THE INFLUENCE OF NITROGEN AND SLUDGE AGE CHANGE IN REACTOR PERFORMANCE AND BIOPOLYMER PRODUCTION IN ACTIVATED SLUDGE

by

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(ABSTRACT)

This study investigated the influence of nitrogen and sludge age change in reactor performance and biopolymer production in activated sludge systems. The qualitative and quantitative analyses of the naturally occurring biopolymers were performed and the results were correlated to sludge a settling characteristic and effluent quality.

In order to obtain the sludge samples for the analyses, two completly mixed, continuous flow activated sludge systems were maintained during this research. Raw wastewater from the Celanese Fiber Plant located at Narrows, Virginia was utilized as the influent. Nitrogen was added in the feed solution as ammonium sulfate. The

sludge age was changed from ten to five days for both systems.

Biopolymers were extracted from the sludge floc matrix using pH-adjustment technique followed by centrifugation. The total biopolymer contents were analyzed for protein and carbohydrate concentrations. High molecular weight biopolymers were also analyzed following gel filtration. The sludge settling characteristics were measured in terms of Sludge Volume Index and effluent quality in terms of effluent turbidity.

The results indicated that the relationship between total biopolymer concentrations and sludge settling characteristics is culture specific. No consistent relationship was observed between total biopolymer concentrations and effluent turbidity and/or SVI.

Additional nitrogen in a reactor system promoted production of high protein content biopolymers. However, no significant improvement in effluent quality of the reactor was noticed by the additional nitrogen. Deficiency of nitrogen in a reactor system promoted the production of high carbohydrate content biopolymers. The high concentration of carbohydrate biopolymers seemed to correspond directly the high effluent turbidity.

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I. INTRODUCTION

The flocculation of microorganisms and other suspended colloidal components into a readily settleable mass is an important phenomenon in the activated sludge system. This inherent characteristic, along with the assimilation of organic matter present in an influent by active biomass, is essential in achieving high-quality effluent. Furthermore, the extent of this bacterial flocculation influences the efficiency of sludge thickening and sludge conditioning processes.

The most important mechanism for the separation of microbial species from their suspending medium after they have fulfilled their metabolic role is the biological agglutination. There has been no straightforward, factual hypothesis that can describe this self-imposed phenomenon; however, prior researchers have shown that the phenomenon may be accomplished by coagulation due to biopolymer bridging. They suggest these naturally produced anionic exocellular biopolymers, reportedly containing polysaccharides, protein, and nucleic acids, cause the bacterial suspension to agglomerate and, ultimately, to settle.

Previous studies addressed the effect of various parameters such as influent composition, the mean cell residence time, and aeration basin configuration on the

biopolymer composition. Emphasis was placed on the first two factors in this study for a better understanding of the fundamental nature of the biopolymers and their role in biological flocculation. It is believed that variation in these parameters would sufficiently alter the biopolymer composition and concentration.

Specific objectives of this study which may elucidate the understanding of the mechanisms of bioflocculation are:

1. to describe the compositional make-up of naturally produced exocellular polymers which may be responsible for microbial aggregation with emphasis on high molecular weight(HMW) fractions;

2. to describe the quantitative production of the exocellular polymer in an activated sludge system; and

3. to correlate exocellular polymer content to degree of settling and effluent quality.

II. LITERATURE REVIEW

2.1 Bioflocculation

Biological flocculation and subsequent phase separation are essential in the efficient operation of the conventional activated sludge process. This biological agglutination is thought to be most important mechanism in producing an acceptable effluent by the separation of microorganisms from their suspending medium after they have fulfilled their metabolic role. There has been no straightforward, factual hypothesis that can explain this self-imposed phenomenon, but prior studies have shown that the phenomenon may be accomplished by naturally occurring exocellular biopolymer(ECP).

This literature review will discuss important aspects of the biopolymers which in turn may explain the mechanism of bioflocculation.

2.2 Origin and Nature of Exocellular Polymer

It may be inferred that cell binding polymeric materials conducive to flocculation are synthesized by some strains of microorganisms under conditions where growth is limited by a nutritional imbalance. Investigations using pure and heterogeneous batch cultures have established the belief that exocellular polymers are specifically produced by bacterial cells as a means

of gaining an ecological advantage (1).

Recent investigations have established that lytic activity is the main mode of release of the polymeric materials. Gulas et al. (2) indicated this autolytic phenomenon is prompted by the presence of an enzyme capable of cleaving the cell wall and, in the process, releasing nondialyzable heteropolymers, dialyzable muco-peptides, amino acids, and glucose. Most of the contents of lysed cells are oxidized by other bacteria or incorporated in the ECP fraction (2). Therefore, it is conceivable that the ECP may contain intracellular material and cell wall polymers which are inherently more resistant to metabolic degredation.

Since a typical biological floc consists of a great variety of bacteria and possibly other organisms such as protozoa, funzi, and virus, as well as abiotic suspended matter, it may comprise a broad spectrum of hydrophobic and hydrophilic interfaces (3). ECP exists as polymeric organic compounds external to the cell wall of the organism. The extracellular fraction can be further subdivided into capsular and slime polymers (4). Capsular polymers are attached to individual cells or floc particles, and slime polymers are unattached polymeric organic compounds in suspension that are free of microorganisms and particles (4).

The results of a study by Pavoni et al. (5)

depicted a direct correlation between ECP production and biological flocculation with maximum agglutination occurring during endogenous growth phase. They observed that bacterial bioflocculation takes place only after the microorganisms have entered their endogenous growth phase. This phenomenon is in agreement with the findings of investigation by Tenney and Stumm (6). A decrease in culture turbidity was observed with an increase in the ECP concentration during the endogenous growth phase.

Busch and Stumm (3) hypothesized that an adequate concentration of polymer can accumulate per unit surface area of bacterial cells only under conditions of declining or endogenous growth stage. During high specific growth phase, new microbial surfaces may be produced faster than surfaces can be covered with polymer for flocculation.

However, the study of Gulas et al. (2) contradicted the theory of the polymer production occurring only during endogenous respiration phase. Their study showed that the ECP produced per unit active biomass versus the sludge age is an U-shaped curve (2). Two regions of interest which indicated production of exocellular polymer content per unit active biomass are shown in Figure 1. The left-most region represents low sludge ages where the polymer contents are decreasing. The presence of relatively large amounts of biopolymer at the low sludge ages in the study is interesting to note. This region contradicts the

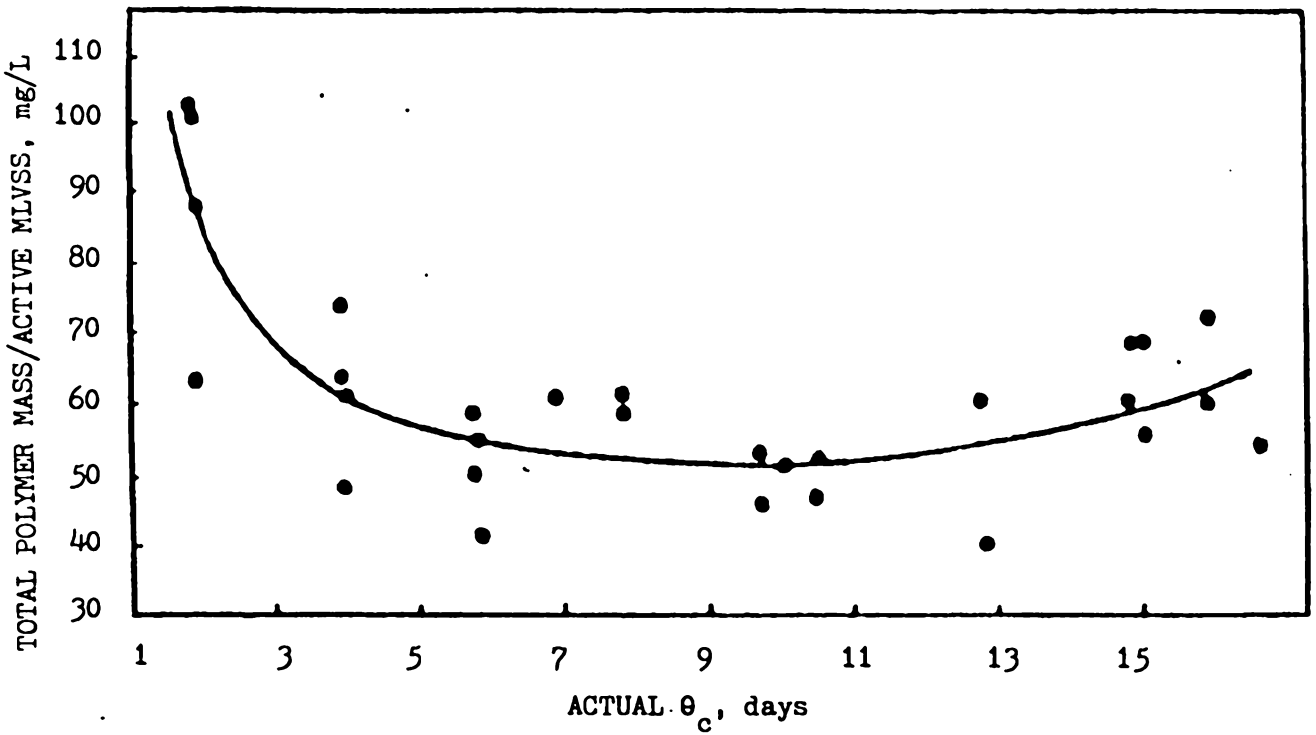


FIGURE 1. Total polymer mass per active mixed liquor volatile suspended solids versus actual sludge age (2).

accepted theory of exocellular polymer formation only during the endogenous growth stage. During this phase of logarithmic growth, the bacterial cells in the system undergo autolytic activity releasing biological polymers into the medium. As the sludge age increases, the autolysis slows. The right-most region in Figure 1 indicates a slow increase in the polymer content. This response more closely agrees with the classical theory of polymer production requiring endogenous phase of growth which was hypothesized by Pavoni et al. (5) and Busch and Stumm (3).

2.3 Bipolymer Composition

The general compositional classification of ECP is polysaccharides, protein, RNA and DNA. An increase in the concentration of these major components, which serve only as general catagories for vastly complex and assorted mixture of organic polymers present, was observed with the sharp increase in culture aggregation (5). Some studies have shown that composition of the exocellular metabolite is solely of polysaccharides, but it would seem illogical that the metabolites produced by a system would be uniform in compositional make-up since the culture that produces the metabolites is composed of a mixed and varied population microorganisms (5).

Tenney and Stumm (6) suggested substances such a

gluctamic acid, lipids, gluco- and muco-polysaccharides were more likely major components than protein after observing the surface isoelectric points of bacterial suspensions (pH 2 - 4) which is lower than that of protein (pH 5 - 8). This was further supported by Forster (7) who also suggested the presence of glucuronic acid following the study of isoelectric points and pK values of the surface of activated sludge particles. He showed that the polysaccharides were composed of galactose, fucose, mannose, and glycuronic acid.

Brown and Lester (8) reported that biopolymers were primarily polysaccharides. Their extraction products include monosaccharides, such as hexose, D-glucose, D-galactose and D-mannose, and protein, nucleic acids, glucuronic acids and uronic acids.

Sato and Ose (9) concluded that the method of polymer extraction determines the fraction of various organic components in the extract. Their data demonstrated that the extractions from pure and heterogeneous cultures had a different compositional make-up.

The results of study by Pavoni et al. (5) showed the presence of the four basic constituents in common at different percentages in the ECP extracted from various heterogeneous cultures with variation in dominant species.

The majority of studies found that ECP are primarily polysaccharides. However, the studies do not rule out the

possibility of protein, RNA and DNA being ECP constituents. The roles of protein and the nucleic acids still need to be evaluated. In this research the relationship between biopolymer protein and carbohydrates with a sludge settling characteristic and effluent quality was studied.

2.4 Mechanism of Bioflocculation

Biopolymers agglomerate individual dispersed colloidal particles into flocs by forming linkages between them. This bioflocculation is a result of complicated physical, chemical, and biological interaction involving many variables that are difficult to control experimentally. At present, no single unifying interpretation may be given for the phenomenon.

Bioflocculation occurs according to accepted coagulation theory where naturally occurring anionic biopolymers coagulate bacteria via the interparticle bridging mechanism (3,6). The mechanism of this interparticle bridging involves interaction of high molecular weight ECP, which have sufficiently accumulated at the microbial surface during endogenous growth (5). These polymers bridge the dispersed cells physically or electrostatically to form a three-dimensional matrix of sufficient magnitude for settling.

Pavoni et al. (5) and Busch and Stumm (3) reported

that extracted ECP may contain functional surface groups that are primarily anionic and nonionic in most neutral pH ranges. Naturally occurring bioflocculation is analogous to flocculation of bacteria caused by synthetic anionic and nonionic polyelectrolytes (10).

The mechanism of attachment of anionic polymeric species to net negatively charged surfaces of bacteria involves chemical forces such as hydrogen bonding, or anion interchange with cations on the colloidal surface (11). Then, effective molecular bridging and alteration of the polymer charge occur (8,10,11). Because of similar charges of the biopolymers (5), the bacteria and inorganic colloidal surfaces, charge reduction or charge neutralization are not believed to be the mechanism of floc formation (5,12).

The reduction of surface potential may not be a prerequisite for bioflocculation. There is definite length requirement of a polymer to bridge the minimum distance separating the particles which is established by electrostatic repulsion of like particle charges (6). In accordance with the proposed model, the optimum destabilization occurs when a certain fraction of available adsorption sites on the surface of bacteria are bridged by polymers. Correspondingly, the optimum polymer dosage should be proportional to the number of microorganisms, i.e., total specific available surface

area of microorganisms (3,6).

2.5 Factors Influencing Bioflocculation

It is believed that sludge age, feed composition, and polymer type produced by a culture have strong influence in the mechanism of bioflocculation. Other physical parameters such as temperature, agitation and pH of the colloidal suspension, which may also influence the bioflocculation are not studied in this research.

Researchers (2,4) have shown a pronounced effect of sludge age on bioflocculation. Gulas et al. (2) postulated the existence of two regions with regard to ECP content per unit biomass and sludge age. At lower sludge ages and high specific growth rates, pin-point floc were present along with a high level of polymer production. A logical explanation for this phenomenon may be provided by speculating on the characteristics of the polymer. The pin-point floc were resulted due to low molecular weight polymers whose agglutative properties are deficient. They are released in the surrounding medium by autolytic phenomenon during low sludge ages. At higher sludge ages and low growth rate, autolysis of cell slows and polymer production again increases as proposed by the classical theory of endogenous phase polymer production. During this time, bacterial cells release high molecular weight polymers which are more

capable of efficient flocculation of microorganisms.

Becarri et al. (13) showed that the effects of biopolymers on the settling were otherwise. At low sludge ages, settling improved as ECP concentration increased. However, the settling deteriorated with increase in the ECP concentration at high sludge ages.

The loading intensity is directly related to sludge age. An increase in sludge age will increase the biomass concentration (provided that the feed rates are constant). This will in turn decrease the loading intensity. It has been generally accepted that settling characteristics improve as sludge age is increased.

Kiff (14) found that the ECP content increased with the loading intensity. However, Brown and Lester (15) claimed that the percent of extracted materials remained "virtually constant" as the sludge age increased from 3 to 18 days. Saunders and Dick (4) found no significant correlation in the evaluation of the "capsular carbohydrate" concentration at the four different sludge ages which they studied.

Studies conducted by Wu et al. (16) emphasized the important relationship between feed composition and the extracted carbohydrate concentration. Wu et al. studied the effects of the food to microorganism ratio (F/M) and feed nitrogen composition on the production of sludge

protein and sludge carbohydrate. The results from the studies indicated the conversion of the carbon source into protein, as opposed to carbohydrate, during the nitrogen rich conditions may have improved the settling (16). The carbohydrate to protein ratio for the nitrogen rich condition almost doubled the ratio for the nitrogen deficient condition.

Many conflicting results in the subject of ECP production versus sludge age can be attributed to both the extraction methods utilized and the operation of individual reactor. Also the actual increase or decrease in ECP concentration may be dependent upon the nature of the particular culture.

2.6 Biopolymer Extraction Methods

Determining the most efficient method for extracting ECP is essential in the study of bioflocculation. The most desired extraction method should yield maximum quantity of biopolymers without promoting bacterial cell lysis.

Brown and Lester (8) concluded high speed centrifugation following pH adjustment and steam treatment to be the most effective methods of extraction among five methods compared.

Novak and Haugan (17) demonstrated the ineffectiveness of high speed centrifugation in stripping polymers

from activated sludge floc. They suggested that the extraction methods utilized in the polymer characterization studies by several other investigators may have been "harsh extraction procedures" which may have promoted cell lysis or may have hydrolized polymeric molecules (17). Thus, not only their data do not repesent the activated sludge flocs, but they may represent confusing and imprecise interpretations.

Study by Kajornatiyudh (18) showed clear advantages of utilizing pH-adjustment method over the steaming method in extraction of biopolymers. The pH-adjustment method clearly gave higher yields of polymers than the steaming method without causing significant cell lysis.

Gel filtration chromatography is considered to be a promising method in determing molecular weight distributions of the supernatant of activated sludge without cell lysis or altering biopolymer properties. Several researchers including Novak and Haugan (17) made molecular weight analyses using Sephadex dextran gels.

Novak and Haugan (17) showed that no significant difference existed between centrifuged sludges and settled supernatant liquors when high and low molecular weight organic fractions were obtained using Sephadex G-25 and G-75. Kajornatiyudh showed both supernatants obtained by pH-adjustment and steaming methods yielded higher quantity of polymers that the supernatant from raw

sludge for high and low molecular weight fractions (18).

A review of the literature pertaining to bacterial flocculation indicates that there are, no straightforward, factual hypotheses which describe observed phenomena. The studies undertaken in this investigation have as their primary goal elucidation of the naturally produced biological flocculants. Such information would ultimately be applicable to the effective operation of prototype biological wastewater treatment processes.

III. METHODS AND MATERIALS

Two activated sludge reactors were used in this study to generate sludge samples. The initial sludge culture was obtained from the aeration basin of the wastewater treatment plant of the Celanese Fiber Plant located at Narrows, Virginia. A detailed description of the reactor operational procedures and analytical techniques used to obtain pertinent data are included in this section.

3.1 Reactor System

To initiate the experimental study, a completely mixed, continuous flow activated sludge reactor (shown in Figure 2) was operated in a constant temperature room (20 °C ± 1 °C) to acclimate the culture to the new environment. Air was introduced through a porous stone diffuser. The gas flow provided sufficient mixing and maintained at least 1.0 milligram per liter (mg/L) of dissolved oxygen concentration in the aeration chamber.

Raw wastewater from the Celanese Fiber Plant was fed at the feed rate of 4 milliliter per minute (mL/min) or 5.8 liters per day. This rate provided approximately 1.5 day hydraulic detention time for the reactor which had an effective volume of 8.5 liter (L). The feed composition will be discussed in a latter

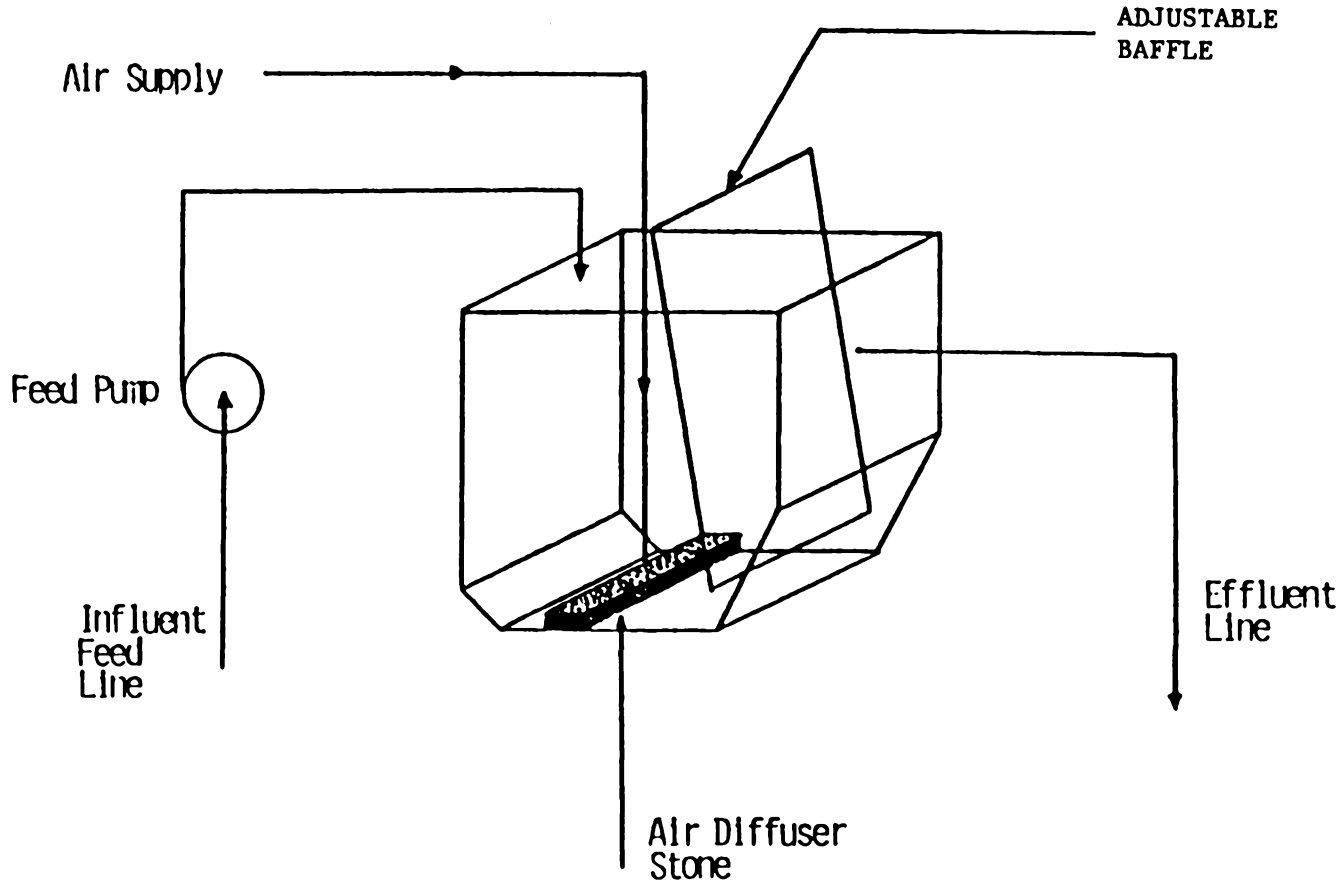


FIGURE 2. A Completely Mixed, Continuous Flow Reactor Set Up.

section. Sludge from the reactor was wasted every day by lifting the baffle, allowing complete mixing, and removing 850 milliliters (mL) from the reactor. Therefore, the average mean cell residence time was set at ten days. The wasted sludge was analyzed for characterization of biopolymer.

Operational parameters, such as total suspended solids, effluent turbidity and Sludge Volume Index (SVI) were measured at each sampling to determine steady state conditions. The influent and effluent chemical oxygen demand (COD) and Total Kjeldahl Nitrogen (TKN) analyses were performed to monitor the reactor performance.

Following thirty days of acclimation, one half of the sludge from the reactor was removed and poured into another reactor of exactly the same configuration and size. Both reactors were then operated without wasting until the total suspended solids concentration remained constant. During this time, a total of 100 mg/L of nitrogen was introduced into the feed of one reactor (referred to as reactor 2). Nitrogen was added as ammonium sulfate.

The reactors were then operated for 75 days under the same operating conditions mentioned above. Following this, the sludge age of each reactor was changed from 10 days to 5 days.

The reactors were monitored for the operational

parameters mentioned above (See Appendix A, Table A-1 and A-2). Also, the sludge from each reactor was analyzed for biopolymer.

3.2 Wastewater Composition

The wastewater from the Celanese Fiber Plant was used as the influent. It was collected from the equalization basin once every two weeks and refrigerated at 4 °C until fed to the laboratory units. The wastewater is generated from a cellulose acetate manufacturing operation. Small amounts of domestic wastewater are also present in the wastewater. Table I shows the relative composition and the characteristics of the wastewater fed to the reactors.

3.3 Chemical Oxygen Demand(COD)

The COD test was performed according to procedures detailed in Standard Methods for the Examination of Water and Wastewater (19). Feed samples were collected directly from the feed line. Effluent samples were collected from near the liquid surface in the clarifiers. The results of COD tests performed throughout the reactor operation are presented in Appendix A, Table A-5.

TABLE I. RELATIVE COMPOSITION AND CHARACTERISTICS OF INFLUENT.

	INFLUENT CONCENTRATION, mg/L
COMPONENT:[1]	
Acetic Acid	800 - 1,200
Ethanol	80 - 250
Methanol	5 - 15
Acetone	60 - 150
Methyl Cyanide	20 - 50
CHARACTERISTICS:	
COD	2,500 - 4,300
Total Nitrogen	10 - 15(mg/L as N)
Total Phosphorus[1]	40 - 60(mg/L as P)
pH	4.3 - 5.2

[1] Reported by the analytical laboratory of the Celanese Fiber Corporation, Narrows, VA.

3.4 Total Kjeldahl Nitrogen(TKN)

The TKN analysis was performed to measure influent and effluent nitrogen concentration as ammonia. The detailed procedure can be found in Standard Methods for the Examination of Water and Wastewater (19). TKN analyses were performed to determine the nitrogen uptake in the reactors. The results of TKN analyses performed throughout the reactor operation are presented in Appendix A, Table A-6.

3.5 Settling Characteristics

Sludge settling characteristics were determined using the Sludge Volume Index (SVI) test. The test involved settling 1,000 mL of sludge in a one-liter graduated cylinder. The settled sludge volume was measured after 30 minutes. The SVI was calculated as (20):

$$SVI = \frac{\text{settled sludge volume, ml} \times 1{,}000}{\text{total suspended solids concentration, mg/L}}$$

3.6 Turbidity Measurement

The effluent quality was measured in terms of turbidity. A 50-mL sample from the upper section of the clarifier was pipetted and then settled in a 50-mL graduated cylinder for 30 minutes prior to measuring the turbidity. The turbidity of the top 20 mL in the graduated cylinder was measured in terms of

Nephelometric Turbidity Unit (NTU) with a Hach Chemical Company (Loveland, Colorado) Model 2100A Turbidimeter.

3.7 Biopolymer Extraction

Quantitative measurements of biopolymer were made in each sampling. ECP extraction by centrifugation following pH adjustment was chosen over other techniques of extraction such as steaming and sonification based on a review of the literature findings. The effectiveness of this method over others was not validated in this experiment; however, it was believed to be the most effective extraction technique (8,18,21).

Numerous studies (including 2 and 3) have relied upon centrifugation alone to release ECP from biological floc to study the relationship between the extracted material and various operational parameters such as sludge age, sludge settling and dewatering characteristics. Brown and Lester (8), Carr and Ganczarczyk (12), and Novak and Haugan (17), all concluded the ineffectiveness of centrifugation in stripping floc of biopolymer. Some studies (8,12,22) have supported the use of heat extraction as the most appropriate method. They concluded that it is the most reliable method of biopolymer extraction. However, other researchers (17,21) suggested that boiling is too severe method of extraction. Kiff and Thompson (21) found that heat treatment "leads to

substantially degraded polymers, substantial cell breakdown, and apparently irreversible surface changes in the residual biomass." The data presented by Fitzgerald (23) showed almost complete inhibition of sludge dewatering following the adjustment to pH 11 of an activated sludge sample. This phenomenon may be attributed to a significant release of ECP from the floc matrix.

The ECP extraction technique utilized in this study was based primarily on the findings of Kajornatiyudh (18). The results from his study of comparing extraction techniques indicated that centrifugation at 5,000 rpm for 10 minutes followed by pH-adjustment to pH 11 and a 1 hour stirring period was most effective method when compared to the steaming extraction and collection of supernatant. An increase in the pH of the sample increased the polymer yield. At pH 12 the highest polymer quantities were released, but polymerization of the low molecular weight to the high molecular weight fraction also seemed to occur. At pH 11 the highest polymer quantities were obtained without significant polymerization. Furthermore, the DNA analysis showed no significant cell lysis occuring for this method whereas extraction by steaming resulted in a considerable DNA generation, suggesting cell lysis had occurred. An increase in the stirring period increased the polymer

yield. However, it was found that stirring beyound 1 hour at pH 11 did not result in a significant increase in the polymer yield. A centrifugal force of 5,000 rpm was chosen because it was sufficient to remove all the bacterial cells and small floc in the supernatant, and yet provided a high yield of polymer.

The sludge samples were extracted as soon as sludges were wasted from the reactors. The detailed procedure is listed below (25):

1. The pH of the 50-ml sludge sample was adjusted to 11 with 1 N NaOH, while stirring gently.
2. The sample was stirred slowly for 1 hour.
3. The stirred sample was then centrifuged at 5,000 rpm for 10 minutes.
4. The supernatant was analyzed for quantity and molecular weight of biopolymer and the precipitate pellet was discarded. The supernatant sample was refrigerated at 4 °C never longer than 6 hours before the analyses.

3.8 Gel Chromatography

Gel chromatography, commonly referred as gel filtration, was used in separating the high and low molecular weight organic fractions of the centrifuged supernatants and raw effluents. The gel used was

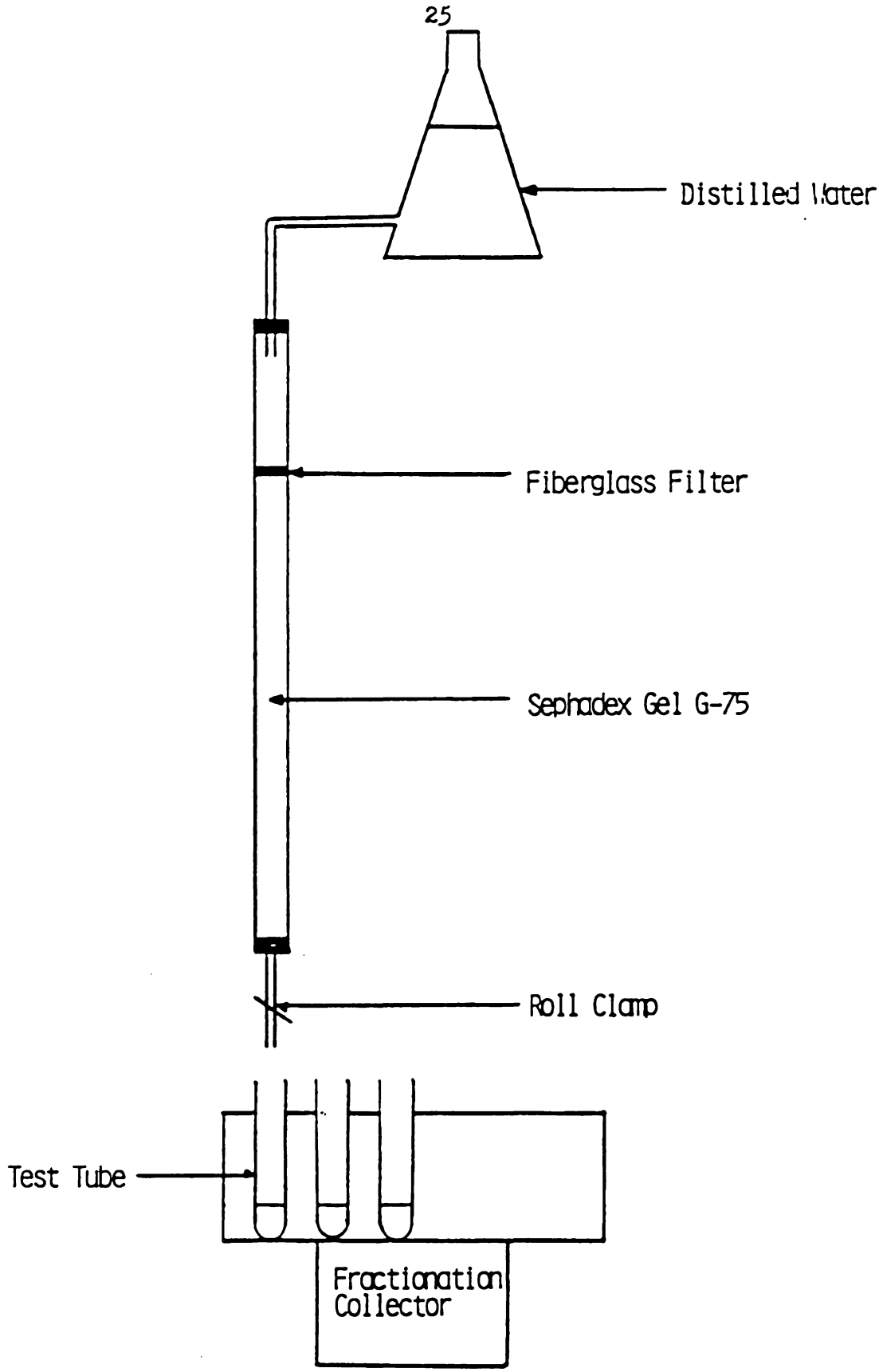


FIGURE 3. Schematic of Gel Filtration Column.

Sephadex G-75 from Phamacia Fine Chemicals (Piscataway, New Jersey). Sephadex, a bead-formed, dextran gel, is capable of separating molecules of different weight. Sephadex G-75 was chosen instead of G-25 and G-50 for its suitable fractionation range as shown in Table II.

The experimental set up of gel filtration is shown in Figure 3. Sephadex G-75 gel, swollen in distilled water for 24 hours, was packed in a cylindrical (1 cm X 50 cm) glass column until the gel level was stabilized at a depth of approximately 30 cm. Once packed, the gel column was used for no more than ten trials before repacking. The procedure of gel filtration is listed below:

1. The column outlet was opened to drain the water above the gel surface.
2. A 2 mL sample was applied at the gel surface gently without disturbing the gel and allowed to drain into the column.
3. The column outlet was closed and distilled water was applied at the gel surface to the top of the column. The eluant line was connected.
4. Sample collection began as soon as the outlet line was opened. The eluant from the column was collected continuously with a fraction collector, exactly 3 mL in each test tube.
5. The samples in the test tubes were measured for

TABLE II. SEPHADEX GELS AND THEIR PROPERTIES.

Gel	Dry Particle Diameter, um	Fractionation Range (molecular weight, g/mole)	
		Peptide and Globular Protein	Dextrans
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

absorbance at a wavelength of 280 nanometer (nm) to determine high and low molecular weight fraction with a Beckman Instruments, Inc (Irvine, California) DU-6 Spectrophotometer.

6. The samples were refrigerated at 4 °C never longer than 6 hours before protein and carbohydrate concentrations could be determined.

Calibration of the gel column using several known molecular weight substances is presented in Appendix A, Figure A-1.

3.9 Carbohydrates

The procedure described by Dubois et al. (25) was used in determining carbohydrate concentration in terms of mg/L as glucose. Standard solutions of glucose (concentration ranging from 0 to 20 mg/L) were used to prepare the standard curve (see Appendix A, Figure A-2). The following method was used in analyzing carbohydrate concentrations of samples:

1. The following reagents were required:
   A. Phenol, 5%(weight/weight) solution in distilled water;
   B. concentrated sulfuric acid, reagent grade.
2. 2 mL aliquots of samples to be analyzed were prepared

in 10 mm X 130 mm test tubes.

3. 1 mL of reagent A was added to each sample and vortexed immediately.
4. Before going to the next sample, 5 mL of concentrated sulfuric acid was added rapidly, directing the stream of acid against the liquid surface, to insure good mixing.
5. The samples were incubated at room temperature for 10 minutes.
6. The samples were shaken and incubated at room temperature for at least 30 minutes and at most 90 minutes before readings were taken.
7. Absorbance of each sample was measured against a reagent blank at the wavelength of 490 nm using a Baush and Lomb (Rochester, New York) Spectronic 20 Spectrophotometer.
8. Carbohydrate concentrations of the samples were determined using the standard curve prepared.

The carbohydrate measuring technique was not as reproducible as the protein measuring technique. The experimental error was ± 5 to 10 percent. This may be a result of evaporation and/or splattering caused by addition of concentrated sulfuric acid in the samples. The variabilities were considered to be within reason for the analysis and uncontrollable.

3.10 Proteins

Protein concentration of all samples were determined by the Lowry procedure (24). Samples analyzed include the fractions from the gel filtration and the extracted and effluent samples. Protein concentration was measured in terms of mg/L as bovine serum albumin (BSA). A standard curve was prepared with various concentrations of BSA ranging from 0 to 300 mg/L (see Appendix A, Figure A-3). The detailed procedure is listed below:

1. The following reagents were prepared:
   A. 100 g sodium carbonate in 1 liter (final volume) 0.5 N NaOH;
   B. 1 g copper sulfate in 100 mL (final volume) distilled water;
   C. 2 g 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.
2. 1 ml aliquots of samples to be analyzed were prepared in 10 mm X 130 mm test tubes.
3. 20 mL of reagent A were mixed with 1 mL of reagent B and 1 mL of reagent C.
4. 1 mL of the mixed solution prepared in step 3 was added to each test tube and vortexed.
5. The samples were incubated at room temperature for

15 minutes.

6. While the samples were incubating, the reagent D was prepared.
7. Following the incubation, 3 mL of the reagent D was added to the samples and vortexed immediately (before going to the next sample).
8. The samples were incubated at room temperature for 45 minutes.
9. The absorbance of each sample was measured against a reagent blank at the wavelength of 660 nm with a Bechman Instruments, Inc (Irvine, California) DU-6 Spectrophotometer.
10. The concentration of protein of each sample was determined using the standard curve prepared with BSA.

The results obtained using this technique were quite reproducible. The variability for the results was less than 5 percent always. Thus, the duplicate samples were not prepared after several analyses.

IV. RESULTS AND DISCUSSION

In order to fulfill the objectives of investigating the role of biopolymers in an activated sludge system, two major operational changes were made to the reactors. The first was a change in the reactor feed nitrogen concentration; the second was a change in sludge age of the reactors. A summary of the operating conditions for reactors 1 and 2 is shown in Table III. The performance data for the reactors are tabulated in Appendix A (Table A-1 and Table A-2).

The following sections in this chapter present the pertinent data collected during this study and correlate the results obtained from this study with previous studies reported in the literature.

4.1 Gel Filtration

The supernatants of the extracted fractions were subjected to gel filtration for molecular size separation of biopolymers. A calibration of gel column was necessary to determine molecular weight distribution as a funciton of the elution volume. A calibration curve, (shown in Figure A-1, Appendix A) was prepared using three organic compounds of known molecular weight. The elution volume of each of these compounds was determined and plotted as a function of the log of its

TABLE III. SUMMARY OF OPERATING CONDITIONS.

DAY	SLUDGE AGE (days)	REACTOR 1 FEED	REACTOR 2 FEED
0 - 34	10	WASTEWATER	WASTEWATER
35 - 116	10	WASTEWATER	WASTEWATER (100 mg/L N)
117 - 171	5	WASTEWATER	WASTEWATER (100 mg/L N)

molecular weight. The plot resulted in a linear relationship. This result was also observed by others (23,27) who have calibrated Sephadex G-50 in their studies.

Separation of molecules by gel filtration technique involves the process of molecular sieving. Small molecules move with the eluent both within and outside of gel particles. Molecules larger than the pore size of the gel particles cannot penetrate the particles; therefore, they move more rapidly down the column with the eluent. The smaller particles are entrapped in the crevices of the gel particles to a varying extent, depending on their shape and size. Therefore, the molecules larger than the rated pore size of the gel will exit the column first.

A typical gel chromatogram is shown in Figure 4. Two peaks were consistently observed for all samples tested. HMW fraction of molecular weight greater than 50,000 at elution volume of 6-9 ml and LMW fraction of molecular weight less than 10,000 at elution volume of 27-30 ml were determined. A third peak was often discernible at elution volume of 21-24 ml with fraction molecular weight between 10,000 and 50,000.

The results agree fairly well with findings of others (23,26,27). Forster (26) also reported three major molecular weight ranges of organic compounds in

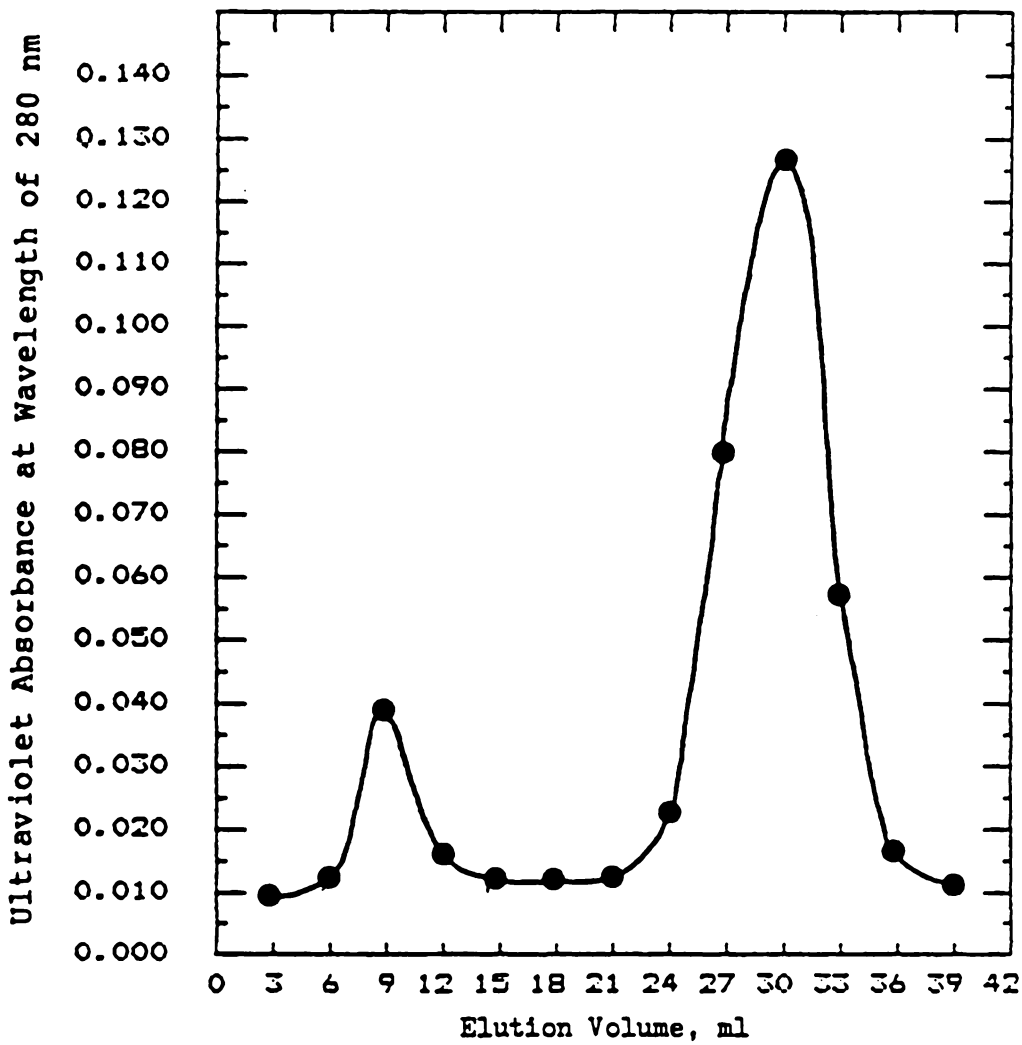


FIGURE 4. Typical gel filtration chromatogram.

activated sludge systems: one around 10,000, one between 26,000 and 78,000 and one greater than 100,000. The differencess in the molecular weights of the three fractions determined and their location in this study with respect to findings of others (23,26,27) may be attributed not only to the gel size used, but also to chemical variations in the systems.

Others have utilized a fracto-scan which measures the absorbance of eluent from the column continuously. Due to unavailability of a fracto-scan, the eluent from the column was collected continuously in 3 mL fractions with a fraction collector in this study. Thus, the absorbance readings of eluent were average absorbance measurements in increment of 3 mL collected in each fraction. Since the exact locations of the peaks could not be determined with the average absorbance values, discrepancies in the molecular weight ranges may have resulted. Secondly, the molecular weights of biopolymers produced may be reactor specific. The specific feed composition and the sludge age in this study may have produced biopolymer of such weight fractions.

4.2 Overall Reactor Performance

A steady state period for the reactors was chosen as the reactor operating time between day 60 and day 110 for

sludge age of 10 days. During this period, suspended solids concentrations, effluent turbidity, and SVI of both reactors remained relatively stable. Even though the responses of the two cultures were quite different, sludges were usually able to provide efficient wastewater treatment by forming a distinct enough sludge-liquid interface to prevent solids loss in the effluents, thus providing low effluent COD values. In order to simplify the data analyses, much of the data for the steady-state operating period are reported as the average values depicted during the chosen steady state period.

Suspended solids concentration measurements of the sludge samples were obtained throughout this study not only to determine the steady state conditions, but also to calculate SVI and normalized ECP components. The average suspended solids concentrations obtained for reactors 1 and 2 were 3,400 and 3,100 mg/L, respectively. This yield difference is an unanticipated result which occurred between the two cultures. The yield for reactor 1 was slightly greater than that of reactor 2 despite the possible nitrogen deficient condition which may have existed in reactor 1. TKN analyses indicated that nitrogen concentrations of reactor 1 effluents never exceeded 1.5 mg/L N during this period. This is further supported by nitrogen utilization of reactor 2 being consistently 2 or 3 times greater than that of reactor 1.

Despite this condition, the suspended solids concentrations of reactor 1 was consistently higher than those of reactor 2 during the steady state period as shown in Figure 5.

Effluent turbidity and SVI were two parameters used in determining the settling characteristic of sludges. As mentioned above, both parameters remained relatively constant during the steady state period. The effluent turbidity and SVI values recorded for both reactors are presented in Figure 6 and 7, respectively. Although the SVI was not as consistent as the other parameters, it did appear to be a good indicator of reactor performance during the period from day 40 to day 65. SVI values during this time period of around 300 corresponded well with high effluent turbidity exhibited by both reactors during this period. During the steady state period (day 85 - 115), the average values for effluent turbidity for reactors 1 and 2 were 29 and 14 NTU, respectively; the average values for SVI were 150 and 250. This result is interesting to note. The better settling sludge (SVI = 150) was expected to produce the lower effluent turbidity. Formation of large, loose floc in reactor 1 and pin-point floc in reactor 2 during this period may account for such results. During the steady state operating period, extremely large biological floc were present in the aeration basin of reactor 1. Even

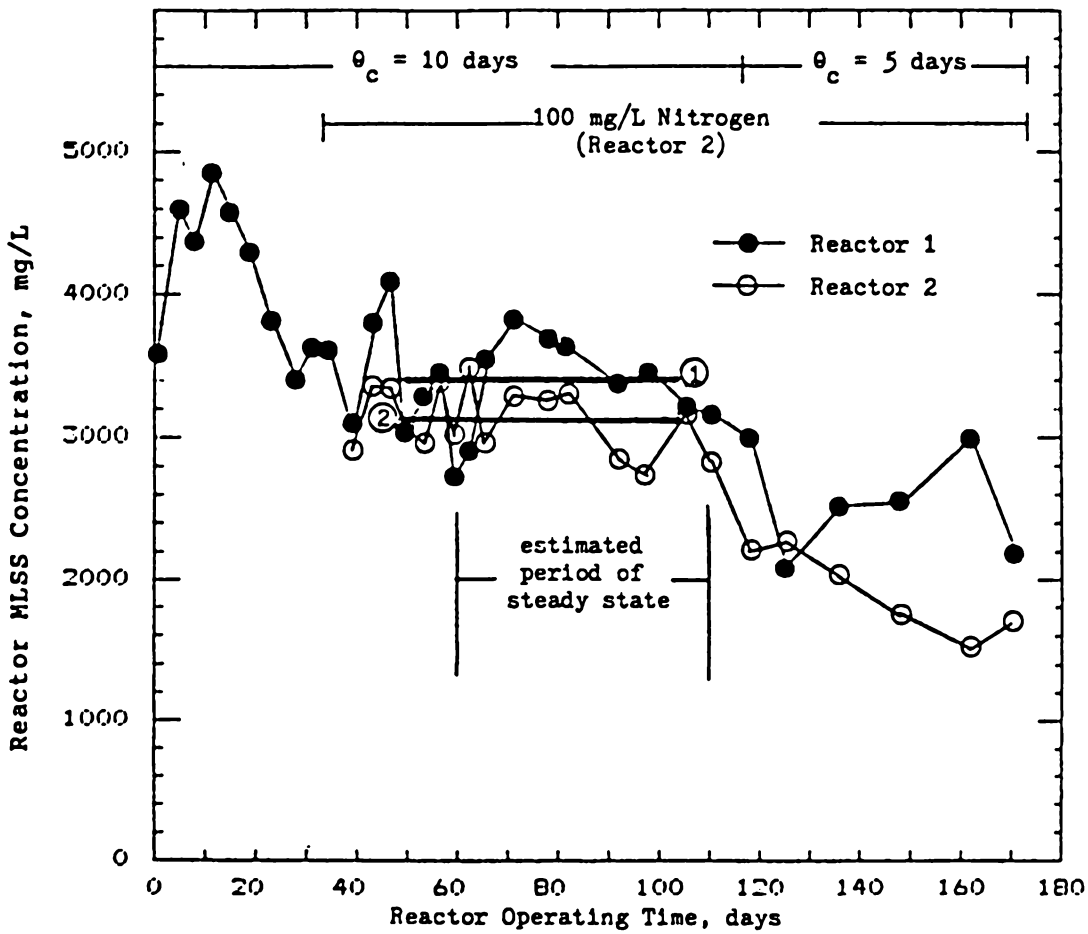


FIGURE 5. Mixed liquor suspended solids concentrations of activated sludge samples from Reactors 1 and 2.

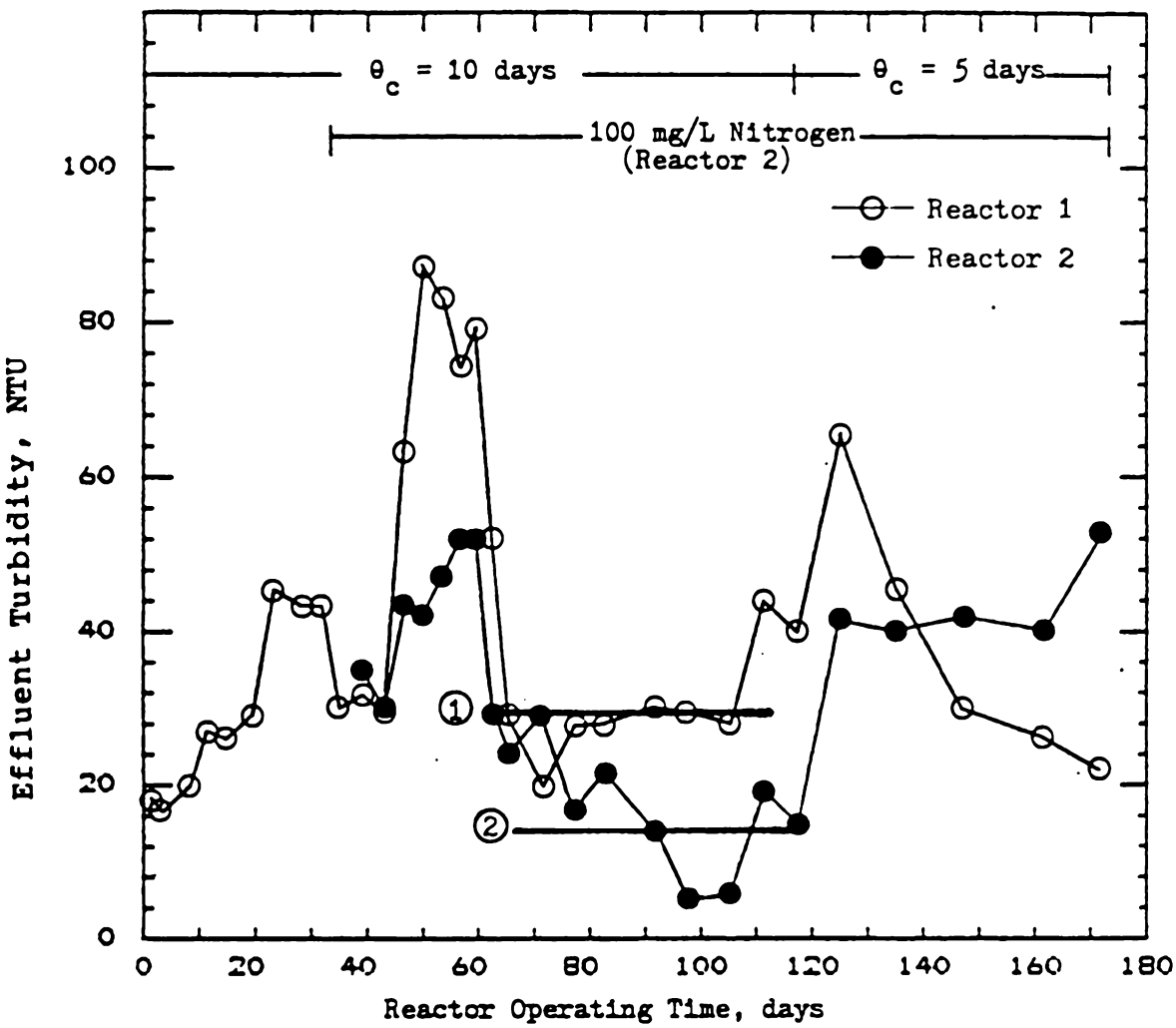


FIGURE 6. Effluent turbidity measurement of Reactors 1 and 2.

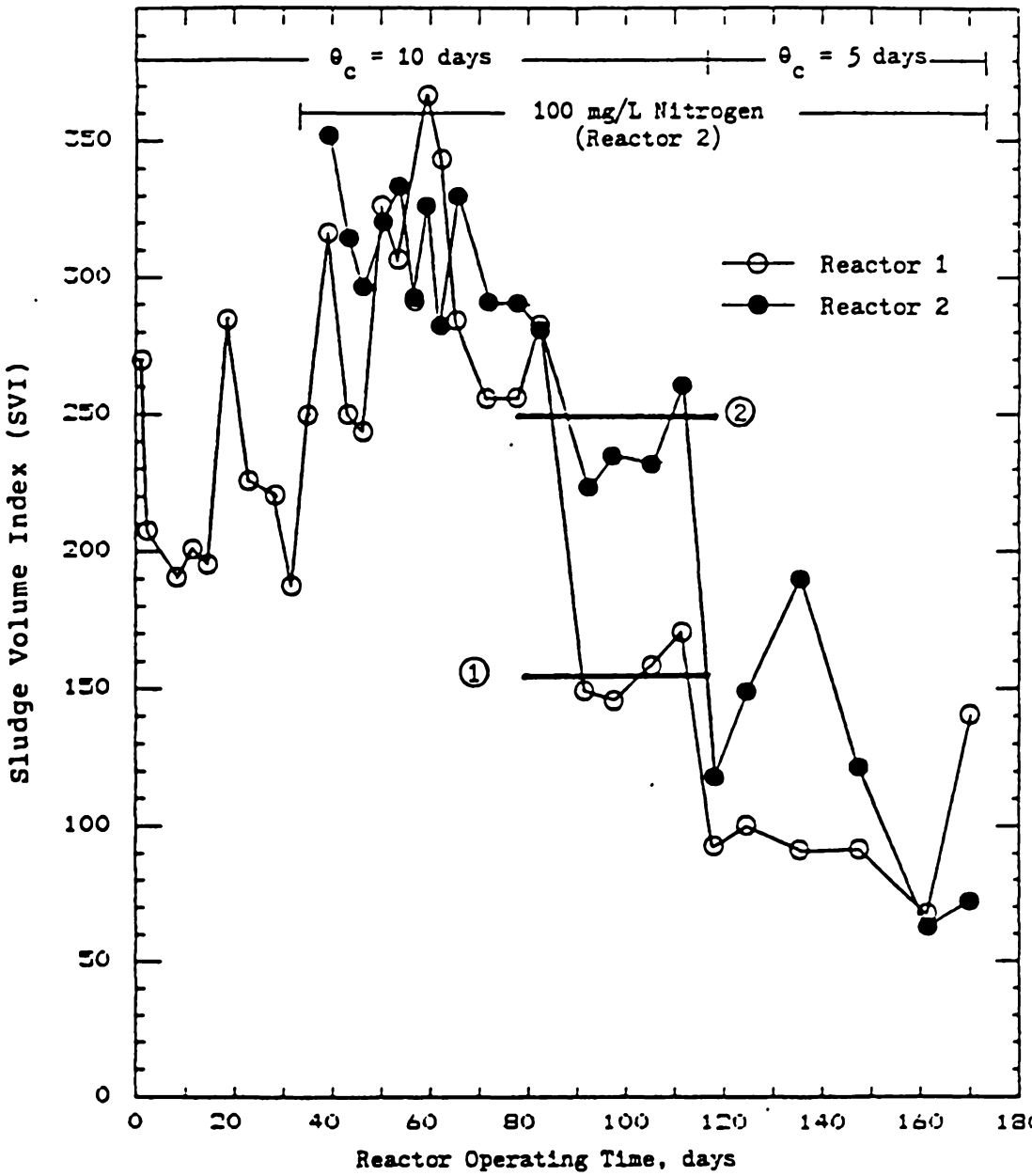


FIGURE 7. Sludge Volume Index measurement of Reactors 1 and 2.

though the sludges settled quite readily, the effluents of reactor 1 were consistently cloudy. The large floc may have been due to loose polymer binding. Although the binding may have been adequate to form floc, these may have been poorly formed and resulted in high biopolymer levels in the effluent. Analysis of the effluent indicated a presence a of high concentration of carbohydrate, in excess of 100 mg/L. This high concentration of carbohydrate in the effluent not only may have caused high effluent turbidity, but also increased the COD of the effluent.

The additional nitrogen in the reactor 2 feed did not prove to have a marked effect on the performance of the activated sludge systems. Perhaps additional nitrogen may have aided in decreasing the turbidity of effluent in reactor 2 slightly, but other than that, no significant improvement in reactor operation occurred.

The change in sludge age from 10 days to 5 days at the reactor operating day 117 resulted in deterioration followed by a slow recovery. It is not clear that steady state was attained even after 60 days of operation at the new sludge age. During this period, the reactors were plagued with problems and the performance worsened.

The most obvious observation made in reactor 1 at the five day sludge age was the immediate rapid decrease in the MLSS concentration. The change in sludge age

decreased the MLSS concentration from the neighborhood of 3,000 to 2,000 mg/L over period of 10 days. This decrease in MLSS concentration was a result of biomass being lost through the effluent as well as the decrease in sludge age. During this time, the effluent turbidity increased sharply from 30 to 60 NTU while the SVI decreased from 150 to 75. The increase in the effluent turbidity was not only a direct result of biomass lost through the effluent, but also may have been from the refractory organics present in the effluent due to insufficient COD removal by the system. The COD values of reactor 1 effluent increased from 300 - 500 mg/L (the steady state value) to 1,700 - 2,000 mg/L during this period. COD removal efficiency decreased from 90 to 50 per cent. The nitrogen concentration in the effluent increased, as well, from 1.5 mg/L N to excess of 6.0 mg/L N during this period. The decrease in SVI value can be attributed not only to the decrease in sludge age, but also to "clumping" of the sludge in the aeration basin. Due to their size, approximately 1/4 inches in diameter, microscopic examination to determine the nature of clumps was futile. During this period, the color of biomass in aeration basin continued to fade from light brown to almost greyish yellow.

A slow recovery was noticeable following the initial period of the sludge age change. The reactor 1 MLSS

concentration increased slightly and the effluent turbidity decreased sharply to the previous values, while the SVI remained relatively stable. The effluent COD decreased considerably to 800 mg/L and nitrogen consumption started to increase. The response of reactor 2 was quite different from that of reactor 1 following the reactor sludge age change. The MLSS concentration of reactor 2 decreased sharply initially, then further decreased gradually to approximately 1,500 mg/L until 50 days following the change. During this period, the SVI decreased erratically, possibly due to the decrease in MLSS concentration, while the effluent turbidity increased to 40 NTU and stayed in that range. The presence of pin-point floc in the settled sludge along with the excessive turbidity in the effluent indicated that the culture was no longer forming adequate floc. Despite the significant loss in microoganisms through the effluent, the culture appeared to be microscopically healthy. COD removal efficiency did not decrease drastically as it did in reactor 1, but nitrogen consumption rate decreased sharply from 40 mg/L N to 20 mg/L. Near the end of the reactor operation, there were some indications of recovery. However, the response was not as pronounced as that noticed for reactor 1.

As mentioned previously, the response of the

reactors were first for deterioration to occur, followed by slow recovery. The speed of recovery following the sludge age change seems to be noteworthy when compared to the recovery times reported by others (23,28). Fitzgerald (23) reported almost immediate recovery of system (less than 20 days) when the sludge age of an activated sludge system was changed from 15 to 5 days. The microscopic observations of the cultures Fitzgerald studied during her research indicated that the reactor failure due to sludge age changes was not due to massive filamentous growth. Her observation failed to recognize any marked change in the floc matrix of sludge, indicating no significant population shift had occurred. Chudoba et al. (28) indicated that their reactors recovered to a nominal steady state condition 74 days and 63 days following the change in the sludge age from 20 to 10 days. Unlike the findings of Fitzgerald, Chudoba et al. reported the reactor failures in terms of filamentous growth. The recovery of an activated sludge system after a severe operational change seems to be reactor and operation specific. Additional nitrogen in the reactor feed did not aid in the recovery of the reactor systems for this study. At sludge age of 5 days, the systems experienced near washout conditions until new steady state condition could be reached.

4.4 Biopolymer and Sludge Settling Characteristics

In this section, the results of biopolymer analyses and possible explanations for the sludge settling behavior of the reactors mentioned in the previous section will be presented in detail. Since previous researchers have suggested that biopolymers are responsible in sludge settling, it was essential to correlate the results of ECP analyses to the parameters which may indicate the sludge settling behavior such as SVI and effluent turbidity. In order to make quantitative comparisons of ECP concentrations of sludges, all data collections were reported as normalized concentrations. Normalized values of protein and carbohydrate biopolymers were obtained by dividing the corresponding values by the MLSS concentrations.

The average concentrations of ECP from the reactor 1 culture during the steady state period of 10 day sludge age were 0.008 mg/L protein and 0.018 mg/L carbohydrate per mg/L MLSS. As illustrated by Figure 8, the total protein biopolymer concentrations were relatively stable. The corresponding carbohydrate concentrations during this period (presented in Figure 9) showed a significant variation. Unlike the sludge from reactor 1, the amount of ECP from the sludge or reactor 2 was less variable, expecially in carbohydrate biopolymer concentration. This greater stability is shown in

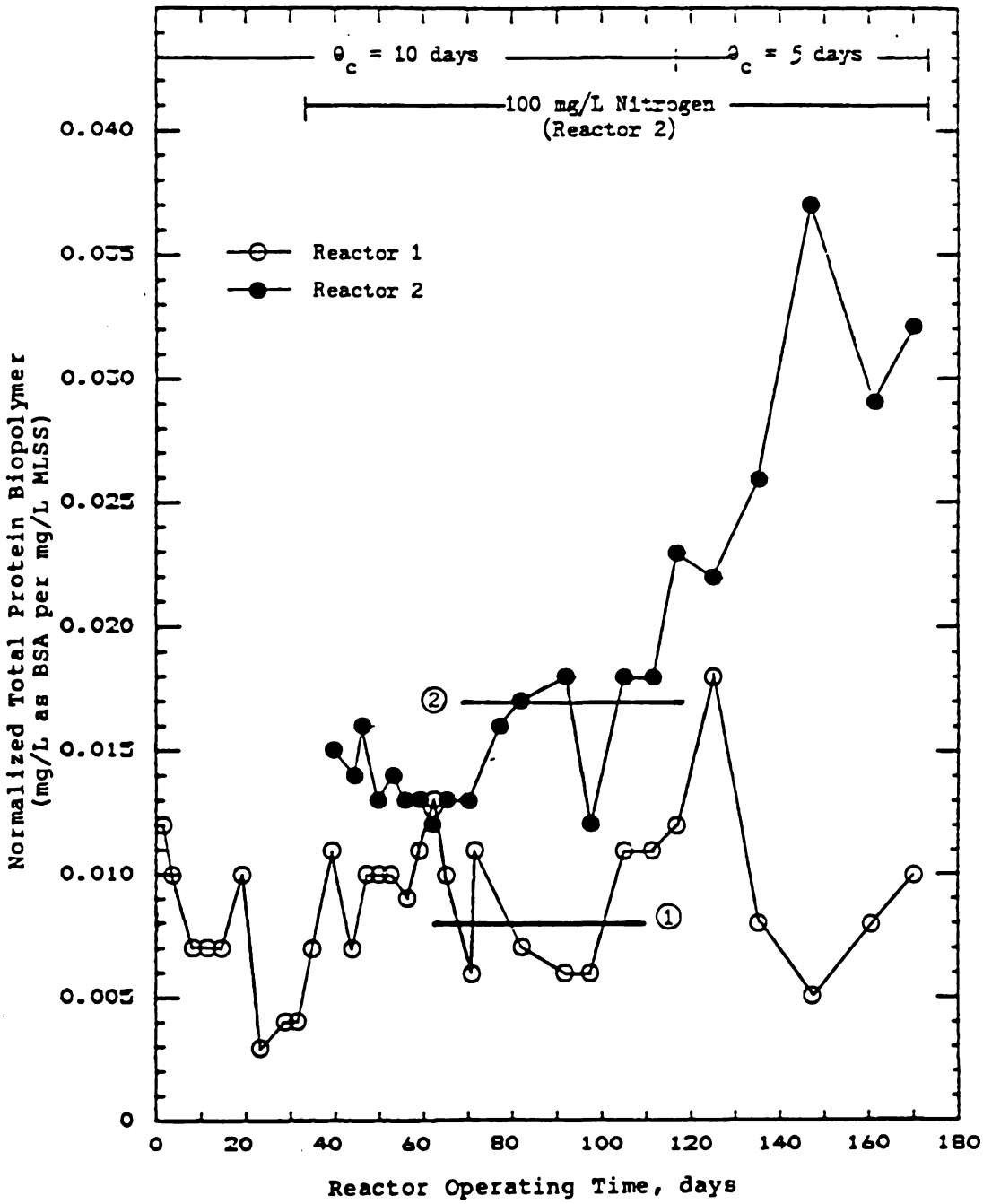


FIGURE 8. Total protein biopolymer concentration of activated sludge samples from Reactors 1 and 2.

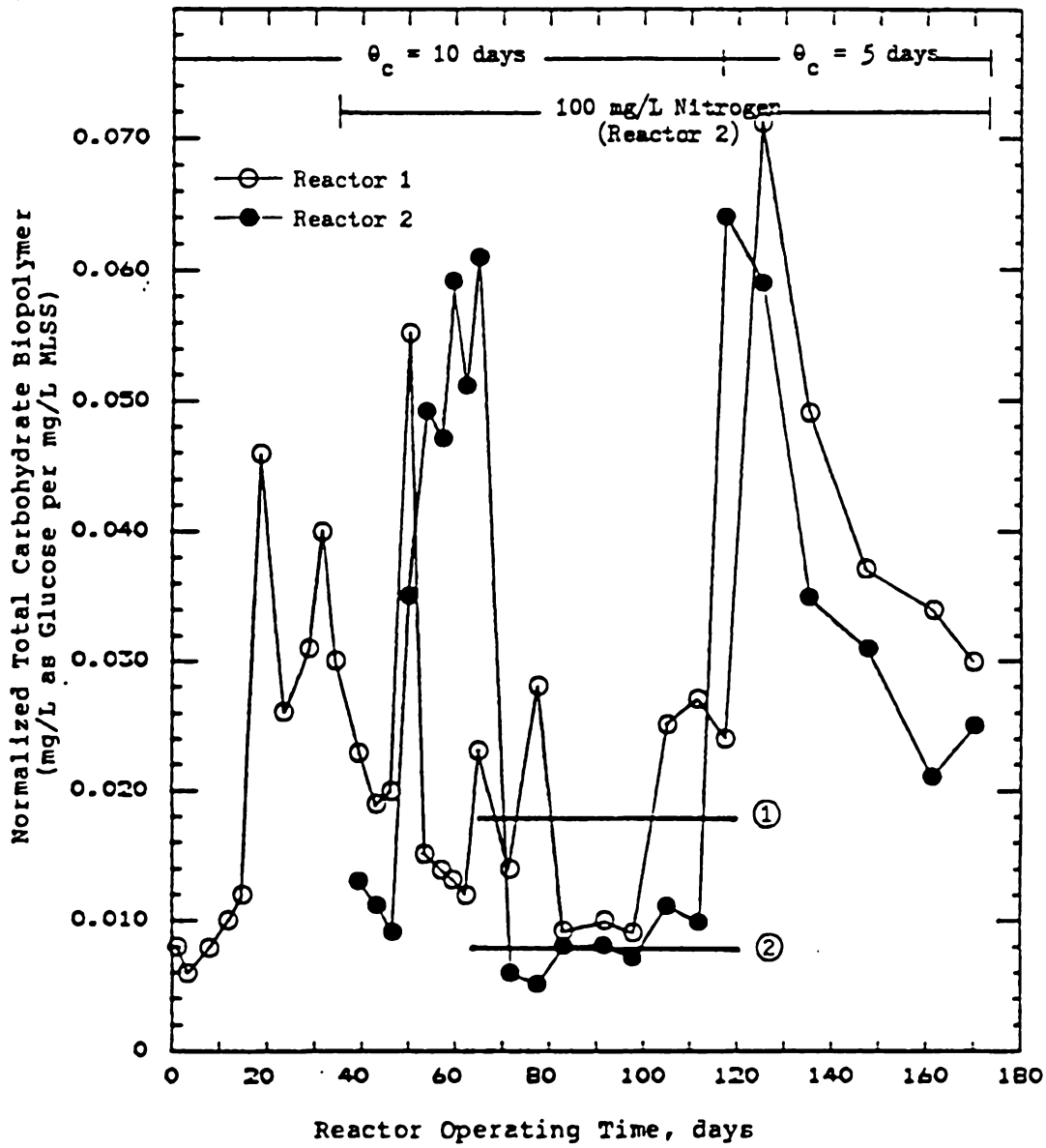


FIGURE 9. Total carbohydrate biopolymer concentration of activated sludge samples from Reactors 1 and 2.

Figure 9 with average steady state value of 0.008 mg/L carbohydrate per mg/L MLSS. The plot of protein biopolymer concentrations exhibited almost the same variability that existed for the corresponding reactor 1 concentrations with a mean of 0.017 mg/L protein per mg/L MLSS during this period.

The relationship between the ECP concentrations and operational change was noticed quite obviously in both cultures. When the sludge age was decreased from ten to five days, both biopolymer concentration of the cultures increased sharply initially, but generally decreased during the recovery period except for the protein biopolymer concentrations of reactor 2 which increased further even after the other parameters started to decrease. A steady state period was not apparent for the 5 day sludge age operating condition. The rebounding values near the end of the reactor operation may indicate that a new steady state period is soon to follow.

It is interesting to note, from Figure 9, that the carbohydrate biopolymer concentration of both reactors exhibited very similar response while divergent response of protein ECP production of reactors was noticed in Figure 8 throughout the study. The ratio of carbohydrate biopolymers to protein polymers during the steady state operating period for reactor 1 was 2.27 : 1 and that for

reactor 2 was 0.47 : 1. This significant difference in the ratio may be a direct result of nitrogen deficiency in reactor 1, as suggested in the previous section, and abundance of nitrogen in reactor 2. Due to nitrogen deficient condition existed in reactor 1, the culture produced a limited amount of amino sugar polymers which requires nitrogen for formation and, instead, diverted the abundant carbon source to production of carbohydrate biopolymers. In reactor 2, the abundance of nitrogen promoted production of protein biopolymers while depressing production of carbohydrate biopolymers.

This observation is in congruence with observations made by others in previous studies. Wilkinson (29) investigated the effects of various nutrients on the polysaccharide production by K. aerogens. He concluded that production of polysaccharide is not limited by nitrogen. Also when nitrogen was limited, the polysaccharide produced per cell was maximized. His results further suggested that nutrients such as nitrogen, phosphorus and sulfur are necessary for growth and when were limited the excess carbon source is diverted to polysaccharide production. This was also supported by a study conducted by Wu et al. (16). They noticed stimulation of carbohydrate production by nitrogen deficient wastes, whereas nitrogen rich

conditions stimulated protein production.

Effluent turbidity and SVI were compared to ECP concentrations to determine if any possible relationship existed between ECP and sludge settling characteristics. The relationship between ECP concentrations and effluent turbidity for reactor 1 is presented in Figures 10 and 11. Almost every increase or decrease in effluent turbidity is matched by an increase or decrease in ECP concentrations. This relationship is particularly well defined starting from the onset of steady state at day 80. For reactor 2 the relationship between ECP and turbidity is not as well defined as indicated in Figures 12 and 13. Figure 12 shows a decrease in effluent turbidity with an increase in protein biopolymer concentration during the steady state period. In Figure 13, carbohydrate biopolymer relationship, a similar behavior to that which was observed in reactor 1 was shown during this time.

No such correlation between ECP carbohydrate production and effluent turbidity has ever been made in any previous studies. Since the ECP is believed to play an important role in coagulation and flocculation of biomass, one would expect to observe lower turbidity during high biopolymer production period and conversely higher turbidity during low

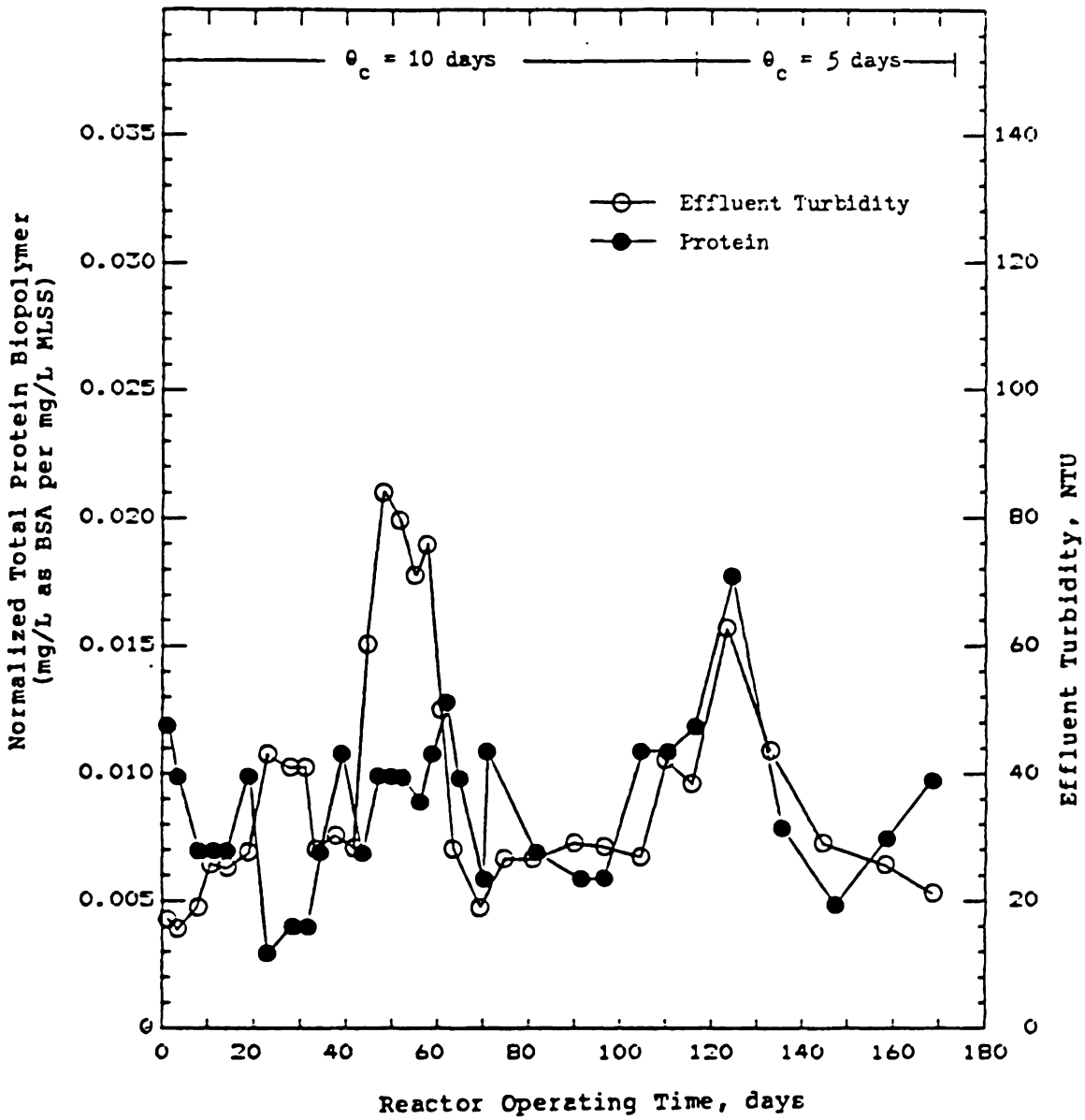


FIGURE 10. Effluent turbidity versus total protein biopolymer concentration of activated sludge samples for Reactor 1.

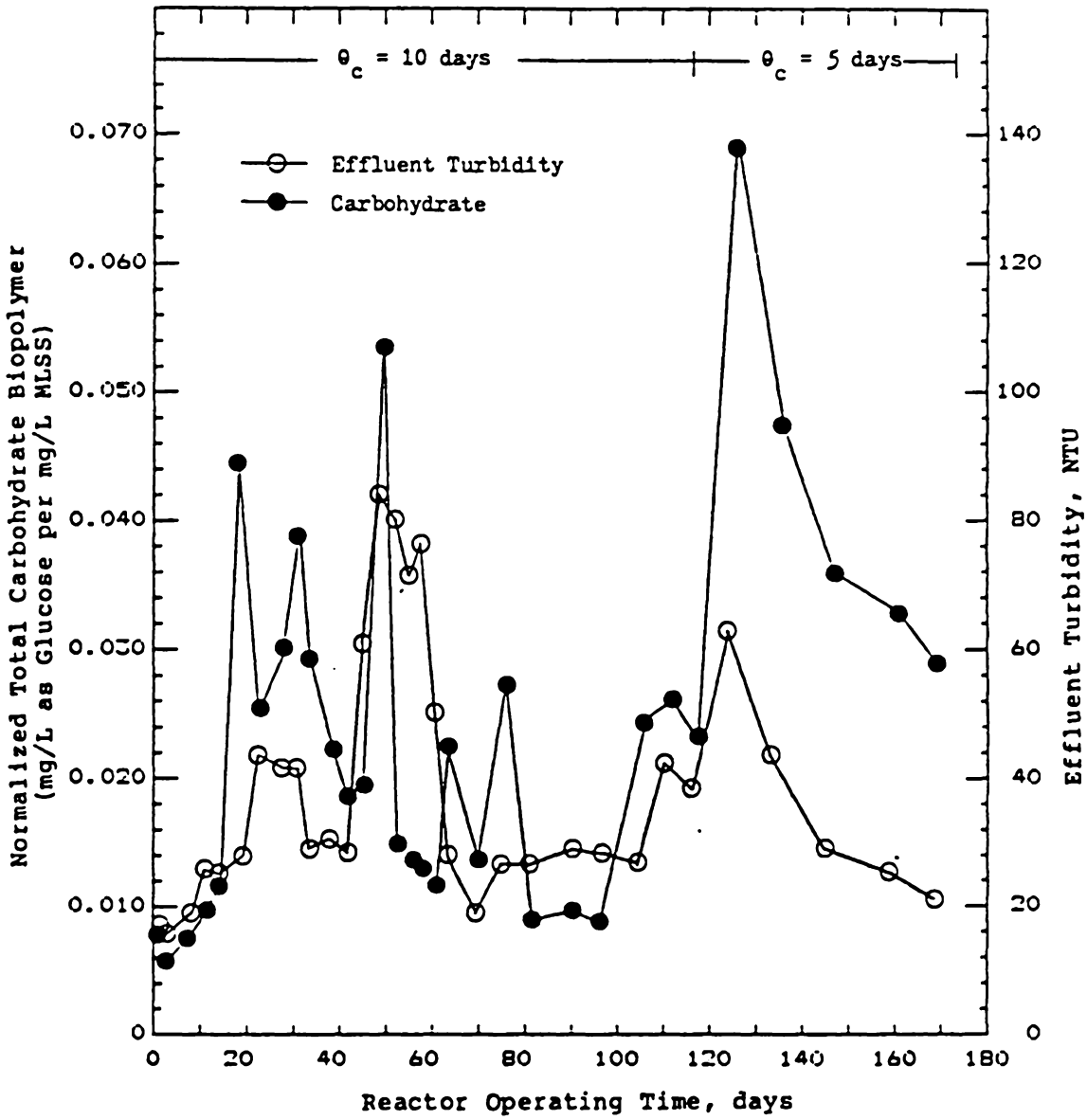


FIGURE 11. Effluent turbidity versus total carbohydrate biopolymer concentration of activated sludge samples for Reactor 1.

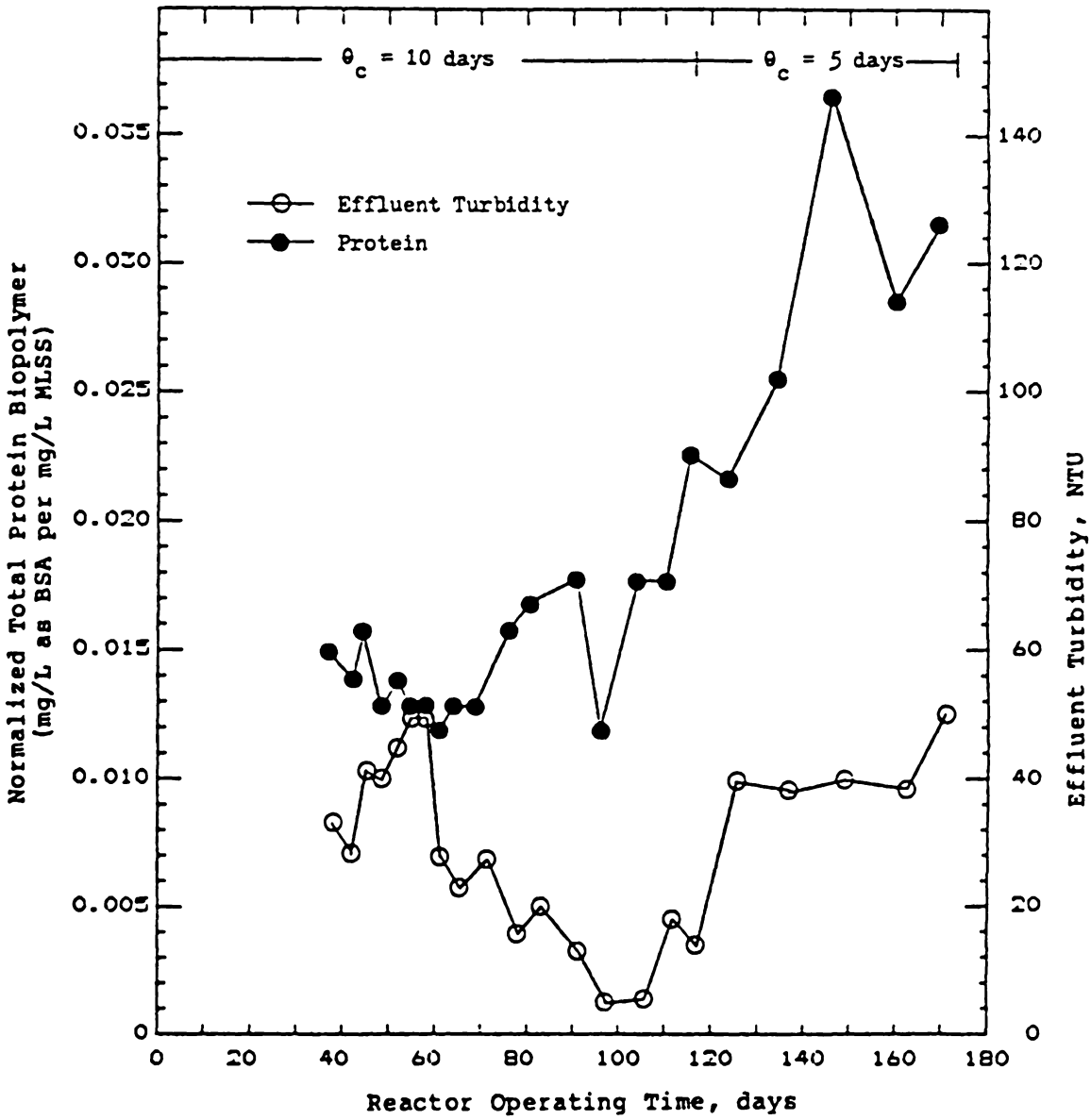


FIGURE 12. Effluent turbidity versus total protein biopolymer concentration of activated sludge samples for Reactor 2.

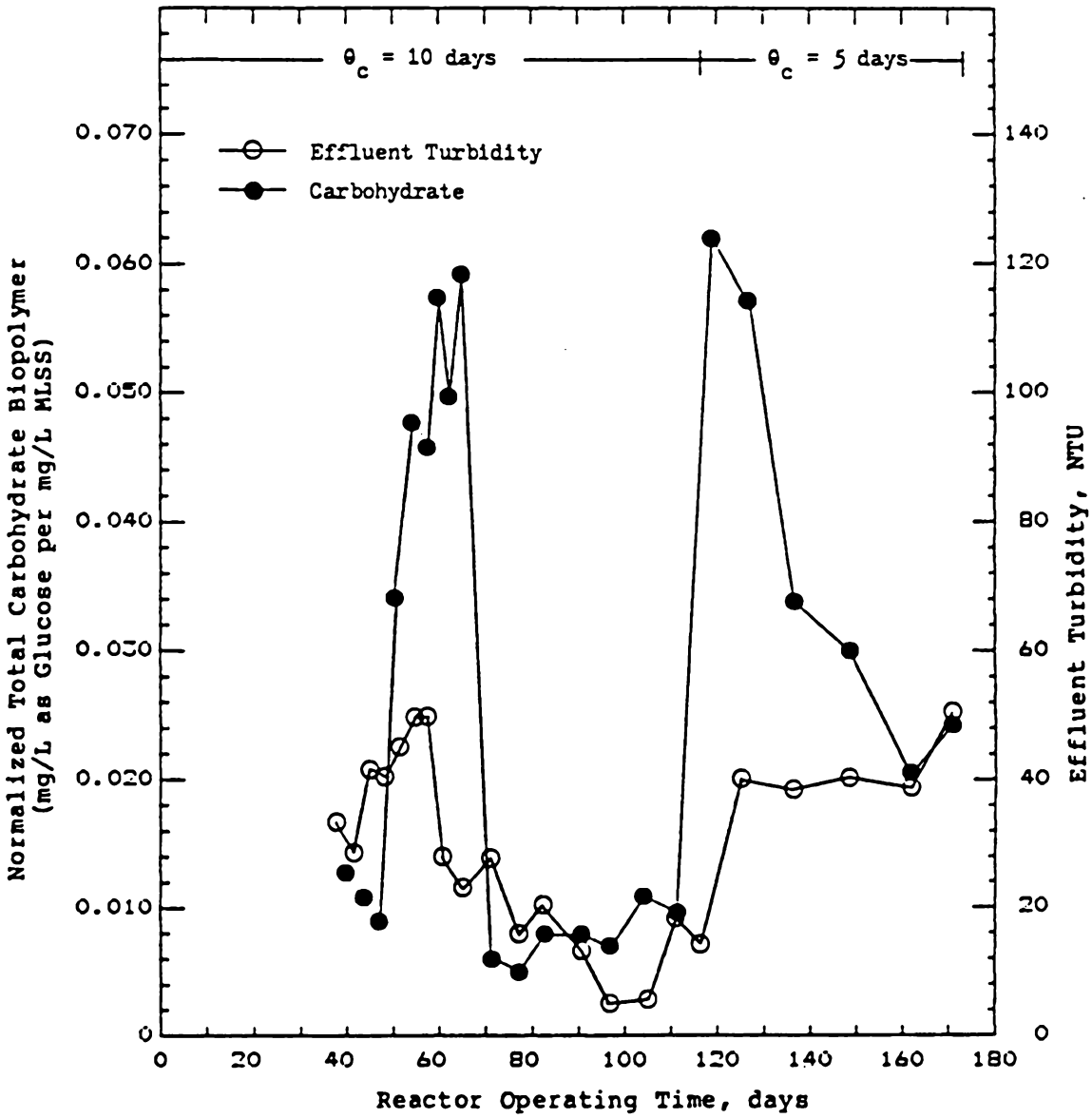


FIGURE 13. Effluent turbidity versus total carbohydrate biopolymer concentration of activated sludge samples for Reactor 2.

polymer production period. However, it is important to remember that the suspending biomass is not the sole source of turbidity of reactor effluent. As indicated in previous section, high turbidity that was exhibited in reactor 1 effluent may have been a direct result of loose carbohydrate biopolymers which were released from the floc. The result of biopolymer analysis following gel filtration of LMW carbohydrate polymer in the reactor 1 effluent during this time. For the reason mentioned above, it is difficult to develop a unified relationship between biopolymer production and effluent turbidity in this study and to explain such contradictory behavior. This may be because not only is the quantity of biopolymer important but also because the binding properties may play a role in floc formation.

The second part of this analysis involved an attempt to correlate the amount of ECP with SVI, a measurement of sludge settling characteristic. As experienced in the previous discussion, there was no unifying correlation which may indicate a direct relationship exists between these parameters. As can be seen in Figure 14 and 15, SVI of reactor 1 decreased steadily throughout the study while the carbohydrate biopolymer concentration showed no significant trend and the protein biopolymer concentration remained

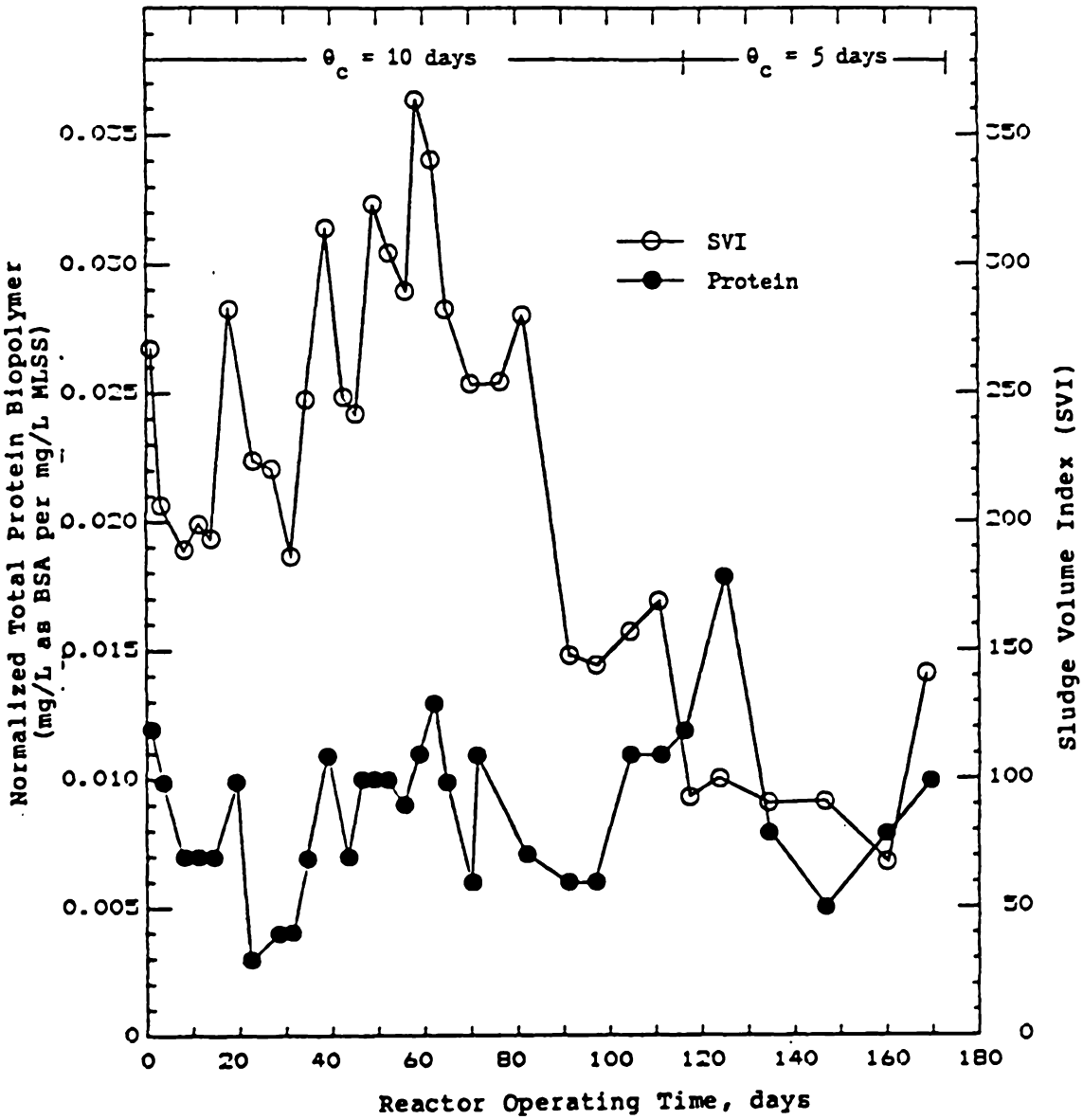


FIGURE 14. SVI versus total protein biopolymer concentration of activated sludge samples for Reactor 1.

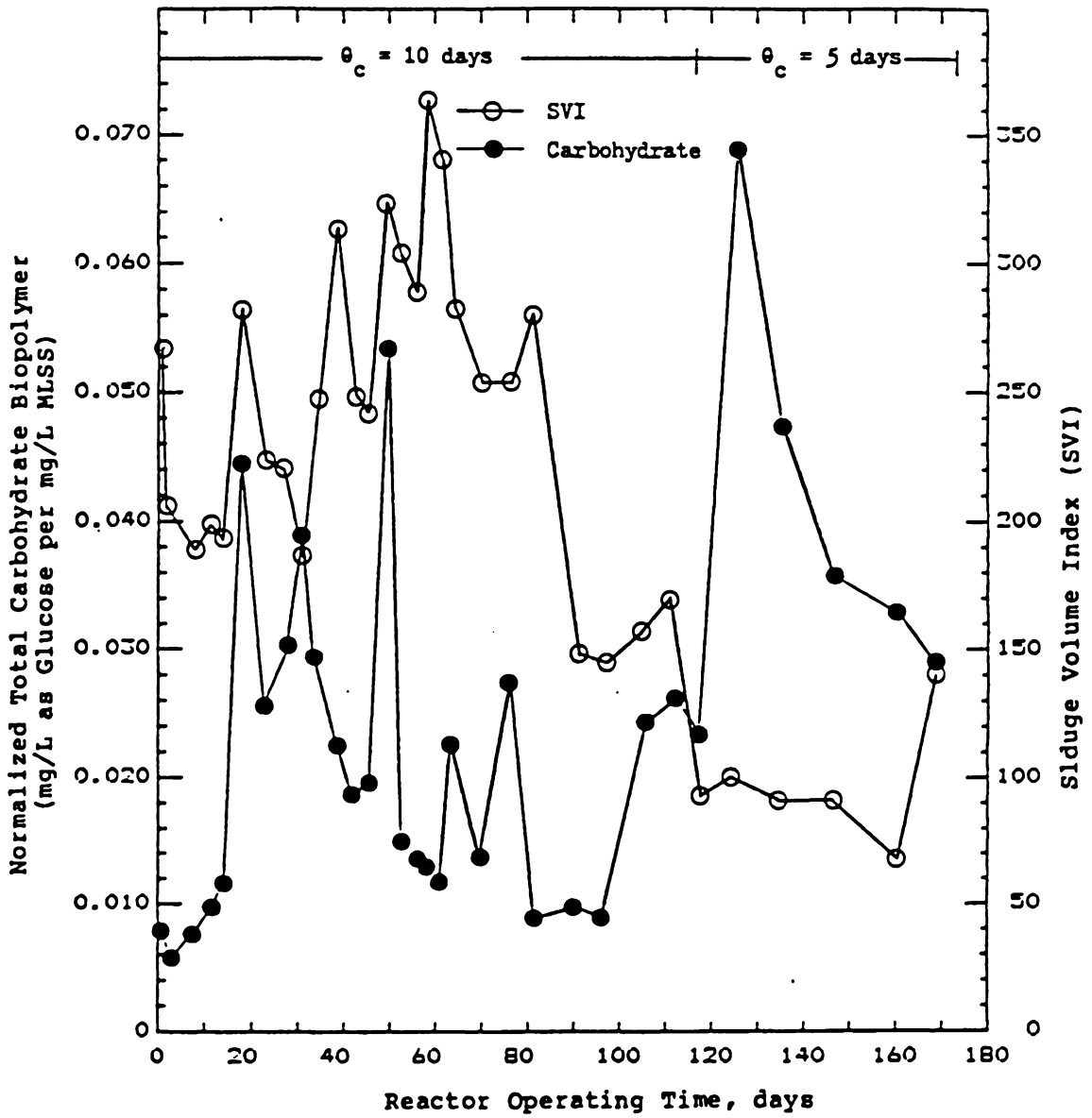


FIGURE 15. SVI versus total carbohydrate biopolymer concentration of activated sludge samples for Reactor 1.

relatively stable. The Corresponding plots of reactor 2 are presented in Figure 16 and 17. In Figure 16, the relationship between protein and SVI indicates a definite trend of increasing protein biopolymer concentration with decreasing SVI throughout entire operating period.

Becarri et al. (13) observed similar correlations between SVI and ECP production in their study. At one period of their experiment every decrease or increase in SVI was promptly matched by a decrease or increase in ECP. In other, the SVI and ECP differed; an increase SVI corresponded to a decrease in ECP and vice versa. Such diverse correspondences between SVI and ECP exhibited in the study (13) may be explained by likening ECP behavior to that of polyelectrolyte in the coagulation-flocculation process of particles suspension. As is known, the maximum agglomeration of particles occurs only with optimal polyelectrolyte dosages: for increasing doses up to the optimal value, system flocculation improves progressively, while for doses above the optimal value, this process worsens gradually upon increasing the dose. Abundance of nitrogen has apparently produced biopolymer of higher protein content. However, the effect of biopolymers on SVI seems to be less pronounced when compared to their effect on effluent turbidity.

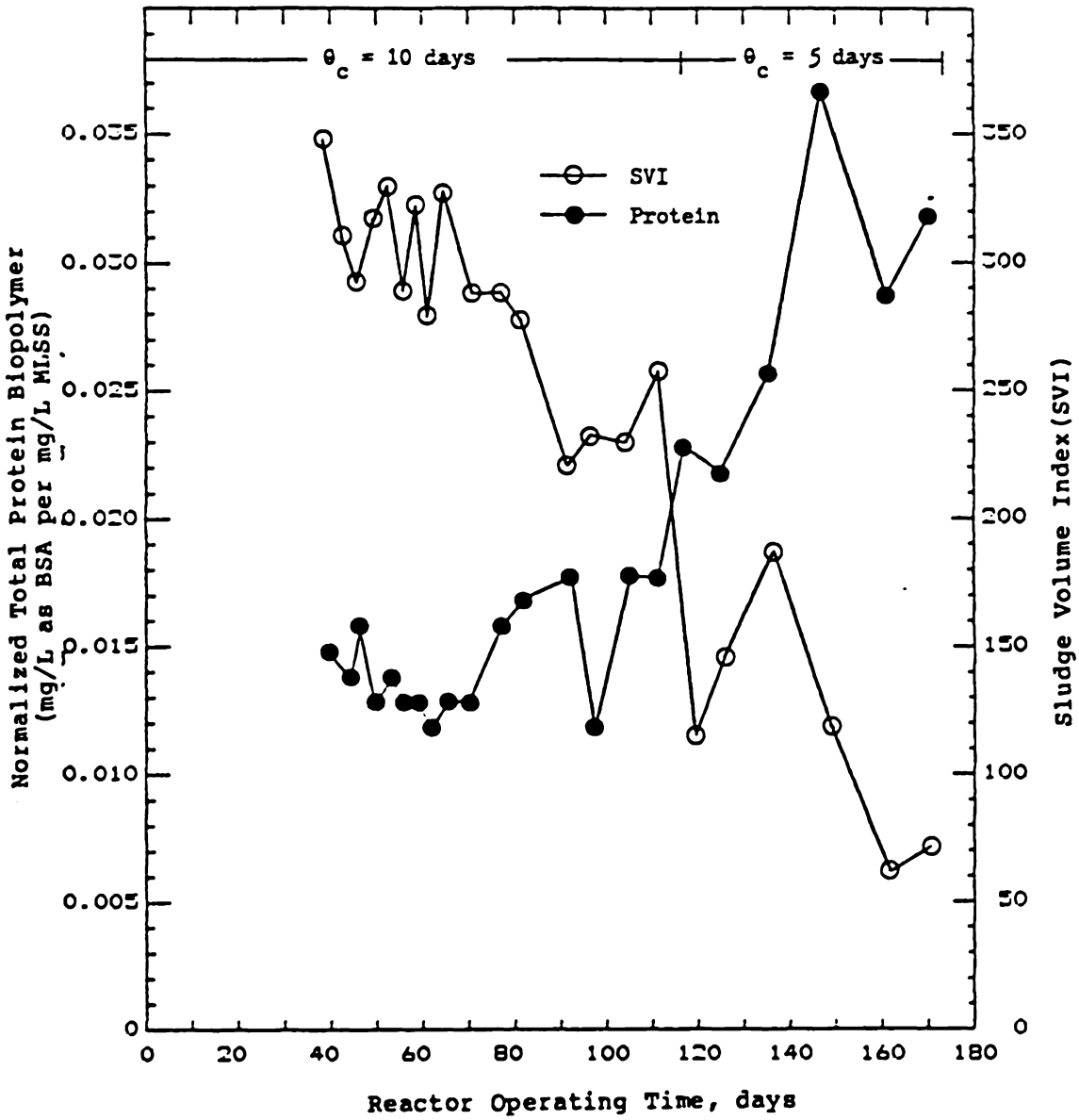


FIGURE 16. SVI versus total protein biopolymer concentration of activated sludge samples for Reactor 2.

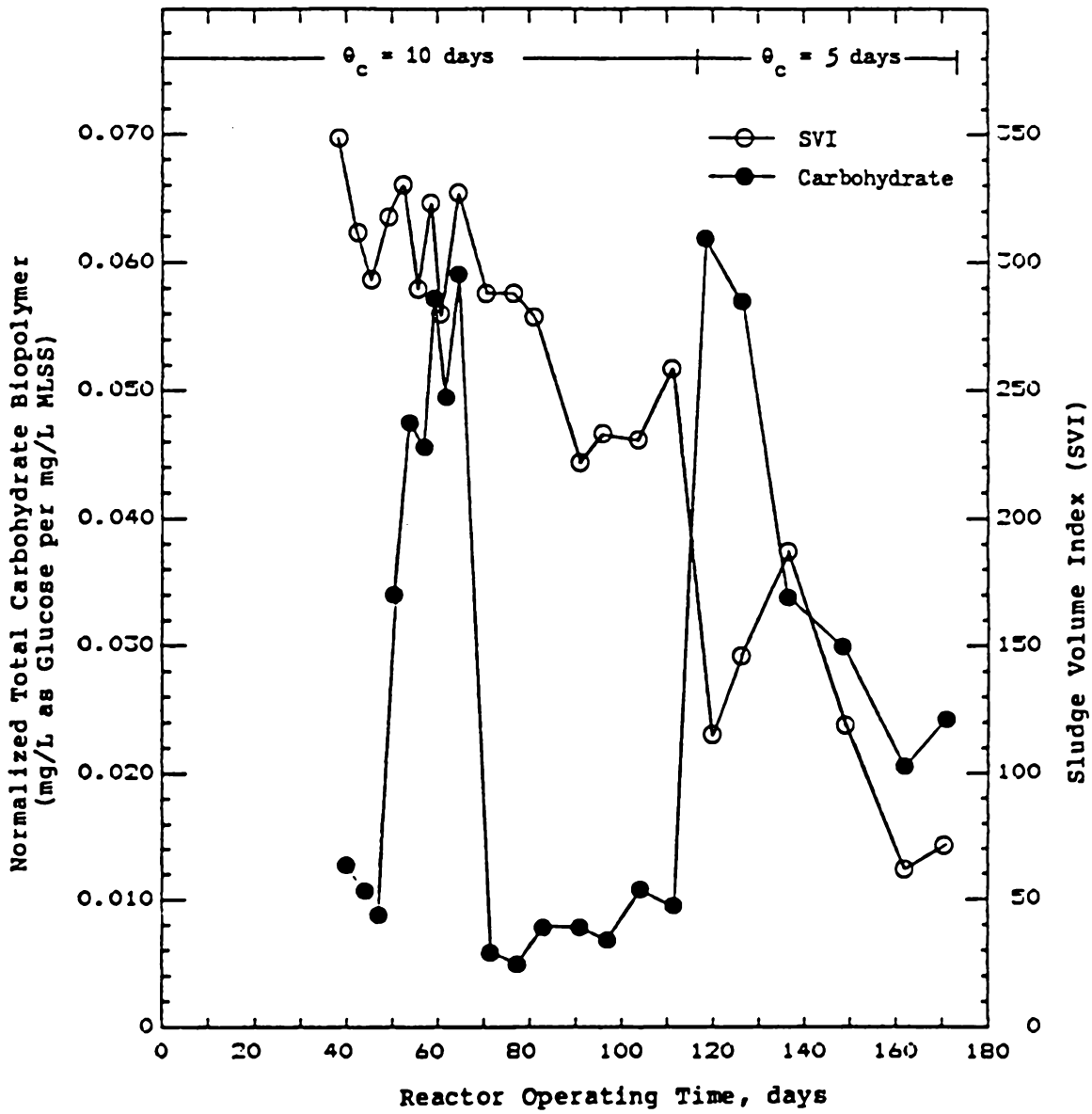


FIGURE 17. SVI versus total carbohydrate biopolymer concentration of activated sludge samples for Reactor 2.

The high molecular weight(HMW) fraction was analyzed for HMW protein biopolymer and HMW carbohydrate biopolymer. The summary of averaged values of HMW biopolymers recorded during the steady state operating period of 10 day sludge age is presented in Table IV.

It is most important to note the ratio of HMW carbohydrate to HMW protein biopolymer in each reactor. The calculated ratio is 10 : 1 and 1.9 : 1 for reactors 1 and 2, respectively. Compared to the corresponding values for the biopolymers, these ratio indicate there is more pronounced effect of feed nitrogen concentration on HMW biopolymer production than the total biopolymer production. The concentrations of HMW biopolymers remained stable during the steady state operating period. Figure 18 and 19 show there were greater stability in HMW protein concentration than HMW carbohydrate concentration for both cultures during this time. This behavior was markedly noticeable upon the change in sludge age.

Effluent turbidity and SVI were correlated with HMW biopolymer concentrations. The relationship between HMW biopolymer concentrations and effluent turbidity is presented in Figures 20 through 23. Figure 20 shows a trend of increase and decrease in effluent turbidity corresponding with increase and decrease in the concentrations of biopolymers. This relationship is less obvious in Figure 21. An inverse relationship

TABLE IV. SUMMARY OF STEADY STATE VALUES FOR REACTOR OPERATING PARAMETERS AND BIOPOLYMER CONCENTRATIONS.

SLUDGE AGE = 10 DAYS

	STEADY STATE VALUES	
	REACTOR 1	REACTOR 2
OPERATING PARAMETERS		
MLSS(mg/L)	3,400	3,100
EFFLUENT TURBIDITY(NTU)	29	14
SVI	150	250
BIOPOLYMER CONCENTRATIONS		
TOTAL BIOPOLYMERS:		
PROTEIN[1]	0.008	0.017
CARBOHYDRATE[2]	0.018	0.008
HMW BIOPOLYMERS:		
PROTEIN[1]	0.0007	0.0017
CARBOHYDRATE[2]	0.0070	0.0032

[1] mg/L BSA per mg/L MLSS

[2] mg/L glucose per mg/L MLSS

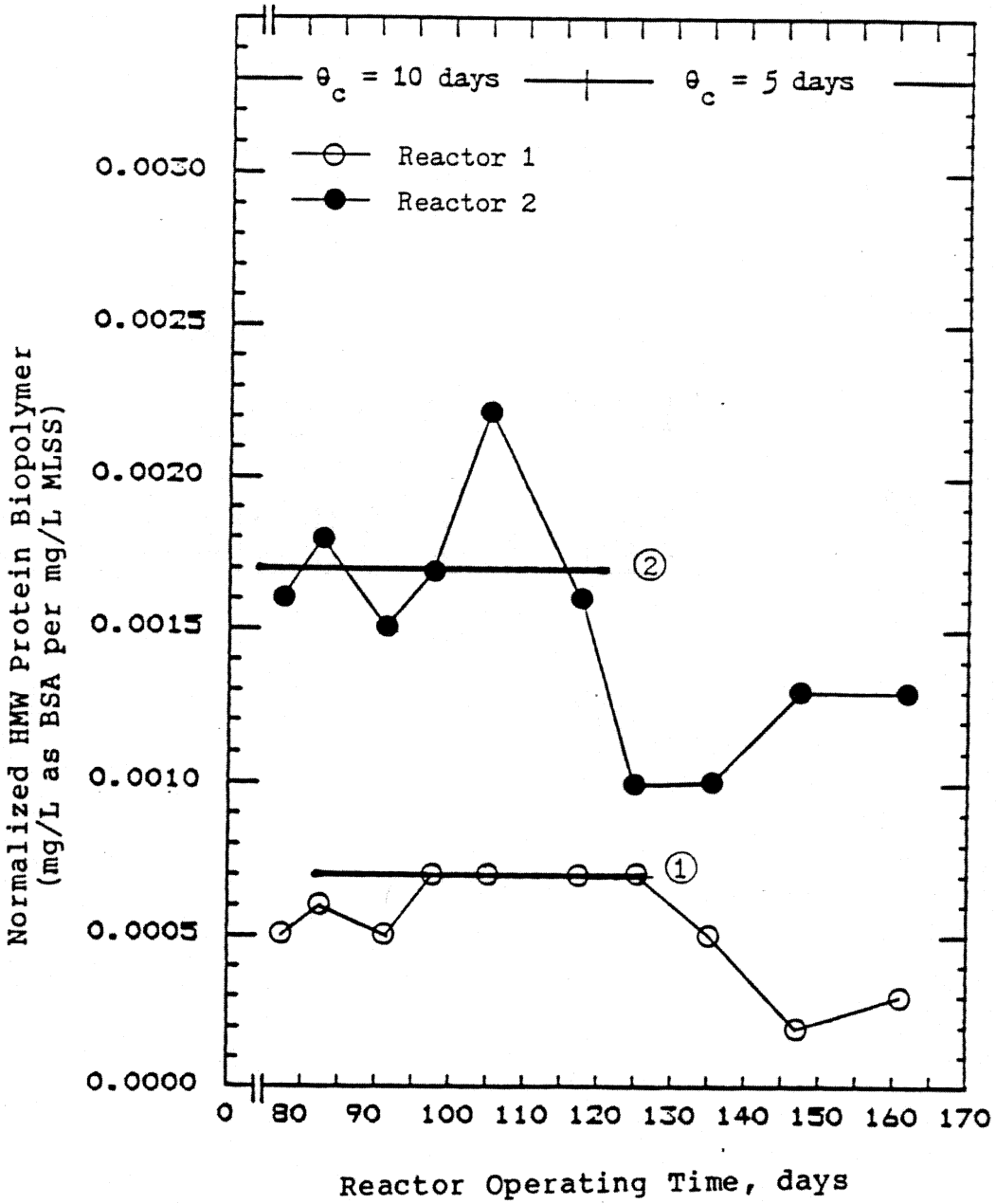


FIGURE 18. Total HMW protein biopolymer concentration of activated sludge samples from Reactors 1 and 2.

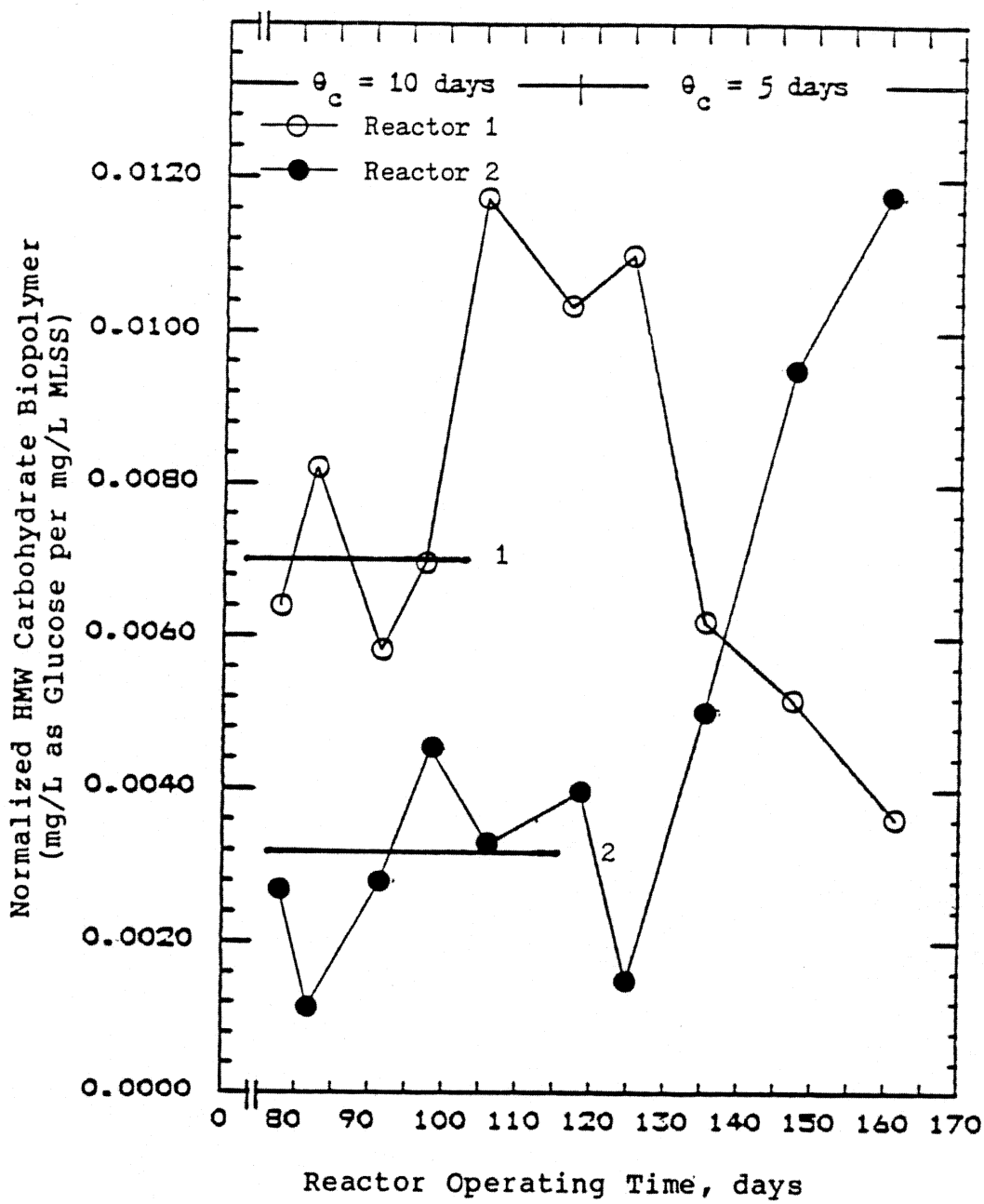


FIGURE 19. Total HMW carbohydrate biopolymer concentration of activated sludge samples from Reactors 1 and 2.

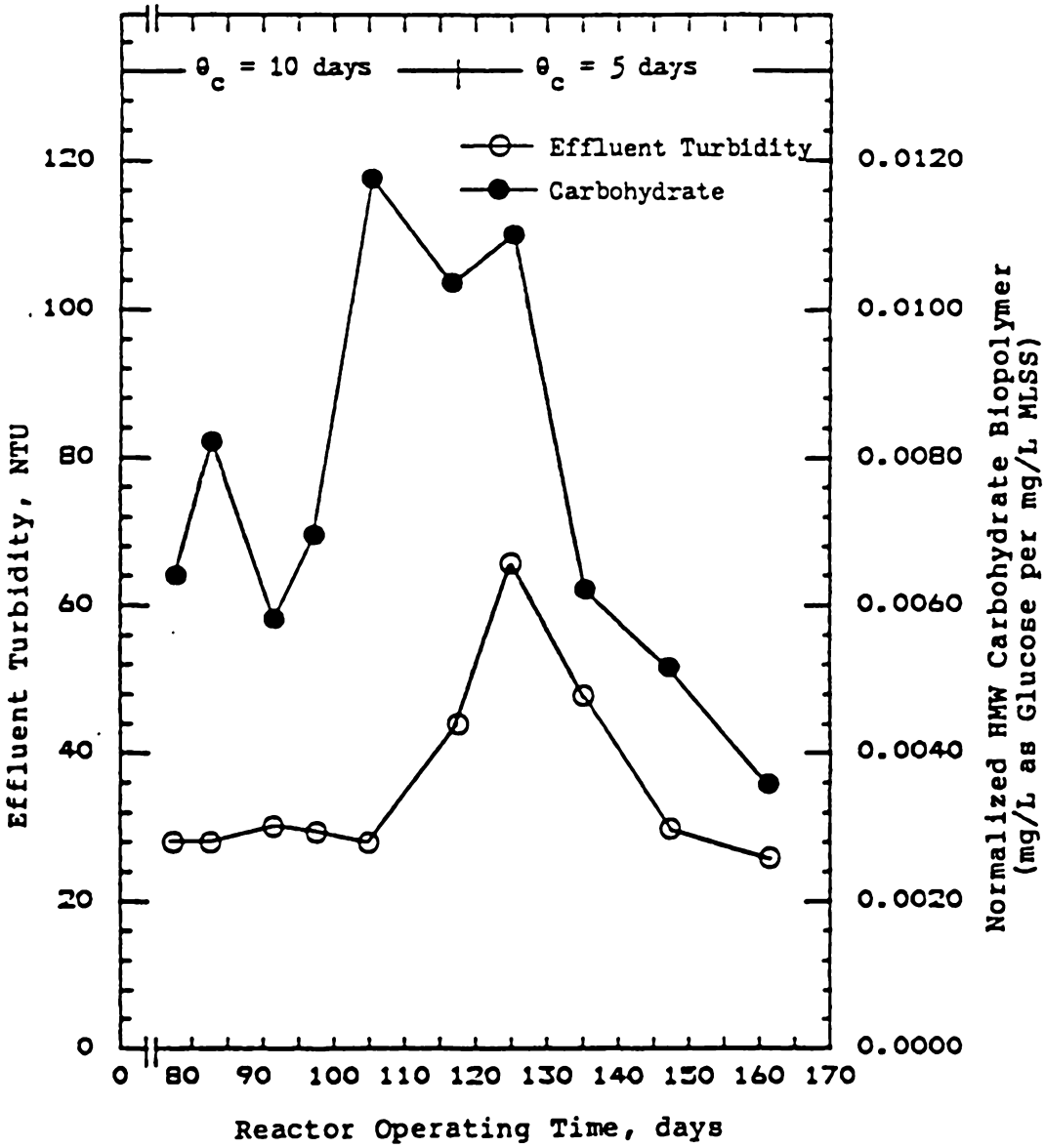
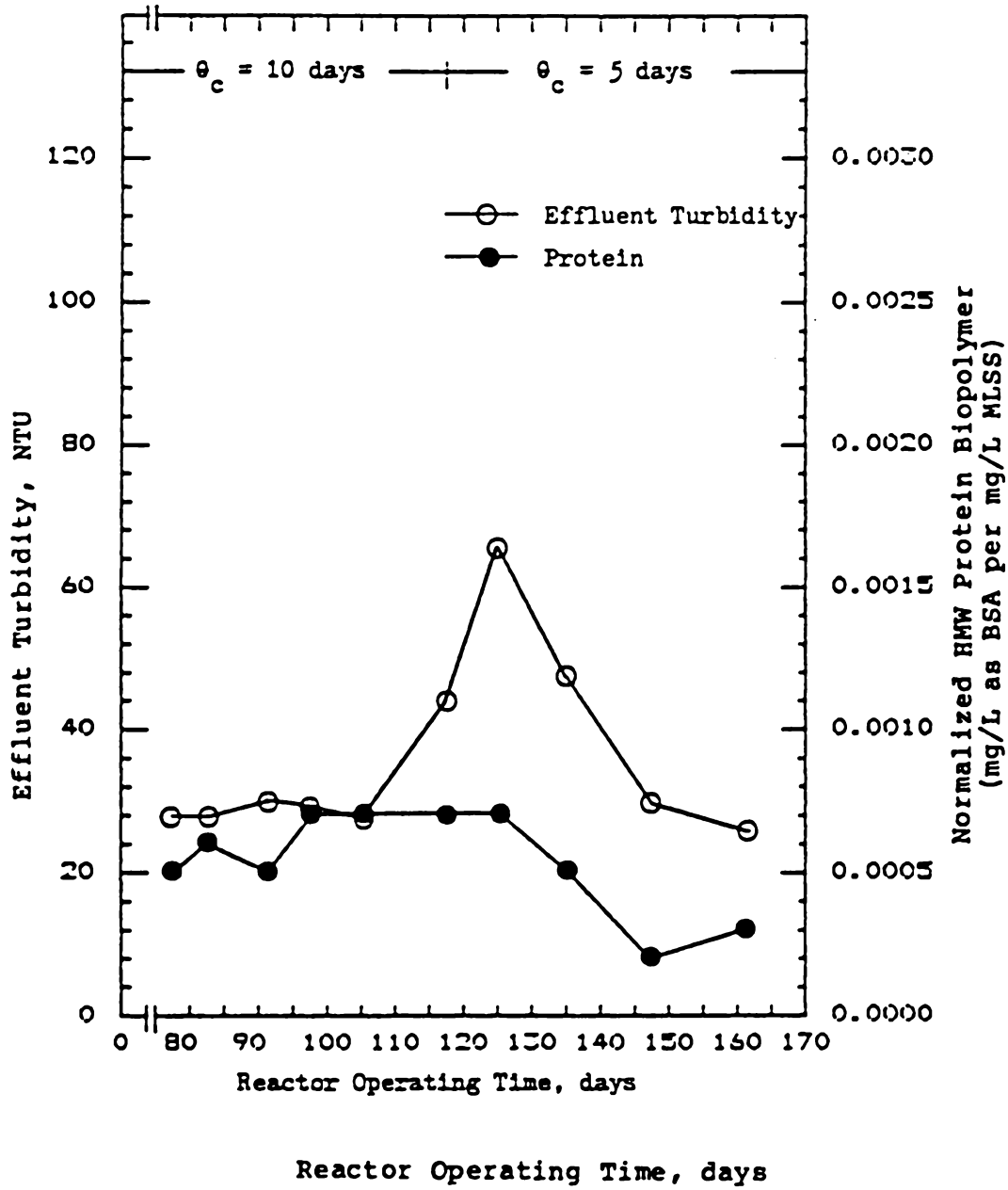


FIGURE 20. Effluent turbidity versus total HMW carbohydrate biopolymer concentration of activated sludge samples for Reactor 1.



Reactor Operating Time, days

FIGURE 21. Effluent turbidity versus total HMW protein biopolymer concentration of activated sludge samples for Reactor 1.

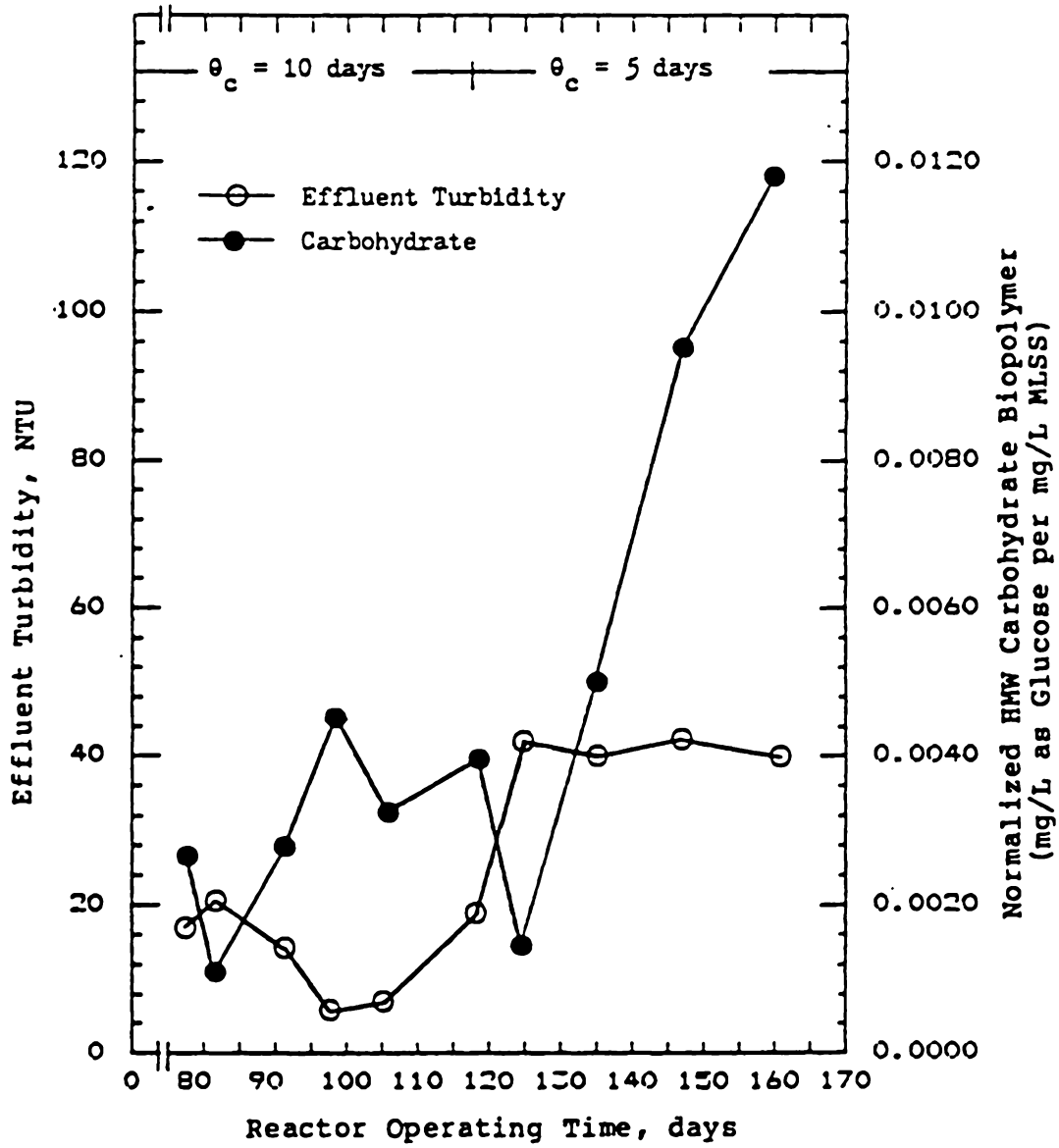


FIGURE 22. Effluent turbidity versus total HMW carbohydrate biopolymer concentration of activated sludge samples for Reactor 2.

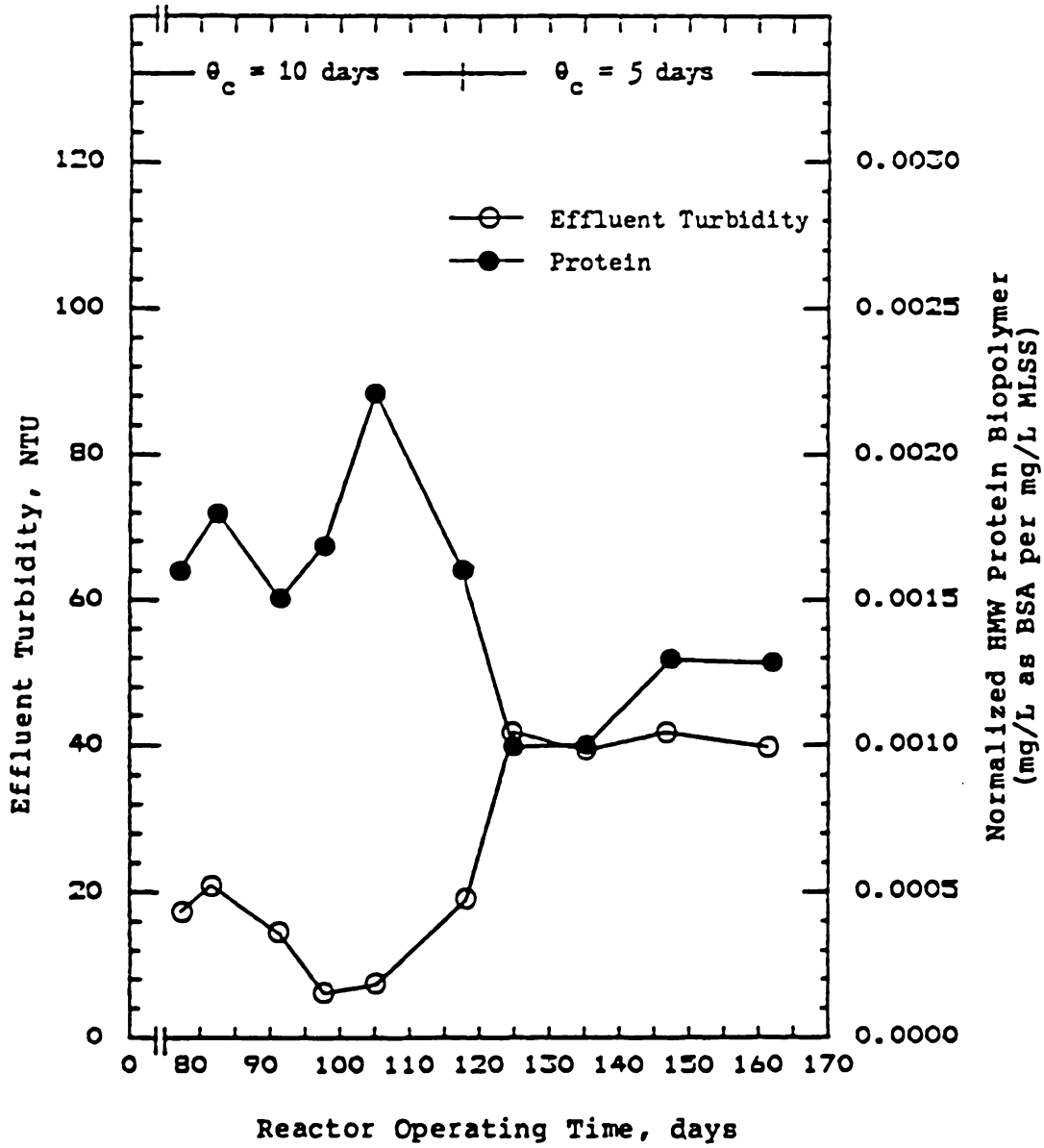


FIGURE 23. Effluent turbidity versus total HMW protein biopolymer concentration of activated sludge samples for Reactor 2.

between effluent turbidity and biopolymer concentrations was observed for reactor 2 as shown in Figures 22 and 23. This observation was particularly noticeable during the steady state period. The inverse relationship in reactor 2, in part, may be due to the relative high concentration of the protein biopolymer which may inhibit such behavior observed in reactor 1. A more pronounced correlation between effluent turbidity and HMW carbohydrate biopolymer concentration was observed when plot of the concentration versus the effluent turbidity was made. As shown in Figure 24, there was clear indication of the effluent turbidity being related directly to the carbohydrate concentration. However, no such correlation was observed when such plot was made for the effluent turbidity versus the protein concentration, so this plot was not included.

No evident correlation was observed between the SVI pattern and that of HMW protein or HMW carbohydrate ECP concentrations. Figures 25 through 28 show markedly inconsistent responses of SVI with respect to the HMW biopolymer concentrations. SVI of both reactors decreased gradually throughout the reactor operating period as shown in the figures. However, the plots for the HMW biopolymer concentrations not only show that there is no unifying correlation between two parameters, but do not indicate any consistency when compared to each other.

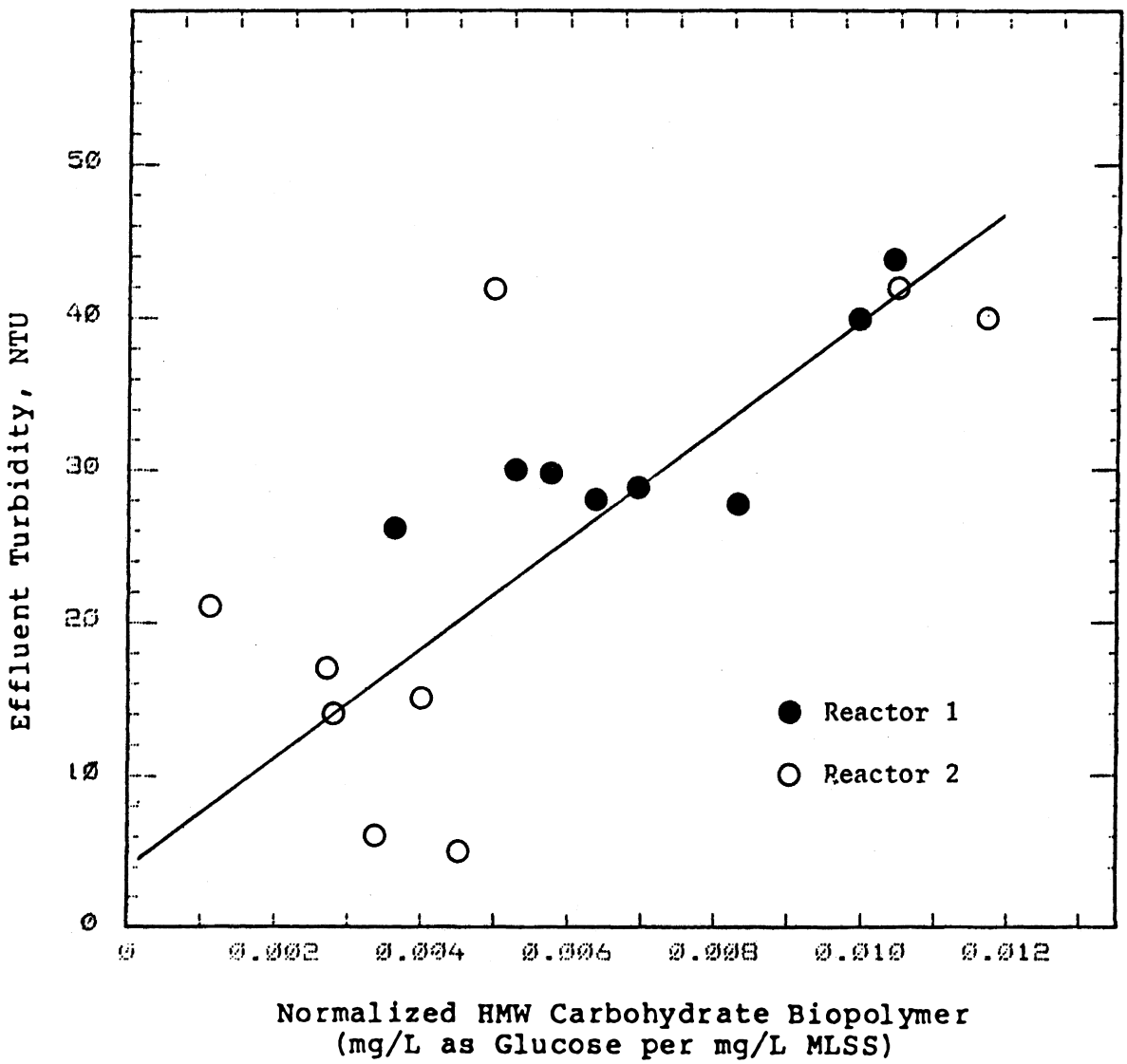


FIGURE 24. Effluent turbidity versus total HMW carbohydrate biopolymer concentration of activated sludge samples.

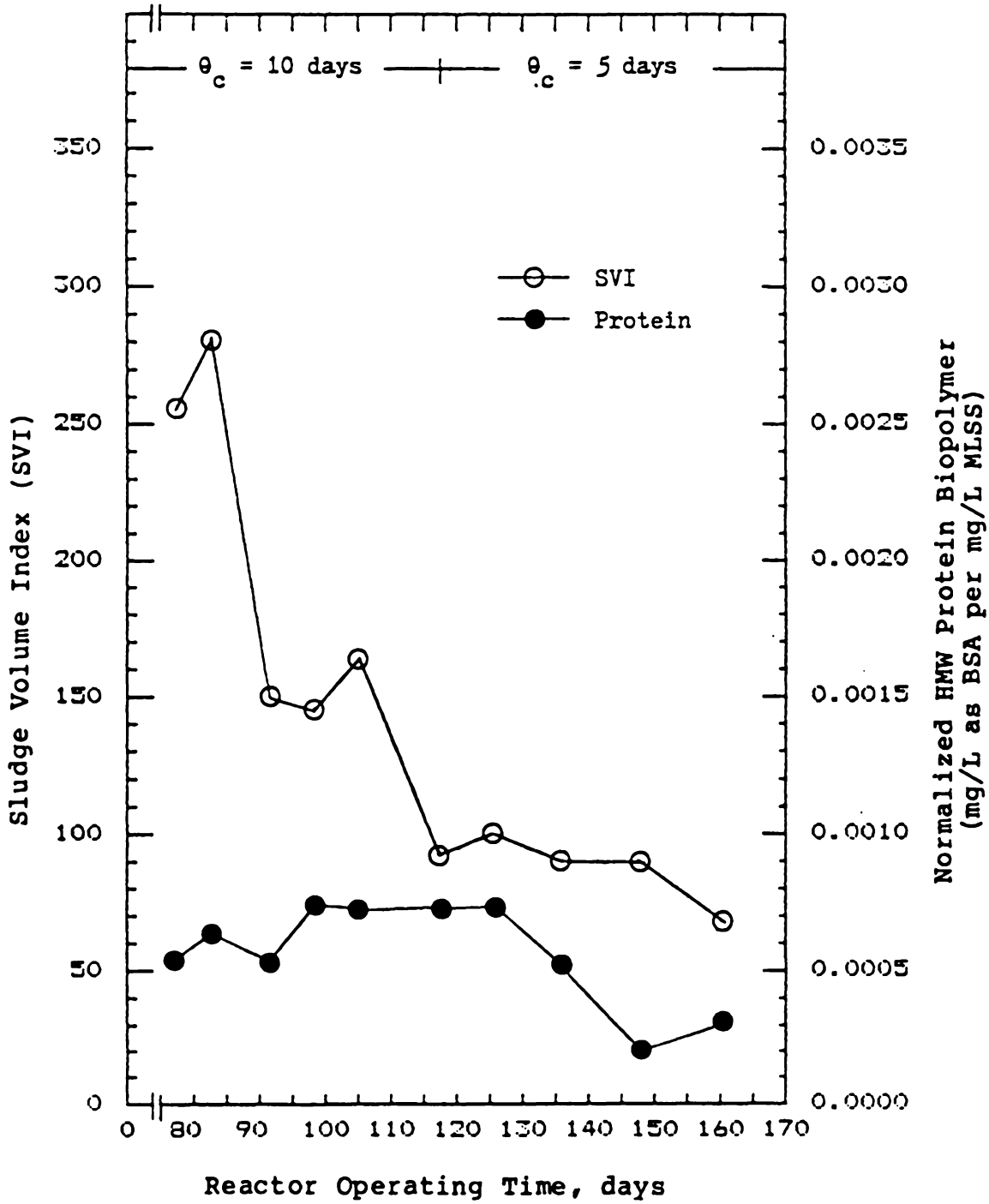


FIGURE 25. SVI versus total HMW protein biopolymer concentration of activated sludge samples for Reactor 1.

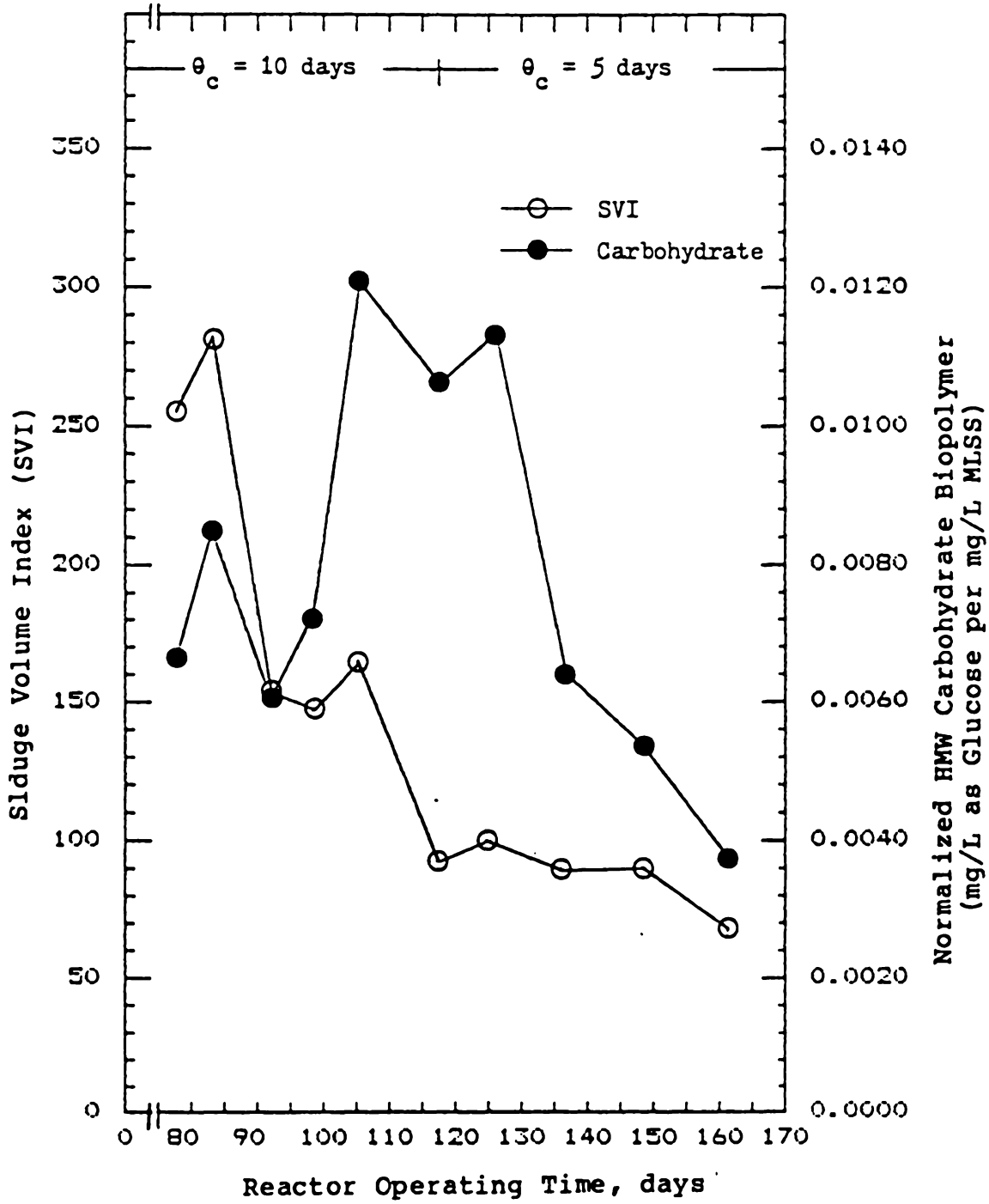


FIGURE 26. SVI versus total HMW carbohydrate biopolymer concentration of activated sludge samples for Reactor 1.

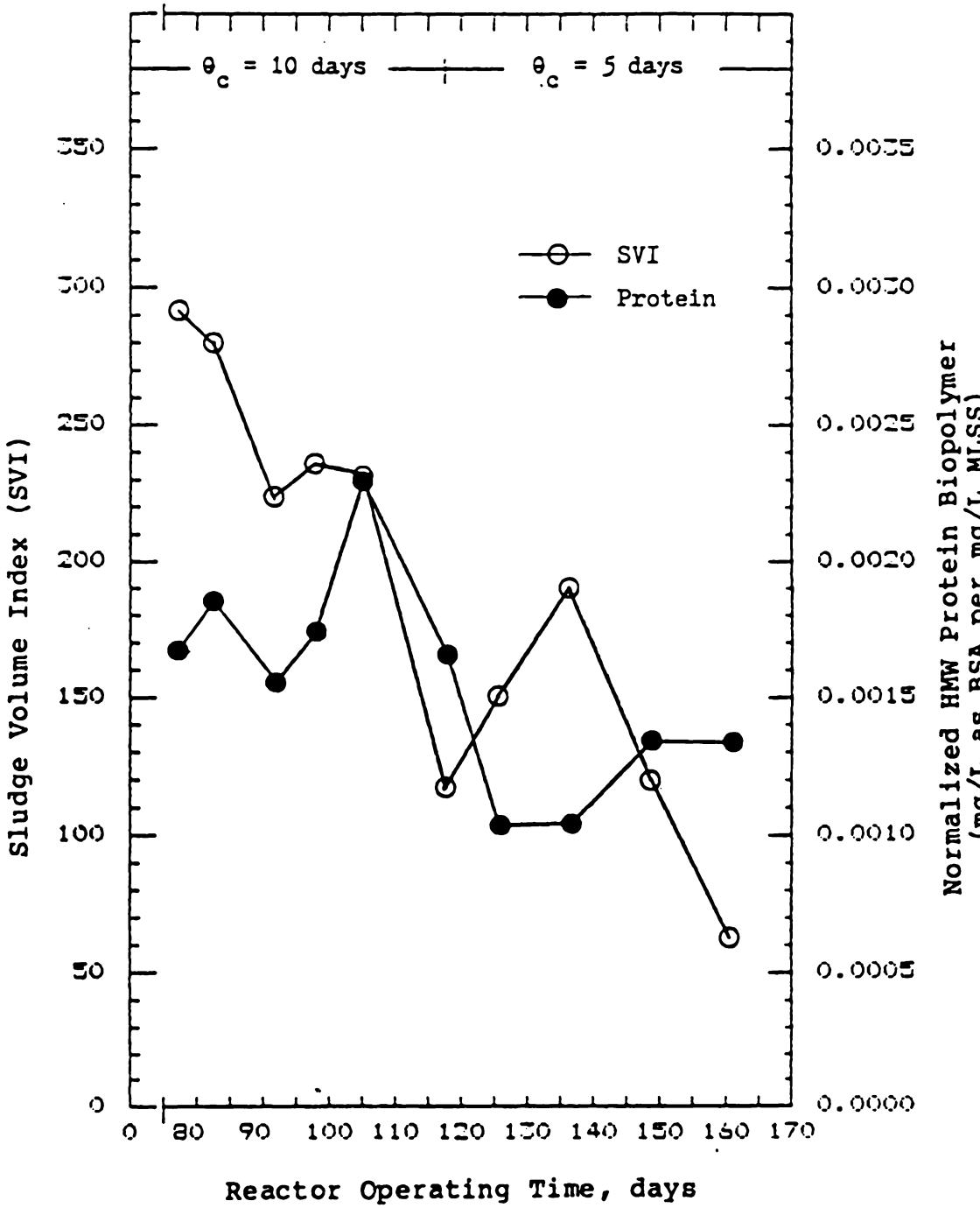


FIGURE 27. SVI versus total HMW protein biopolymer concentration of activated sludge samples for Reactor 2.

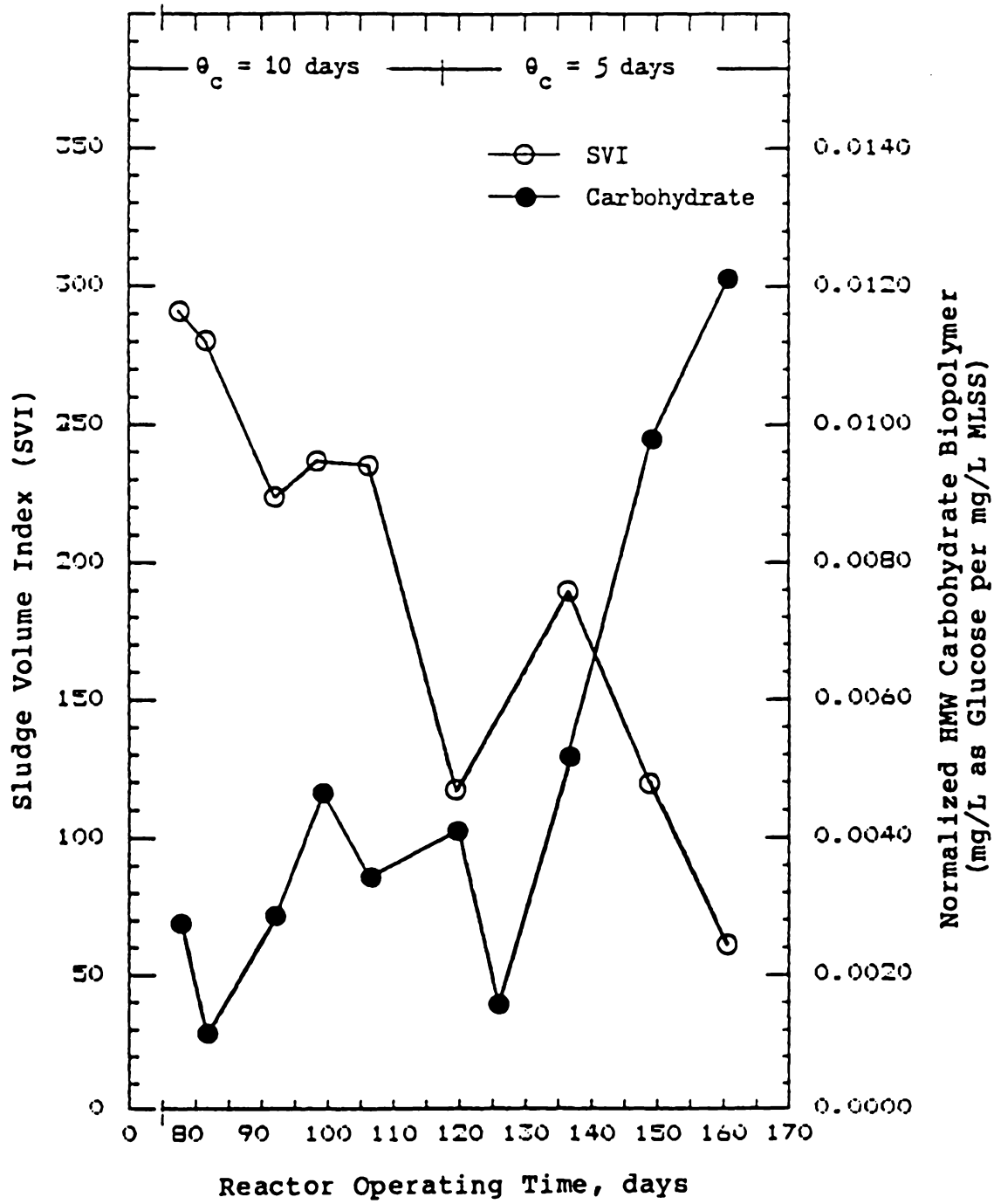


FIGURE 28. SVI versus total HMW carbohydrate biopolymer concentration of activated sludge samples for Reactor 2.

It is not likely that the increase in effluent turbidity of reactors was due solely to the increase in the amount of HMW carbohydrate biopolymer. However, it is conceivable that a certain combination of HMW protein and HMW carbohydrate biopolymer concentration produced a pronounced effect in effluent turbidity. Less pronounced correlations were derived from the comparison of HMW biopolymer production with SVI.

Throughout this study both HMW and LMW fractions of biopolymers were analyzed. The LMW concentrations were generally higher than the HMW values and consistent. The data for LMW fractions are presented in Appendix A (Table A-3 and A-4). These LMW fractions may be comprised of low molecular weight volatile acids and cell wall fragments, including numerous polymeric compounds such as proteins, polysaccharides amd nucleic acids excreted by microorganisms (4). Due to their size limitation, they are considered to have an insignificant effect on sludge settling characteristics (23).

The significance of proteins and carbohydrate in ECP seems apparent. However, it should be noted that the effectiveness of polymers is dependent on its binding characteristics. It has been seen from other studies (18) that biopolymers, containing a substantial protein content, possess stronger binding ability than those of high carbohydrate content. Also, floc of high

carbohydrate content tend to possess higher percent floc bound water which in turn may decrease sludge settling rates (30). As noted by Busch and Stumm (3), without sufficient cations, polymers will not aid in agglomeration. Therefore, it may be imperative to provide sufficient cations to avoid possible misinterpretations of biopolymer data. During this study additional cations, other than ammonium ions, were not provided in the feed solutions. However, operation of full scale reactors for this wastewater treatement does not require additional cations, thus it is assumed that sufficient cations are provided in the wastewater.

V. CONCLUSIONS

The expected improvement in sludge settling characteristic and effluent quality of reactor systems due to nitrogen addition was not seen in this study. The results suggest that binding properties of ECP are equally or more important than their quantitative production. Unfortunately the binding characteristics were not measured, but it appears that binding strength of ECP is different for settled and pH-adjusted sludge.

The following conclusions were inferred or derived from the data trends identified in this study.

1. The relationship between extracted biopolymers and sludge settling characteristics is culture specific. In one system, correlations may be observed between extracted biopolymers and effluent turbidity and/or SVI while the same relationship may be absent in another system.
2. Additional nitrogen in reactor 2 promoted production of high protein content biopolymers. Deficiency of nitrogen in reactor 1 promoted the production of highcarbohydrate content biopolymers.
3. No consistent improvement in effluent quality of reactor was noticed by additional nitrogen.

4. The effluent turbidity was related directly to the carbohydrate biopolymer concentration. Quantitative analysis of ECP indicates high carbohydrate content biopolymer was associated with high turbidity.

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APPENDIX A

TABLE A-1. PERFORMANCE DATA FOR REACTOR 1.

DAY	MLSS (mg/L)	ECP concentrations PROTEIN	ECP concentrations CARBOHYDRATE	TURBIDITY (NTU)	SVI
protein(mg/L as BSA per mg/L MLSS) carbohydrate(mg/L as glucose per mg/L MLSS)					
1	3,595	0.012	0.008	18	270
4	4,580	0.010	0.006	17	207
8	4,190	0.007	0.008	20	191
11	4,920	0.007	0.010	27	201
14	4,780	0.007	0.012	26	196
18	4,300	0.010	0.046	29	285
23	3,830	0.003	0.026	45	227
28	3,430	0.004	0.031	43	220
31	3,650	0.004	0.040	43	187
34	3,630	0.007	0.030	30	250
39	3,130	0.011	0.023	32	317
43	3,930	0.007	0.019	29	250
46	4,080	0.010	0.020	63	243
50	3,040	0.010	0.055	87	326
53	3,260	0.010	0.015	83	305
56	3,420	0.009	0.014	74	290
59	2,730	0.011	0.013	79	363
62	2,890	0.013	0.012	52	341
65	3,520	0.010	0.023	29	281
71	3,850	0.006	0.014	20	255
77	3,720	0.011	0.028	28	255
82	3,620	0.007	0.009	28	280
91	3,370	0.006	0.010	30	151
97	3,450	0.006	0.009	29	146
105	3,190	0.011	0.025	28	163
111	3,130	0.011	0.027	44	170
117	2,990	0.012	0.024	40	91
125	2,050	0.018	0.071	65	100
135	2,500	0.008	0.049	45	90
147	2,520	0.005	0.037	30	90
161	2,990	0.008	0.034	26	68
170	2,160	0.010	0.030	22	140

TABLE A-2. PERFORMANCE DATA FOR REACTOR 2.

DAY	MLSS (mg/L)	ECP concentrations PROTEIN	CARBOHYDRATE	TURBIDITY (NTU)	SVI
protein(mg/L as BSA per mg/L MLSS)					
carbohydrate(mg/L as glucose per mg/L MLSS)					
Data for days(1 - 34) is same as those of the Reactor 1.					
39	2,890	0.015	0.013	35	351
43	3,360	0.014	0.011	29	315
46	3,350	0.016	0.009	43	295
50	3,060	0.013	0.035	42	320
53	2,960	0.014	0.049	47	331
56	3,380	0.013	0.047	52	290
59	3,010	0.013	0.059	52	326
62	3,480	0.012	0.051	29	280
65	2,970	0.013	0.061	24	330
71	3,270	0.013	0.006	29	291
77	3,230	0.016	0.005	17	291
82	3,310	0.017	0.008	21	280
91	2,820	0.018	0.008	14	223
97	2,710	0.012	0.007	5	236
105	3,250	0.018	0.011	6	234
111	2,800	0.018	0.010	19	260
117	2,210	0.023	0.064	15	117
125	2,250	0.022	0.059	42	150
135	2,010	0.026	0.035	40	190
147	1,730	0.037	0.031	42	120
161	1,480	0.029	0.021	40	61
170	1,690	0.032	0.025	53	72

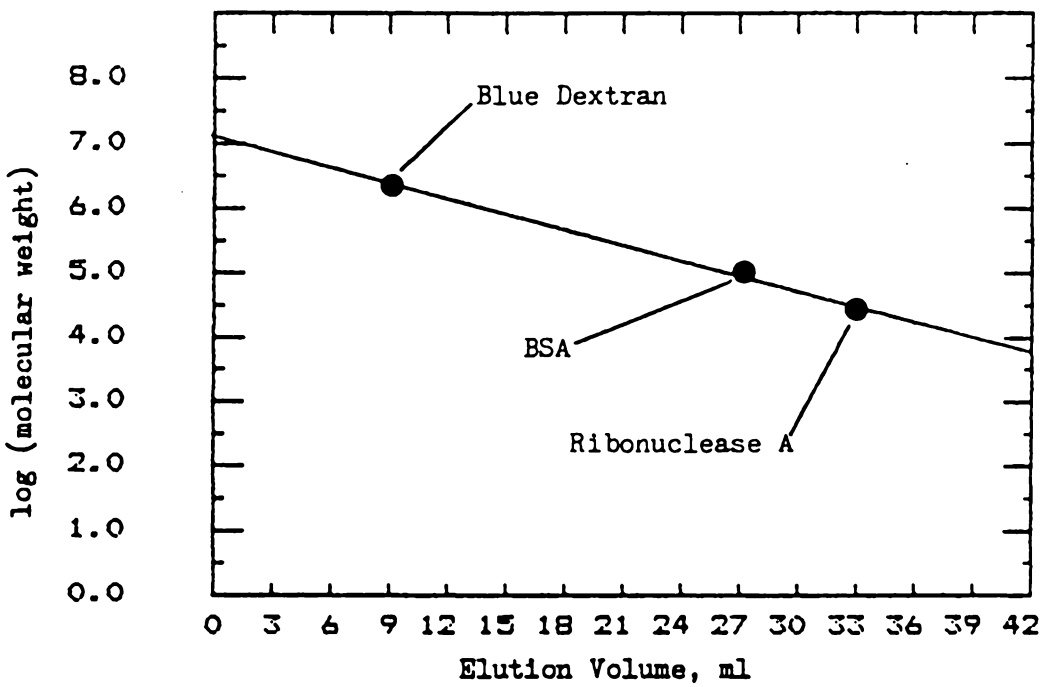

Figure A-1. Calibration of Sephadex G-75 column using organics of known molecular weight.

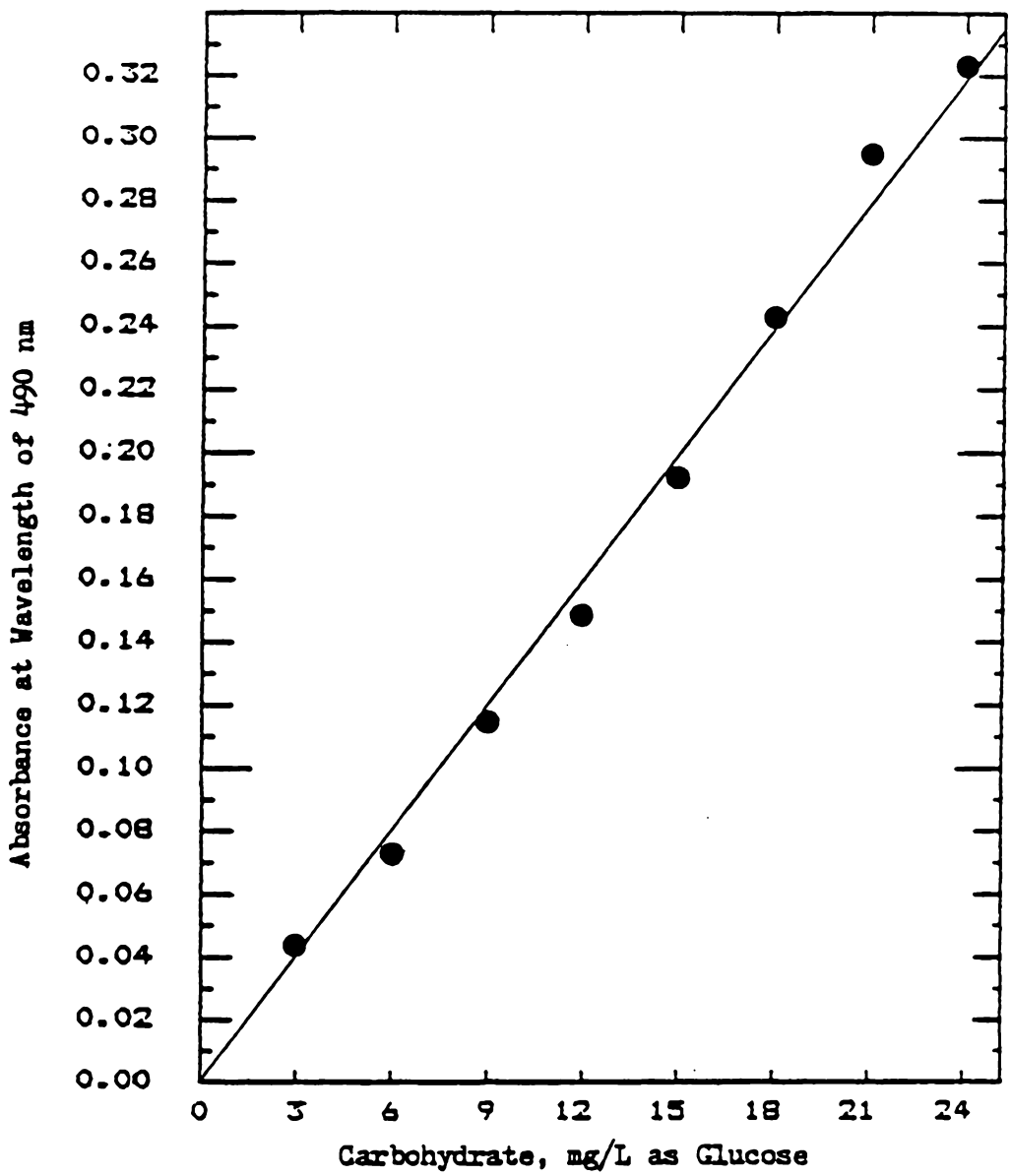

Figure A-2. Standard curve for carbohydrate determination.

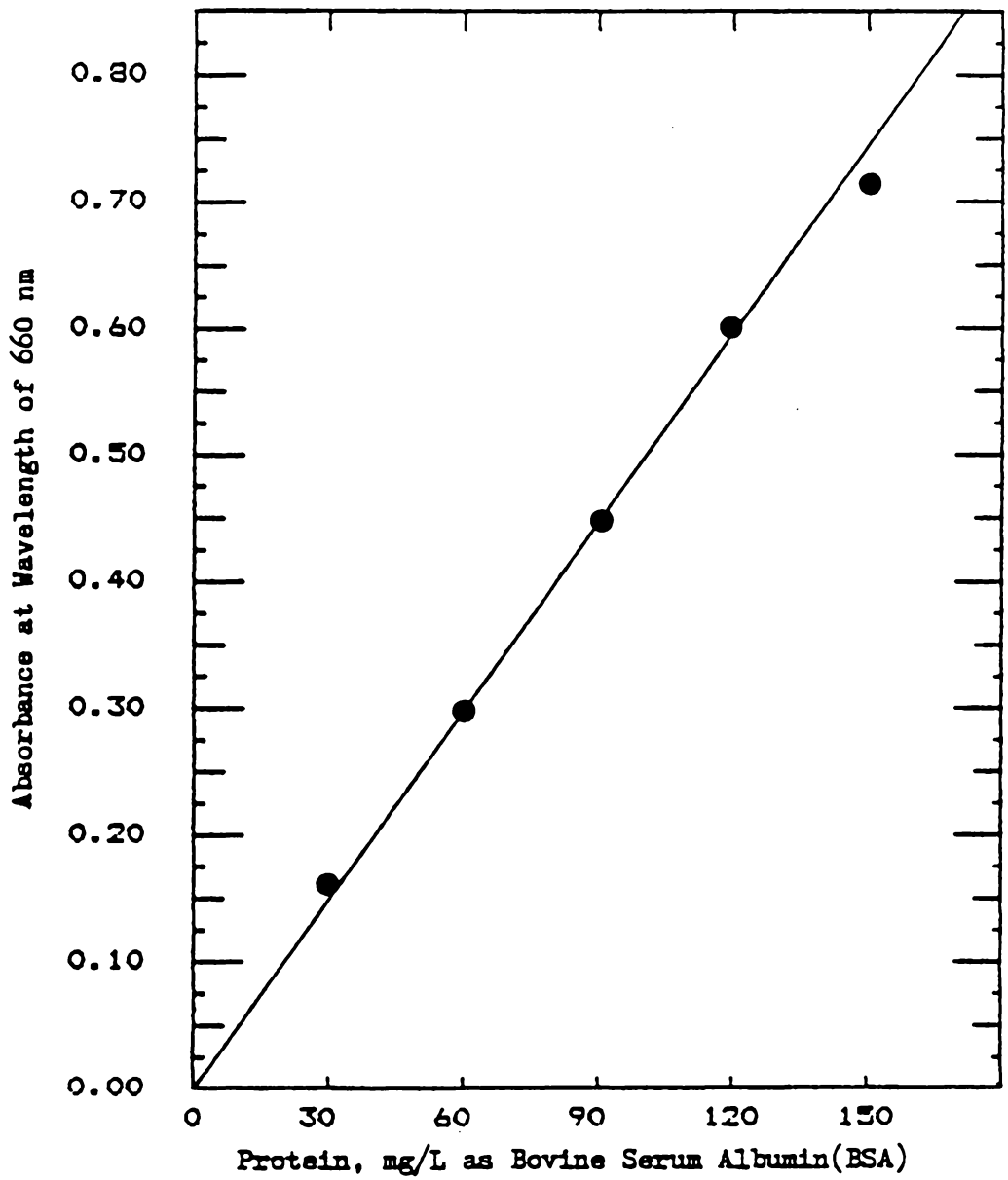

Figure A-3. Standard curve for protein determination.

TABLE A-3. NORMALIZED CARBOHYDRATE CONCENTRATION FOR GEL FILTRATION PEAKS.

DAY	REACTOR 1		REACTOR 2	
	HMW	LMW	HMW	LMW
	(mg/L as glucose per mg/L SS)			
77	0.0064	0.0048	0.0027	0.0030
82	0.0082	0.0030	0.0011	0.0022
91	0.0058	0.0049	0.0028	0.0034
97	0.0070	0.0044	0.0045	0.0035
105	0.0116	0.0016	0.0033	0.0028
117	0.0103	0.0020	0.0040	0.0026
125	0.0110	0.0030	0.0015	0.0057
135	0.0062	0.0042	0.0050	0.0033
147	0.0052	0.0022	0.0105	0.0012
161	0.0036	0.0068	0.0117	0.0028

TABLE A-4. NORMALIZED PROTEIN CONCENTRATION FOR GEL FILTRATION PEAKS.

DAY	REACTOR 1		REACTOR 2	
	HMW	LMW	HMW	LMW
	(mg/L as BSA per mg/L SS)			
77	0.0005	0.0015	0.0016	0.0050
82	0.0006	0.0024	0.0018	0.0049
91	0.0005	0.0019	0.0015	0.0042
97	0.0007	0.0028	0.0017	0.0026
105	0.0007	0.0033	0.0022	0.0048
117	0.0007	0.0036	0.0016	0.0047
125	0.0007	0.0027	0.0010	0.0068
135	0.0005	0.0036	0.0010	0.0041
147	0.0002	0.0011	0.0013	0.0054
161	0.0003	0.0015	0.0013	0.0060

TABLE A-5. RESULTS OF COD ANALYSIS

DAY	INFLUENT	EFFLUENT	
		REACTOR 1	REACTOR 2
		(mg/L COD)	
2	2,950	400	-
9	3,100	400	-
19	3,050	450	-
29	3,800	600	-
43	2,840	430	570
52	3,360	1,470	1,120
65	4,300	600	300
73	4,500	750	350
109	2,800	640	240
131	3,340	720	300
148	3,510	2,110	1,120
173	2,710	800	670

TABLE A-6. RESULTS OF TKN ANALYSIS

	REACTOR 1		REACTOR 2	
DAY	INFLUENT	EFFLUENT	INFLUENT	EFFLUENT
	(mg/L TKN as NH_3)			
9	10.7	1.4	-	-
23	8.1	1.0	-	-
40	13.4	1.1	97.4	62.7
61	14.1	1.3	100.2	65.3
84	10.1	1.1	99.7	62.7
101	13.2	1.4	99.9	63.7
123	12.9	1.2	98.9	61.5
146	13.6	8.3	99.8	72.3
170	14.6	7.8	101.2	88.5

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