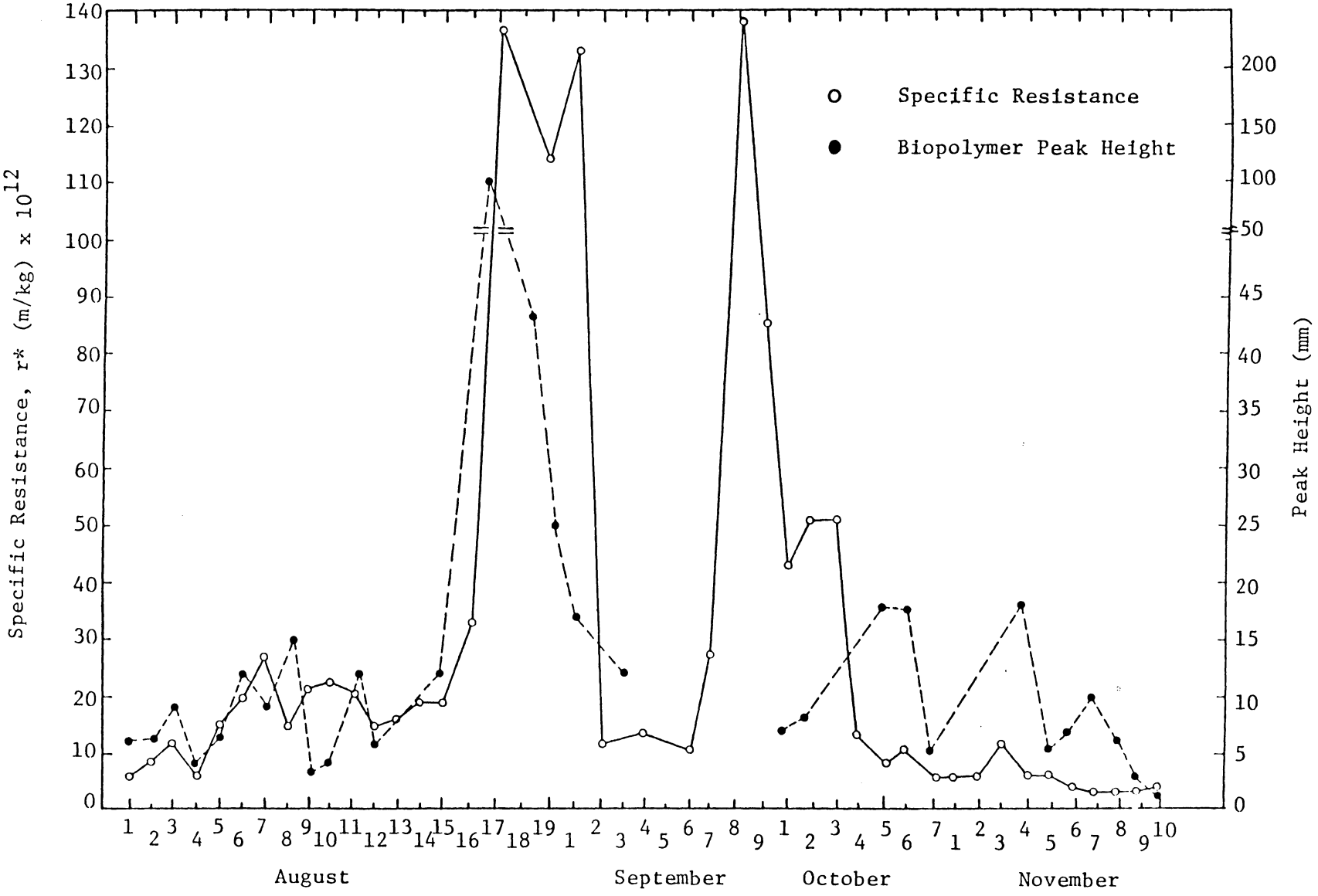

Figure 10. Change in resistance and biopolymer peak height from August-November for Reactor #1.

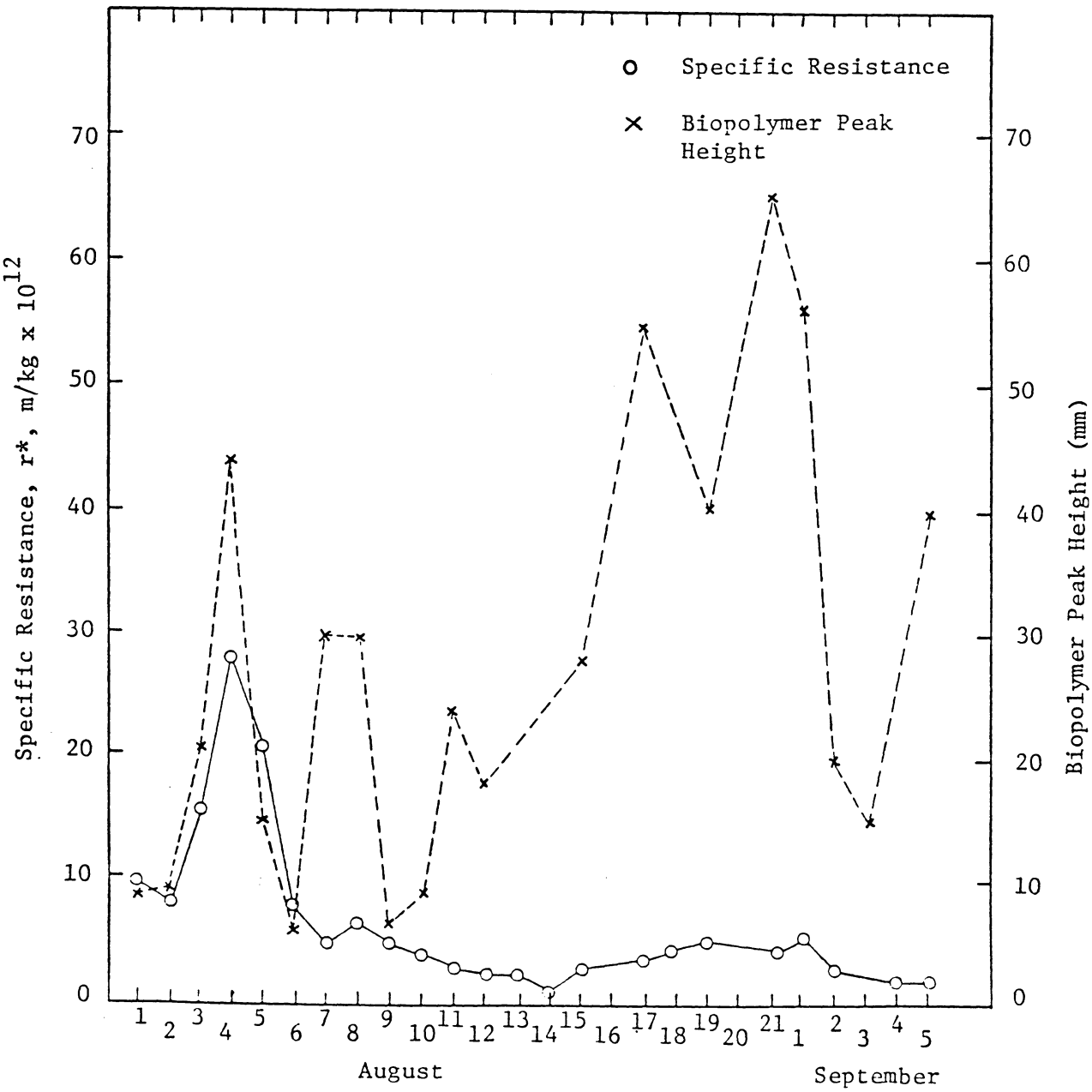

Figure 11. Changes in resistance and biopolymer concentration from July-September for Reactor #2.

were the same, at times they exhibit divergent dewatering properties. For reactor #1, changes in the supernatant biopolymer concentration correlated fairly well with changes in specific resistance values. On most days, high biopolymer concentrations in the sludge supernatant were matched by high specific resistance values. However, for Reactor #2, a similar correlation was not observed. Biopolymer concentrations and specific resistance values followed an initial similar trend. However, during the end of August and beginning September, high biopolymer concentrations in the sludge supernatant corresponded to low specific resistance values. During this period, sludge bulking was observed along with a decrease in total suspended solids in the reactor. Long lengthed filamentous microorganisms were predominant and the SVI was close to 250. The data seem to indicate a relationship between biopolymer and r* which is culture specific and not universally applicable.

Sludge settling behavior in Reactors #1 and #2 and its relation to supernatant biopolymer concentration is shown in Figures 12 and 13. There is no relationship between the two parameters although Beccari et al. (20) have observed an increase in SVI when biopolymer concentration decreased. The influence of biopolymer on sludge settling and dewatering appears culture specific and it may be difficult to develop a unified model which could explain this kind of contradictory behavior.

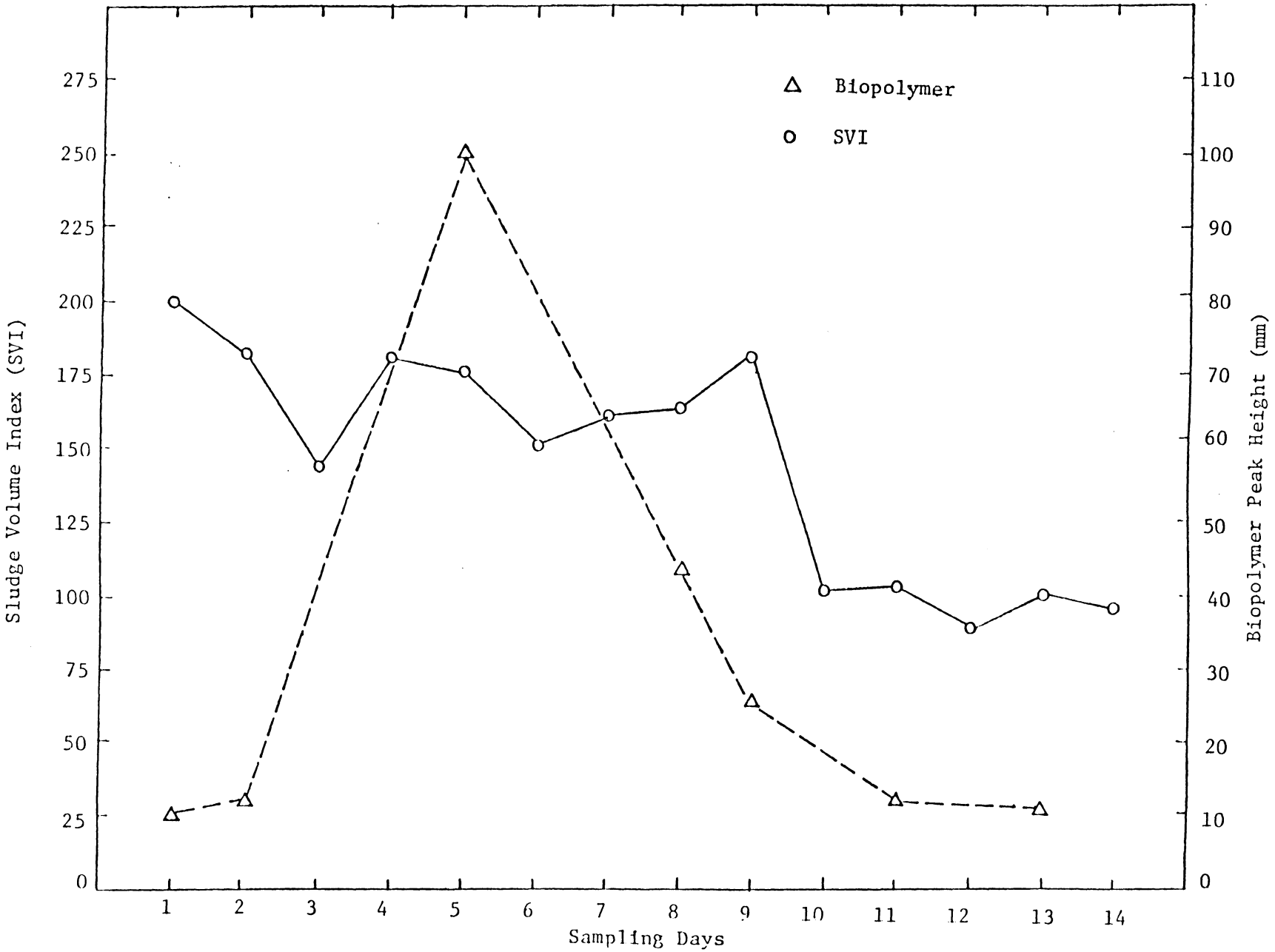

Figure 12. SVI versus biopolymer concentration for Reactor #1.

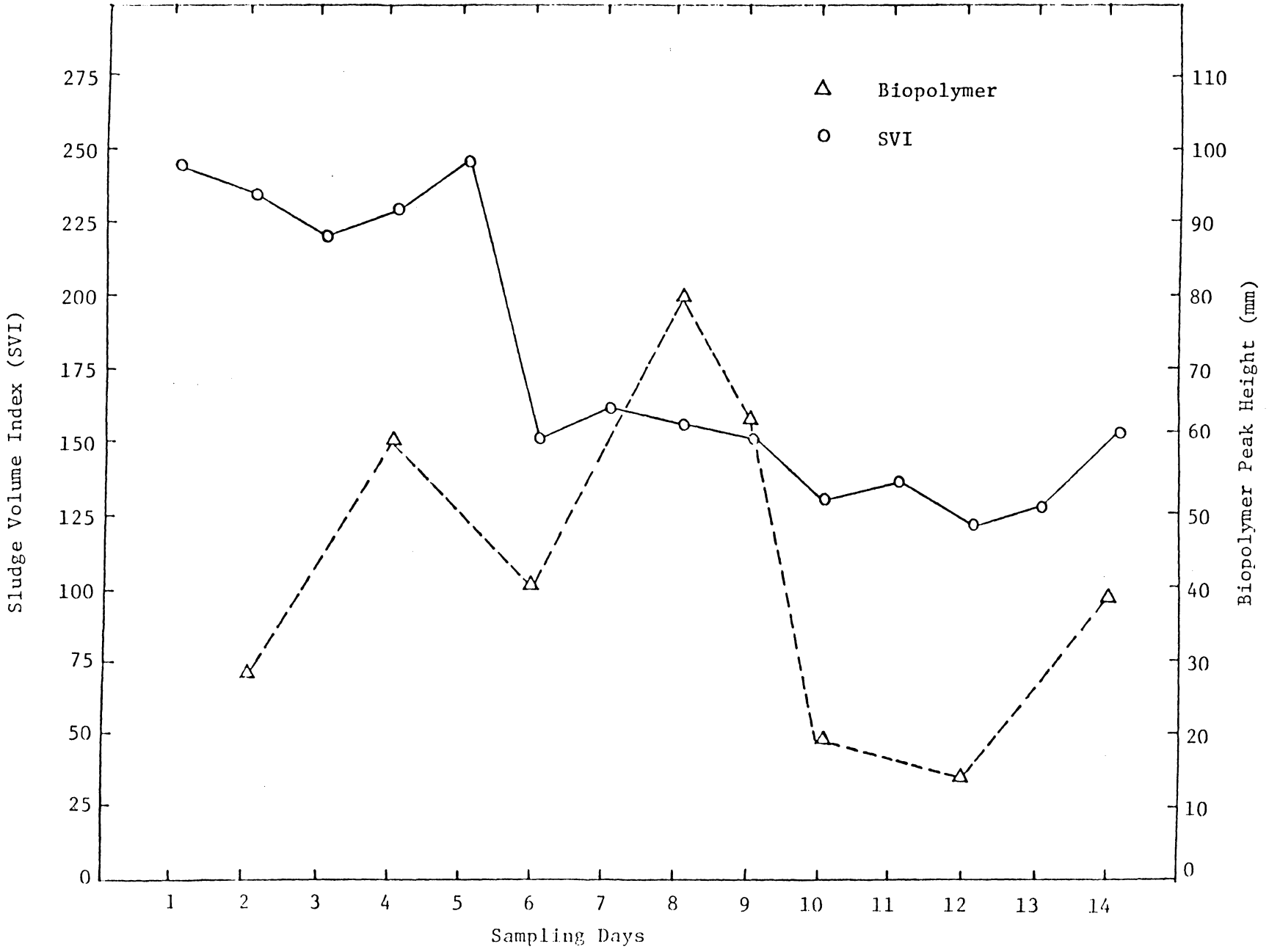

Figure 13. SVI versus biopolymer concentration for Reactor #2

4.4 Effect of pH on Sludge Dewatering Properties

As the pH of activated sludge was increased from pH 7.0 to pH 11.0, distinct changes in specific resistance values and supernatant biopolymer concentration were observed (Figure 14). At pH 7.0 and pH 8.0, no change was observed regarding dewatering rates and biopolymer concentration. However, from pH 9.0 to 11.0, both specific resistance and biopolymer content increased dramatically. Poor dewaterability of sludge at high pH could be due to several factors. The addition of sodium hydroxide to sludge could cause cellular disruption accompanied by the release of cellular contents into sludge supernatant. Pavoni et al. (3) have postulated that as pH is elevated from pH 2.0 to pH 10.0, cell surface charge becomes increasingly negative. This could cause electrostatic repulsion among negatively charged bio-colloids, resulting in a more dispersed colloidal suspension at high pH. These dispersed colloids and lysed cell products could interfere with vacuum filtration by clogging the drainage pores in the filter medium and cake solids, thus giving unusually high values for specific resistance at pH 10.0 and 11.0. An examination of the filtrate from dewatering showed higher concentration of biopolymer in the filtrate than sludge supernatant, up to pH 9.0. This could be attributed to the shearing action on the biomass under vacuum which causes the release of high molecular weight organics from cell surfaces, through cell lysis and floc breakup. At pH 10.0 and 11.0, biopolymer con-

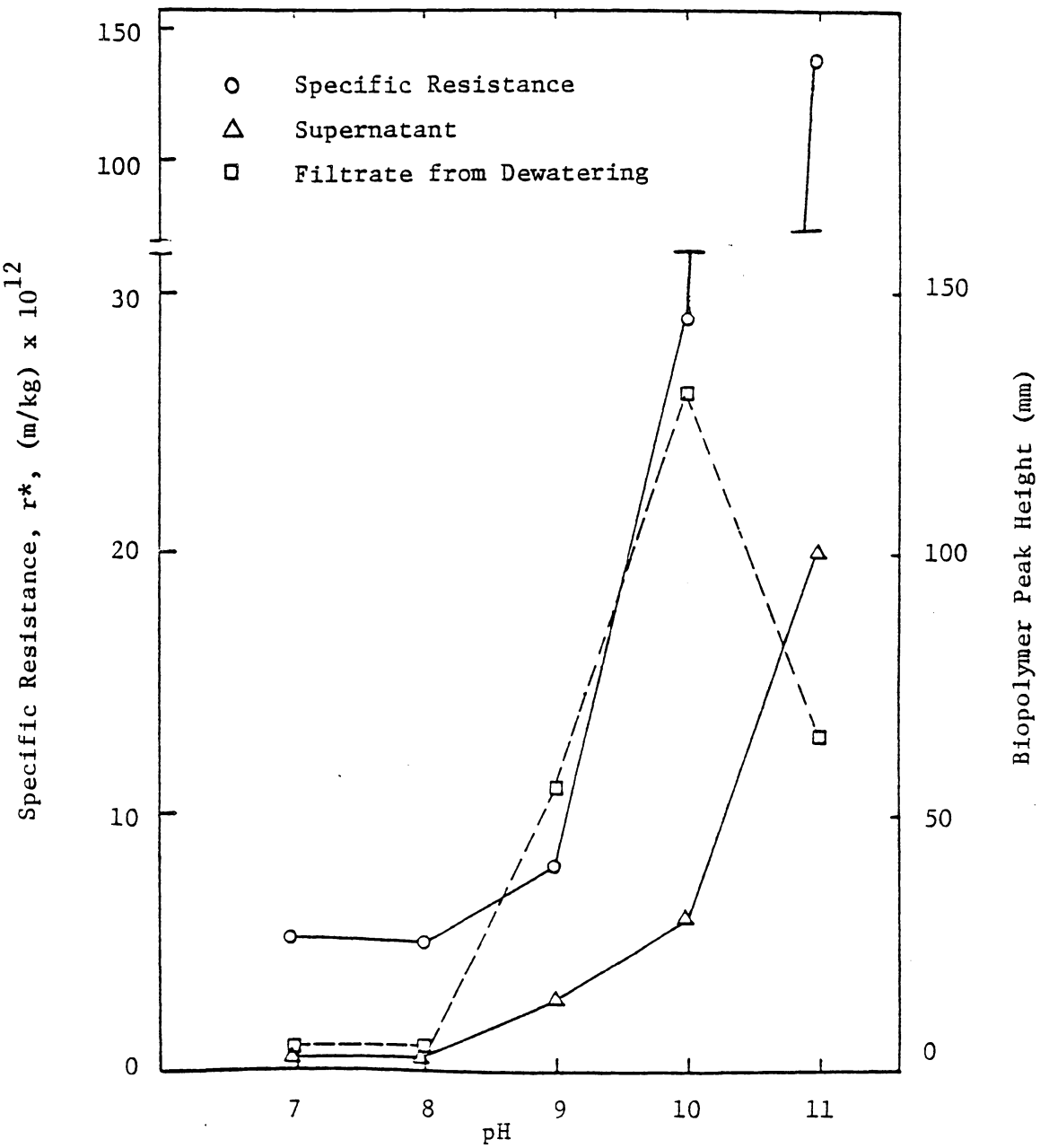

Figure 14. Variation of biopolymer peak height and specific resistance with pH for Reactor #1.

centration in the filtrate was much lower than in the sludge supernatant. Sludge samples dewatered very slowly at this pH since biopolymeric solution colloids may be clogging the filter medium, thus restricting the flow of water.

Further investigation of the effect of pH variation from pH 3.0-11.0 on sludge dewatering and supernatant biopolymer content for #1 is shown in Figure 15. Specific resistance values increased from pH 3.0-5.0, remained fairly constant from pH 5.0-8.0 and increased rapidly from pH 9.0-11.0. Biopolymer concentration was very low at pH 3.0, did not vary much between pH 4.0-9.0 and increased beyond pH 9.0. Other investigators (3,15) have shown that the point of zero charge or the isoelectric point for naturally occurring colloidal biopolymer is near pH 3.0. These anionic biopolymers that interfere with sludge filtration could possibly be coagulated and precipitated by acid addition. The improved sludge filtration at pH below pH 4.0 could also be due to reduction in the electrophoretic mobility of the biological floc (15). As pH was increased from pH 4.0-8.0, increased surface negativity of the colloidal suspension did not significantly influence the filtering properties of sludge. Biopolymer concentrations did not change much in this pH range. Novak and Haugan (12) have proposed an adsorption model in which an equilibrium was found to exist between adsorbed anionic particles or biopolymers and free or solution biopolymers. The adsorbed organics were thought to be weakly adsorbed and easily

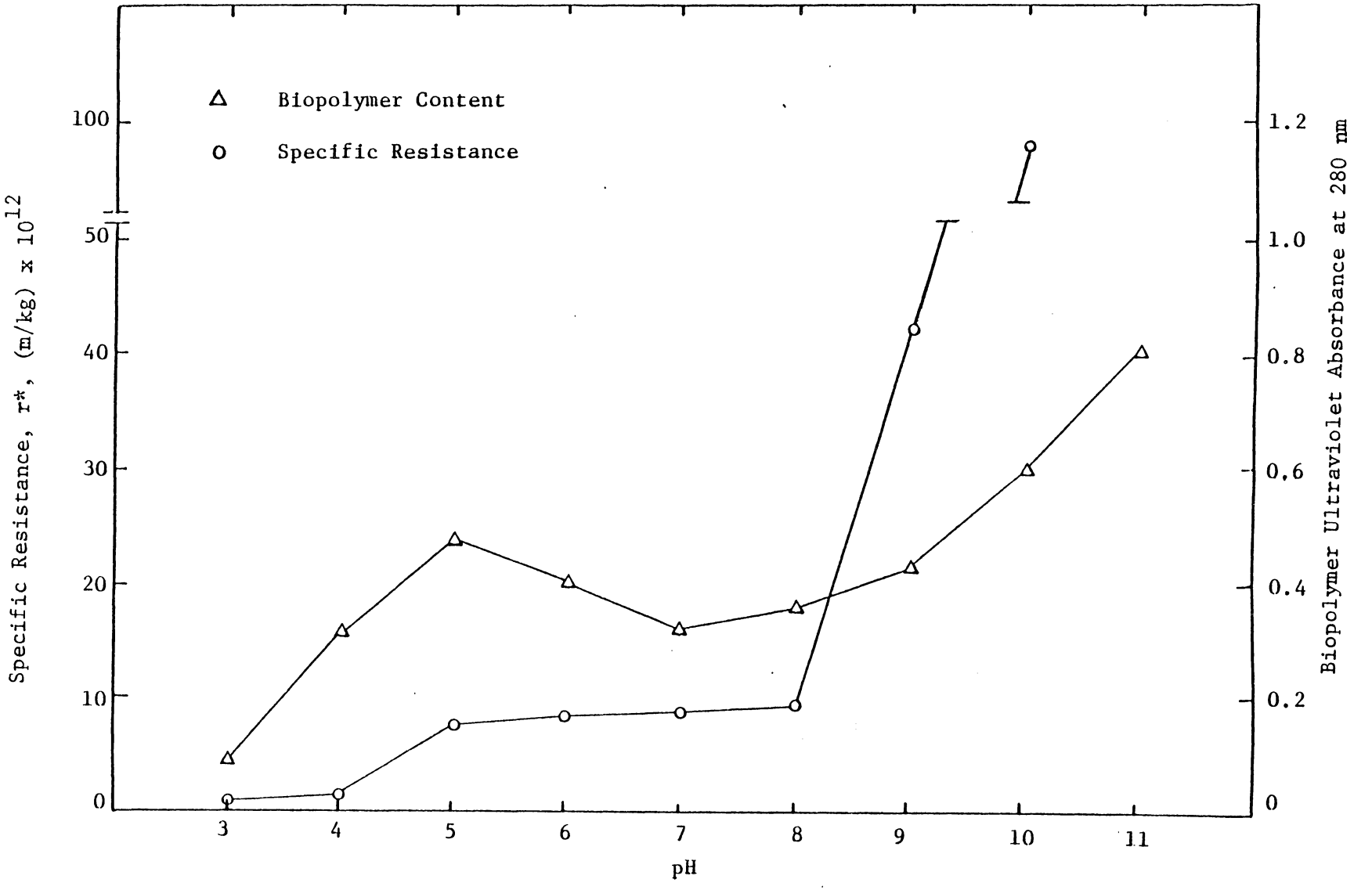

Figure 15. Specific resistance and Biopolymer concentration at different pH for Reactor #1.

dislodged by mixing. Increasing the pH of sludge could cause a shift in the equilibrium concentration of adsorbed solution biopolymers by causing desorption of biopolymer from floc surface. The increased concentration of colloidal biopolymers in the sludge supernatant may have caused a reduction in sludge filtering rates. This phenomenon was observed when the time to filter (TTF) 100 ml of sludge supernatant was measured as a function of pH for sludge samples from Reactor #1 (Figure 16). The TTF increased gradually from pH 3.0 to pH 9.0 and rapidly beyond pH 9.0. When pH is reduced below pH 5.0, adsorption of anionic biopolymer followed by coagulation and precipitation of floc may have taken place. Moore (33) conducted a similar study of the pH effect on sludge supernatant. He concluded that more important than the adsorption-desorption mechanism were biopolymer structural changes from a coiled structure at low pH to an uncoiled structure at high pH accompanied by the release of large quantities of biopolymer into the supernatant liquor. The biological polymers that control sludge properties appear to be contained in the supernatant liquor and not associated with the biological flocs.

To facilitate a better understanding of the nature of biopolymers, the high and low molecular weight fractions of gel chromatography were analyzed for carbohydrate and protein content. When the pH was increased from pH 3.5 to pH 10.0, a characteristic increase in low and high molecular weight organics

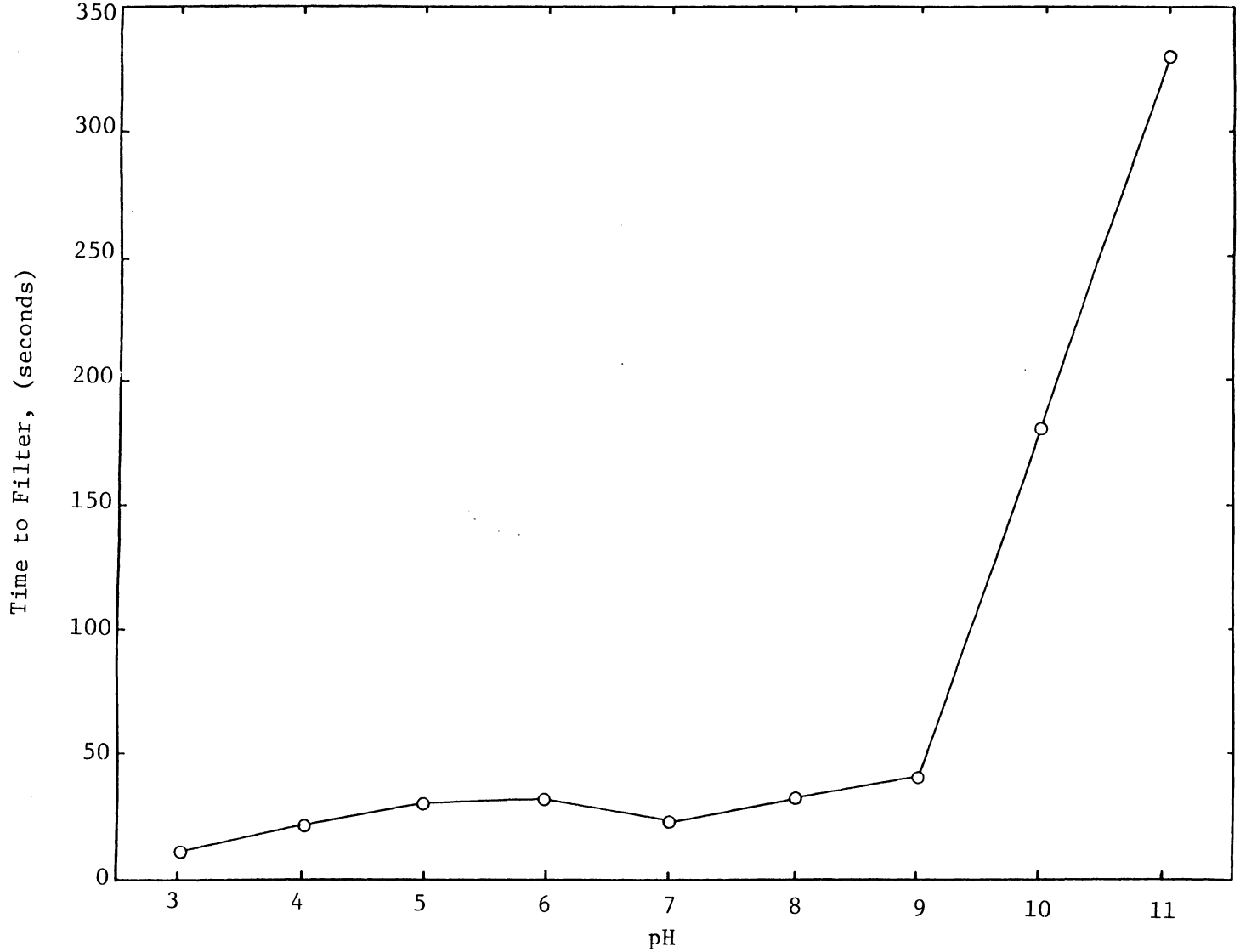

Figure 16. Time to filter (TTF) one hundred milliliters of supernatant through a 0.45 μm Whatman filter at different pH values.

(measured as ultraviolet absorbance at 280 nm) was observed as shown in Figure 17. Carbohydrate concentration (in mg/l as glucose) was quite large at pH 10.0, although the fractions collected at pH 3.5 and pH 7.5 contained similar quantities of carbohydrates in both the HMW and LMW fractions (Figure 18). A similar relationship was observed for protein concentration (in mg/l as Bovine Serum Albumin) in each fraction (Figure 19). This similarity in the concentrations of carbohydrate present in biopolymer fraction at pH 3.5 and pH 7.5 may be due to experimental errors in their determination because of the low concentrations present. Protein concentration in both the HMW and LMW fractions increased as the pH was increased from pH 3.0 to pH 10.0. Increasing the pH could cause the release of large quantities of proteins due to cell lysis. However, the results do indicate that both the biopolymer fraction and the low molecular fraction contains organics which are partly composed of carbohydrates and proteins.

Humic acid concentrations in the supernatant after pH variation showed a tremendous increase in humic acid concentration as pH was increased from pH 3.0-10.0 (Figure 20). Also shown are changes in protein and carbohydrate concentration in the supernatant and the concentration of biopolymer itself. It may be concluded with reasonable certainty that the high molecular weight organics which interfere with sludge filtration may be partly composed of proteins, carbohydrates and humic acids.

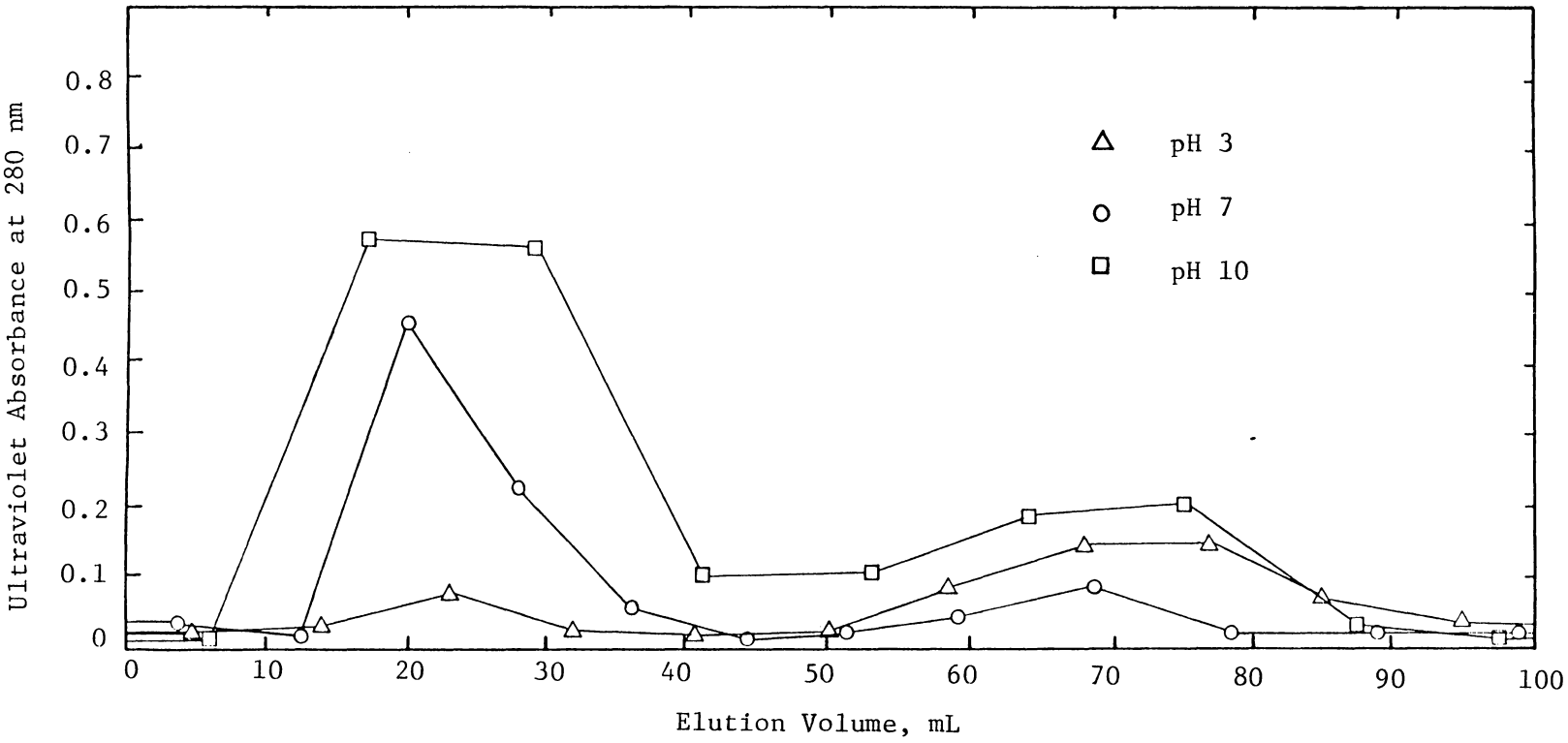

Figure 17. Biopolymer concentration in fractions collected from the gel filtration column at different sludge sample pH values.

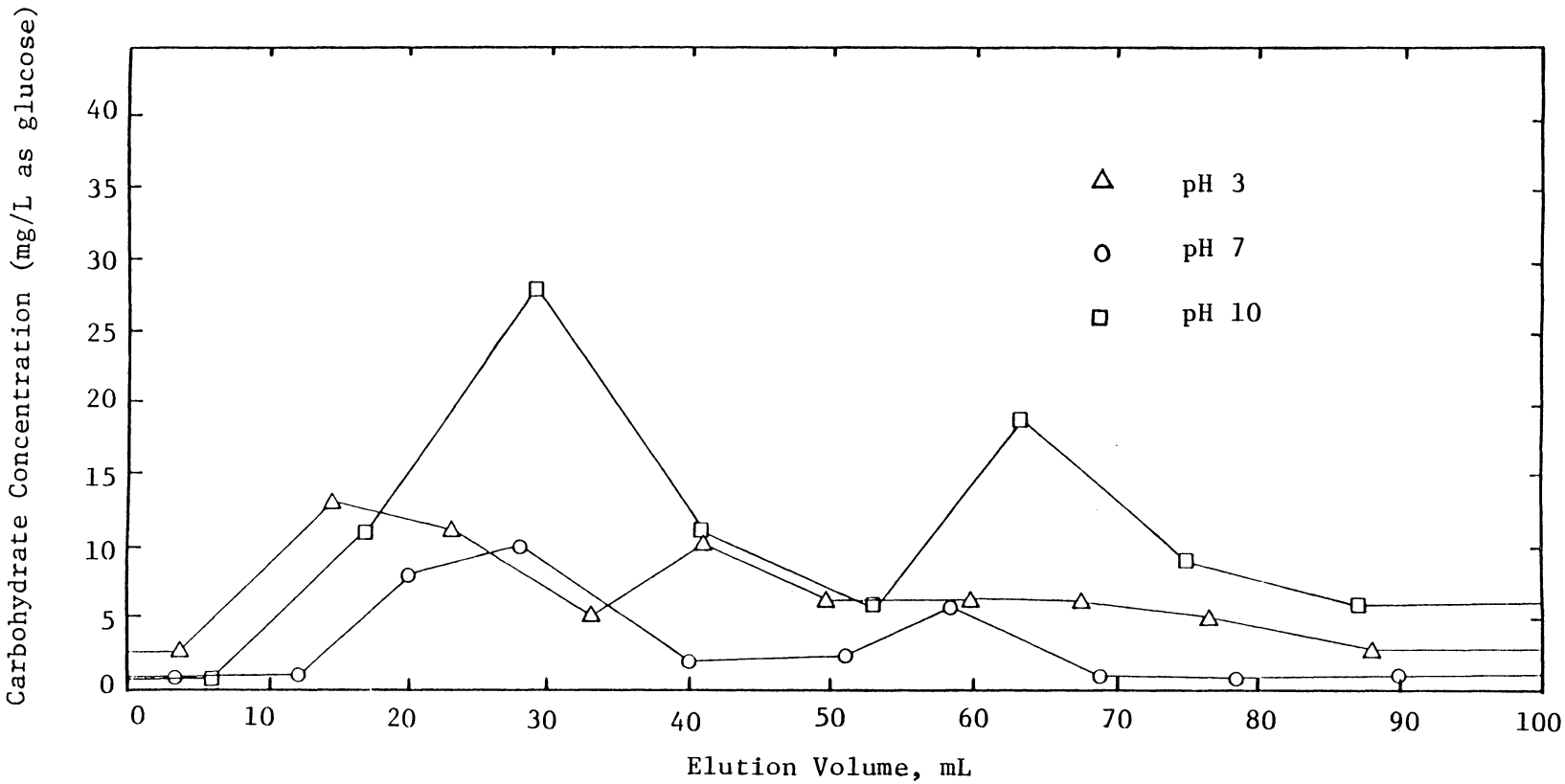

Figure 18. Effect of pH on carbohydrate content of biopolymer fractions collected in eluant.

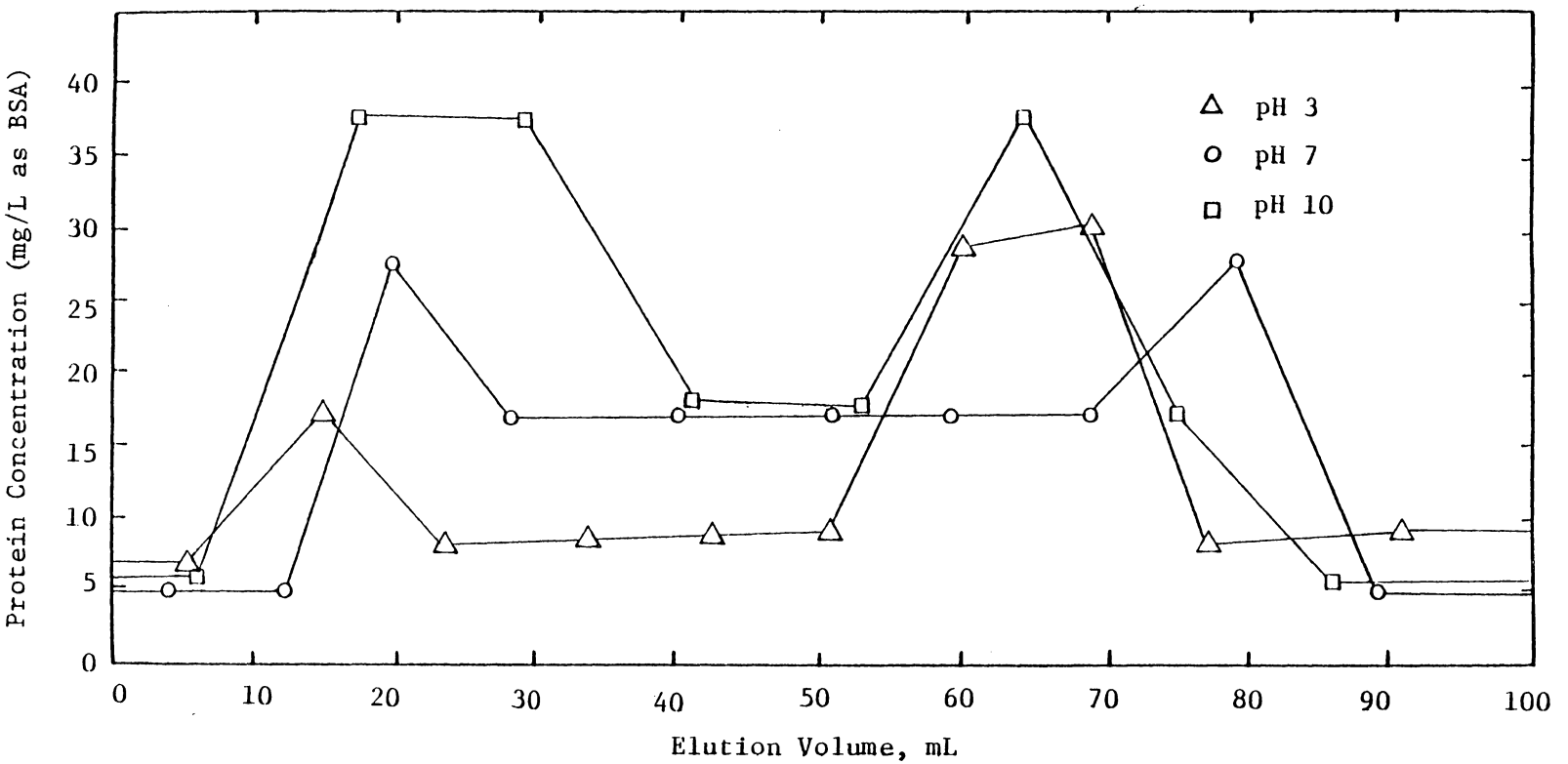

Figure 19. Effect of pH on protein content of biopolymer fractions colledted in eluant.

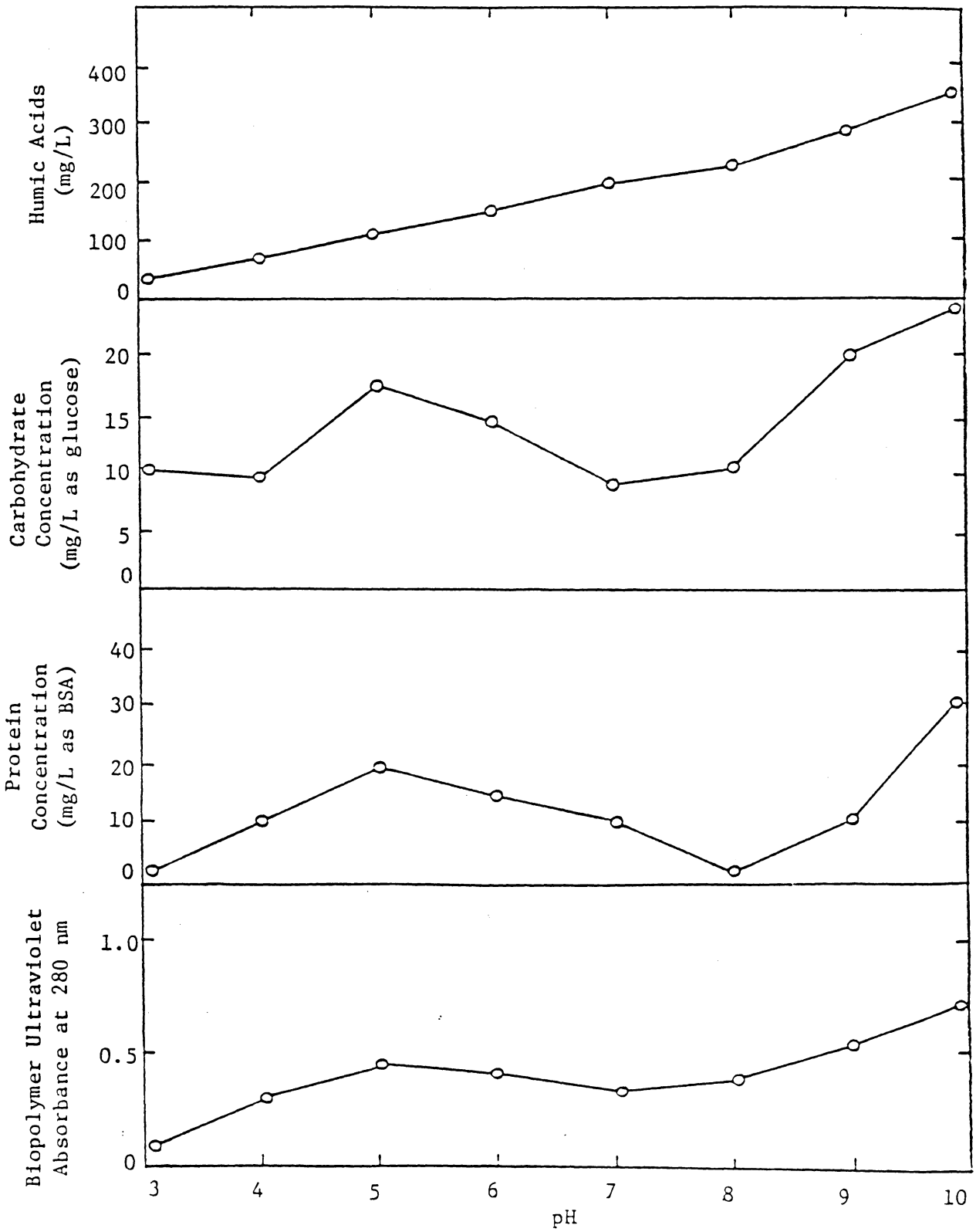

Figure 20. Biopolymer, protein, carbohydrate, humic acids concentrations in supernatant as a function of pH.

Blacksburg and Christiansburg sludge samples behaved in a similar manner at different pH (Figures 21, 22) values. This lends support to the earlier data on pH effects on activated sludge and has already been discussed in detail.

4.5 Particle Size Analysis

The various particle size ranges analyzed in the supernatant are shown in Table V. Sludge specific resistance is very sensitive to the mean sludge floc size in the range 8 to 20 mirons, and less sensitive at higher mean size values (34). The mean pore size of the filter medium used in dewatering studies was approximately 8 μm. The lower particle sizes (less than 10μm) may increase r* by blinding the filter medium or the filter cake. When the pH of the sludge sample was reduced from pH 7.0 to pH 3.0, the percentage of total volume occupied by particles of size less than 10 microns was reduced from 96 per cent at pH 7.0 to 83 percent at pH 3.0 (Figure 23). A reduction in specific resistance values accurately reflects this change in supernatant particle size distribution. The decrease in particles in this size range at pH 3.0 could be due to coagulation of anionic biopolymer followed by precipitation. The reduction of biopolymer responsible for interferring with sludge filtration results in an improved dewatering rate for the sludge.

When sludge sample pH was raised from pH 7.0 to pH 10.0, the percentage of total volume occupied by particles of size less than 10 microns increased from 75 percent at pH 7.0 (to 84 percent at

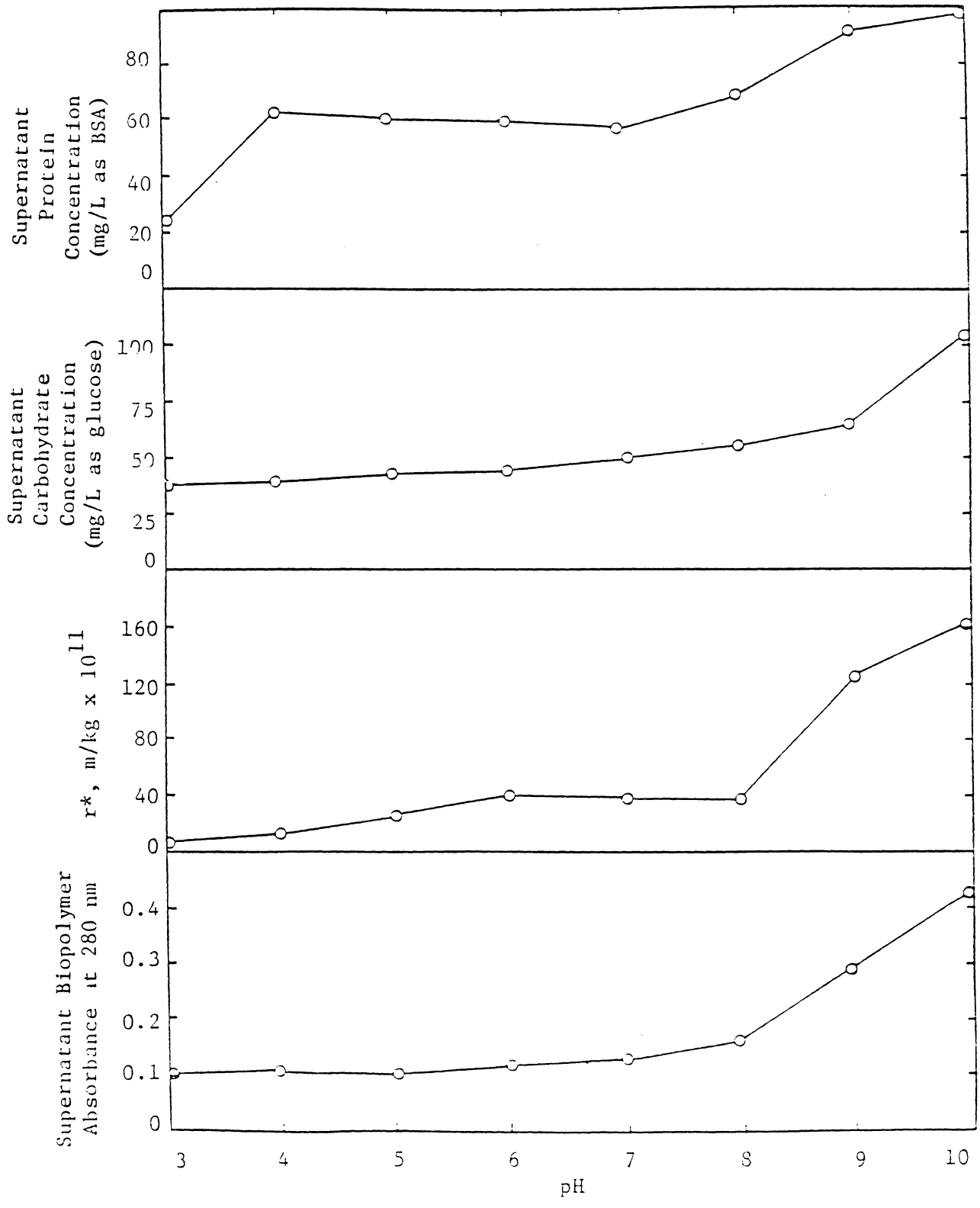

Figure 21. Effect of pH on sludge dewatering characteristics of Blacksburg Wastewater Treatment Plant.

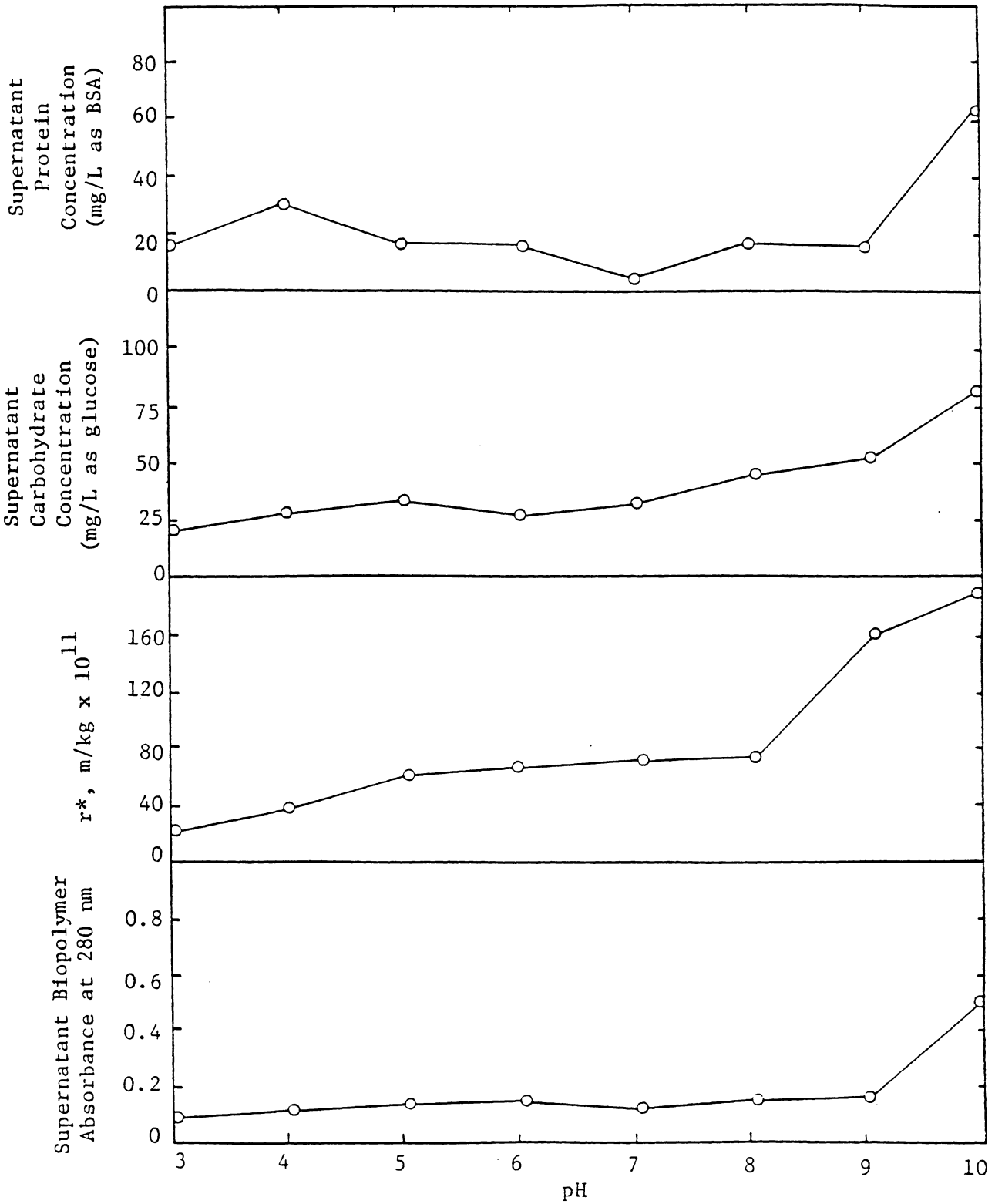

Figure 22. Effect of pH on sludge dewatering characteristics of Christiansburg wastewater treatment plant.

TABLE V. PARTICLE SIZE RANGES UTILIZED FOR HIAC SIZE ANALYSIS

Range #	Size Fraction Within Range (μm)
1	1-4
2	4-7
3	7-13
4	13-17
5	17-20
6	20-25
7	25-30
8	30-35
9	35-40
10	40-46
11	46-55
12	Greater Than 55

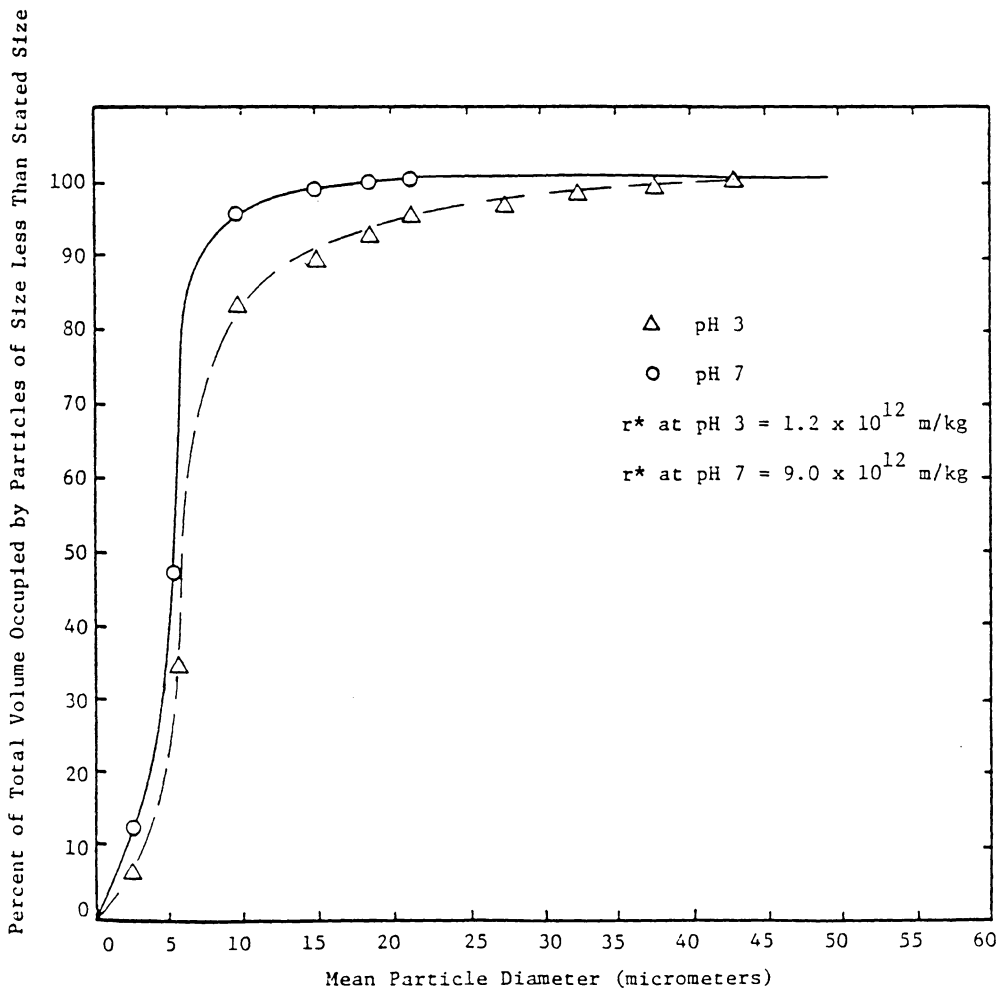

Figure 23. Particle size distribution of sludge supernatant at two different pH.

pH 10.0 (Figure 24). Resistance values, r*, are also seen to increase at high pH. At pH 10.0, an increase in particles of size less than 10 microns could be due to desorption of adsorbed biopolymer on flocs into the superantant. This increase in colloidal biopolymer causes the sludge dewatering rate to decrease.

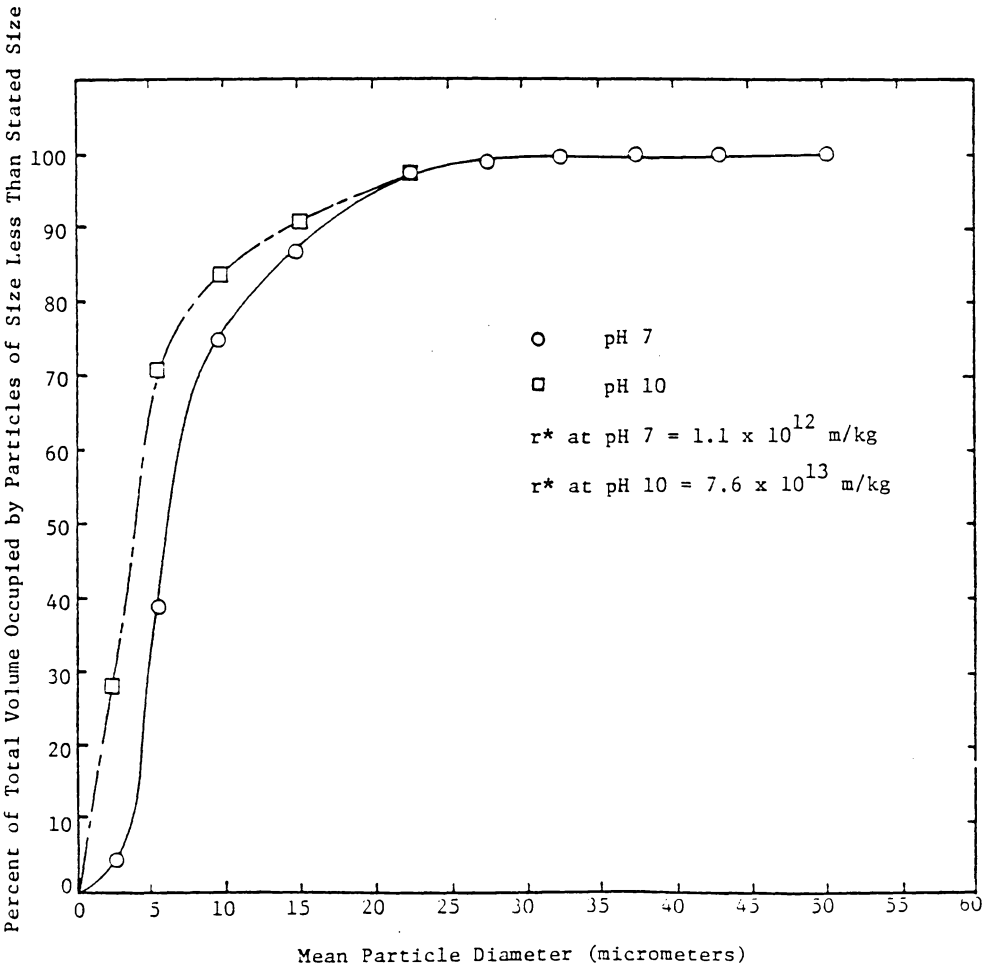

Figure 24. Particle size distribution of sludge supernatant at two different pH

V. CONCLUSIONS

From the results of the research described in this thesis, the following conclusions seem warranted:

1. The relationship between supernatant biopolymer and sludge settling and dewatering characteristics is system specific. In one system, a direct correlation may be observed between sludge specific resistance and biopolymer while the same relationship may not exist for another microbial system.
2. The biopolymer that interferes with sludge filtration is a high molecular weight ($>10^5$) anionic biocolloid composed partially of proteins, carbohydrates and humic acids.
3. Sludge dewatering characteristics and supernatant biopolymer content vary drastically as pH is increased from pH 3.0-10.0. Supernatant biopolymer content increases with sludge specific resistance. Changes in supernatant biopolymer could be due to (i) alternation of the equilibrium between adsorbed polymer on floc surface and solution colloids; (ii) structural changes of biopolymer from coiled structure at pH 3.0 to uncoiled structure at pH 10.0; and (iii) coagulation followed by precipitation due to acid addition. Sludge dewatering rate decreased gradually from pH 3.0 to pH 8.0 and decreased rapidly beyond pH 8.0.
4. The percent of total volume occupied by particles with mean size less than 10 microns increases as the pH is raised from pH 3.0 to pH 10.0. This could be due to an increase

in supernatant biocolloids at higher pH. Particles in this size range could affect sludge dewatering rate by clogging filter medium, thus increasing r*.

5. Gel filtration chromatography allows qualitative characterization of high and low molecular weight organics in biological sludge samples without causing cellular disruption or lysis. However, further refinements are considered necessary to improve the quantitative aspects of this test.

VI. REFERENCES

1. Tenney, M. W., and Stumm, W., "Chemical Flocculation of Microorganisms in Biological Waste Treatment." Journal of the Water Pollution Control Federation, 37, 1370 (1965).

2. Busch, P. L., and Strumm, W., "Chemical Interactions in the Aggregation of Bacteria." Environmental Science and Technology, 2, 49 (1968).

3. Pavoni, J. L., Tenney, M. W., Echelberger, W. F., "Bacterial Exocellular Polymers and Biological Flocculation." Journal of the Water Pollution Control Federation, 44, 414-431 (1972).

4. Forster, C. F., "Activated Sludge Surfaces in Relation to the Sludge Volume Index". Water Research, 5, 861-870 (1971).

5. Forster, C. F., "The Surface of Activated Sludge Particles in Relation to their Settling Characteristics." Water Research, 2, 767-776 (1968).

6. Wilkinson, J. F., "The Extracellular Polysaccharides of Bacteria." Bacteriological Review, 22, 46-69 (1958).

7. Gulas, V., Bond, M., Benefield, L., "Use of Exocellular Polymers for Thickening and Dewatering Activated Sludge." Journal of the Water Pollution Control Federation, 51, 798-807 (1979).

8. Saunders, F. M., and Dick, R. I., "Effect of Mean-Cell Residence Time on Organic Composition of Activated Sludge Effluents". Journal of the Water Pollution Control Federation, 53, 201-215 (1981).

9. DeWalle, F. B., Chain, E. S. K., 'Kinetics of Formation of Humic Substances in Activated Sludge Systems and Their Effect on Flocculation." Biotechnology and Bioengineering, 16, 739-755 (1974).

10. Peter, G., Wuhrmann, K., "Contribution to the Problem of Bioflocculation in the Activated Sludge Process." Proceedings of the 5th International Water Pollution Research Conference, Pergamon Press Ltd., New York (1971).

11. Brown, M. J., and Lester, J. N., "Role of Bacterial Extracellular Polymers in Metal Uptake in Pure Bacterial Culture and Activated Sludge-II". Water Research, 16, 1549-1560 (1982).

12. Novak, J. T., and Haugan, B. E., "Chemical Conditioning of Activated Sludge." Journal of the Environmental Engineering Division, Proceedings of the American Society of Civil Engineers, Vol. 105, No. EE5, 993-1008 (1979).

13. Parker, D. S., Kaufman, W. J., and Jenkins, D., "Physical Conditioning of Activated Sludge Floc," Journal of the Water Pollution Control Federation, 44, 2206-2077 (1972).

14. Roberts, K., and Olsson, O., "Influence of Colloidal Particles on Dewatering of Activated Sludge with Polyelectrolyte." Environmental Science and Technology, 9, 945-948 (1975).

15. Novak, J. T., Becker, H., Zurow, A., "Factors Influencing Activated Sludge Properties". Journal of the Environmental Engineering Division, Proceedings of the American Society of Civil Engineers, EE5, 103, 815-828 (1977)

16. Stumm, W., and O'Melia, C. R., "Stoichiometry of Coagulation". Journal of the American Water Works Association, 514-539 (1968).

17. Wu, Y. C., SMITH, E. D., and Novak, R., "Filterability of Activated Sludge in Response to Growth Conditions." Journal of the Water Pollution Control Federation, 54, 444-457 (1982).

18. Pitman, A. R., "Bioflocculation as Means of Improving the Dewatering Characteristics of Activated Sludges". Water Pollution Control, 688-699 (1975).

19. Friedman, B. A., Dugan, P. R., Pfister, R. M., Remsen, C. C., "Structure of Exocellular Polymers and their Relationship to Bacterial Flocculation". Journal of Bacteriology, 98, 1328-1334 (1969).

20. Beccari, M., Mappelli, P., and Tandoi, V., "Relationship Between Bulking and Physio -Chemical-Biological Properties of Activated Sludges". Biotechnology and Bioengineering, 22, 969-979 (1980).

21. Eriksson, L., Axberg, C., "Direct Influence of Wastewater Pollutants on Flocculation and Sedimentation Behavior in Biological Wastewater Treatment-I." Water Research, 15, 421-431 (1981).

22. Brown, M. J., and Lester, J. N., "Comparison of Bacterial Extracellular Polymer Extraction Methods." Applied and Environmental Microbiology, 40, 179-185 (1980).

23. Novak, J. T., and Haugan, B. E., "Polymer Extraction from Activated Sludge." Journal of the Water Pollution Control Federation, 53, 1420-1424 (1981).

24. Sachdev, D. R., Ferris, J. J., and Clesceri, N. L., "Apparent Molecular Weights of Organics in Secondary Effluents." Journal of the Water Pollution Control Federation, 48, 570-578 (1976).

25. Manka, J., Rebhun, M., Mandelbaum, A., and Bortinger, A., "Characterization of Organics in Secondary Effluents". Environmental Science and Technology, 8, 1017-1020 (1974).

26. Rebhun, M., and Manka, J., "Classification of Organics in Secondary Effluents." Environmental Science and Technology, 5, 606-609 (1971).

27. Manka, J., and Rebhun, M., "Organic Groups and Molecular Weight Distribution in Tertiary Effluents and Renovated Waters." Water Research, 16, 399-403 (1982).

28. Standard Methods for the Examination of Water and Wastewater.

29. Vesilind, P. A., Treatment and Disposal of Wastewater Sludges, Ann Arbor Science Publishers, Inc., Ann Arbor, Michigan, (1980).

30. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., "Protein Measurement with the Folin Phenol Reagent." Journal of Biological Chemistry, 265-275 (1951).

31. Dubois, M., Gilles, K. A., Hamilton, J. K., Roberts, P. A., and Smith, F., "Colorimetric Methods for Determination of Sugars and Related Substances." Analytical Chemistry, 28, 350 (1956).

32. Tenney, M. W., Echelberger, W. F., Coffey, J. J., and McAloon, T. J., "Chemical Conditioning of Biological Sludges for Vacuum Filtration." Journal of the Water Pollution Control Federation, 42, R1-R20 (1970).

33. Moore, B. D., "pH Effect on Activated Sludge Dewatering and Biopolymers", M.S. Thesis, University of Missouri (1982).

34. Knocke, W. R., "The Characterization of Metal Hydroxide Suspensions". Ph.D. Thesis, University of Missouri (1979).

APPENDICES

APPENDIX A1. Effect of concentrating effluent samples on TOC (total organic carbon, mg/L) in front and back peaks in the gel chromatography eluant.

Elution Volume mL	Concentrated Sample TOC, mg/L		
	2:1	5:1	10:1
0-12	0.5	1.0	1.0
12-24	2.5	4.6	9.0
24-36	1.5	1.7	3.2
36-48	1.0	1.8	3.4
48-60	3.1	5.2	13.2
60-72	2.8	4.0	4.4
72-84	1.1	1.2	1.3
84-96	0.5	1.0	1.0
96-108	0.5	1.0	1.0

Sample Concentration Factor	Total TOC, µg	Front Peak %	Back Peak %
2:1	162	33	67
5:1	215	40	60
10:1	375	40	60

APPENDIX A2. Relationship between effluent Total Organic Carbon (TOC) and biopolymer for Reactor 1.

Date	Effluent TOC (mg/l)	Peak Height (mm)
10/6	14.1	7.0
10/7	13.0	
10/8	19.6	10.6
10/9	16.8	
10/10	14.9	8.0
10/13	18.1	
10/21	20.1	18.0
10/23	15.4	8.6
10/24	23.2	17.5
10/29	20.05	5.0
10/31	19.2	5.0

APPENDIX A3. Total Organic Carbon (TOC) analysis of eluant from gel chromatography for effluent samples from Reactor 1 (Samples concentrated by a factor of 3:1).

Elution Volume mL	U.V. Absorbance	TOC ppb	Adjusted TOC ppb	TOC, g
-10	.03	714	0	
0-13	0.025	1534	820	10.66
13-23	0.055	2215	1501	15.01
23-37	0.04	2183	1469	20.57
37-48	0.03	1611	897	9.87
48-58	0.06	3757	3043	30.43
58-68	0.085	4182	3468	34.68
68-82	0.045	1162	448	6.27
82-99	0.03	913	199	3.38
			TOTAL	130.87

Before passing through column:

$$TOC = 26.38 \text{ mg/L} \times 8 \text{ mL} \times \frac{1L}{1000 \text{ mL}}$$

$$= 211 \text{ g}$$

Percent of effluent TOC accounted for by peaks

$$= \frac{130.87}{211} \times 100 = 62\%$$

High mol wt. fraction

from elution volume 0 to 37 mL = $\frac{46.24}{130.37} \times 100 = 35\%$ of total TOC

Low mol fraction

37-99 mL = 65% of total TOC

APPENDIX B1. Reactor #1 operating conditions from July to November, 1982.

Date	pH	TSS (mg/L)	ESS (mg/L)	r* (mg/kg) x 10^{12}	Peak Height (milli metres)
7/29	6.7	2260	5	5.46	2.5
8/3	6.5	2530	13	8.28	2.0
8/4	6.5	2520	22	11.5	3.0
8/5	7.1	2530	9	5.6	1.0
8/6	7.0	2610	5	14.8	2.0
8/10	7.0	2570	20	20.0	4.0
8/11	7.1	2430	8	25.6	3.0
8/12	7.0	2550	13	14.4	5.0
8/13	7.2	2625	4.0	21.2	1.0
8/17	7.2	2500	--	22.1	1.0
8/20	7.3	2500	73	19.8	4
8/21	7.3	--	--	14.3	2
8/22	7.3	2840	30	15.6	--
8/23	7.5	2500	--	18.6	--
8/24	7.4	2570	119	17.6	12.3
8/25	7.5	2610	--	--	--
8/26	7.4	2600	24	33.0	107
8/28	7.4	2840	--	--	--
8/29	7.4	2470	31	175.0	43
8/30	7.5	2500	--	--	30
8/31	7.4	2660	70	115.0	25.5
9/1	7.3	2580	--	147	17
9/9	7.5	1890	--	11.0	--
9/10	7.4	1880	28	-	12.0

APPENDIX B1. (Cont.)

Date	pH	TSS (mg/L)	ESS (mg/L)	r* (mg/kg) x 10^{12}	Peak Height (milli metres)
9/11	7.4	1980	40	12.8	--
9/12	7.4	2170	--	--	--
9/13	7.5	2450	36	9.76	--
9/17	7.4	2510	18	27.1	--
9/21	7.4	2320	11	185.0	--
10/5	7.7	2480	5	84.5	--
10/6	7.6	2570	32	43.4	7.0
10/10	7.5	2500	21	50.6	8.0
10/15	7.5	2750	19	51.3	--
10/18	7.5	2590	26	13.7	--
10/21	7.5	2470	16	8.0	18.0
10/25	7.5	2520	31	11.1	17.5
10/30	7.6	2550	23	5.4	5.0
11/3	7.6	2550	22	5.3	--
11/6	7.5	2610	48	6.0	--
11/12	7.4	2530	26	12.0	--
11/21	7.4	2530	25	4.9	17.5
11/22	7.5	2540	6	6.3	5.0
11/23	7.5	2570	5	3.5	7.0
11/24	7.5	2540	6	2.8	10.0
11/25	7.5	2690	8	2.9	5.5
11/26	7.5	2690	5	2.6	2.5
11/27	7.5	2640	20	3.6	1.0

APPENDIX B2. Reactor #2 operating conditions from July to November, 1982.

Date	pH	TSS (mg/L)	ESS (mg/L)	r* (mg/kg) x 10^{12}	Peak Height (milli metres)
7/29	6.8	2120	2.0	9.96	3.0
8/3	6.8	2290	13.0	7.74	3.0
8/4	6.8	2340	8.0	16.1	7.0
8/5	7.1	2880	12.0	27.6	13.0
8/6	7.0	2710	--	21.0	5.0
8/10	7.0	2320	6.0	8.26	2.0
8/11	7.0	2390	15.0	5.25	11.0
8/12	7.1	2450	3.0	6.59	11.0
8/13	7.1	2405	13.0	4.74	2.0
8/17	7.2	2360	5.0	3.89	3.0
8/20	7.3	2580	11	3.14	8
8/21	7.3	--	--	2.62	6
8/22	7.4	2660	10	2.60	--
8/23	7.4	2630	--	1.15	--
8/24	7.3	2360	16	3.19	27.6
8/25	7.4	2560	--	--	--
8/26	7.4	2610	31	4.3	70
8/28	7.5	2600	--	--	--
8/29	7.7	3030	10	5.31	40.5
8/30	7.6	3010	--	--	94
*8/31	7.5	3130	34	4.43	64
9/1	7.3	3000	--	5.26	20.5
9/9	7.4	2880	--	2.58	--
9/10	7.3	2750	19	--	15.0
9/11	7.3	2840	14	1.71	--

APPENDIX B2. (Cont.)

Date	pH	TSS (mg/L)	ESS (mg/L)	r* (mg/kg) x 10^{12}	Peak Height (milli metres)
9/12	7.3	2980	--	--	40
9/13	7.4	2740	18	2.21	--
9/17	7.5	2750	19	5.2	
9/21	7.5	2500	22	7.5	
10/5	7.7	2440	5	2.3	
10/6	7.6	2800	103	4.0	
10/10	7.6	2610	36	16.0	
10/15	7.6	2760	18	19.7	
10/18	7.5	2610	23	18.9	
10/21	7.5	2520	11	13.1	
10/25	7.6	2140	17	12.3	
10/30	7.5	2300	19	7.7	
11/3	7.5	2150	9	12.7	
11/6	7.5	2240	47	6.8	
11/12	7.6	2310	29	10.9	
11/20	7.5	1800	31	9.4	
11/21	7.5	1710	25	7.1	
11/22	7.6	1840	10	6.6	
11/23	7.5	1660	50	7.3	
11/24	7.6	1810	40	7.1	
11/25	7.6	1800	42	4.6	
11/26	7.6	1700	11	8.0	
11/27	7.6	1700	14	19.9	

*Observed large number of filamentous microorganisms.

APPENDIX C. Sludge Volume Index and biopolymer concentration for Reactor 1 and Reactor 2

Day	SVI		Biopolymer Peakheight mm	
	1	2	1	2
1	200	245	10	--
2	182	235	12	28
3	140	220	--	--
4	180	230	--	60
5	175	245	100	--
6	150	150	--	40
7	160	160	--	--
8	165	155	43	80
9	180	150	25	63
10	100	130	--	20
11	105	135	12	--
12	90	120	--	15
13	98	125	10	--
14	95	150	--	40

APPENDIX D1. Analysis of sample eluant from gel chromatography column for biopolymer, proteins and carbohydrates (sample from Reactor #1).

pH 3.5		pH 7.5		pH 10.0	
Elution Volume mL	Absorbance at 280 nm	Elution Volume mL	Absorbance at 280 nm	Elution Volume mL	Absorbance at 280 nm
-10	.30	-10	0.01	-10	0
0-9	.03	0-8	.02	0-12	0
9-18	.03	8-16	.02	12-23	0.57
18-27	.07	16-24	.45	23-35	0.56
27-36	.03	24-32	.22	35-47	0.10
36-45	.02	32-40	.04	47-58	0.10
45-54	.02	40-48	.01	58-69	0.18
54-63	.08	48-54	.02	69-81	0.20
63-72	.14	54-64	.04	81-92	0.03
72-81	.15	64-74	.08	92-103	0.01
81-90	.06	74-84	.01		
90-101	.04	84-94	.01		
		94-104	0		

APPENDIX D2. Analysis of eluant for protein concentration (as mg/L Bovine Serum Albumin) at different pH.

pH 3.5		pH 7.5		pH 10.0	
Elution Volume (mL)	Protein (mg/L as BSA)	Elution Volume (mL)	Protein (mg/L as BSA)	Elution Volume (mL)	Protein (mg/L as BSA)
0-9	18	0-8	0	0-12	0
9-18	28	8-16	0	12-23	38
18-27	18	16-24	28	23-35	38
27-36	18	24-32	18	35-47	18
36-45	18	32-48	18	47-58	18
45-54	18	48-54	18	58-69	38
54-63	38	54-64	18	69-81	18
63-72	38	64-74	18	81-92	0
72-80	18	74-84	28	92-102	0
80-90	18	84-94	0		
90-101	18	94-104	0		

APPENDIX D3. Analysis of eluant for carbohydrate concentration (as mg/L glucose) at different pH.

pH 3.5		pH 7.5		pH 10.0	
Elution Volume (mL)	Carbohydrate (mg/L as glucose)	Elution Volume (mL)	Carbohydrate (mg/L as glucose)	Elution Volume (mL)	Carbohydrate (mg/L as glucose)
0-9	2.5	0-8	0.5	0-12	0.5
9-18	29	8-16	1.0	12-23	11
18-27	11	16-24	8	23-35	28
27-36	5	24-32	10	35-47	11
36-45	10	32-48	1.5	47-58	6
45-54	6	48-54	2	58-69	19
54-63	6	54-64	6	69-81	9
63-72	6	64-74	1	81-92	6
72-81	5	74-84	0.5	92-102	0.5
81-90	2	84-96	0.5		
90-100	2	96-106	0.5		

APPENDIX D4. Experimental Procedure for Determining Protein and Carbohydrate Concentration in Solution

Lowry Method for Protein Determinations (30)

1. Reagents

Reagent A: 100 g sodium carbonate in 1.0 liter of 0.5 N sodium hydroxide copper sulfate

Reagent B: 1.0 g in 100 ml distilled water

Reagent C: 2.0 g Potassium Tartarate in 100 ml distilled water

Reagent D: 5.0 g ml of 2N Folin-Phenol reagent to 50 ml distilled water in 125 ml Erlenmeyer flask.

II. Procedure

a) Prepare standard solution containing 30 mg Bovine Serum Albumin (BSA) in 100 ml distilled water. Ad 0,0.1, 0.2, 0.4, 0.6, 0.8, 1.0 ml of this solution into a set of test tubes and bring the total volume to 1.0 ml with distilled water.

b) Mix 15 ml A, 0.75 ml B, 0.75 ml, C in 50 ml Erlenmeyer flask. Add 1.0 ml of this solution into each of the test tubes and mix thoroughly.

c) Incubate at room temperature for 15 minutes

d) Add 3.0 ml of D into each test tube and immediately mix tubes thoroughly.

e) Incubate sample at room temperature for 45 minutes. Determine absorbance at 540 nm.

Phenol Sulfuric Acid Method for Carbohydrate Determinations (31):

(I) Reagents

Concentrated sulfuric acid (H_2SO_4) reagent grade phenol, 80 percent by weight.

(II) Procedure

a) Prepare standards from 50 mg/l glucose stock solution. Add 0,0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 2.0 ml of this solution into a set of test tubes and bring the total volume to 2.0 ml with distilled water.

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 stand at room temperature for 30 minutes.

c) Measure absorbance at 488 nm.

APPENDIX E. Effect of pH on sludge dewatering characteristics for Blacksburg wastewater treatment plant.

pH	Specific Resistance, (mg/kg) x 10^{12}	Biopolymer (Absorbance at 280 nm)	Proteins (mg/L as BSA)	Carbohydrate (mg/L as glucose)	Humic Acids (mg/l)
3.0	1.0	0.10	30.7	32.0	182
5.0	3.1	0.11	31.4	79.2	201.1
6.0	5.0	0.10	31.4	79.2	210
7.0	4.4	0.12	31.4	67.2	178
8.0	4.6	0.16	35.5	84.9	186
9.0	15.7	0.29	53.4	115.1	211
10.0	150	0.43	83.3	125.0	251

APPENDIX F. Effect of pH on sludge dewatering characteristics for Christiansburg wastewater treatment plant.

pH	Specific Resistance $_{12}$ (mg/kg) x 10^{12}	Biopolymer (Absorbance at 280 nm)	Proteins (mg/L as BSA)	Carbohydrate (mg/L as glucose)	Humic Acids (mg/l)
3.0	6.2	0.10	16.0	22.6	350
4.0	9.6	0.12	31.4	28.1	384
5.0	15.0	0.14	15.7	34.0	404
6.0	17.0	0.13	15.7	28.4	416
7.0	18.0	0.11	5.0	34.0	457
8.0	18.7	0.14	15.7	44.2	419
9.0	39.0	0.15	16.0	52.0	486
10.0	135.0	0.45	63.0	82.0	480
11.0	260.0	0.79	101.0	113.2	540

APPENDIX G1. Particle size analysis of sludge supernatant for sludge pH 3.0 to pH 7.0.

Mean Particle Diameter (micrometers)	Particle Counts	Percent of Total Surface Area	Percent of Total Volume
pH 3.0			
2.5	49,251	17.81	6.11
5.3	23,613	38.37	27.91
9.5	7,200	37.59	49.01
14.9	223	2.86	5.86
18.4	72	1.41	3.56
22.4	32	0.93	2.86
27.4	7	0.30	1.14
32.4	7	0.43	1.89
37.4	1	0.08	0.42
42.9	2	0.21	1.254
50.3	0	0	0
pH 4.0			
2.5	127,907	19.01	7.21
5.3	64,179	42.87	34.46
9.5	16,599	35.63	51.33
14.9	239	1.26	2.85
18.4	71	0.57	1.60
22.4	32	0.38	1.30
27.4	7	0.13	0.52
32.4	6	0.15	0.74
37.4	0	0	0
42.9	0	0	0
50.3	0	0	0
pH 5.0			
2.5	216,039	24.45	10.93
5.3	109,600	55.74	52.82
9.5	11,218	18.33	31.13
14.9	188	0.76	2.01
18.4	50	0.31	1.01

APPENDIX G1. (Cont.)

Mean Particle Diameter (micrometers)	Particle Counts	Percent of Total Surface Area	Percent of Total Volume
22.4	29	0.26	1.06
27.4	4	0.05	0.27
32.4	1	0.02	0.11
37.4	0	0	0
42.9	1	0.03	0.26
50.3	1	0.05	0.41
pH 6.0			
2.5	237,664	32.22	14.82
5.3	74,894	45.63	44.51
9.5	10,588	20.73	36.24
14.9	216	1.04	2.85
18.4	27	0.20	0.67
22.4	11	0.12	0.49
27.4	0	0	0
32.4	0	0	0
37.4	2	0.06	0.42
42.9	0	0	0
50.3	0	0	0
pH 7.0			
2.5	142,871	29.72	12.59
5.3	41,027	38.36	34.44
9.5	10,145	30.47	49.05
14.9	161	1.19	3.00
18.4	8	0.09	0.28
22.4	10	0.17	0.63
27.4	0	0	0
32.4	0	0	0
37.4	0	0	0
42.9	0	0	0
50.3	0	0	0

APPENDIX G2. Particle size analysis of sludge supernatant for sludge pH 7.0 to pH 11.0.

Mean Particle Diameter (micrometers)	Particle Counts	Percent of Total Surface Area	Percent of Total Volume
pH 7.0			
2.5	73,080	13.51	4.58
5.3	57,751	47.98	34.49
9.5	10,539	28.13	36.25
14.9	905	5.94	12.01
18.4	254	2.54	6.35
22.4	91	1.35	4.10
27.4	16	0.36	1.32
32.4	5	0.16	0.68
37.4	1	0.04	0.21
42.9	0	0	0
50.3	0	0	0
pH 8.0			
2.5	79,254	11.34	3.86
5.3	71,887	46.22	33.39
9.5	16,724	34.55	44.74
14.9	973	4.95	10.04
18.4	252	1.95	4.90
22.4	82	0.94	2.88
27.4	3	0.05	0.19
32.4	0	0	0
37.4	0	0	0
42.9	0	0	0
50.3	0	0	0
pH 9.0			
2.5	152,912	50.54	26.39
5.3	23,248	34.53	38.22
9.5	2,360	11.26	22.35
14.9	199	2.34	7.27
18.4	38	0.68	2.61

APPENDIX G2. (Cont.)

Mean Particle Diameter (micrometers)	Particle Counts	Percent of Total Surface Area	Percent of Total Volume
22.4	20	0.53	2.48
27.4	3	0.12	0.68
32.4	0	0	0
37.4	0	0	0
42.9	0	0	0
50.3	0	0	0
pH 10.0			
2.5	135,237	52.05	28.03
5.3	21,862	37.82	43.18
9.5	1,068	5.94	12.15
14.9	171	2.34	7.50
18.4	41	0.86	3.39
22.4	3	0.62	2.98
27.4	2	0.14	0.82
32.4	0	0.13	0.90
37.4	1	0	0
42.9	0	0.11	1.05
50.3	0	0	0
pH 11.0			
2.5	157,532	31.15	11.46
5.3	31,073	27.61	21.53
9.5	11,001	31.41	43.90
14.9	1,113	7.82	17.14
18.4	160	1.71	4.64
22.4	5	0.08	0.26
27.4	4	0.10	0.38
32.4	2	0.07	0.32
37.4	0	0	0
42.9	1	0.06	0.37
50.3	0	0	0

The vita has been removed from the scanned document

THE ROLE OF BIOPOLYMERS IN THICKENING AND DEWATERING OF ACTIVATED SLUDGE

by

Jaidev Kunjur

(ABSTRACT)

The purpose of this study was to investigate the qualitative and quantitative characteristics of high molecular weight exocellular materials (biopolymer) produced in biological waste treatment systems and examine the relationship between biopolymer and sludge settling and dewatering properties.

The biopolymer that interferred with sludge filtration was a high molecular weight ($>10^5$) anionic biocolloid composed partially of proteins, carbohydrates and humic acids. The relationship between supernatant biopolymer and sludge settling and dewatering characteristics is system specific.

Sludge dewatering rates and supernatant biopolymer concentration vary drastically as sludge pH was increased from pH 3.0 to pH 10.0. Supernatant biopolymer and sludge specific resistance increased as sludge pH increased.

Particle size analysis of biological sludge showed an increase in particles with mean size less than 10 microns as sludge pH was increased.

No significant relationship was observed between sludge settling and biopolymer concentration in the sludge supernatant.