OPERATIONAL AND EXOCELLULAR BIOPOLYMER CHARACTERISTICS OF SLUDGES GENERATED FROM AN AIR PRODUCTS AND A CONVENTIONAL ACTIVATED SLUDGE SYSTEM

by

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Thesis submitted to the Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirement for the degree of
MASTER OF SCIENCE
in
Environmental Engineering

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July, 1987

Blacksburg, Virginia

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(ABSTRACT)

This study compared the sludge characteristics of a lab-scale Air Products (A/O) and a conventional activated sludge system. The sludges were analyzed for operational properties and the exocellular biopolymers of each sludge was characterized.

Operational analysis mainly consisted of measurements of settling (SVI) and dewatering (specific resistance, CST) parameters at each sludge age. Biopolymers were measured using pH extraction followed by gel filtration and subsequent analysis for carbohydrate and protein concentrations.

The results showed a high degree of similarity between the two systems both operationally and with respect to biopolymer characteristics. The A/O system did produce a lower effluent soluble COD at sludge ages below 5 days. Also, the A/O system showed some consistent differences in the distribution of bound and unbound fractions of protein and carbohydrate ECP but these had no discernible effect on sludge operational characteristics.

In addition relationships between unbound ECP and effluent BOD, bound HMW ECP and SVI, and phosphorus and Mg uptake were observed, with varying degrees of consistency, in this study.

ACKNOWLEDGEMENTS

Thanks to Dr. John T. Novak for tutoring, appropriate (mostly) criticism, and being amiable in general despite my all too frequent treks across the hall to Norris 318. Thanks also to Dr. Clifford W. Randall (an acquaintance of some years) who also endured repeated violations of his inner sanctum. My sincere thanks is also extended to Dr. William R. Knocke whose timely assistance and suggestions contributed considerably to the analysis and understanding of this study.

Special thanks to for typing this manuscript and for many enlightening conversations. Also, thanks to for her tremendous contribution to the actual implementation of this study, and for goading me into working so hard. To for patience with the ignorant.

Most of all, thanks to my friends, who have made graduate school a pleasant and memorable experience.

W.L. - for helping me to keep off balance.

P.S. - for long phone calls and "festivals".

M.S. - for being a good friend and mercy in arm wrestling.

P.I.G. - for friendship, patience, and temporary insanity.

S.McC. - for friendship and letting me use his bathroom.

A.G. - groovie movie guru.

R., B., S., C., Z., etc.. - "life is good".

L.N.s - for taking the pressure off (just kidding).

M.H. - Wahoowa!

And, of course, Mom, who will always take me in...

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I. INTRODUCTION

The microorganisms composing activated sludge form a thermodynamically unstable colloidal system which will tend towards aggregation (1). However, the rate of aggregation can be very slow as well as quite rapid. In wastewater treatment using activated sludge, the rate of aggregation will influence the efficiency of solid-liquid separation in the clarifier. When aggregation and settling do not take place within the hydraulic retention time of the clarifier, suspended biomass will be discharged in the effluent. As a result, adequate flocculation and settling is critical for achieving a high quality effluent.

The ability of activated sludge to flocculate also impacts on its dewatering characteristics. Filtration properties are dependent on floc structure determining the ease with which water passes through the floc matrix. The ability of the sludge to dewater readily is of great operational and, ultimately, economic importance.

The role of exocellular biopolymers in flocculation and settling has been recognized for some time (2,3,4). To date, however, the literature contains numerous apparent contradictions. This is explained in part by the differences inherent in the multitude of extraction techniques used. There is evidence that some methods are inadequate, while others are too harsh or affect the composition of the extract. Other problems involve the comparison of batch studies with studies of continuous flow systems, variations in substrate, and the enormous complexity of describing a process which has proven to be a function of many parameters.

In spite of these difficulties, the importance of exocellular biopolymer quantity and composition to floc formation is well established. Much research has been conducted on both batch and lab-scale conventional activated sludge systems. However, there have been no studies of the exocellular biopolymers (ECP) of the activated sludge in biological phosphorus removal systems. Evidence has been presented that such systems may yield a sludge of superior settling characteristics (5). It is possible that improved settling and dewatering could be related to ECP composition that differs from ECP obtained from conventional systems, either as the result of population differences or physiological changes. This research is intended to examine this hypothesis, determining the characteristics of ECP from sludges generated in an anaerobic-aerobic sequence, and defining the consequent settling and dewatering characteristics.

II. LITERATURE REVIEW

Origin of ECP

Exocellular biopolymers occurring in nature give microbes able to produce them a selective advantage in a number of environments (6). These external polysaccharides, or branching sugar molecules, allow adhesion to surfaces as diverse as lung tissue, rocks, and plastic. The ECPs keep the microbes located in a desirable environment and also may aid bacterium in obtaining nutrients. An additional possibility is that ECP allows microbes to associate together, possibly for protection, or to locate in a favorable environment (such as a stream bottom). All of these possible functions would aid bacteria in obtaining substrate, and it may be this survival mechanism which causes the production of ECP in substrate limited conditions, such as occur in batch reactors during the endogenous phase (2,3,7). This environmental condition is approximated in activated sludge systems as sludge age increases and may be the mechanism involved in the increased ECP production and improved settling observed as the microbial population is forced to rely increasingly on endogenous respiration for survival (8).

Gulas, et al. (8), and Vallom and McLoghlin (7) both implicated lysis as an important factor in the production of ECP. Valom and McLoghlin studied a batch reactor and found that as the dry weight of cells decreased during the endogenous phase, the percentage of DNA in the ECP increased, and the ration of ECP mass to biomass decreased after a short period in the endogenous phase. In a continuous-flow system study by Gulas and co-workers, a relatively high amount of total ECP mass per active MLSS was found both at low sludge ages and at high sludge ages. The authors speculated that the high ECP concentrations at low sludge ages were due to "autolytic" activity. Their observations of ECP production at low sludge ages, or high growth rate, is unique in the literature known to this writer. However, there have been very few publications of data specifically comparing sludge age and ECP production over a broad range, most studies having been conducted with batch systems (which showed no ECP production at high growth rates) or for only a few sludge ages.

One study which does compare ECP production over a broad range of sludge ages was completed by Brown and Lester (9) who found no increase in ECP at low sludge ages corresponding to the data of Gulas and co-workers. Their study did yield an increase of ECP at high sludge ages, corresponding to the work of Gulas, et al., and the batch reactor data of numerous workers (2,3,7).

Hejzlar and Chudoba (10) extracted measurable quantities of refractory components of ECP (mainly polysaccharides) from primary and secondary effluent, streams, and tap water, concluding that these compounds were ubiquitous in the aquatic environment. Thus it is apparent that, at the very least, refractory biopolymers will be present in wastewater influents.

Composition of ECP

Hejzlar and Chudoba (11) found unbound ECP to contain sugars, amino sugars, uronic acids and amino acids. They concluded that ECP was produced during all physiological stages and they found it to be highly refractory.

Horan and Eccles (12), using heat extraction, detected glucose, galactose, mannose, gluconic acid and galacturonic acid with high molecular weight polysaccharide fractions. The technique they used eliminated protein fractions intentionally.

According to Forster, the behavior of polysaccharides as polyelectrolytes may be attributed to the strong negative charge of glucuronic acid, a major ionogenic component of ECP, at neutral pH values (4). The charge of bacteria is the result of acid-base interactions and is thus strongly pH dependent (2). The isoelectric point for the microbial surface is at low pHs and that of proteins is at pH 5-8. This, according to Tenney and Stumm (2), implies that substances with low isoelectric points such as glutamic acid, lipids, and gluco- and mucopolysaccharides, rather than proteins, will be found on the cell surface.

Vallom and McLoghlin (7) discovered that protein was the largest component of the ECP in their study, followed by carbohydrates, DNA and RNA. This is a different result from that of Pavoni, et al. (3) who found carbohydrate as the major constituent, then RNA, protein, and DNA. Vallom and McLoghlin used bovine serum albumin as substrate while Pavoni, et al. used glucose and nutrient broth for the bulk of their feed. Lee (13) induced production of high protein biopolymers through addition of nitrogen, and high carbohydrate biopolymers resulted from nitrogen deficient conditions. Fitzgerald (14) found glucose caused increased ECP production of both proteins and carbohydrates. Substrate apparently has a strong effect on the total and relative amounts of the components making up ECP.

Extraction of ECP

A review of the literature indicates that extraction techniques differ substantially and are not necessarily comparable from one study to another when the method used differs. The studies of Gulas, et al. (8), Busch and Stumm (15), and Pavoni, et al. (3) used centrifugation in order to shear off exocellular polymers associated with bacterial cells and flocs. However, Brown and Lester (16), Carr and Ganczarczyk (17), and Novak and Haugan (18) all found centrifugation to be inadequate for stripping floc of biopolymers, with the ECO obtained by the procedure being unassociated with the floc structure. As a result, the biopolymers obtained in the studies using centrifugation only corresponds to the "unbound" fraction of the exocellular biopolymers described in the research of Kajornatiyudh (19), Fitzgerald (14), and Lee (13).

Kajornatiyudh (19) conducted a comparison of alkaline pH adjustment and steaming measuring the increase in biopolymer yield and cellular disruption with respect to untreated supernatant. Alkaline adjustment proved to be the superior method, greatly increasing both the carbohydrate and protein fraction obtained with a relatively small increase in cellular disruption measured as the increase in DNA concentration. In addition, molecular weight distributions obtained through gel filtration were similar to those of the raw sludge supernatants for the technique developed by him.

Other techniques used in past research include heating followed by centrifugation, extraction with organic solvents, and acid extraction. Brown and Lester (16), Kiff and Thompson (20), and Carr and Ganczarczyk (17) have all published papers comparing a number of these and other methods for biopolymer extraction. Both Carr and Ganczarczyk, and Brown and Lester concluded that heating was the most appropriate method. Kiff and Thompson (20), however, found cellular breakdown to be significant with this method and the data of Kajornatiyudh (19) supports this. In contrast the data of Brown and Lester shows that while alkaline extraction greatly increases biopolymer yields, DNA (and therefore cellular disruption) increases markedly as well. However, their data are inconsistent with regard to this observation. Steaming raised DNA significantly for activated sludge fed a synthetic substrate while the DNA increase for alkaline extraction was at a very low level for this sludge. This was in contrast to the data for activated sludge generated from non-synthetic waste. It should be noted that their alkaline extraction technique is substantially different from the method adopted by Kajornatiyudh. Extraction took place in a 2 N solution of NaOH over a five hour period as opposed to a pH of 11 for one hour for the procedure recommended by Kajornatiyudh.

An examination of Kajornatiyudh's data shows alkaline treatment to be superior to steam treatment, but the DNA content for pH 11 extracted supernatant compared to raw sludge supernatant varies from 140% (steamed supernatant was 340% greater) to only 3% greater than the control concentrations. In addition, only one additional comparison between alkaline extraction and the control which yielded the 3% increase in DNA.

Goodwin and Forster (21) found that extraction temperatures used in heating determined the relative quantities of biopolymer fractions obtained. Proteins and lipids yielded were reduced relative to polysaccharides for temperatures above 80 degrees C. The authors also comment that while thermal extraction may cause a measurable contribution of lytic materials, these materials will exist on the cell surface in any case and will not alter the qualitative composition of the biopolymer studied.

The superiority of either heating or alkaline extraction is difficult to establish conclusively since there are substantial conflicts in the literature and limited data on both sides of the debate. Both methods remain in current use (12-14,19,21,22).

Bioflocculation, Settling, and Dewatering

Past theories for bioflocculation have included special floc-forming bacteria, and flocculation through slime excreted by protozoa in order to trap bacteria. Further conceptual development led to the consideration of bacteria as a dispersion of hydrophilic biocolloids. However it was observed that very stable suspensions of bacteria occur at the isoelectric point, eliminating the concept of bioflocculation as a result of favorable zeta potentials (2). The ability of anionic and non-ionic polymers to destabilize bacterial dispersions led to the hypothesis of flocculation via adsorption of biogenic, anionic polymers to the cell surface, followed by interparticle bridging (2,15). The role of particular matter in floc formation can also be important in activated sludge systems (23).

Horan and Eccles (12), citing Eriksson and Hardin (1984), suggest that flocculation takes place when exocellular polysaccharides bridge the distance between electrostatically stabilized cells to form a weak, elongated floc. Additional polysaccharides bind the cells more firmly, until a point is reached beyond which they will have a dispersing effect.

Beccari, et al. (22), discovered a relationship between ECP and settling properties which corresponds to optimum dose phenomena observed with anionic polymers. Weber (1), citing La Mer and Healy (1963), had previously stated that if a polymer dosage is sufficient to saturate the colloidal surface, restabilization will occur since there are no sites available for interparticle bridging. The observations of Beccari and co-workers correspond well with this type of mechanism. They discovered that at high sludge ages (where the total quantity of ECP was greater than at the low sludge ages) additional ECP caused an increase in SVI. However, at low sludge ages, ECP caused an improvement in settling. In addition they attempted to correlate SVI and the polysaccaride content of the ECP since this had been observed in the literature but were unable to do so.

Novak, et al. (24) observed that activated sludge responds to both anionic and cationic polymers like a chemical sludge overdosed with anionic polymer. They suggest that activated sludge may be a slurry with an excess of anionic polymers, some of which are of sufficient size to effect interparticle bridging. It was noted that the anionic polymers could have a detrimental effect on sludge filtration rates through pore clogging. In a separate study, Novak and Haugan (25) found that excess ECP in effect exerted a demand on conditioner, increasing the dose necessary for optimum dewatering.

Horan and Eccles (12), citing Walles (1986), point out that there are three potential mechanisms of aggregation which can operate between polyelectrolytes and colloidal surfaces: charge neutralization, bridging, and mutual dehydration. The importance of each mechanism is a function of the hyrophobic and hydrophilic nature of the sludge surface.

According to Busch and Stumm (15), microbial aggregation occurs through anionic polymers excreted by microbes or exposed on the cell surface. Furthermore, reduction of surface potential is not necessary for agglomeration to occur. They speculate that the polymers may attach through several possible mechanisms: hydrogen bonding; anion interchange with adsorbed anions (example, OH); or interactions with cations near the cell surface. In their view divalent cations form complexes or ion pairs between the functional groups of the polyelectrolyte and the "counter ion" (their quotes) near the cell surface. Since cation concentrations are greater near the negatively charged cell (1,15), cations are able to fulfill this role even when bulk solution concentrations are insufficient for complex formation. Significantly, they note that flocculation with polymers is possible at much lower concentrations of electrolyte than in solutions where polymers are absent. However, satisfactory agglomeration has been observed only when the concentration of divalent cations exceeds a certain minimum, even with polymers present.

According to Costerton, et al. (6) lectins (a type of protein) may allow polysaccaride adsorption, fulfilling much the same role as divalent cations.

Fitzgerald (14) found that poor settling and dewatering corresponded to an increase in high molecular weight proteins and carbohydrates, and that this type of material resulted from using glucose feed.

Kajornatiyudh (19) determined that ECP consists of bound and unbound fractions, both of which affect settling and dewatering properties. However, he concluded that ECP binding has the greatest impact on sludge properties. His conclusions are as follows: good flocculation and settling corresponds to a high degree of bound carbohydrate and protein ECP, with the binding ability of carbohydrate dependent on the presence of proteinacious ECP; specific resistance decreases as bound ECP content increases; deflocculation occurs when bound protein is low and/or free carbohydrate ECP is high; nitrogen rich substrate promotes production of proteinacious ECP, low nitrogen content, high glucose substrate causes overproduction of carbohydrate ECP resulting in poor dewatering.

Lee (13) found the relation between ECP and settling characteristics to be culture specific. His results confirmed the observation by Kajornatiyudh that nitrogen limitations resulted in the production of carbohydrate ECP while high nitrogen promoted protein ECP. He also related high carbohydrate ECP content to high turbidity effluent.

Kunjur (26), studying unbound ECP, found that the relationship between ECP and dewatering and settling characteristics was system specific, but that in general when supernatant ECP was high, specific resistance was high as well. Kunjur stated that ECP interfering with filtration consists of high molecular weight ($>10^5$) anionic biocolloids composed of proteins, carbohydrates and humic acids. He found that dewatering characteristics and supernatant ECP vary drastically as pH increases. He speculated that this could be the result of changes in the equilibrium between adsorbed polymer on the floc and solution colloids, alternation of the ECP from a coiled to an uncoiled structure, or coagulation and precipitation due to acid addition. He found the dewatering rate decreased from pH 3-8, with a sharp decrease above pH 8. His results showed that particles of less than 10 microns increased in number with increasing pH, possibly clogging the filter and increasing specific resistance.

Implications of Anaerobic-Aerobic Sequencing

There are two reasons to expect distinct ECP characteristics resulting from microbes originating in an anaerobic-aerobic sequence. Either a unique population shift takes place or there is a physiological difference in the microbes resulting from acclimation to different environments. A combination is possible too.

According to Tracy, et al. (5) the anaerobic zone of the A/O process, a patented system consisting of a simple anaerobic-aerobic sequence, may prevent bulking in part because it favors a small population of microbes. This can be the result of the altered hydraulic regime, and the change in environmental conditions in which microbes must compete. This reduces the chance of filamentous growth, eliminating the consequent bulking problems associated with such growth. In addition, data are presented showing superior settling characteristics for A/.O sludge over non-filamentous conventional sludge under equivalent operating conditions.

Kroiss and Ruider (27) found that a plug flow regime yielded a sludge of superior settling characteristics in a pilot plant study conducted in Vienna, Austria. Daigger, et al. (28) found a selector, creating in effect a substrate gradient and approximating to an extent the conditions of plug flow, to be effective in eliminating filamentous populations in pilot tests in Northern Virginia.

Fitzgerald (14) found plug flow cultures more stable and less likely to fail as a result of excessive ECP than a completely mixed reactor. She speculated that this may be the result of a period of low substrate aeration where biopolymers are stabilized.

Novak, et al. (24) found that there was consumption of ECP for a few hours after sludge was exposed to aerobic conditions following anaerobiosis, after which ECP accumulation increased as would be expected during aerobic conditions. A minimum time of anaerobic exposure was necessary for this phenomena to take place.

Malnou, et al. (29) conducted a study of the microbes in a "Phoredox" (Anaerobic-Anoxic-Aerobic) pilot plant and found that *Pseduomonas*, *Acinetobacter*, and *Aeromonas* were the dominant bacteria in the system. *Acinetobacter* was also confirmed to be responsible, at least in part, for the excess phosphorus removal (phosphorus removal above normal stoichiometric requirements) taking place in the plant. it was noted that an important characteristics of *Acinetobacter* is its property of producing agglomerates covered by an extracellular capsule of "unknown composition."

A selective advantage for *Acinetobacter* in an anaerobic-aerobic sequence should be expected from current information concerning the ability of some aerobic organisms, particularly Acinetobacter, to uptake volatile fatty acids (fermentation products) and store PHB (a polymer well known as a storage compound for microbes (30)) in the anaerobic zone (31,32). The PHB storing microbes thus have the first opportunity to uptake substrate in the system. In the aerobic zone the substrate stored as PHB is metabolized for growth and energy requirements while other microbes must compete for remaining substrate. According to

Buchan (32) the dominance of Acinetobacter has been shown in many biological phosphorus plants.

Chudoba, et al. (33) have developed a model which predicts that the predominant microorganism in a mixed culture will be those which have the highest accumulation capacities and the highest accumulation rates of substrate. This corresponds to the situation of aerobic microorganisms capable of accumulating substrate in the anaerobic zone and metabolizing it in the aerobic zone.

Data have been presented in the literature asserting that sludges originating from the A/O system have significantly better settling and dewatering properties (5). Also, numerous studies have shown that settling and dewatering characteristics are related to the presence and composition of exocellular biopolymers. This study is intended to observe any differences in settling and dewatering characteristics between the A/O and A.S. systems and allow comparison of biopolymer characteristics to determine if any observed operational differences are the result of dissimilarities in the exocellular biopolymers of those two sludges. Comparison of biopolymer data and operational characteristics in order to compare the results of this study with those in the literature will be conducted as well. Although there is still reason to question alkaline extraction with respect to the degree of lysis and possible chemical alteration of the extracted compounds, this technique will be used in this study since there is no clearly superior method documented in the literature, and to allow comparison with other research conducted at this university.

III. METHODS AND MATERIALS

Two activated sludge systems were operated simultaneously to generate the sludge analyzed in this study. Initial cultures were obtained from the and A/O plant at the York River Sewage Treatment plant in Yorktown, Virginia for the A/O lab system, and from the Blacksburg-Christiansburg Regional Treatment Plant near Blacksburg, Virginia for the conventional system. Detailed descriptions of the reactor systems and operations as well as analytical techniques utilized in this study to obtain data are described in this chapter.

Reactor Systems

The conventional activated sludge system consisted of a completely mixed, continuous flow activated sludge reactor with internal recycle and a volume of 17.3 liters. Similar reactors have been used in our laboratory and are described in detail by Lee (13). Air was supplied through a diffuser stone in sufficient quantities to assure mixing and to maintain a a high (normally above 5 mg/L) DO concentration in the reactor. All reactors were located in a constant temperature room (20°C) throughout the study. It should be noted that reactor temperatures could be as much as 5 degrees less than the room temperature due to heat stripping in the aeration zones.

The A/O activated sludge system consisted of two reactors and a clarifier, with a recycle line going from the clarifier to the head of the system. The first reactor was an enclosed, completely mixed anaerobic reactor, with a volume of 6.2 liters. This was followed by a separate completely mixed aerated reactor of 8.5 liters. A clarifier of roughly 2.5 liters received the effluent from the aeration zone. The reactors and the clarifier were interconnected with flexible tubing. At sludge ages of 11.9 and 2.9 days the DO in the aeration zone of the A/O system was operated at a DO of 2-3 mg/L but previous to that DO was maintained near saturation.

Operations

Influent consisted of an artificial substrate composed of the substances shown in Table 3.1. Throughout the study, influent flow through each system was maintained at 17.3 liters/day, resulting in an approximate hydraulic detention time of 24 hours. The organic loading rate was roughly 8700 mg COD/day for the given MLVSS. Typical loadings were roughly 0.5 d^{-1} with a range of approximately 0.4-1.90 d^{-1}. In the A/O system typical anaerobic loading was about 1.7 d^{-1} and typical aerobic loading about 0.2 d^{-1}. Sludge was wasted from the aerobic zone of each reactor. The mean cell residence time was calculated considering both the mass of mixed liquor volatile suspended solids wasted and the mass of effluent volatile suspended solids, which were assumed to be entirely biomass. Total biomass was calculated from the average MLSS for each system, using data from both the anaerobic and aerobic reactors for the A/O system.

The parameters for which analysis were regularly conducted in this study were COD, TOC, effluent suspended solids, SVI, CST, specific

Table 3.1. Composition of Synthetic Wastewater

Component	Influent Nutrient Sol. Grams per10 L Water	Influent Solution Grams per 40 L Water	Conc. (mg/L)
Nutrient Broth	--	16.0	400
Yeast Extract	--	1.2	30
Dextrose	--	0	0
Sodium Acetate	--	10.0	500
Ammonium Chloride	--	4.6	115
$MgCl_2 \cdot 6 H_2O$	66.63	1.0 L*	167
KH_2PO_4	16.00	1.0 L*	40
$NaHCO_3$	60.00	1.0 L*	150
Approximate COD	--	--	500
Approximate TOC	--	--	225

*Add 1 liter of Inorganic Nutrient Solution resistance, settling velocity vs. solids concentration, and influent and effluent ortho-phosphorus (soluble). The cations Ca, Mg, Na, and K were also measured throughout, and floc density was included part way into the study. Other measurements such as Oxidation Reduction Potential and MLVSS were taken as deemed necessary. Anaerobic zone effluent was measured for COD, TOC, cations, and ortho-phosphorus (soluble).

The biopolymer of each sludge was characterized with waste sludge obtained from the aerobic zone of each system.

Steady state was determined by measurement of stable concentrations of MLSS in the system, and relatively constant effluent suspended solids (see Figure 3.1). Once steady state was established all parameters were measured over a period of several days. After the data were obtained the quantity of waste sludge was changed to a new value in order to reach a new sludge age. Occasionally small periods of non-wastage were used to shorten the time necessary to build up the MLSS concentration when changing to higher sludge ages.

Analytical Procedures

Treatment of Samples

All samples were stored according to guidelines described by the EPA (Methods for Chemical Analysis of Water and Wastes). Biopolymer samples were refrigerated when it became necessary to store them and were analyzed within 16 hours. All samples to be analyzed for ortho-phosphorus, COD, TOC, and cations, with the exception of influent, were filtered immediately after collection.

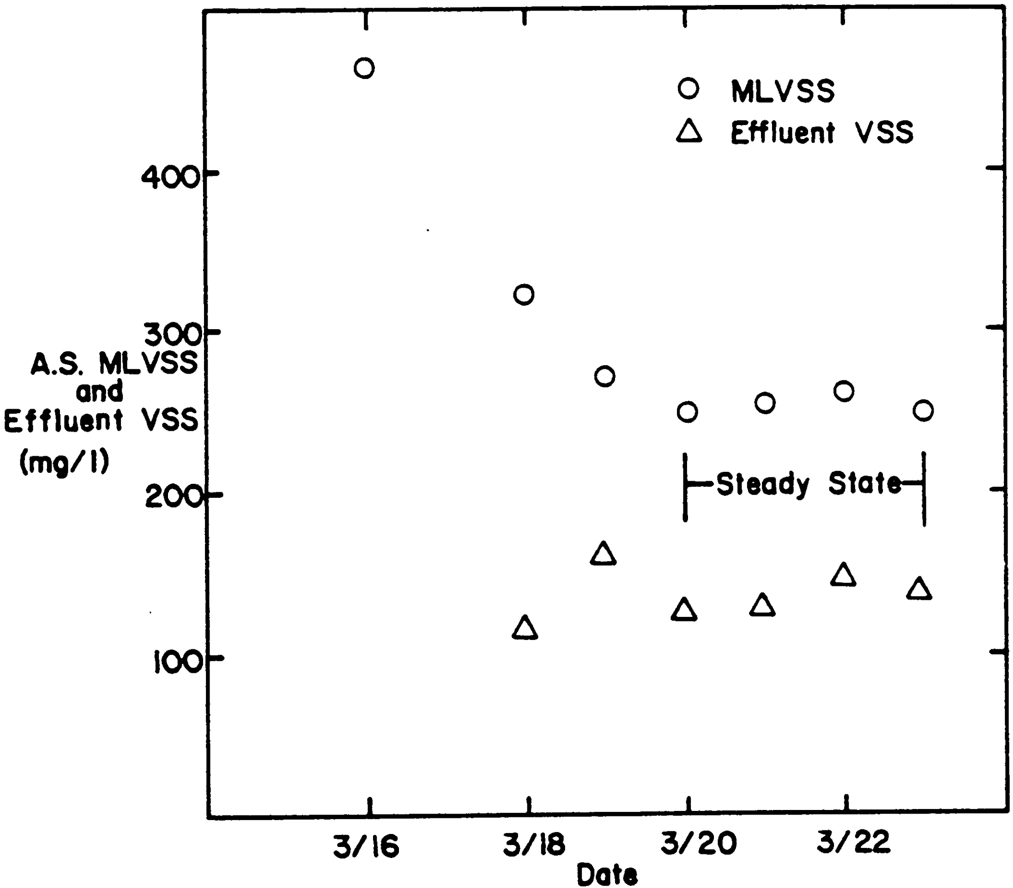

Figure 3.1 Establishment of Steady State

Chemical Oxygen Demand (COD)

The COD test was performed according to the procedure described in Standard Methods for the Examination of Wastewater (1985) using the dichromate method. Samples were collected from the influent line, the tubing connecting the anaerobic and aerobic reactors of the A/O system, and the effluent lines of both the A/O and Activated Sludge systems.

Ortho-Phosphorus (PO_4-P)

Ortho-phosphorus was determined by using a Dionex 2010 Ion Chromatograph (Dionex Corporation, Palo Alto, CA). The eluent was 0.028 N sodium bicarbonate ($NaHCO_3$) and 0.0022 N sodium carbonate ($NaCH_2CO_3$) and the regenerant used was 0.025 H_2SO_4. Output was recorded by a Spectra-Physics 4270 integrator. A stock solution of KH_2PO_4 at 1000 mg/L PO_4 was used to prepare standards for each ortho-phosphorus analysis. Standards of 10 mg/L PO_4 and 30 mg/L PO_4 were prepared and examined each time samples were examined in order to generate the data necessary to calculate sample concentrations. Samples were collected from the same points as the COD samples.

Very inconsistent phosphorus values were obtained with stored samples and the analysis of phosphorus in this study is based entirely on unstored samples as a consequence. In some cases early in the study this resulted in a single set of data for a given sludge age. Generally consistent values were obtained during steady-state conditions later in the study by measuring only unstored samples (see Table 3.2).

Table 3.2. Phosphorus Data for the A/O System (Sludge age 2.9 days).

Date	4/1	4/2	4/4	4/10	4/12
Influent	8.8	8.8	9.1	5.9	9.6
Anaerobic Effluent	8.7	8.6	8.3	5.9	7.3
System Effluent	6.4	8.2	6.6	5.4	7.0

Total Organic Carbon

Total organic carbon was measured with a Dohrman DC-80 Automated Laboratory Total Organic Carbon Analyzer (Xertex Corporation, Santa Clara, CA). Samples were purged of CO_2 with phosphoric acid before examination, injected into the machine, and the results were recorded by an attached printer. Collection sites were the same as for COD.

Magnesium, Potassium, Calcium, and Sodium

Analysis for cations took place utilizing a Model 703, Perkin-Elmer (Norwalk, CN) Atomic Adsorption Spectrophotometer.

Settling and Dewatering Characteristics

Treatment of Samples

All sludge samples examined for settling and dewatering characteristics were taken from the mixed liquor of the aerobic zone of each system. Examination typically followed immediately.

SVI

The Sludge Volume Index test was performed by settling 1000 ml of sludge for 30 minutes in a 1 L graduated cylinder. The volume of the settled sludge was measured after 30 minutes. The SVI was then calculated as follows:

$$\mathrm{SVI} = \frac{\text{Settled sludge volume, ml} \times 100}{\text{MLSS concentration (mg/L)}}$$

C.S.T.

The Capillary Suction Time was measured using a Triton W.R.C. Type 165 C.S.T. Apparatus. C.S.T. is measured directly by the machine after the metal cylinder included is filled with mixed liquor so that the liquid may flow through the filter paper supplied with the instrument.

Specific Resistance

Specific Resistance was measured using a Buchner funnel and a Whatman 40 filter of 9.0 cm diameter. 100 ml of sample was timed as it passed though the filter into a graduated cylinder.

Specific resistance was calculated using the equation below.

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

where

r^* = specific resistance, m/kg

P = pressure, N/m^2

A = area of filter paper, m^2

b = slope of Time/Vol vs. Volume

y = dynamic viscosity, $Nsec/m^2$

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

Velocity vs. Concentration

The volume or height of settled mixed liquor was measured with time in 250 ml graduate cylinders. Dilutions and concentrations of each mixed liquor were created by using supernatant and settled sludge to decrease or increase the concentration of solids in a given sample. Total solids were measured for each sample studied in order to observe the change in settling characteristics over a range of solids concentrations and allow graphical analysis.

Suspended Solids

Suspended solids were measured for both the mixed liquor and the effluent in order to permit calculation of both the total biomass present in each system as well as the mass wasted per day from each system.

Floc Density

Floc density was measured during the latter portion of this study using Percoll from Pharmacia Fine Chemicals (Piscatanay, NJ). Layers of Percoll were carefully placed in test tubes so as to form discrete boundaries according to density ranging in specific gravities from 1.05-1.01. A layer of distilled water was placed on top and a small amount of floc material was injected into this upper layer. The tubes were then centrifuged for 5 minutes to cause the flocs to migrate to an equivalent density layer.

Estimation of MLVSS from MLSS

The ratios of MLVSS to MLSS were calculated for A/O mixed liquor and effluent and A.S. mixed liquor and effluent (see Table 3.3). Approximately 90 percent of the suspended solids were volatilized in all four measurements obtained. This value was used to convert suspended solids measurements to volatile suspended solids throughout the study.

Biopolymers

Sample Treatment

The initial supernatant extracted from both the pH adjusted and non-pH adjusted samples was fractionated using the gel column. Protein and carbohydrate concentrations were obtained for both the initial supernatants and for the high molecular weight (HMW) and low molecular weight (LMW) fractionation samples.

Extraction

Biopolymer extraction was effected by pH adjustment followed by centrifugation. This technique was chosen because it is one of the most effective techniques according to much of the literature (18,19) and its use will allow comparison between this study and the results obtained in previous similar studies which used the same technique (13,14,19). While the efficacy and specificity of this extraction procedure is a subject for debate, especially in comparison to heat extraction (9),

Table 3.3. Estimation of MLVSS from MLSS

System	MLSS	MLVSS	MLVSS/MLSS
A/O System			
Anaerobic Zone	1828	1670	0.91
Aerobic Zone	1208	1103	0.91
A/O Effluent	141	123	0.87
A.S. System			
Aerobic Zone	936	843	0.90
A.S. Effluent	533	478	0.90

there is no reason to believe from the literature that there is any better method.

The ECP extraction technique used in this research is derived from the findings of Kajornatiyudh (19). Mixed liquor from the aerobic zones of each system was adjusted with 1 N NaOH to pH 11 and gently stirred for 1 hour. Then both pH adjusted and non-pH adjusted samples were centrifuged at 5000 rpm for 10 minutes and the supernatant collected. Analysis typically followed immediately but on one occasion some supernatant may have been stored as long as 16 hours at 4 degrees C.

Effects of pH Alteration on ECP Release

As can be seen in Figure 3.2, there is release of ECP as pH is altered in either direction, with a particularly large release as pH approaches 11.

Gel Chromatography

Gel chromatography or gel filtration was used to separate high and low molecular weight organic fractions of both the pH adjusted and non-pH adjusted mixed liquor samples. Sephadex G-75 gel from Pharmacia Fine Chemicals (Piscataway, New Jersey) was used in this study. The gel retains small molecules within the gel matrix longer than high molecular weight compounds, thus differentiating the substances exiting in the eluent at a given volume by molecular weight. Sephadex G-75 was chosen over Sephadex G-25 and G-50 because its fractionation range is more appropriate for this study (See Table 3.4).

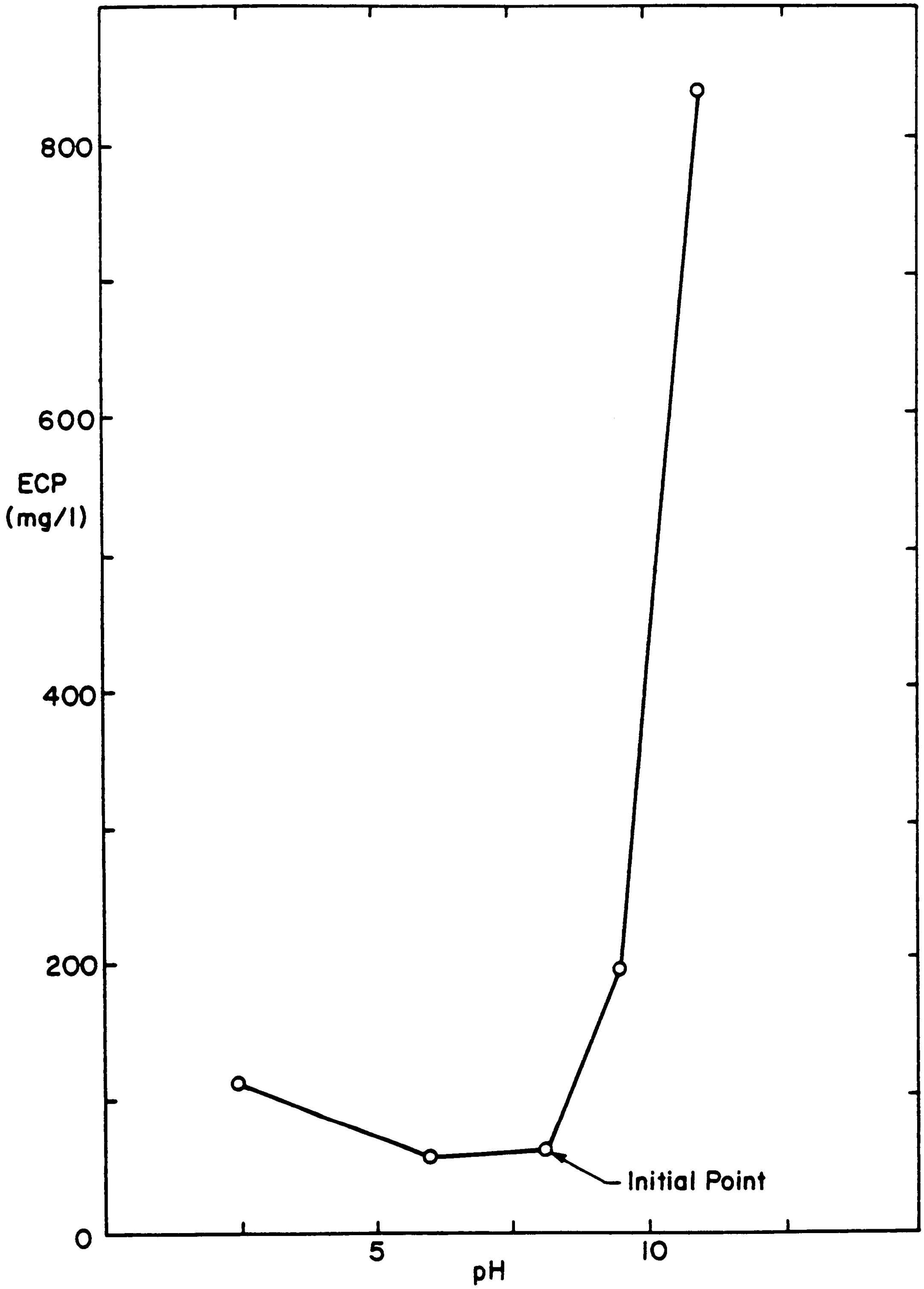

Figure 3.2 ECP Vs pH For A.S. Mixed Liquor, Sludge Age 14.6 days

Table 3.4. Fractionation Range of Sephadex Gels

Gel	(molecular wt, g/mole) Protein	Dextran
G-25	1000- 5000	100- 5000
G-50	1500-30000	500-10000
G-75	3000-70000	1000-50000

The configuration of the gel filtration apparatus is shown in Figure 3.3. The gel, after swelling in distilled water for a minimum of 24 hours, was packed in a buret from 25 mls to 6 mls with 3 mls of free space above the gel. The gel column was used until flow rate began to decrease noticeably, usually after 6 trials. The procedure followed for fractionation with the gel column in this study is listed below:

1. The water above the gel surface was drained by opening the buret valve.
2. A 2 ml sample was gently poured on the gel surface and allowed to drain into the column.
3. The valve was closed and eluent was applied to the gel surface, after which the buret was sealed with the eluent line connected.
4. The valve was opened and sample collection commenced. Approximately 5 ml was collected in each test tube.
5. The samples in each test tube were measured for absorbance at a wavelength of 280 nanometer (nm) using a Beckman Instruments, Inc. (Irvine, California) DU-6 Spectrophotometer.
6. The samples with peak absorbances corresponding to the high molecular weight and low molecular weight fractions were saved for further analysis.

Carbohydrates

The procedure described in Dubois _et al_. (34) was used to determine carbohydrate concentration in terms of mg/L as dextrose. Standard solutions of dextrose (from 0 to 50 mg/L) were used to obtain the

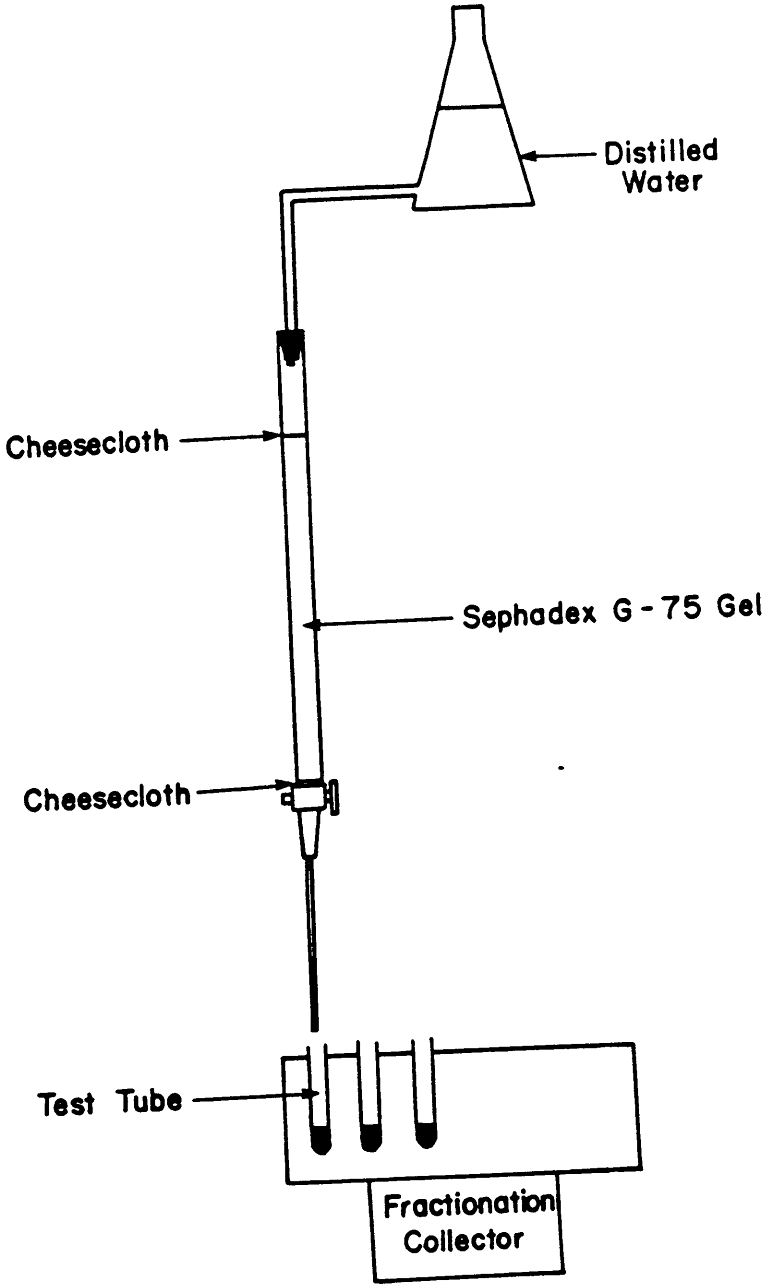

Figure 3.3 Schematic of Gel Filtration Column

standard curve (see Figure A.2). The following method was used to analyze carbohydrate concentrations of samples:

1. The following reagents were applied:
   A. 5% (by weight) phenol solution in distilled water;
2. 2 ml of samples was used for analysis.
3. 0.8 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 and vortexed immediately.
5. After 30 minutes at room temperature, absorbance of each sample was measured against a blank at 488 nm using a Beckman Instruments, Inc. (Irvine, CA) DU-6 Spectrophotometer.

Proteins

Protein concentrations of all samples were analyzed using the Lowry procedure (35). The standard curve was generated from standards made with bovine serum albumin (BSA) in concentrations ranging from 0 to 400 mg/L (see Figure A.1). The procedure is described below:

1. The reagents used were:
   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 (premixed) added to 50 ml of distilled water.

2. 1 ml of sample was used for analysis.
3. 20 ml of reagent A was mixed with 1 ml of reagent B and 1 ml of reagent C. Greater volumes in the same proportions were used when necessary.
4. 1 ml of the mixed reagents prepared in step 3 was added to each test tube and vortexed.
5. The samples were allowed to incubate at room temperature for 15 minutes.
6. Reagent D was prepared while the samples incubated.
7. After the 15 minutes was up, 3 ml of reagent D was added to the samples and vortexed immediately (before going to the next sample).
8. The samples sat at room temperature for an additional 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, CA) DU-6 Spectrophotometer.

Repeatability of Protein and Carbohydrate Analysis

Both the Lowry (protein) and Dubois (carbohydrate) procedure were tested for repeatability with a single sample of mixed liquor obtained from an A/O full-scale plant near Yorktown, Virginia. Both techniques showed very consistent absorbance values for split samples of both pH adjusted and non-adjusted mixed liquor supernatant (see Table 3.5).

Distilled water blanks taken from the analysis of sludges generated in this study showed a high level of repeatability between different

Table 3.5. Comparison of Split Samples, York River Sludge.

Absorbance	660 nm (Protein)	488 nm (Carbohydrate)
Blank (water)	0.025	0.023
	0.031	0.021
Unadjusted	0.127	0.770
	0.127	0.771
pH Adjusted	0.941	0.180
	0.947	0.188

batches of reagent (see Table 3.6). The Lowry (protein) method was slightly more consistent than the Dubois (carbohydrate) method.

Table 3.6. Comparison of Reagent Blanks Used During ECP Measurements

Absorbance	660 nm (Protein)	488 nm (Carbohydrate)
Date		
2/16	0.038	0.012
2/23	0.035	0.028
3/9	0.045	0.032
4/10	0.038	0.015
5/2	--	0.027
5/4	0.032	0.013
Standard Deviation	0.006	0.009

IV. RESULTS AND DISCUSSION

Data obtained in this study may be categorized into two major groups; operational data, and biopolymer data. Operational data were obtained in order to compare the settling and dewatering characteristics of the A/O and conventional activated sludge systems. Biopolymer data allowed comparison of exocellular biopolymer (ECP) characteristics of the A/O and activated sludge systems, and correlation of ECP and operational characteristics.

In this chapter the differences in operational and ECP characteristics between the two systems are presented. In addition correlations between ECP and operational parameters which were consistent for both systems are reported. Comparisons of parameters which suggested relationships between ECP and sludge properties in previous studies but which may not have shown a relationship in this study are included as well. Operational data are summarized in Table 4.1 for both systems.

Operational Differences Between the A/O and the A.S. Systems

Data were obtained at four different sludge ages for the A/O system and five sludge ages for the conventional system in order to compare the operational and biopolymer characteristics of the two systems (see Table 4.1). Data have been presented in the literature, most notably by Tracy, et al. (5), which indicate that sludges produced by A/O systems have superior settling and dewatering characteristics to those of conventional activated sludge systems. Since settling and dewatering

Table 4.1. Operational and Biopolymer Data

System-Approximate Date	Sludge Age (days)	Effluent VSS (mg/L)	MLVSS (mg/L)	SVI (mg/L	CST (sec)	Specific Resistance $(m/kgx10^{12})$	Soluble Eff. COD (mg/L)	Total ECP (mg/L)	Total ECP (mg/L)	
									Prot	Carb
A/O										
3/26-4/10	2.9	270	870	950	12.6	1.4	60	470	410	57
2/13-25	3.3	90	740	120	8.8	1.1	60	290	250	42
3/3-33	6.6	50	680	120	10.2	0.5	80	260	200	66
2/16-22	11.9	50	1000	80	11.5	0.1	70	330	270	56
A.S.										
3/16-22	1.7	140	260	80	8.6	1.8	120	240	220	21
2/13-25	4.2	50	1030	90	16.1	2.2	90	260	210	51
3/3-11	6.2	60	850	70	12.0	0.5	80	290	270	25
4/20-5/5	8.3	10	1190	350	8.4	0.3	80	710	650	62
4/20-5/5	14.6	10	1270	310	34.3	15.1	70	580	550	25

Continued)

Table 4.1. Operational and Biopolymer Data (Continued)

System-Approximate Date	Sludge Age (days)	Unbound ECP (mg/L)		Total Prot (mg/L)		Total Carb (mg/L)		Unbound Prot (mg/L)		Unbound Carb (mg/L)	
		Prot	Carb	HMW	LMW	HMW	LMW	HMW	LMW	HMW	LMW
A/O											
3/26-4/10	2.9	50	34	85	28	43	10	18	18	7	7
2/13-25	3.3	52	29	48	39	10	15	9	22	1	6
3/3-33	6.6	54	57	54	38	5	20	11	10	4	19
2/16-22	11.9	53	44	47	36	13	15	22	7	20	14
A.S.											
3/16-22	1.7	93	9	57	35	24	4	16	7	38	5
2/13-25	4.2	78	47	33	36	5	20	10	13	13	13
3/3-11	6.2	87	11	50	56	9	6	12	15	1	2
4/20-5/5	8.3	50	12	113	121	19	9	0	23	6	9
4/20-25	14.6	46	9	122	108	10	22	14	14	6	8

Continued)

properties have been related to biopolymers (13,14,19), this study was conducted to investigate differences in sludge characteristics resulting from differences in biopolymer quantity, composition, and binding between the A/O and A.S. systems.

Soluble effluent COD values for the A/O system were lower than those from the activated sludge system at the two lowest sludge ages (see Figure 4.1). Above a sludge age of six days, values were similar. Higher organic removals than side-by-side conventional systems have been observed (in terms of BOD) for the A/O system by Tracy, et al. (5), and for an alternating aerobic/anaerobic activated sludge system by Ip, et al. (37). The reason for the lower A/O values is not clear from the data but can be seen from Table 4.1 to correspond to lower quantities of unbound carbohydrate in the ECP compared to the other two A/O data points.

The effluent VSS concentration was generally greater for the A/O system (see Figure 4.2). This may have been due to several factors including discharge of floating biomass in the A/O system resulting from denitrification in the clarifier, or better binding of bacterial polymers in the A.S. system. However, the data do not provide any information that would substantiate either of these reasons. Both systems had high effluent VSS values at low sludge ages and lower values at high sludge ages.

The SVI varied greatly for both systems and no relationship with sludge age or differences between the two systems could be found (see Table 4.1). This is in contrast to the findings of Tracy, et al. (5)

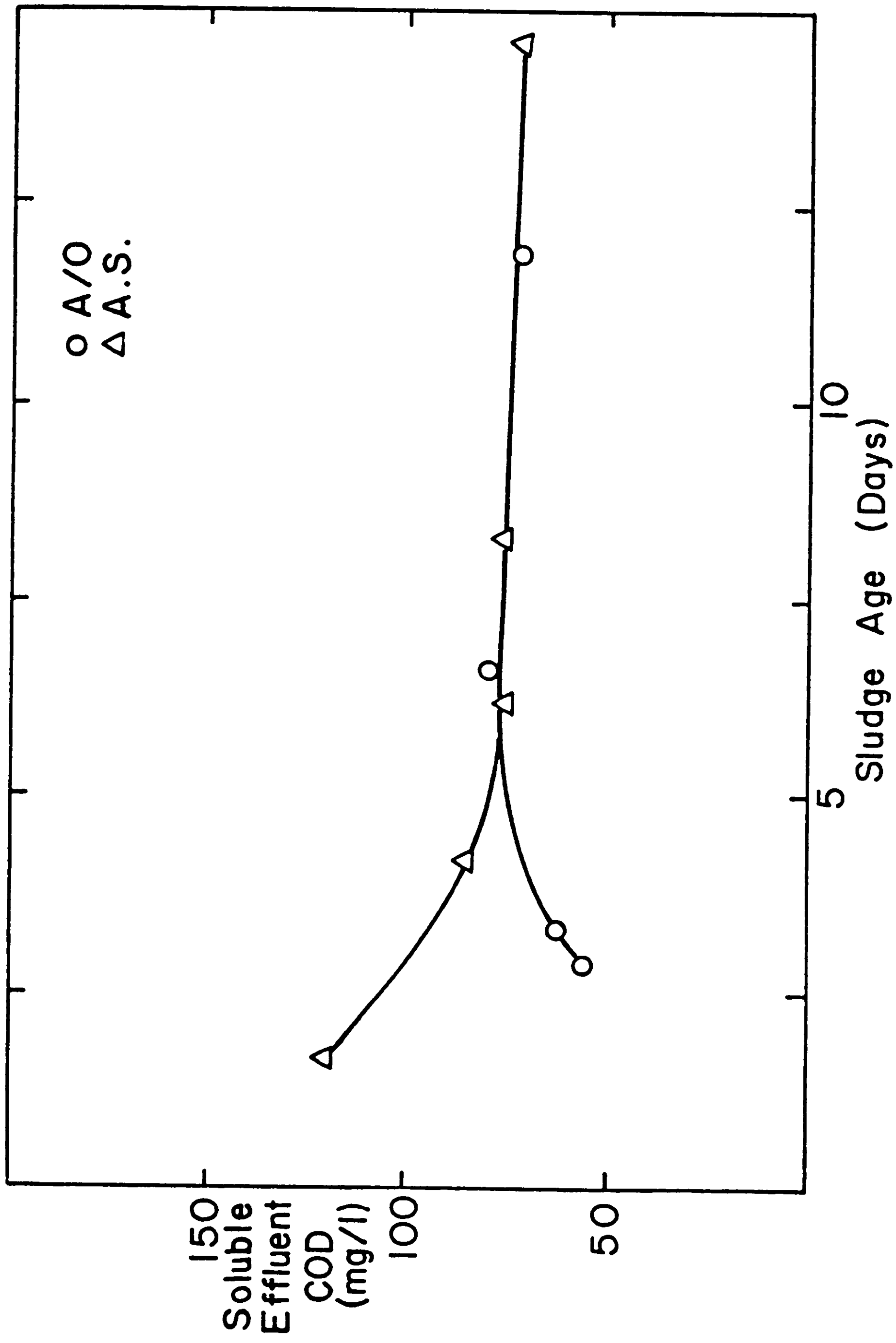

Figure 4.1 Soluble Effluent COD Vs Sludge Age

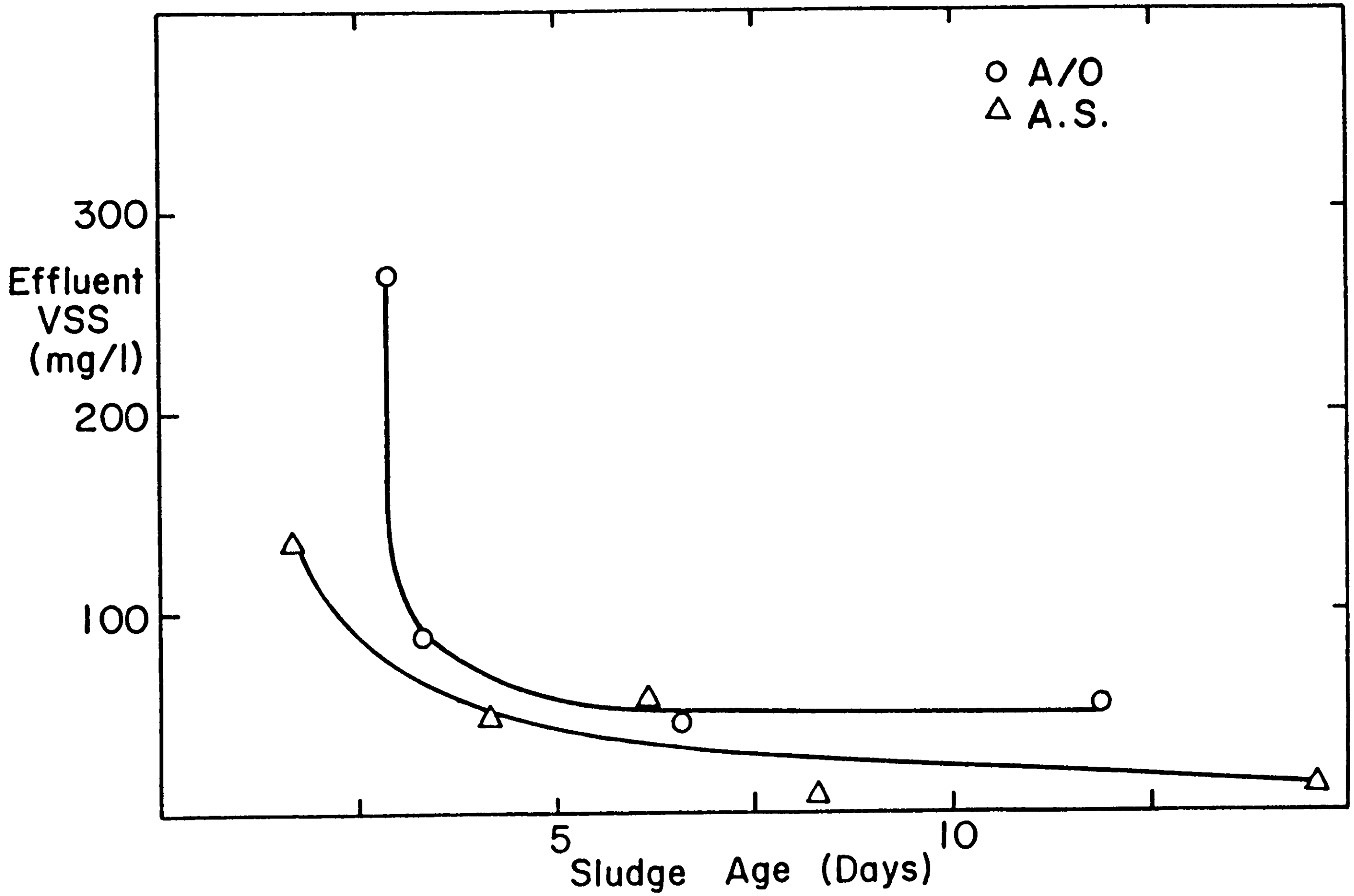

Figure 4.2 Effluent VSS Vs Sludge Age

who found that the SVI of sludge from an A/O system was consistently better than the SVI of sludge from parallel conventional A.S. systems.

The specific resistance (R*) of the mixed liquor was usually similar, at a given sludge age, for the two systems. The specific resistance tended to decrease with increasing sludge age for both systems (see Figure 4.3). Improved dewatering with increasing sludge age has been observed by Bisogni and Lawrence (38). One notable exception to this general trend in this study occurred at a sludge age of 14.6 days in the A.S. system, which had a R* of 15.1x1012 m/kg. This sludge had unique dewatering characteristics with an R* an order of magnitude higher than any other data obtained and as such it is not included in Figure 4.3. Although the dewatering properties of this sludge could not be entirely understood in terms of ECP quantity and composition as measured in this study, the UV absorbance from the gel column fractionation data of this sludge was unusally high (see Section on gel column fractionation by molecular weight of ECP for discussion) and indications were that substantial deflocculation had occurred.

The value of the CST/MLVSS, often used as an index of the dewatering rate, tended to decrease with sludge age for the A.S. system but was constant over a broad range of sludge ages for A/O mixed liquor (see Figure 4.4). The A/O system tended to have a lower value for CST/MLVSS at low sludge ages.

Interfacial sludge thickening data could not be obtained because it was necessary to concentrate sludges into compression in order to obtain a well-defined interface. As a result only three data points were

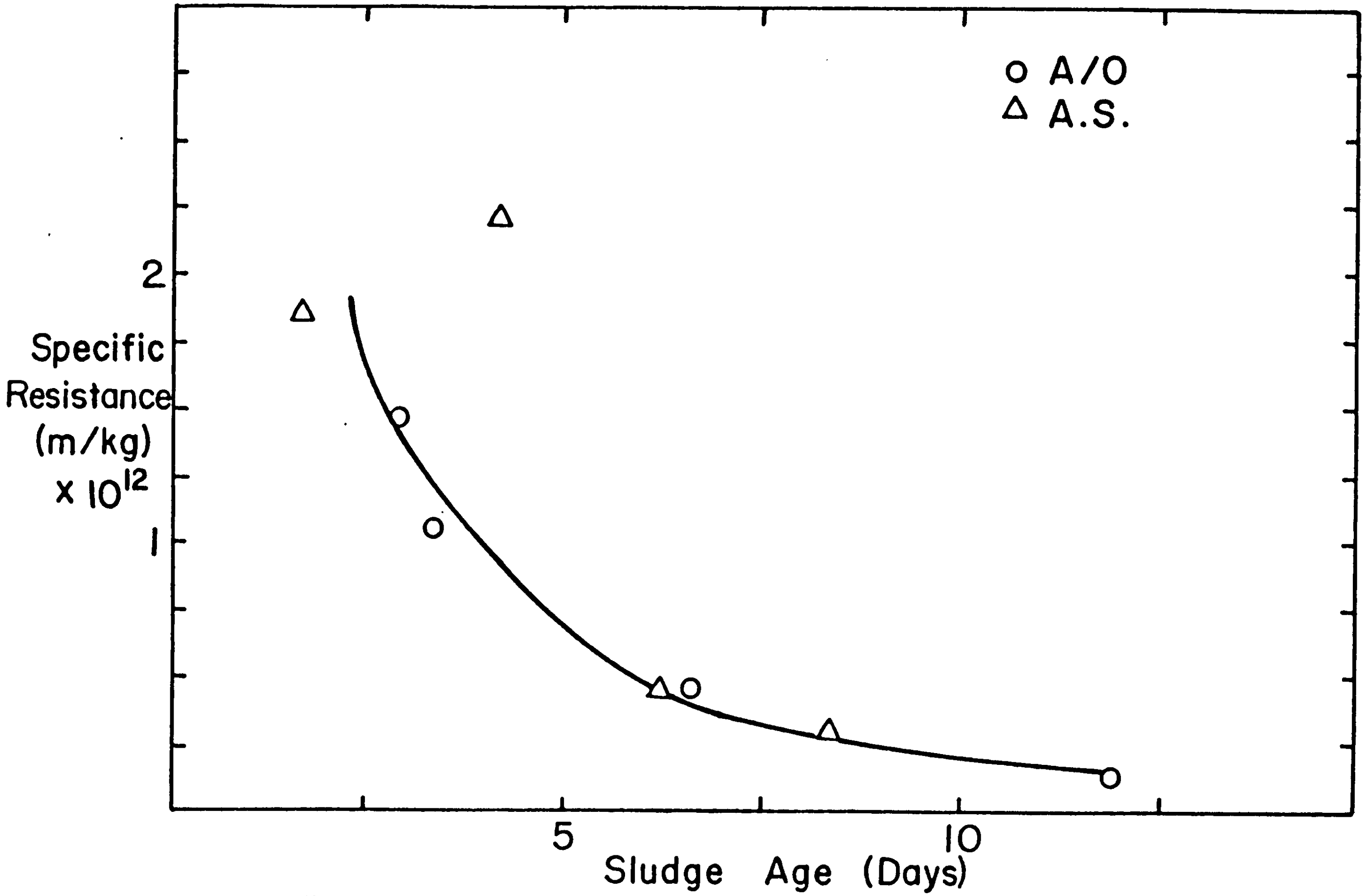

Figure 4.3 Specific Resistance Vs Sludge Age

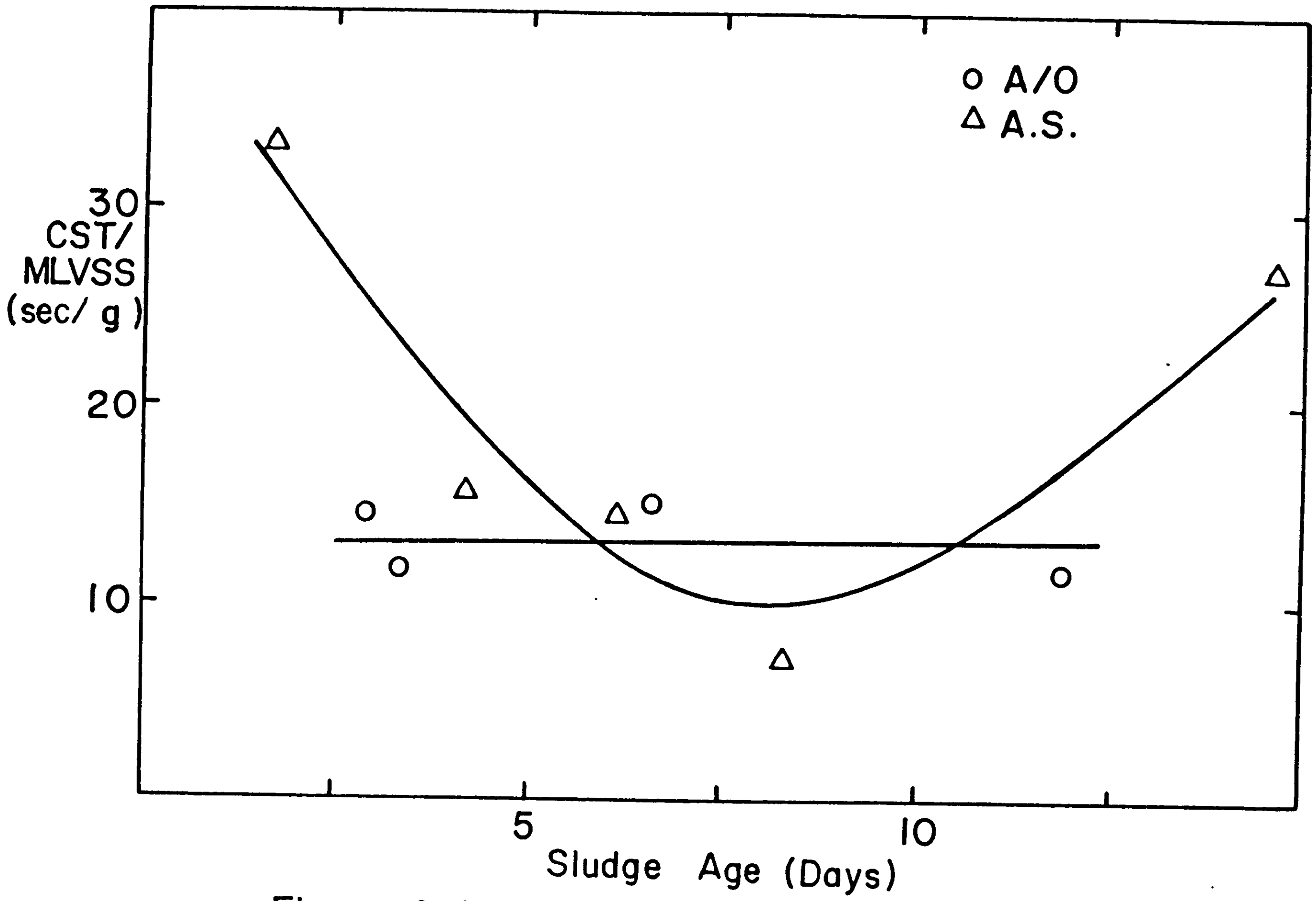

Figure 4.4 CST/MLVSS Vs Sludge Age

obtained and these are discussed in the section entitled "Suggestions for Future Research".

These data show that the two systems had very similar operational characteristics with the exception of the high COD removals achieved by the A/O system at low sludge ages.

Phosphorus Removal

Reasonable phosphorus mass balances were obtained for sludge ages above 5 days for both the A/O and A.S. systems (see Table 4.2). A/O data indicated that excess biological phosphorus removal took place at sludge ages of 6.6 and 11.9 days. Activated sludge data at a sludge age of 6.2 days resulted in the expected cellular growth stoichiometric assimilation of phosphorus (2.2%) based on an assumed biomass nutrient ratio of $C_6OH_{87}O_{23}N_{12}P$. At low sludge ages the quantity and variability of effluent suspended solids in the A/O system made an adequate mass balance impossible. For these sludge ages it was not possible to say if excess biological phosphorus removal took place. Oxidation-reduction (ORP) data were taken for these sludge ages to determine if anaerobic conditions were maintained.

Anaerobic release of phosphorus was observed in the A/O system at a sludge age of 3.3 days. Data at a sludge age of 2.9 days indicates that no anaerobic phosphorus release occurred and this coincides with a small amount of anaerobic COD removal. It is likely, judging from these observations, that excess P removal was maintained at a sludge age of 3.3 days but was failing or had failed at a sludge age of 2.9 days. The

Table 4.2. Phosphorus Data

		A/O Phosphorus Data		
Sludge Age (days)	2.9	3.3	6.6	11.9
P influent (mg/L)	9.7	7.2	10.3	11.3
P effluent (mg/L)	7.1	5.2	5.7	7.0
P removal (mg/L)	2.6	2.0	4.6	4.3
Calculated % P of sludge	0.5	1.2	4.5	5.7
ORP (platinum)	-219	-190	not measured	
P influent (w/recycle) (mg/L)	9.0	5.9	7.4	8.6
P anaerobic effluent (mg/L)	7.4	16.1	9.2	8.3
		A/S Phosphorus Data		
Sludge Age (days)	1.7	4.2	6.2	8.3
P influent (mg/L)	11.3	7.2	10.3	6.4
P effluent (mg/L)	5.7	6.7	7.2	4.1
P removal (mg/L)	5.6	0.5	3.1	2.3
Calculated % P of sludge	4.3	0.7	2.2	1.9

failure to measure phosphorus release at a sludge age of 11.9 days is believed to be a non-representative data point. Only one measurement was made in this case because of accidental decomposition of stored samples. Other data and the results of the system mass balance indicate that at this sludge age the reactor was functioning as expected for the A/O system.

The fact that the expected phosphorus content of the A.S. system could be obtained by conducting a mass balance at a sludge age of 6.2 days and could not be obtained at lower sludge ages indicates that a good mass balance was only possible at medium and high sludge ages. From the results of mass balances and from the observed data for sludge ages where this was not possible, it may be concluded that biological phosphorus removal occurred at sludge ages of 3.3, 6.6, and 11.9 days but not at 2.9 days.

Cation Data

Several investigators have suggested a linkage between phosphorus uptake and release of Mg, K, and Ca (36,40). Comeau, et al. (40), suggest that these cations may remain bound to phosphate molecules when they are released into solution until they are taken up again by bacteria capable of excess biological phosphorus removal. In this study the uptake of phosphorus and Mg is linked for both the A/O and A.S. system with respect to the net system uptake of phosphorus (see Figure 4.5). The simultaneous release and uptake of magnesium and phosphorus

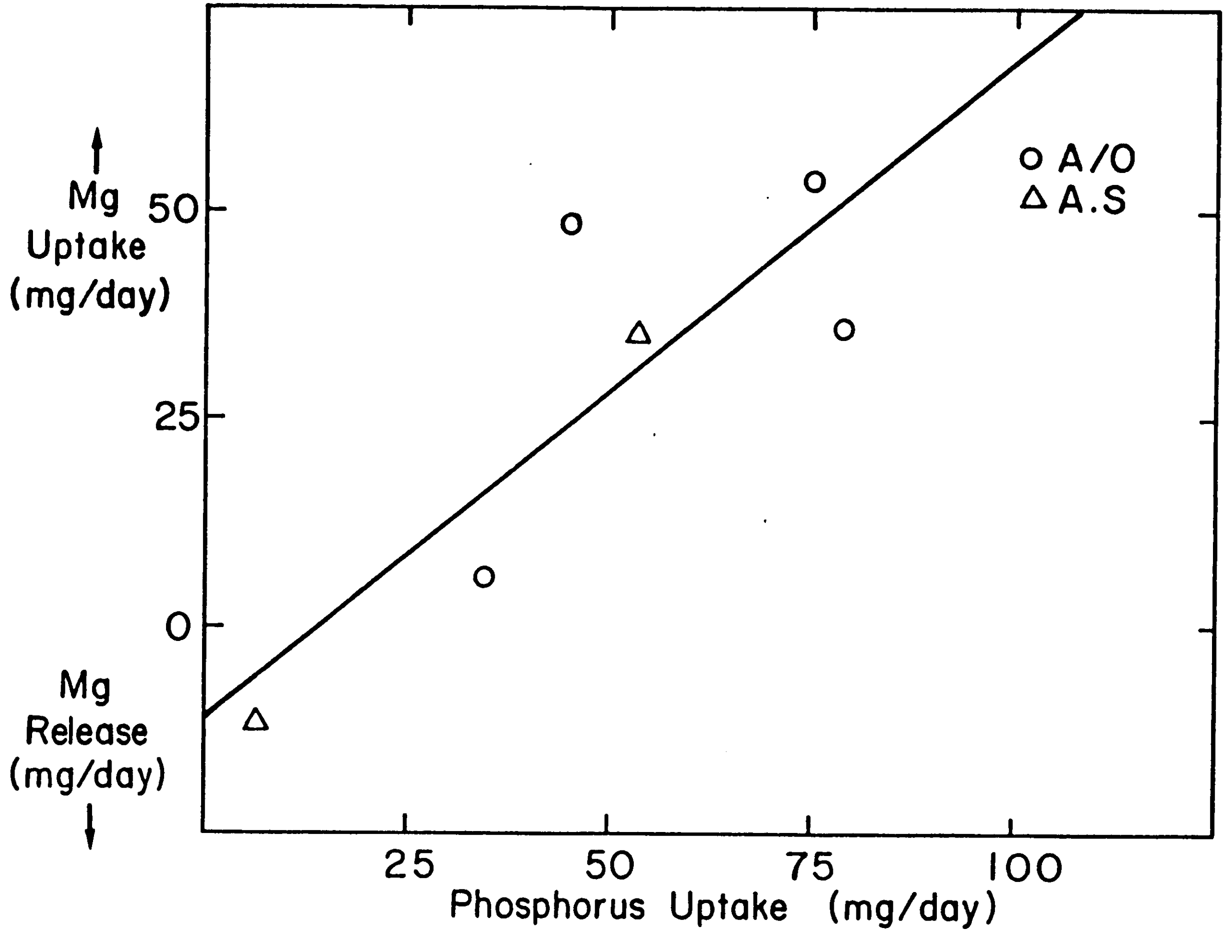

Figure 4.5 Mg Vs Phosphorus

in the anaerobic and aerobic zones observed by Brannan (36) and in other previous studies was not consistently observed here.

According to Lehninger (41) intracellular Mg complexes with both ATP and ADP and phosphate. The observed uptake of Mg and phosphorus over the systems may be related to this phenomena, with Mg being cotransported with phosphorus across the cell membrane and complexed within the cell.

Although Ca, K, and Na were measured in this study, no correlation with phosphorus was observed (see Figure 4.6). A relationship between Na and Mg was observed, however (see Figure 4.7). It is well-known that most cells maintain potassium and magnesium concentrations within the cell which are higher than concentrations outside the cell, while sodium concentrations are typically lower within the cell than without (41). The apparent link is most likely the result of magnesium uptake with phosphorus while sodium is maintained at very low concentrations within the cells relative to the mixed liquor.

Novak, et al. (10) observed that activated sludge often behaves as a slurry overdosed with anionic polymer. They found that activated sludge dewatering improved with the addition of divalent cations (corresponding to release) and worsened as the dosage of divalent cations decreased (uptake) or monovalent ions increased. The results of this study shown in Figure 4.8 indicate that no relationship exists between Mg changes and CST. This may be because the changes were not significant enough to alter biopolymer binding. Although a substantial amount of Mg was removed by some of the sludges, the Ca concentration

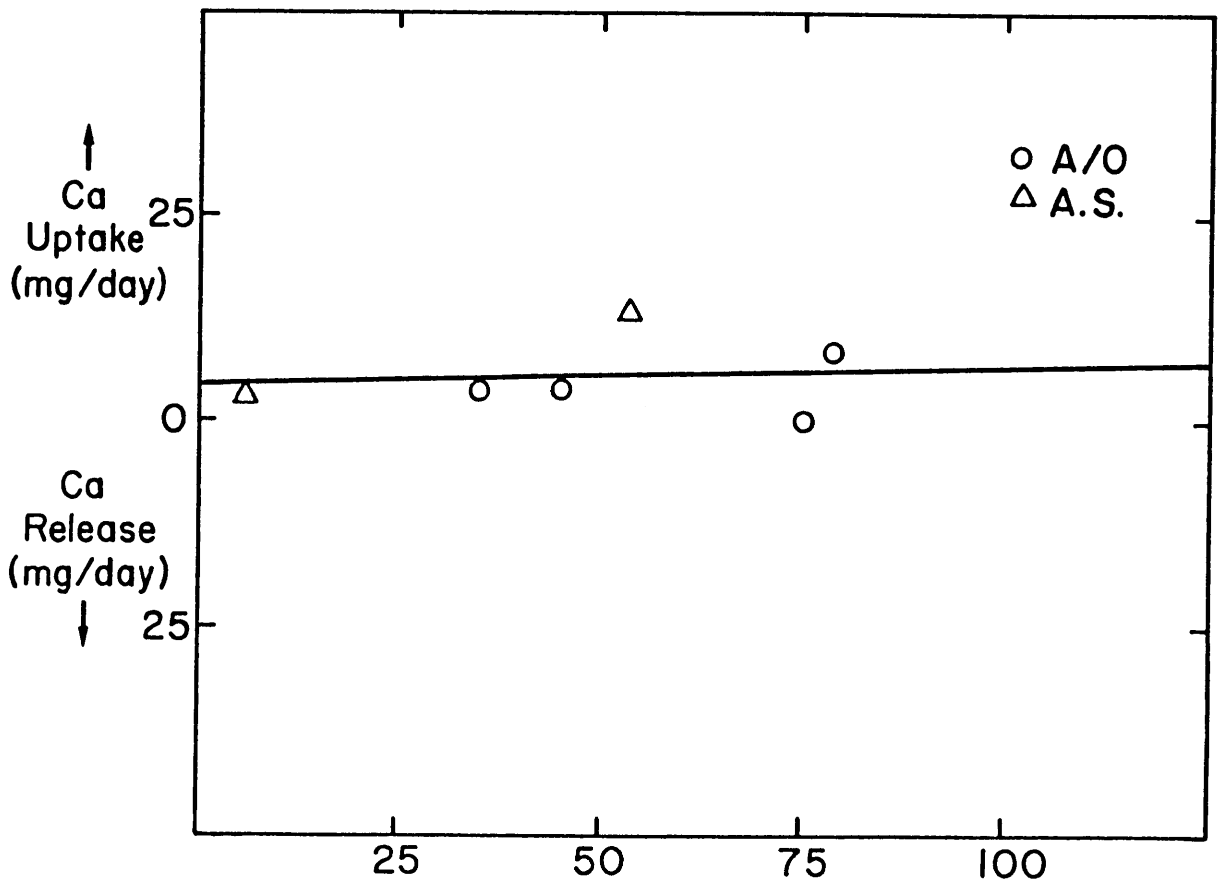

Figure 4.6 Ca Vs Phosphorus

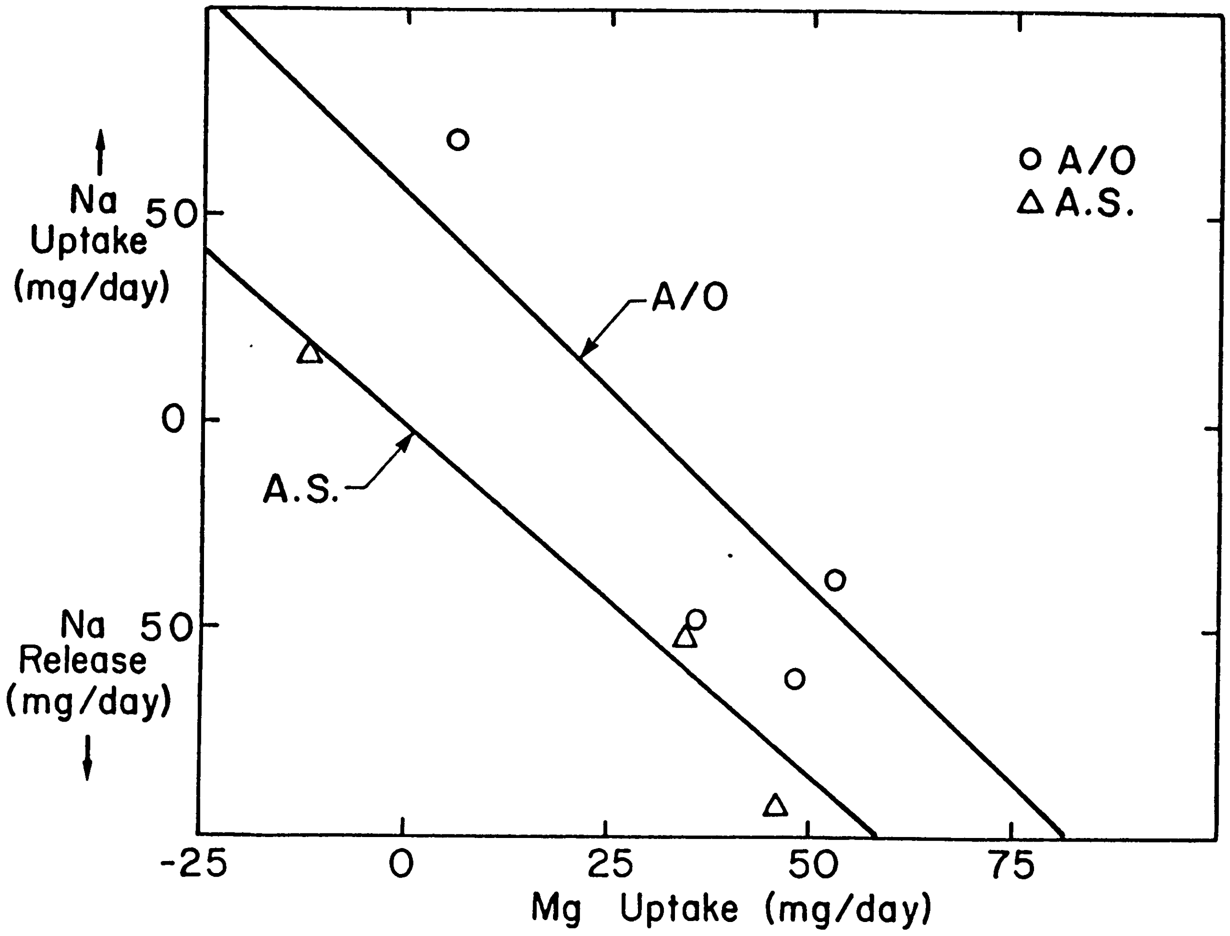

Figure 4.7 Na Vs mg

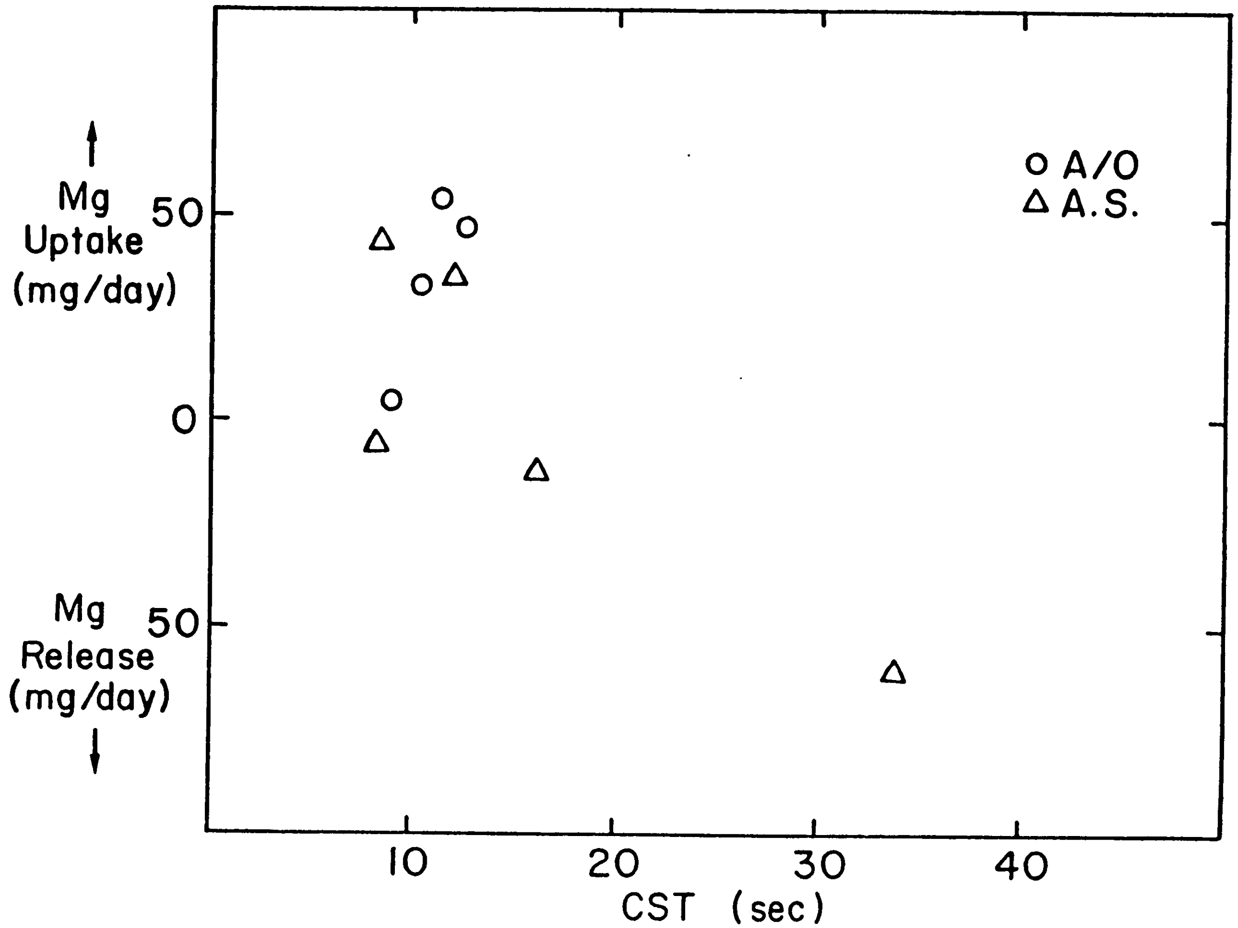

Figure 4.8 Mg Uptake/Release Vs CST

remained virtually unchanged as shown in Figure 4.6. The results suggest that differences in sludge properties between the A/O and A.S. systems could be due to differences in cation content if the overall cation level is significantly changed by the A/O process. In particular, if Mg was significantly removed as phosphorus removal occurred and the Ca content was low, some deflocculation could occur.

Differences in ECP Characteristics Between A/O and A.S.

ECP characteristics for both systems did not show an obvious relationship with sludge age. This was in contrast to a continuous-flow study conducted by Gulas, et al. (8) in which the quantity of ECP produced showed a dependence with sludge age. Their results showed an increase of ECP at both low and high sludge ages. Brown and Lester (9) observed an increase in ECP concentrations at high sludge ages only. It is intersting to note that in this study UV absorbance (see Figure 4.14) did increase with sludge age while measured ECP concentrations did not.

It is important to observe that total ECP was roughly equal to the quantity of protein, since protein quantity was almost an order of magnitude higher than that of carbohydrate (compare Figures 4.9 and 4.10). As a result ECP production was mirrored closely by total proteins. While there was a large increase in ECP for the A.S. data at high sludge ages, this is also associated with high SVI values (see Section 4.6). Since increasing SVI was not associated with increasing sludge age in the literature (38) it is unlikely that this increase in ECP was related to sludge age but rather to these specific sludges.

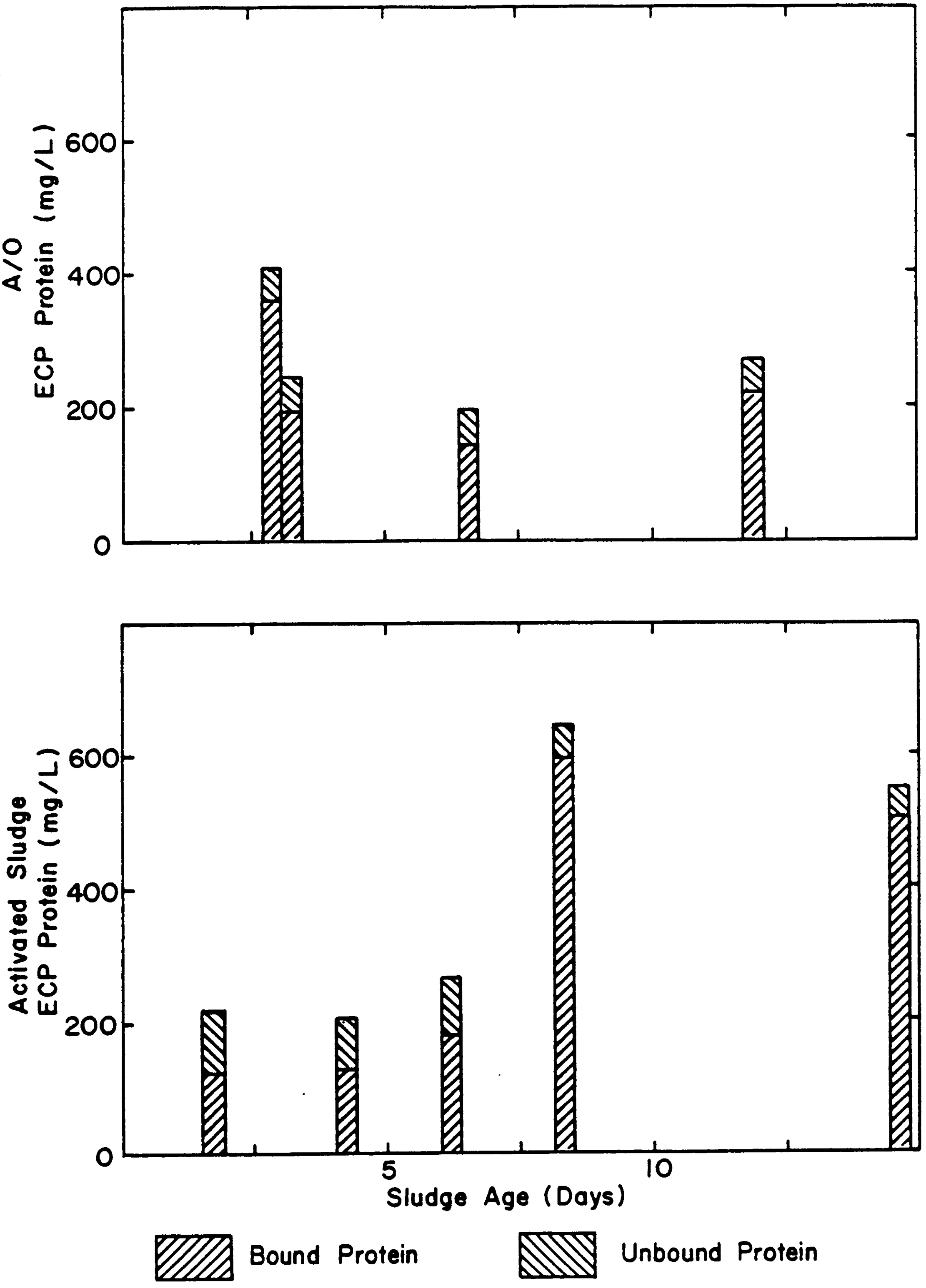

Figure 4.9 Exocellular Protein Vs. Sludge Age

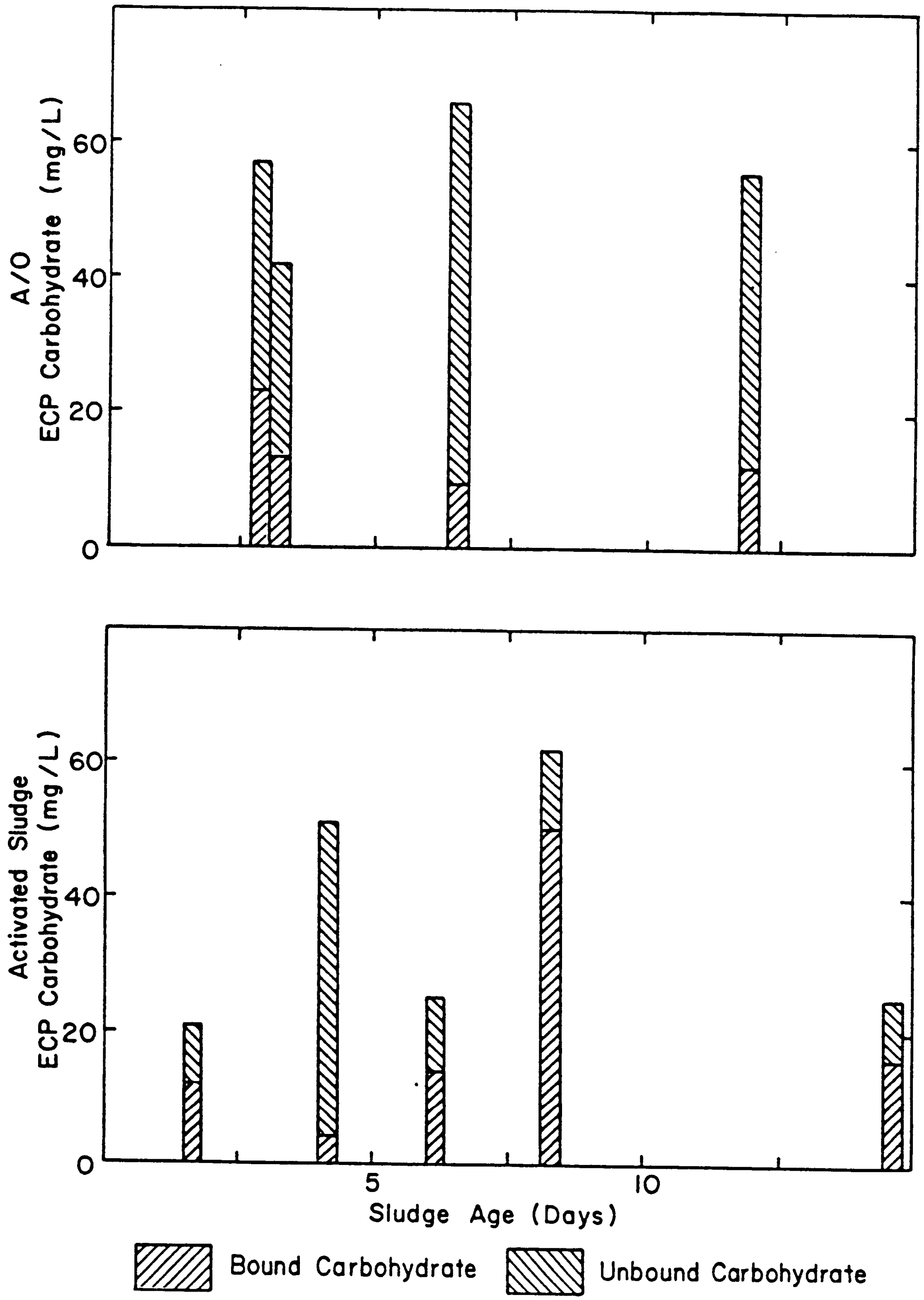

Figure 4.10 Exocellular Carbohydrate Vs Sludge Age

The production of both protein ECP and carbohydrate ECP varied over roughly the same ranges for the A/O and A.S. systems. The only exceptions are the two higher protein concentrations for the A.S. system (see Figure 4.9), which had higher values than any A/O sludge studied.

Although no consistent differences in total ECP or protein and carbohydrate production were observed there were differences in the proportions of ECP between the A/O and A.S. systems (see Figures 4.9 and 4.10). In general, the A.S. system yielded greater concentrations of unbound protein than the A/O system (see Figure 4.9). The A/O system yielded much higher concentrations of unbound carbohydrate than the A.S. system (see Figure 4.10).

The significantly higher proportion of unbound carbohydrate in the A/O system may be the result of any one, or a combination, of several differences between the two systems. First of all it should be noted that total production of carbohydrate for the A.S. system varied much more than the A/O system, tending to be significantly lower than A/O production. Lower unbound carbohydrate concentrations can be related to lower total carbohydrate concentrations in three of four cases for the A.S. system. However, inspection of Figure 4.10 makes it obvious that the degree of binding of carbohydrate was less for the A/O system with an average of 27% bound carbohydrate versus an average of 53% for the A.S. system. The percentage of carbohydrate binding was a more consistent difference between the two systems than total carbohydrate quantity.

Protein fractions cannot be explained on the basis of either binding or total production. For three of the five sludge ages, the degree of protein binding for the A.,S. system was significantly less than that of the A/O system. However, the two lowest values of percent unbound protein for the A.S. system correspond to the two highest productions of total protein ECP. It is unlikely that the increased degree of total protein production and binding observed at the two high sludge ages for the A.S. system was a function of sludge age. Corresponding carbohydrate data show no dependence on sludge age.

Carbohydrate binding was consistently poorer for the A/O system while protein binding for the A.S. system was worse than that of the A/O system in a significant portion of the data. These differences could be due to either different populations of microorganisms within the systems or to differences in the synthesis and chemistry of the cell surfaces and biopolymers within each reactor. Either is a plausible explanation because of the differing environmental conditions existing in the systems.

The only consistent difference between the two sludges was the higher fraction of unbound carbohydrates found in A/O sludge. This difference has little or no implications on bulk sludge behavior and characteristics.

Gel Column Fractionation by Molecular Weight of ECP.

Standardization of the gel column using Sephadex gel G-75 and Blue Dextran, a compound with a molecular weight of 2x106, resulted in a UV peak in the range of 6-11 ml (see Figure 4.11). Organics which elute at this volume are considered to have weights greater than 50,000 daltons and are referred to as the high molecular weight fraction.

Molecular weight data were quantified in two ways, using the UV intensity and by measuring the carbohydrate and protein content of the eluent volumes. Both measures have been used before and neither is considered to be an exact quantitative measure of ECP.

UV data for quantification of ECP consistently showed two major peaks at elution volumes of 6-11 ml and 22-27 ml (see Figure 4.12), constituting what are referred to in this study as the HMW and LMW fraction, respectively. The similarity of the fractionation data between the A/O and A.S. systems can be seen from the comparative UV absorption versus elution volume plots shown in Figure 4.13. The quantitative similarities between the two systems is made even more evident by using the heights of the HMW peaks in terms of UV absorbance as an index of the quantity of ECP versus sludge age (see Figure 4.14). For an operational range of sludge ages of 3 to 12 days, UV absorbance of fractionation samples showed nearly identical characteristics for both systems. The increase in UV absorbance with sludge age is analogous to the accumulation of biopolymer observed by Bisogni and Lawrence (38). However, the measured protein and carbohydrate concentrations in the Sephadex eluent frequently did not correspond to UV data and therefore, the extent to which UV data may be interpreted quantitatively requires further study.

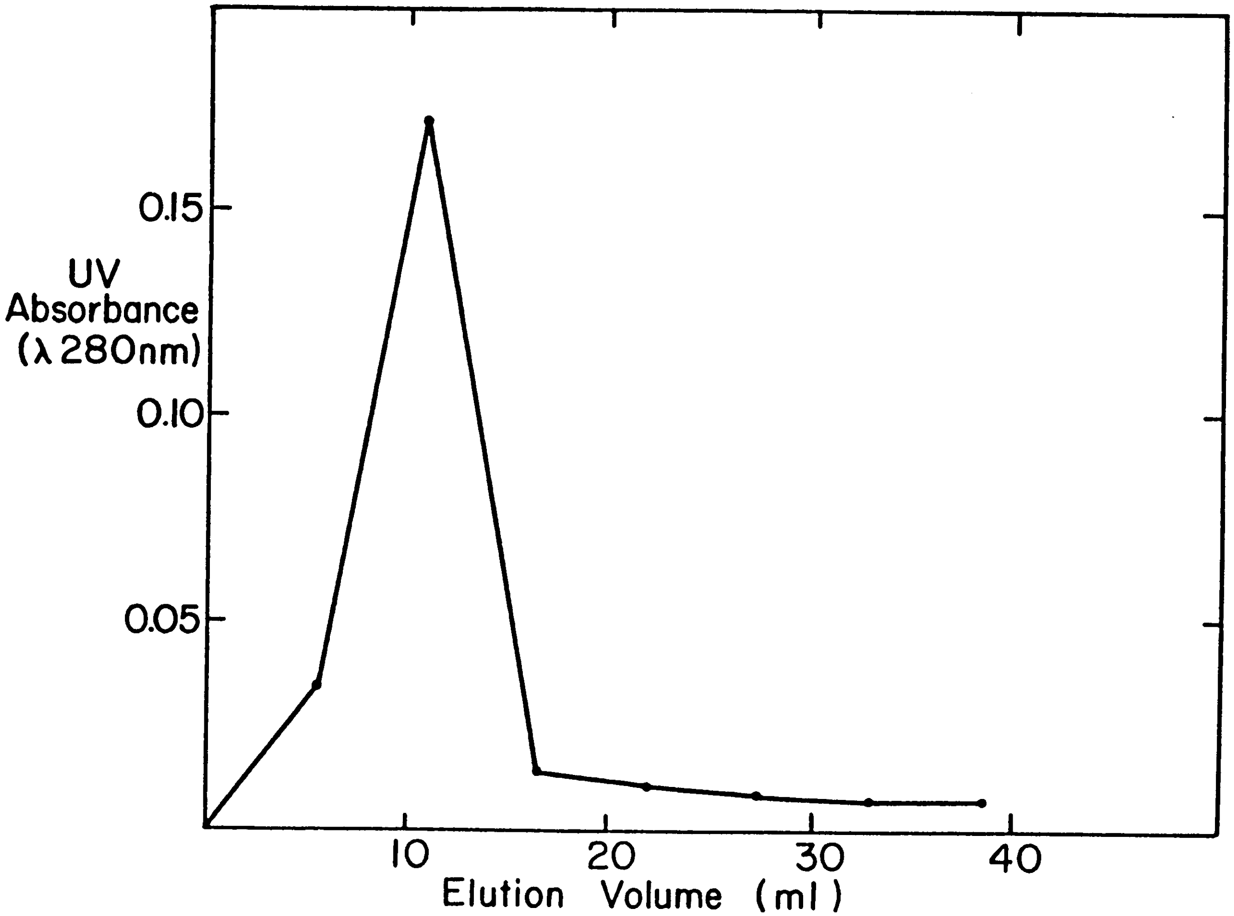

Figure 4.11 Fractionation Data of Dextran

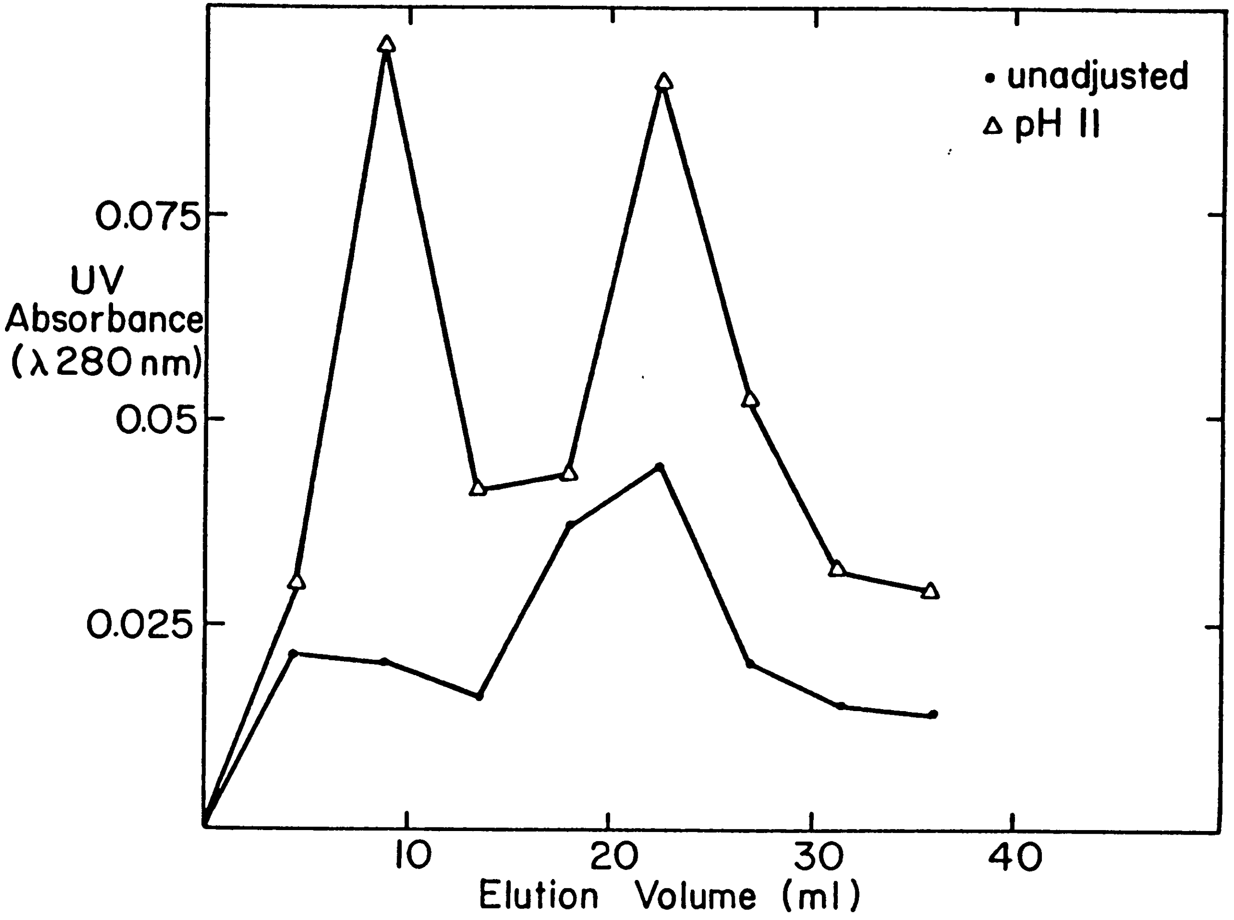

Figure 4.12 A/O Fractionation Data Sludge Age 3.3 days

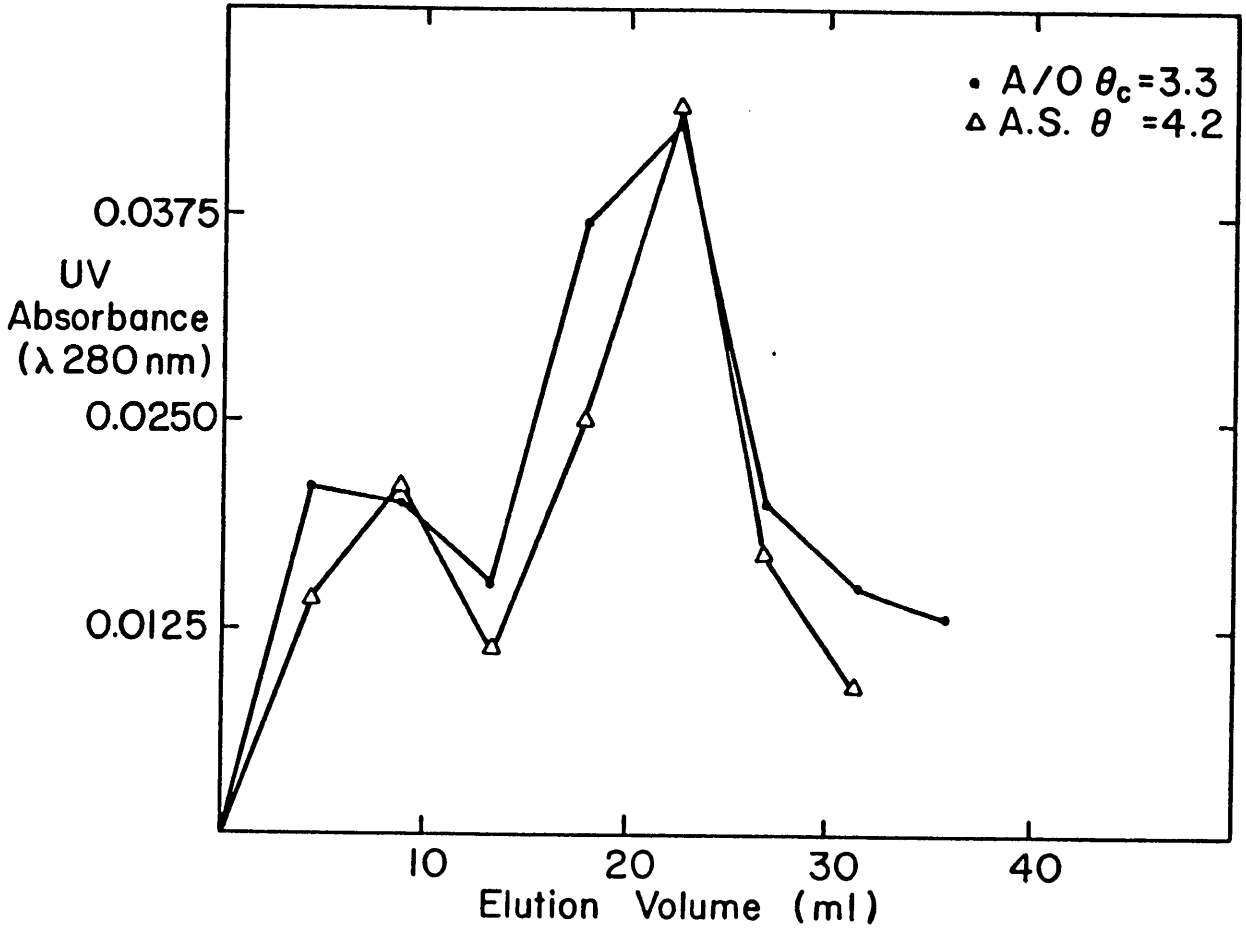

Figure 4.13 Superimposed Non-pH Adjusted Fractionation Data

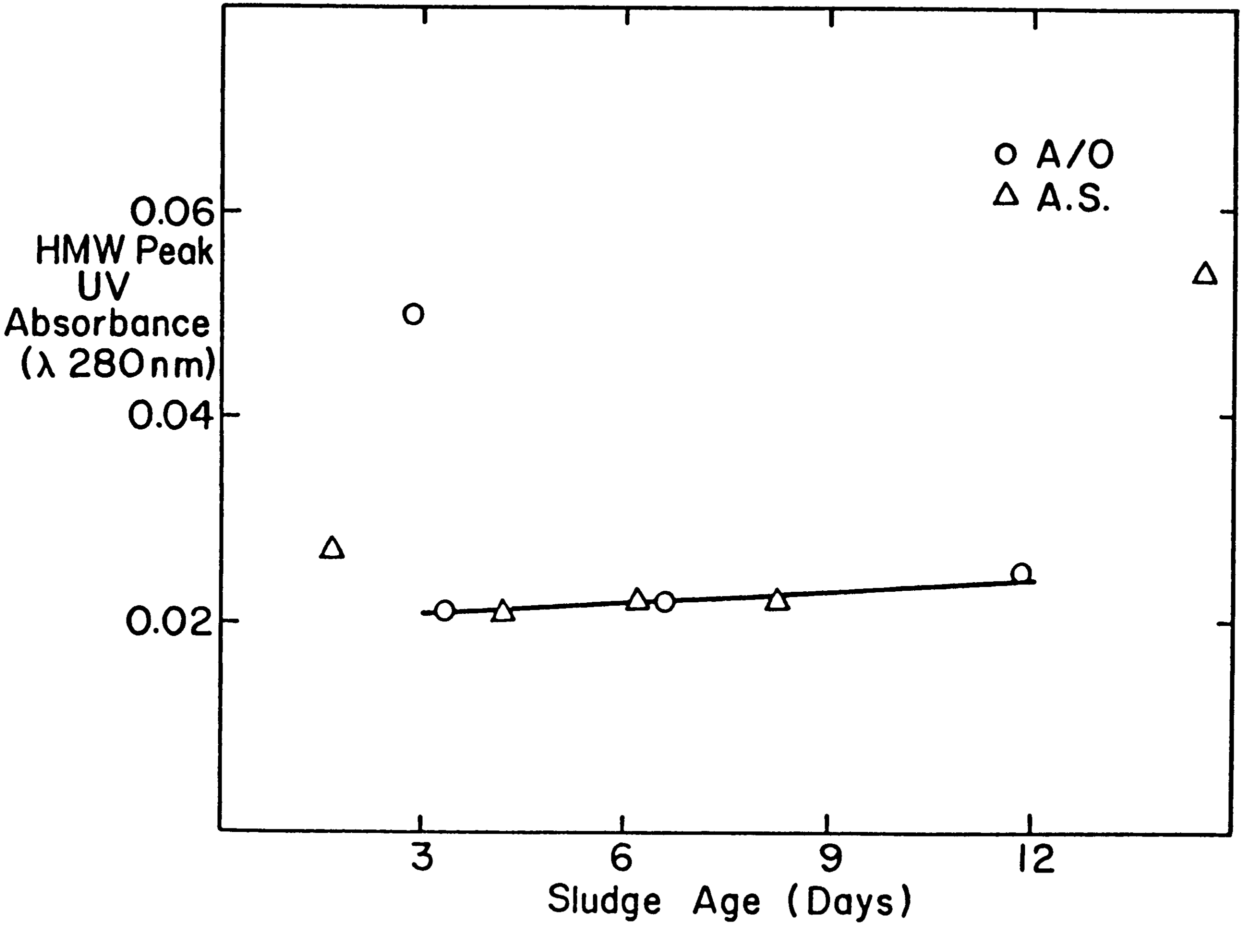

Figure 4.14 HMW Peak Height Vs Sludge Age

The high degree of UV absorbance for the low sludge ages may be due to the presence of undegraded substrate since influent gel fractionation yields similar peaks in terms of molecular weight to those of the samples (see Figure 4.15). Also the highest UV point (corresponds to the lowest sludge age) for the A/O system was filamentous and this may have resulted in different ECP absorption characteristics. The high level of UV absorbance at the sludge age of 14.6 days occurred for a sludge that was operationally unique, with a dewatering rate an order of magnitude higher than any other sludge sample in this study. Because the high UV response did not show a corresponding high ECP concentration, it may be that high UV absorbance reflects a change in ECP structure or chemistry, or a component of ECP, which was not reflected well in the crude ECP measurement techniques used in this study. The gel filtration data, particularly as measured by the UV response, suggests that little difference exists between the A/O and A.S. systems with regard to either the quantity or characteristics of unbound biopolymer. Also, over a wide range of sludge ages, the biopolymer quantity is similar. To the degree that the non-degradable COD in activated sludge systems consists of unbound biopolymer, this suggests that a relatively constant value of residual COD can be expected over the same sludge age range.

Relationships between ECP and Operational Characteristics

Effluent COD was found to correspond best with unbound ECP for the A/O system while there was only a poor correspondence for the A.S.

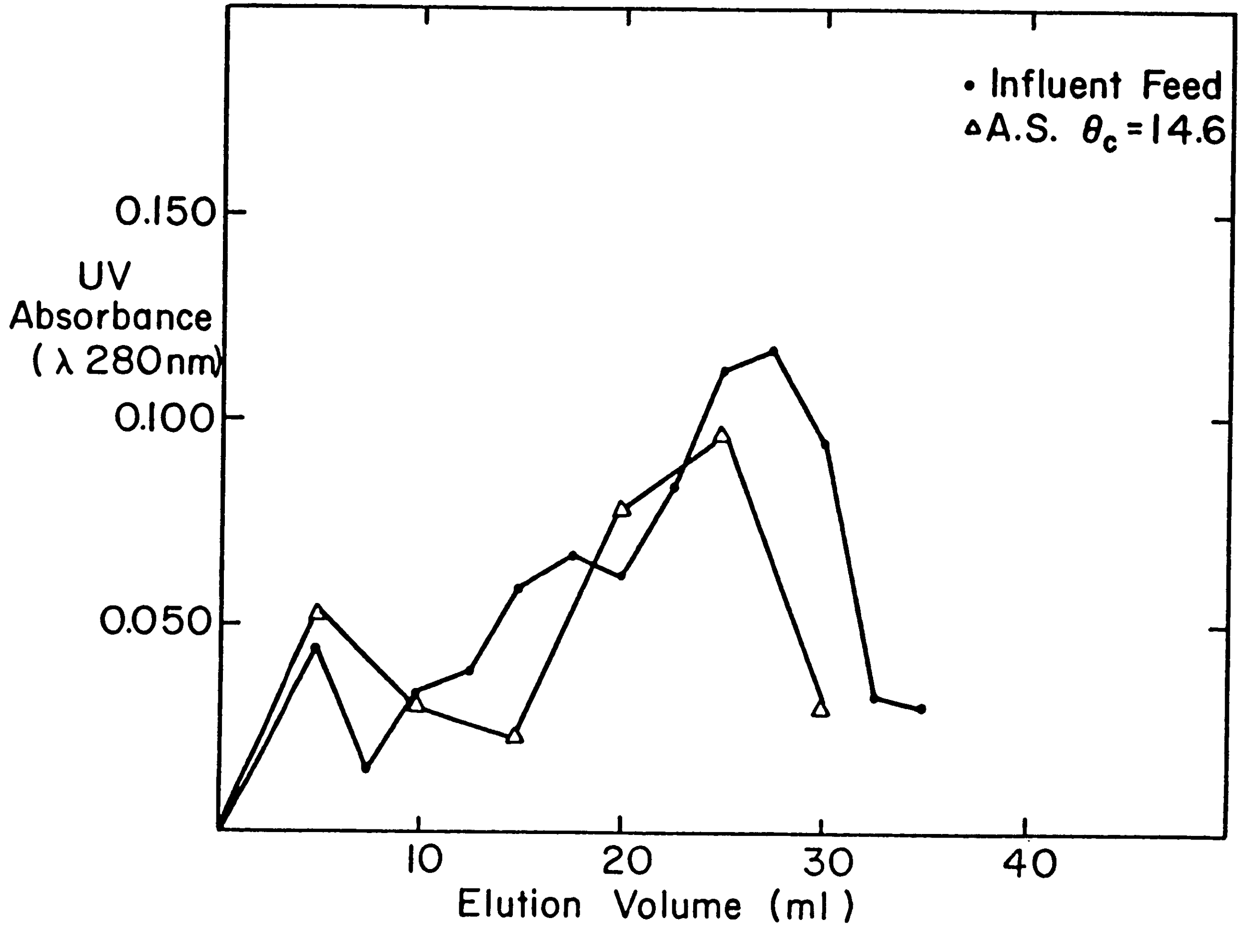

Figure 4.15 Superimposed Influent and Non-pH Adjusted Fractionation Data

system (see Figure 4.16, 4.17, and 4.18). The low concentrations of both unbound ECP and effluent COD at low sludge ages for the A/O system differs from the results of Saunders and Dick (39). They observed a decrease in "slime organic matter" (which corresponds roughly with unbound ECP) and effluent COD as the sludge age increased. The results obtained for the A.S. system (see Figure 4.17) corresponded much more closely with their observations, with ECP decreasing with increasing sludge age.

A plot of effluent COD and unbound ECP versus sludge age shows how similar each parameter was for the A/O system (see Figure 4.16). The A.S. system showed similar trends but the quantitative similarity between ECP and COD was not as good as for the A/O system. The differences in COD and ECP data indicate differences in biopolymer characteristics between the two systems as well as operational differences related to sludge age.

Effluent VSS showed a relationship with SVI (see Figure 4.19). High SVI values were associated with low effluent VSS values while lower SVIs were found as effluent VSS increased. Knocke (42) also found that SVI was a good "surrogate indicator" of changes in floc water content with increasing SVI values corresponding to high incorporation of water in the floc structure. Increased floc water content was found to decrease dewatering rates but to improve coagulation. Knocke, et al., (43) observed in a separate study that for alum sludge resulting from coagulation of clay solutions, high effluent turbidities were associated

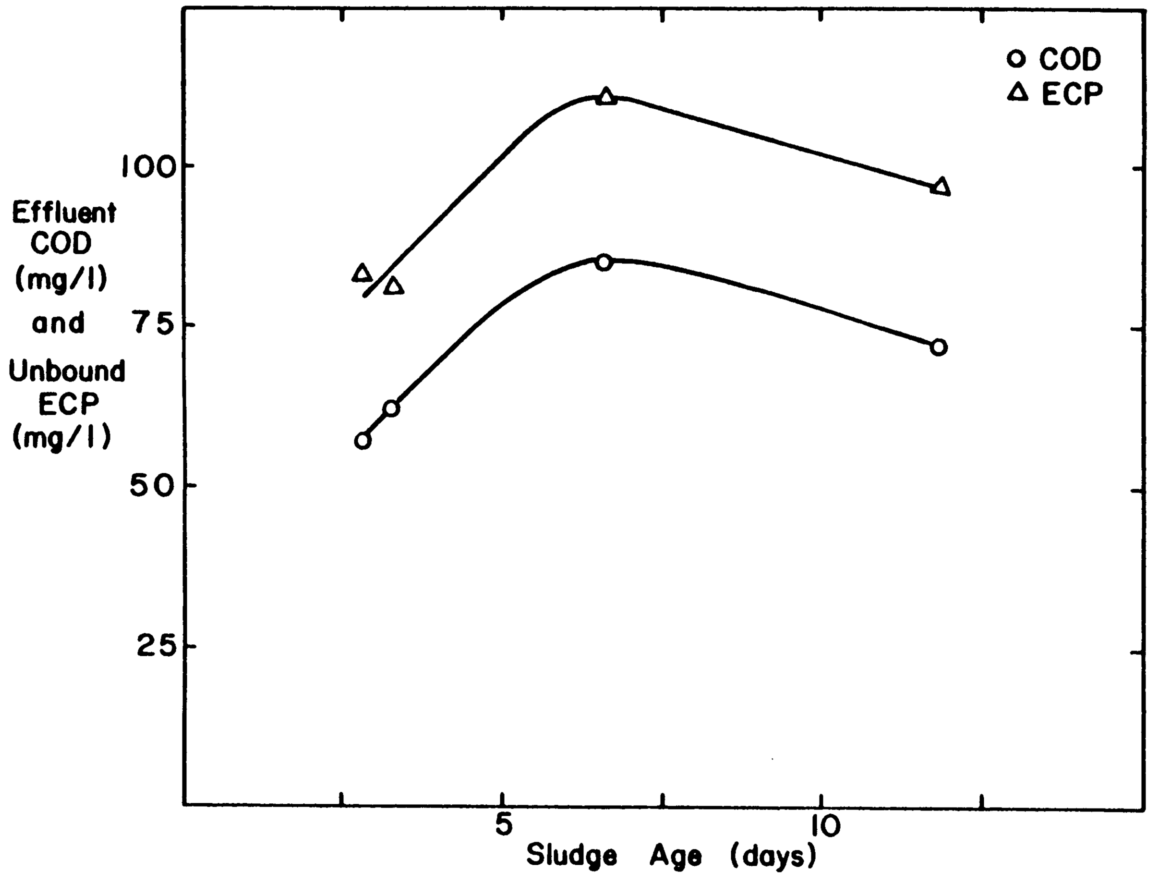

Figure 4.16 Effluent COD and Unbound ECP Vs Sludge Age
A/O System

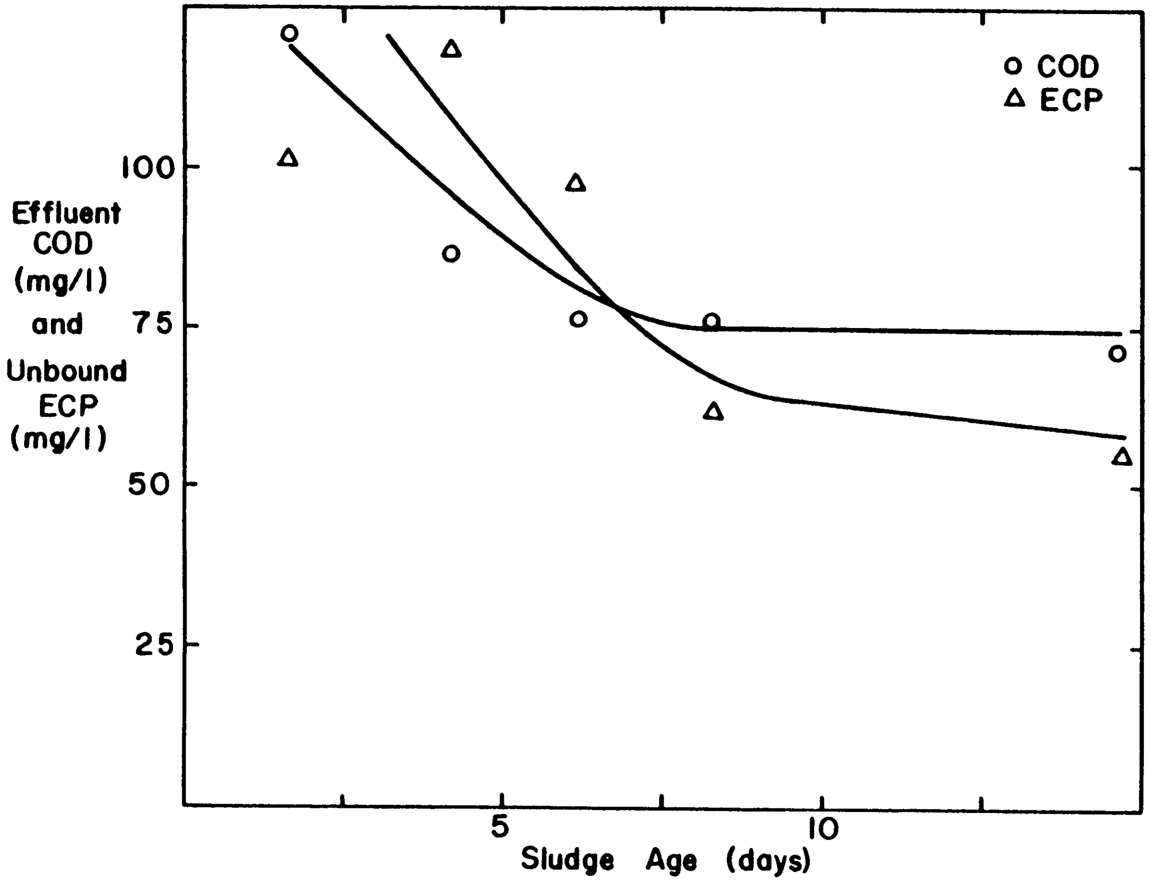

Figure 4.17 Effluent COD and Unbound ECP Vs Sludge Age A.S. System

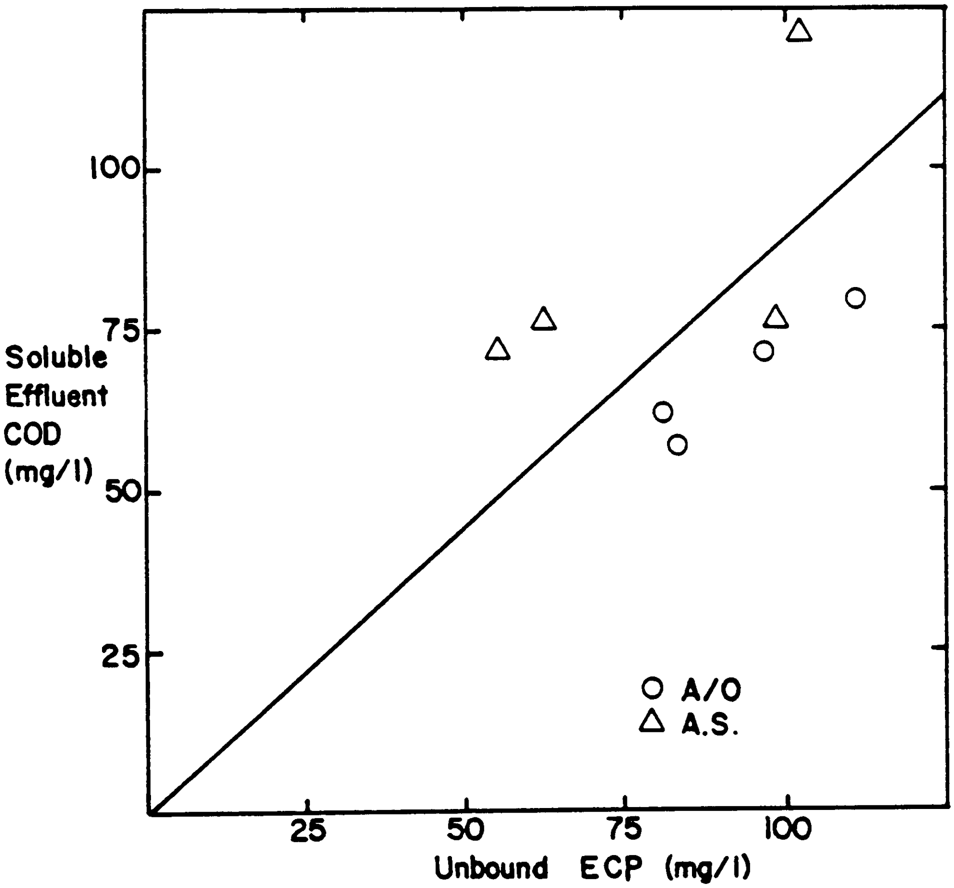

Figure 4.18 Soluble Effluent COD Vs Unbound ECP

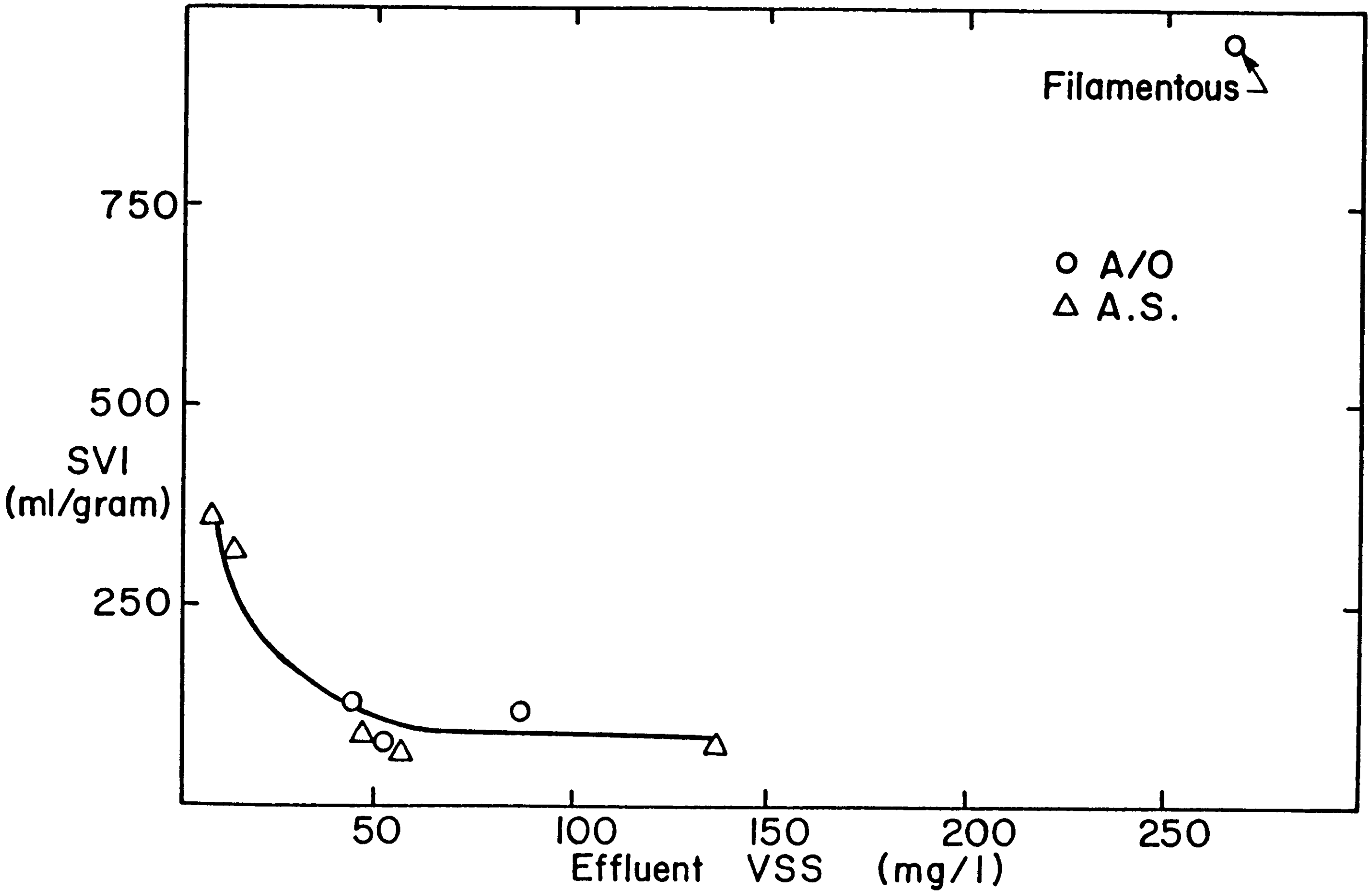

Figure 4.19 SVI Vs Effluent VSS

with good dewatering properties. This relationship was not consistent in this study, however (see Figure 4.20).

If similar responses were found for activated sludge, it would be expected that at high SVIs the floc structure which is capable of incorporating considerable amounts of water, would also allow few solids to leave in the effluent. At low SVIs the flocs contain less bound water but leave uncoagulated a considerable amount of effluent solids. Dewatering rates would be proportional to the amount of bound water, reflecting the difficulty of extracting water contained within the floc matrix.

SVI data showed a relationship with bound HMW ECP except for the filamentous sludge (see Figure 4.21). This correspondence coexisted with an almost identical relationship between SVI and bound HMW proteins. Because of the relatively small quantity of bound HMW carbohydrates it is not possible to assess their importance in determining settling characteristics relative to the bound HMW protein fraction from this data alone. Fitzgerald (14) observed that poor settling corresponded to an increase in both HMW proteins and carbohydrates. It is important to realize that a large portion of the ECP produced in this study existed in the form of bound proteins of high molecular weight. This non-filamentous sludge bulking may be the result of ECP overproduction and the fact that SVI corresponds with bound HMW ECP and specifically protein may be due to the fact that nitrogen rich systems such as those in this study produce high proportions of bound proteins (13).

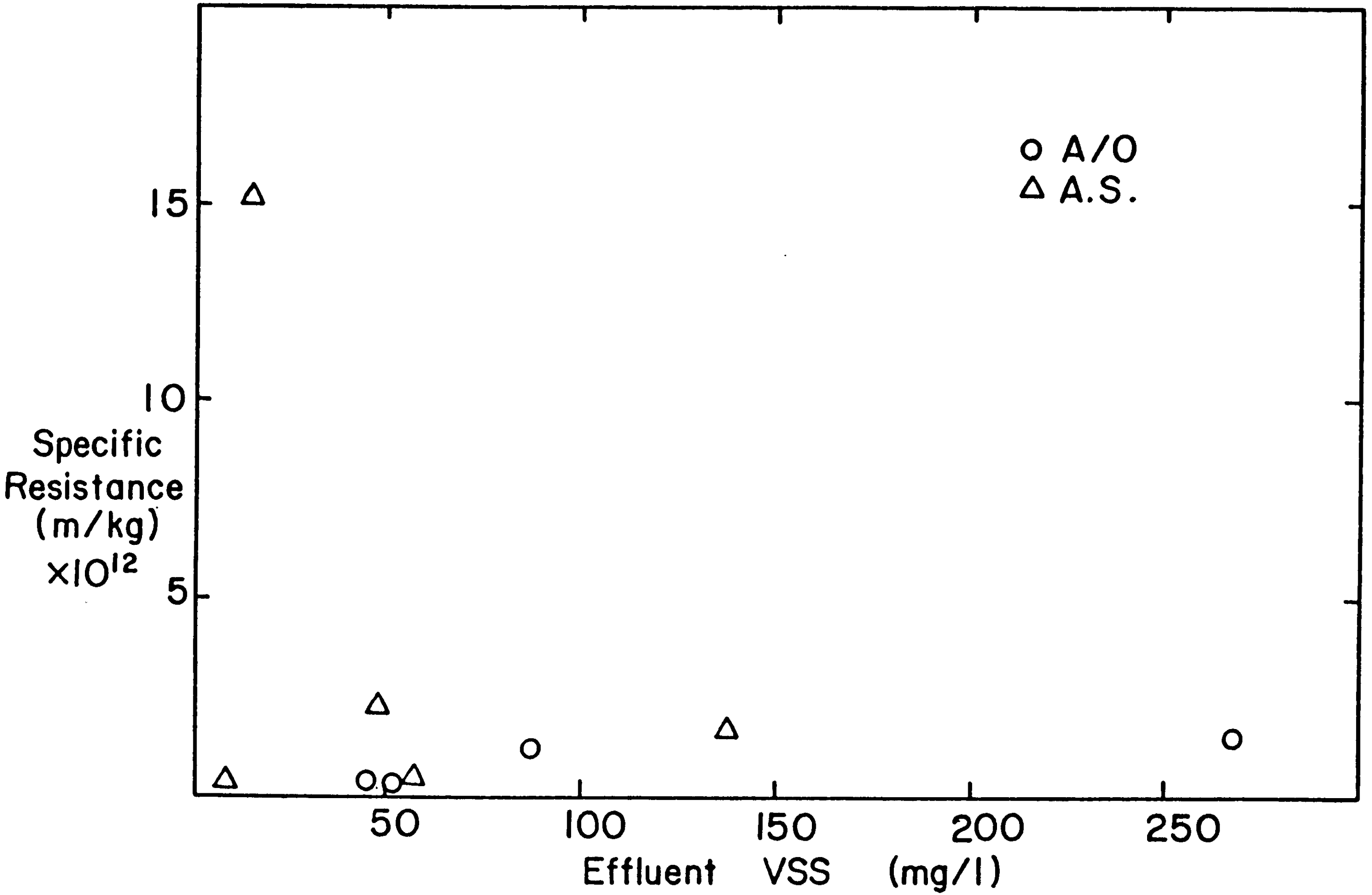

Figure 4.20 Specific Resistance Vs Effluent VSS

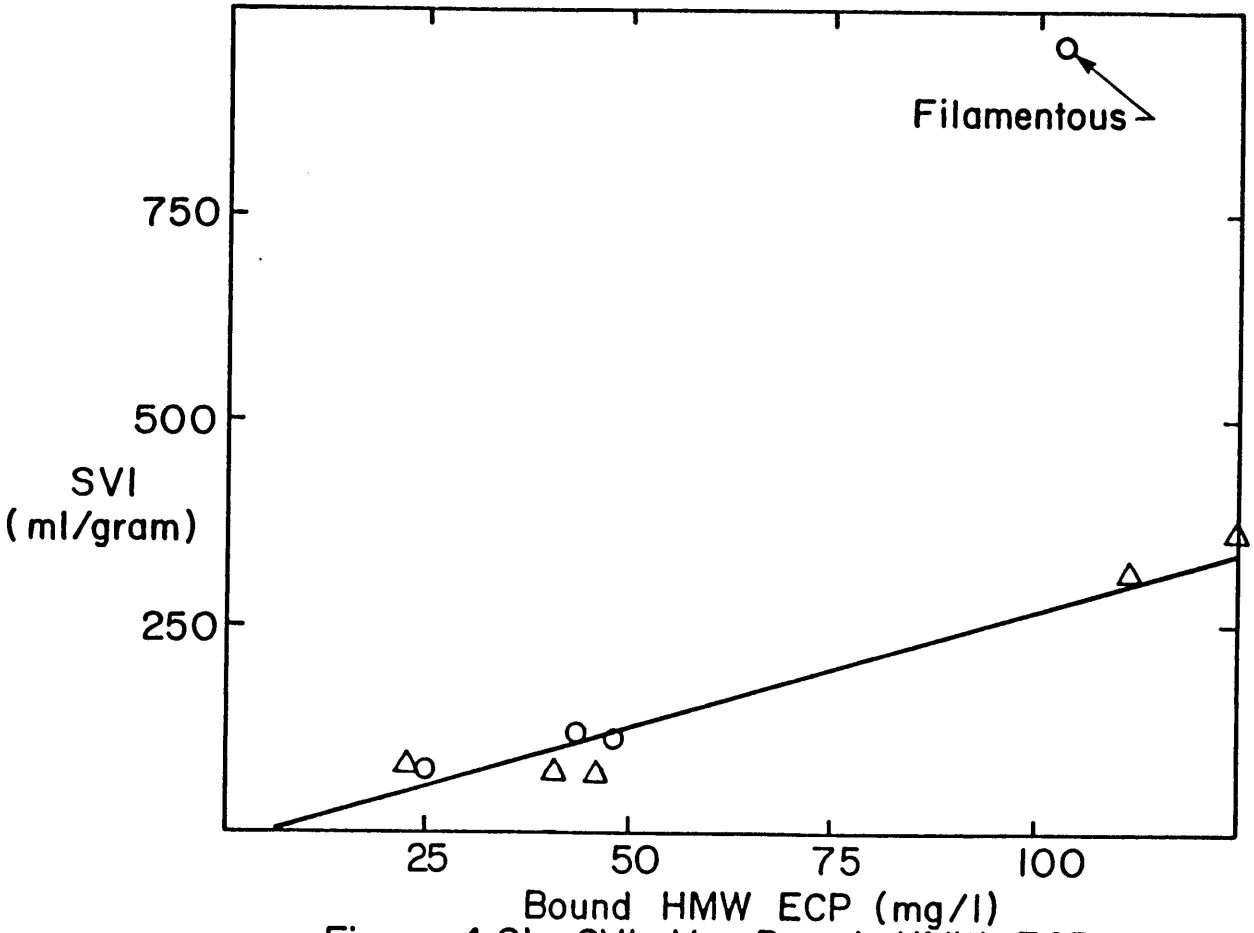

Figure 4.21 SVI Vs Bound HMW ECP

Although specific resistance (R*) corresponded with sludge age (see Figure 4.3), no relationship with ECP data was established. Increasing R* was shown to correspond with low amounts of bound ECP, and bound protein in particular, by Kajornatiyudh (19). As can be seen in Figures 4.22 and 4.23, this correspondence is neither confirmed or disputed by this study since no data were obtained for a sludge with low quantities of bound proteins and ECP. Fitzgerald (14) determined that R* was related to the amount of HMW ECP. In Figure 4.24, the data from this research show no relationship. Since Fitzgerald's data were normalized for mixed liquor concentration, R* was also compared to HMW ECP divided by MLVSS (see Figure 4.25). Normalized ECP concentrations did not yield any additional information or relationships in any other comparisons either and are thus not used in the analysis of this study. The greatest R* value, at 15.1x1012 m/kg, does not fit the data trends of the other sludges and no explanation can be offered for the unique dewatering properties of this sludge.

ECP data showed a relationship to settling (SVI) but did not show a consistent relationship to dewatering. The relationship of SVI with bound HMW ECP may reflect the increased incorporation of water in the floc structure. The bound HMW biopolymers may cause a more structurally stable floc capable of including a greater volume of water and decreasing the extent of settling possible. It was expected that this property of ECP would adversely affect dewatering as well but this is not reflected in the data. In addition, the contrast of the two A.S. sludges in this section showed marked differences in dewatering

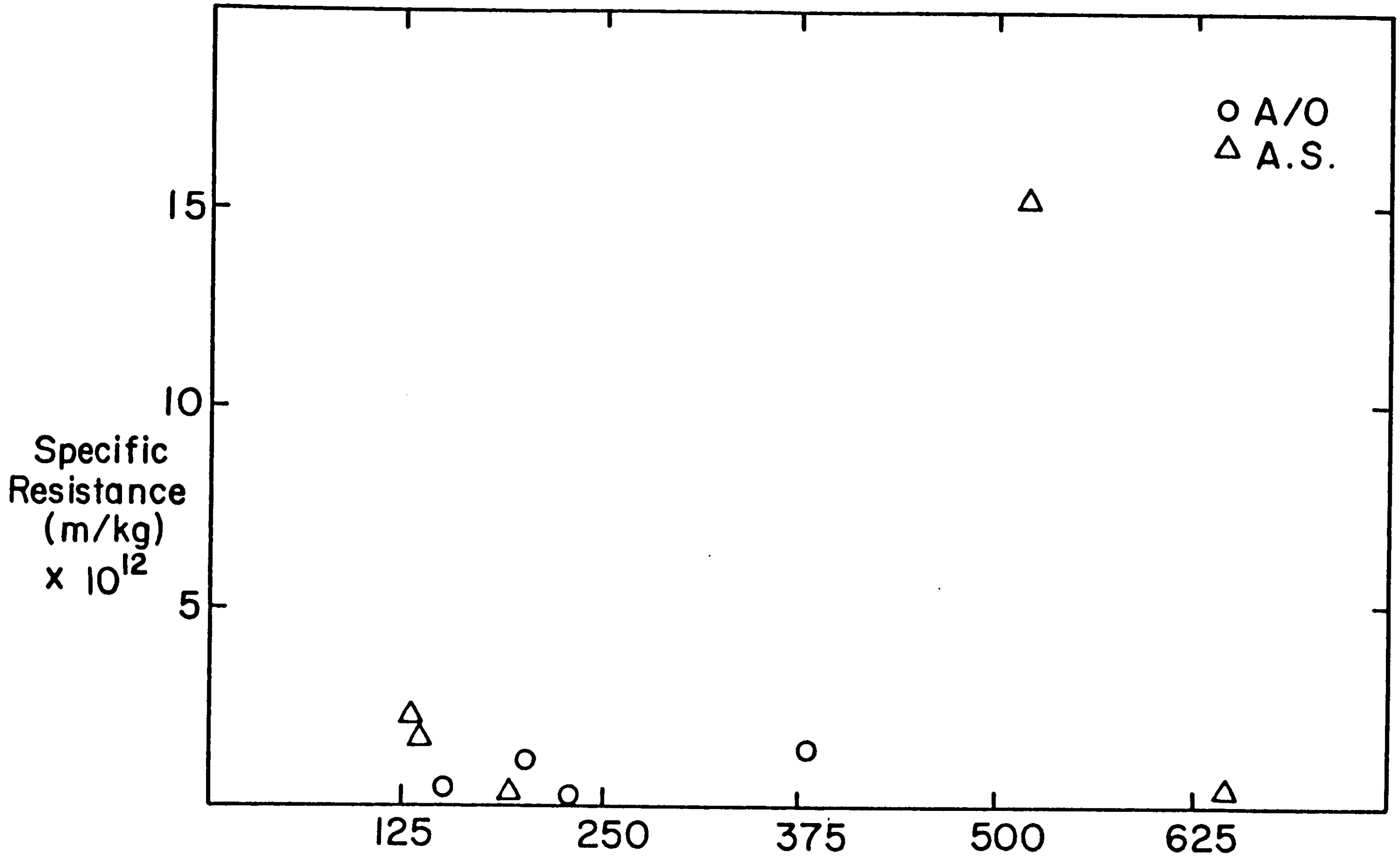

Figure 4.22 Specific Resistance Vs Bound ECP

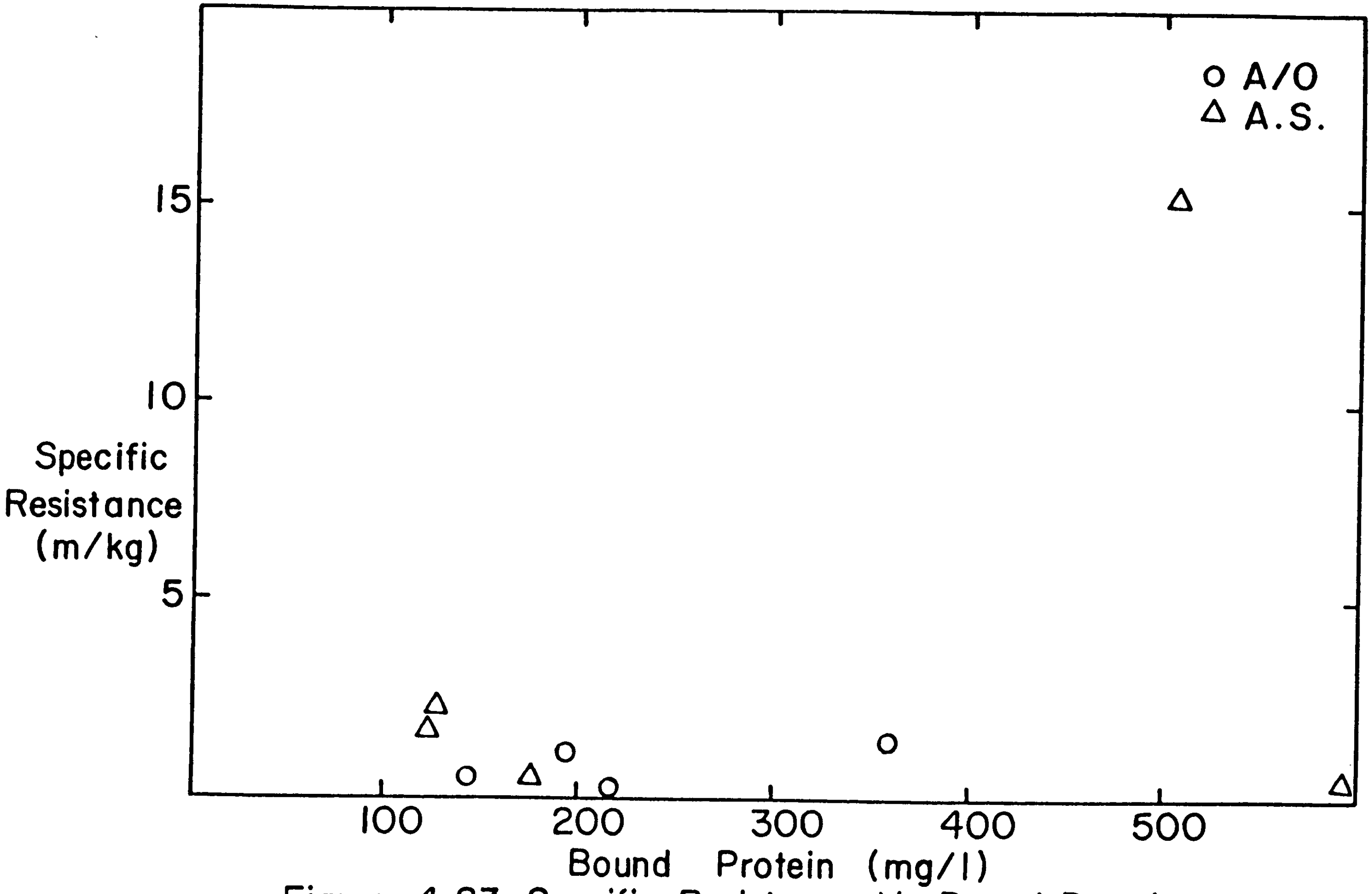

Figure 4.23 Specific Resistance Vs Bound Protein

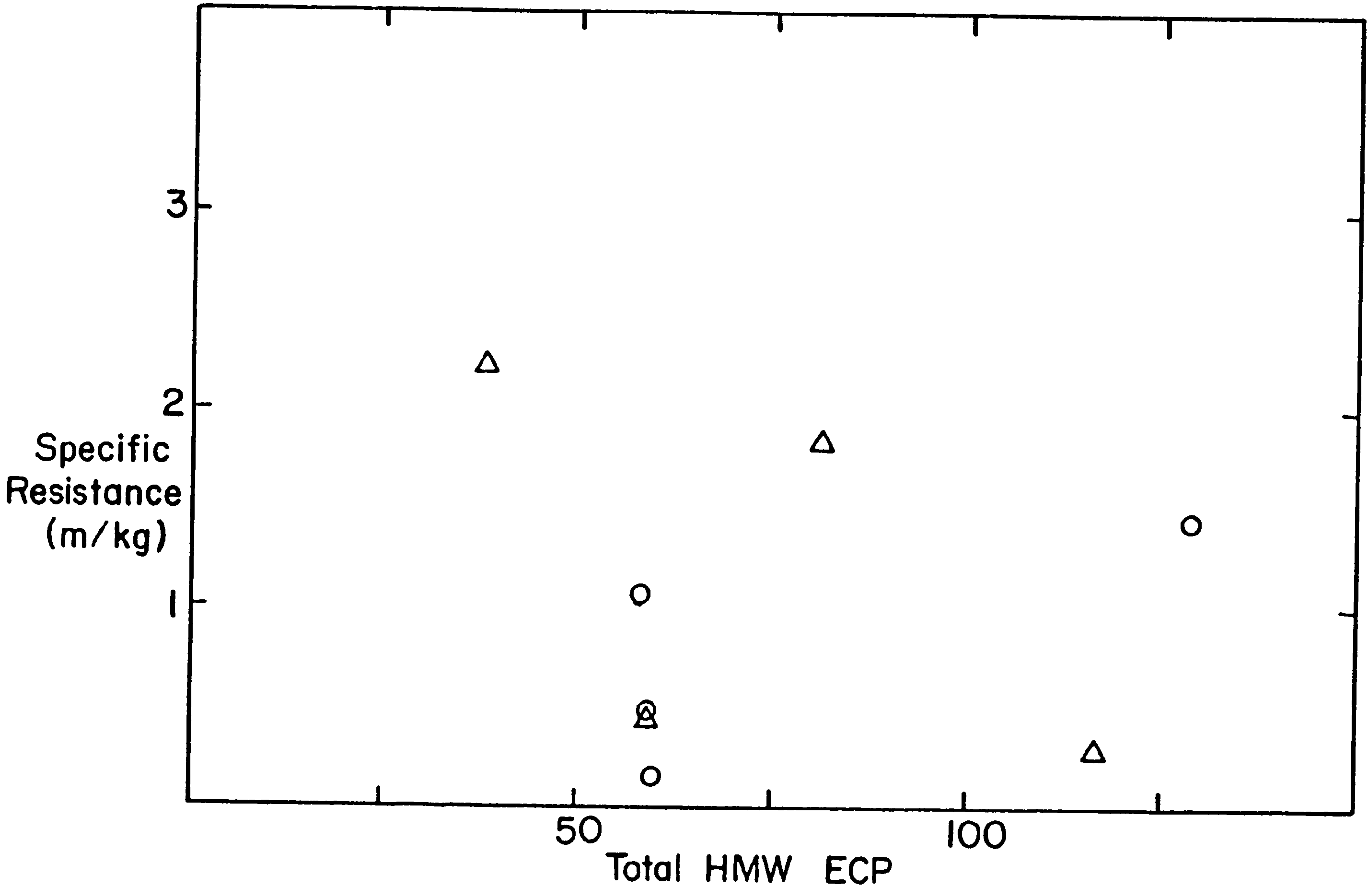

Figure 4.24 Specific Resistance Vs Total HMW ECP

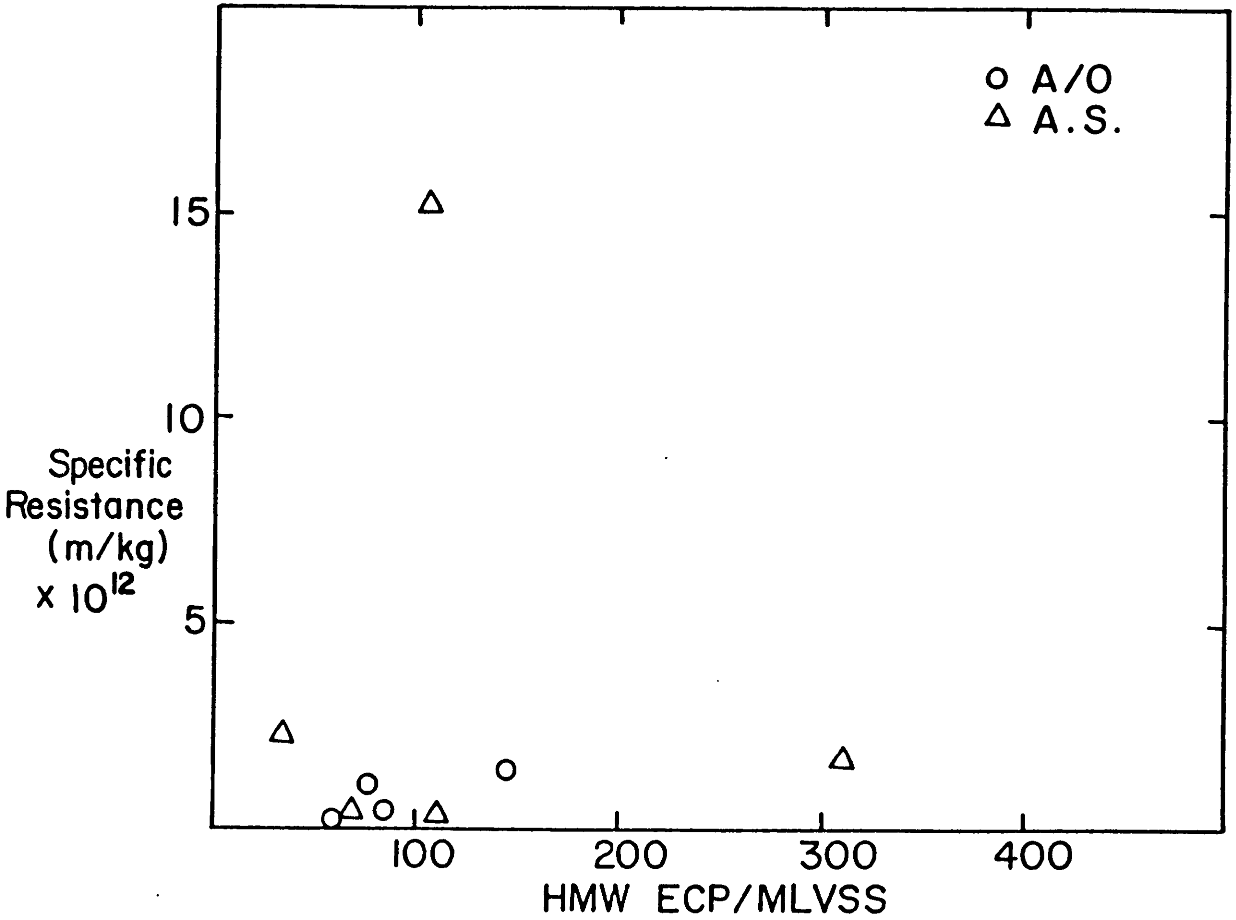

Figure 4.25 Specific Resistance Vs HMW ECP/MLVSS

characteristics for the two sludges but no related differences in ECP characteristics. It was expected that both settling and dewatering could be related to ECP characteristics and it is uncertain why this was not the case for dewatering.

V. CONCLUSIONS

The expected differences in settling and dewatering characteristics between the A/O and A.S. system which were reported by Tracy, et al. (5) were not observed in this study. Due to the relatively small quantity of data generated, as well as operational differences between laboratory and full-scale units, which allowed only short periods of steady-state data to be obtained for the laboratory units, this study may not be representative of full-scale A/O and A.S. systems, especially where operational parameters related to dewatering and settling are concerned. However, the A/O unit was found to remove excess phosphorus and alter cation concentrations in manners similar to full-scale units. Therefore, the data are expected to reflect trends found in operational units receiving a similar substrate.

The conclusions resulting from this study are as follows;

1. The A/O and A.S. systems yielded approximately equal values, at a given sludge age, for the dewatering characteristics (specific resistance and CST) measured in this study. Sludge dewatering characteristics improved with increasing sludge age for both systems.
2. ECP characteristics and quantity did not show any clear pattern relating to sludge age. Although both total and bound ECP quantities varied significantly with sludge age, there was no consistent increase or decrease in the quantity or in the proportion of bound ECP or any other trend which allowed prediction of ECP characteristics.

3. Differences in polymer concentration and distribution between bound and unbound fractions existed between the two systems but these had no discernible effect on sludge operational characteristics.
4. An increase in soluble effluent COD corresponded to increases in unbound ECP and effluent COD may be largely composed of unbound biopolymers.
5. High SVI values corresponded to an increase in bound HMW in the floc. It may be that a high bound HMW ECP fraction results in increased aggregate size and decreased solids concentration of the settled biomass.

VI. SUGGESTIONS FOR FUTURE STUDY

Effects of pH Alteration and Subsequent Neutralization

Adjustment of mixed liquor to pH eleven increased CST and ECP release (see Figure 6.1 and 6.2). Acidification of mixed liquor decreased CST and increased ECP release. As pH neared eleven, there was a large increase of both ECP and CST. Original CST values were recoverable with neutralization to the original pH. ECP release, in contrast, was irreversible, and additional ECP release occurred with neutralization of the pH 11 sample..

It may be that chemical and structural changes of exocellular ECP associated with pH alteration of mixed liquor cause changes in sludge dewatering properties (CST) rather than a simple relationship between the quantity of ECP in solution and the ability of the supernatant to pass through a filter. This would be a complicated area of research because it would be necessary to study structural and chemical changes in the biopolymers as well as molecular weights. Many techniques may be helpful including chromatography, with recent applicatiosn being described in the literature in the field of microbiology.

AVI and Floc Density Data

Velocity versus concentration data yielded three aggregate volume index (AVI) data points (see Figure 6.3). The AVI has been defined and described in detail by Knocke, et al. (44) and is the ratio of the volume occupied by sludge aggregates to the volume occupied by the dry

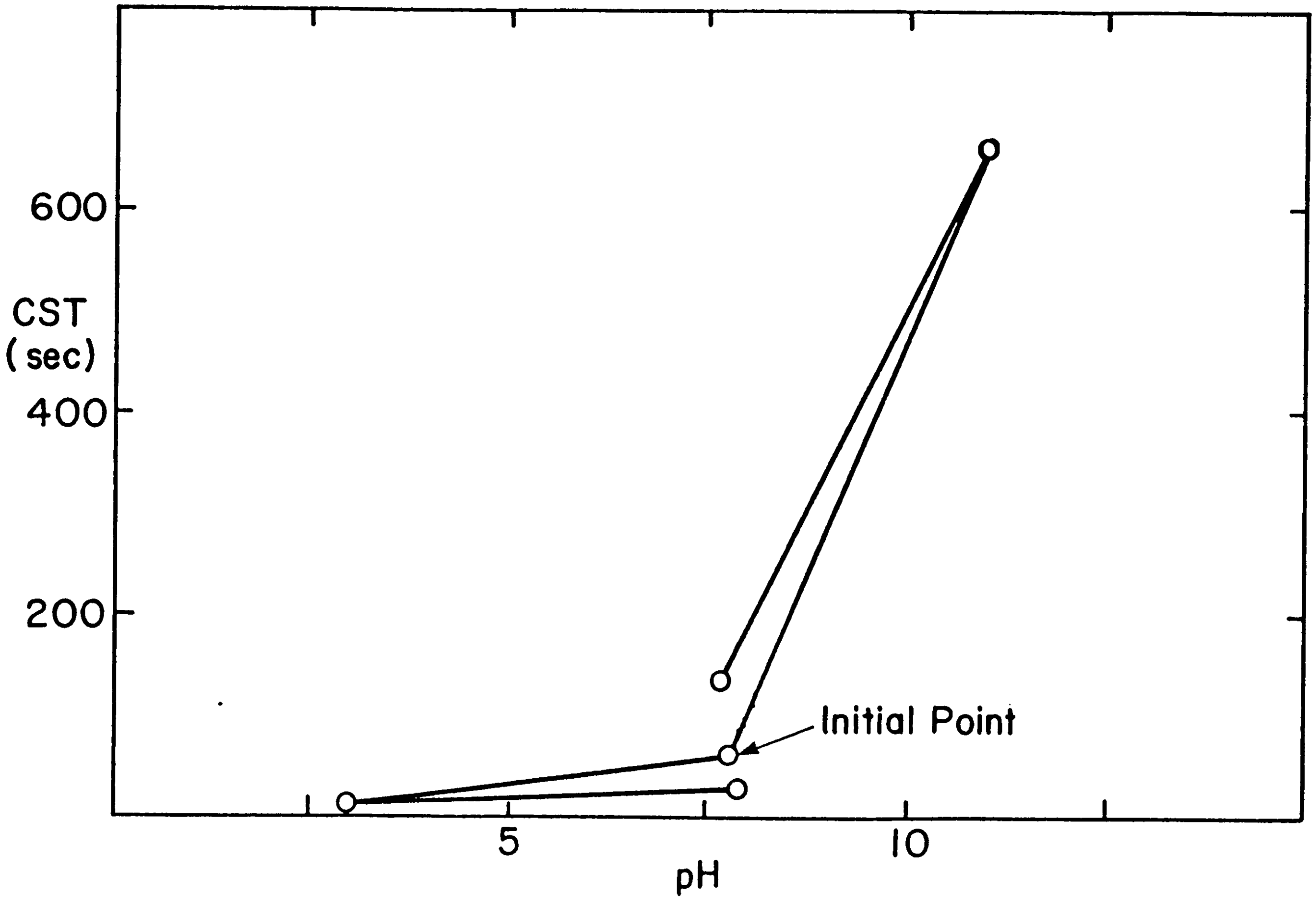

Figure 6.1 CST Vs pH for A.S. Mixed Liquor, Sludge Age 14.6 days

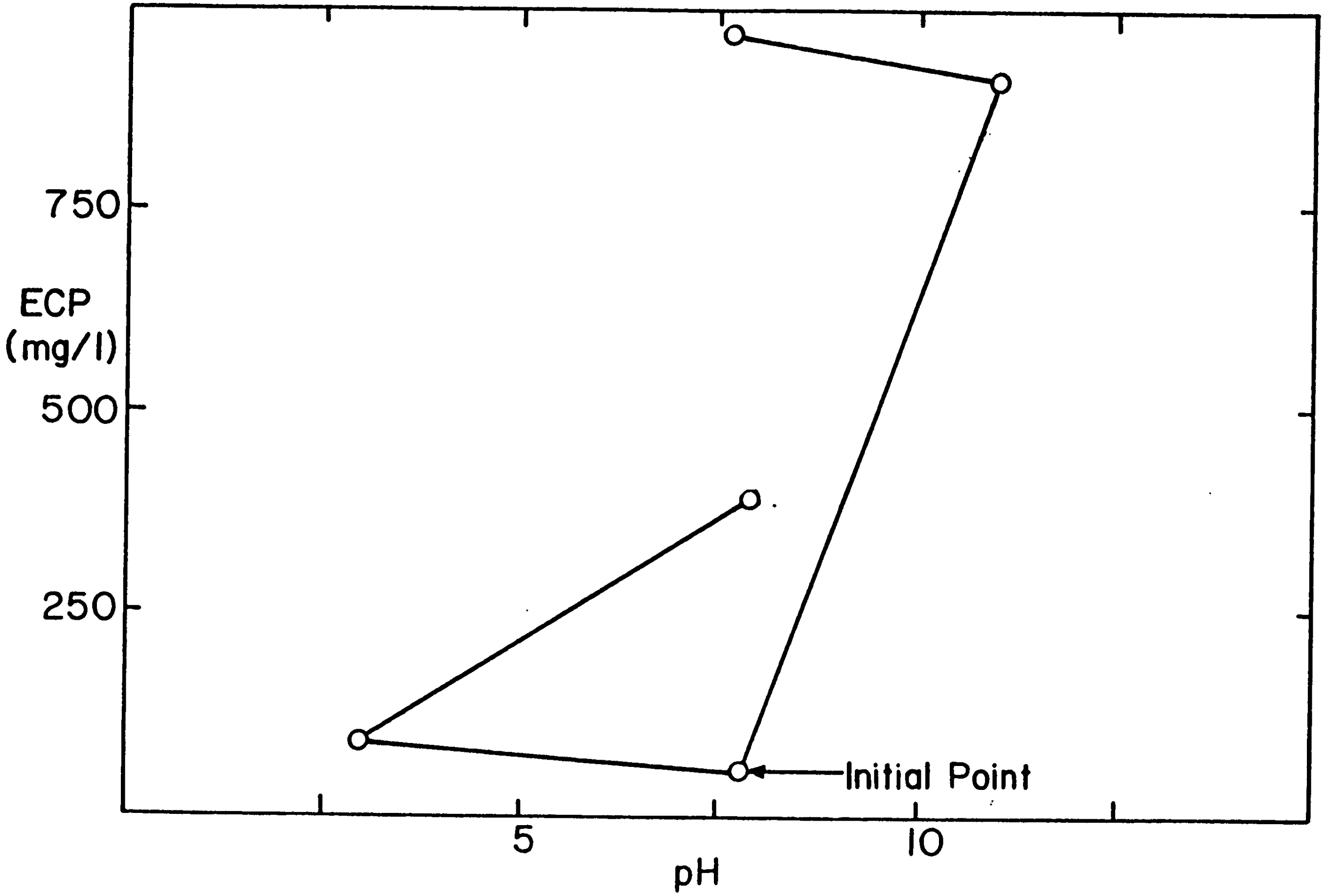

Figure 6.2 ECP Vs pH for A.S. Mixed Liquor, Sludge Age 14.6 days

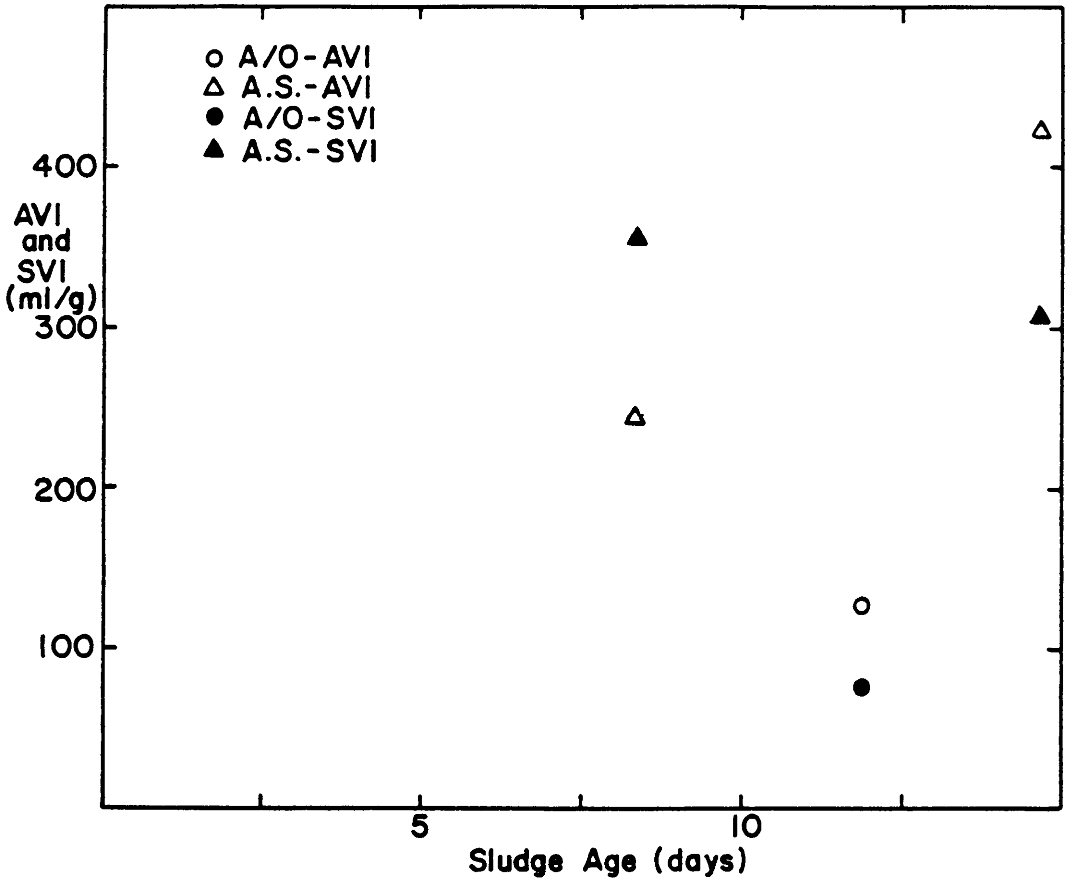

Figure 6.3 AVI and SVI Vs Sludge Age

sludge solids alone. SVI values in this study corresponded fairly well with the AVI data obtained. This is supportive of Knocke's (42) conclusion that SVI can serve as a "surrogate indicator" of floc water content.

Floc density data were taken for several sludges during the later part of the study. This data may be seen in Table 6.1.

Both AVI and floc density data are inconclusive because of the paucity of data collected in this study. However, these would be useful parameters to measure in future studies. AVI values in particular give a reliable estimation of floc density because they are not based on a single measurement.

Table 6.1. Floc Density Data

System	Sludge Age (days)	Floc Density (g/cm^3)
	2.9	1.02-1.03
A/O	3.3	1.02-1.03
	6.6	1.01-1.02
	4.2	1.01-1.02
A.S.	6.2	1.01-1.02
York River (A/O)	-	1.01-1.02
Blacksburg Regional (A.S.)	-	1.02-1.03

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APPENDIX A

Table A.1. Cation Data

	System-Sludge Age(days)	Anaerobic Release	Net System Uptake
	A/O		
Phosphorus (mg/day)	2.9	38	45
	3.3	470	35
	6.6	83	79
	11.9	-14	74
MG (mg/day)	2.9	-11	47
	3.3	100	5
	6.6	-34	34
	11.9	-30	53
K (mg/day)	2.9	-20	- 2
	3.3	148	28
	6.6	13	- 3
	11.9	47	0
Ca (mg/day)	2.9	4	3
	3.3	-2	2
	6.6	-25	7
	11.9	-19	0
Na (mg/day)	2.9	34	-62
	3.3	-23	69
	6.6	-62	-48
	11.9	195	-38

(continued)

Table A.1. Cation Data (Cont'd)

	System-Sludge Age(days)	Anaerobic Release	Net System Uptake
	A.S.		
Phosphorus (mg/day)	1.7		286
	4.2		7
	6.2		54
	8.3		11
	14.6		-76
Mg (mg/day)	1.7		45
	4.2		-12
	6.2		35
	8.3		- 5
	14.6		-60
K (mg/day)	1.7		-21
	4.2		- 3
	6.2		-17
	8.3		12
	14.6		-14
CA (mg/day)	1.7		- 2
	4.2		3
	6.2		14
	8.3		-26
	14.6		-12
NA (mg/day)	1.7		-93
	4.2		17
	6.2		-52
	8.3		3
	14.6		-90

Table A.2. TOC and COD Data

	TOC			COD		
System-Sludge Age (days)	Influent (mg/L)	Anaerobic Effluent (mg/L)	System Effluent (mg/L)	Influent (mg/L)	Anaerobic Effluent (mg/L)	System Effluent (mg/L)
A/O						
2.9	213.8	127.3	16.9	507	278	60
	219.0	136.0	16.1	553	256	53
3.3	-	24.5	17.3	515	108	68
	239.0	39.8	13.0	575	112	56
6.6	220.6	58.7	17.2	450	152	72
	225.2	50.7	19.4	450	124	88
11.9	221.6	66.8	20.4	517	156	62
	226.8	69.0	19.7	517	144	82
A.S.						
1.7	221.6		17.3	517		125
	226.8		17.1	517		117
4.2	-		30.2	515		68
	239.0		29.5	575		104
6.2	220.6		22.9	450		64
	225.2		14.2	450		88
8.3	-		-	571		101
	222.8		14.7	562		50
14.6	-		-	571		101
	222.8		12.6	562		43

Table A.3. Ortho-Phosphorus Data

Sludge Age (days)	Ortho-Phosphorus ($mg/L-PO_4$) Influent (mg/L)	Anaerobic Effluent (mg/L)	System Effluent (mg/L)
A/O			
2.9	26.3	26.0	19.3
	26.4	25.7	24.7
	27.4	25.0	19.7
3.3	21.8	48.8	15.7
6.6	--	18.7	21.8
	31.1	27.8	17.3
11.9	--	17.0	15.0
	34.2	25.2	21.3
A.S.			
1.7	--	--	11.5
	34.2	--	17.6
4.2	21.8	--	20.5
6.2	--	--	19.2
	31.1	--	21.7
8.3	19.4	--	12.5
	17.7	--	13.5
14.6	19.4	--	25.4
	17.7	--	31.0

Table A.4. Operational Parameters

System	Sludge Age (days)	Wastage Volume (liters)	Recycle Rate (%)	Hydraulic Detention Time (days)
A/O	2.9	1.000	39	1
	3.3	3.450	167	1
	6.6	1.725	167	1
	11.9	1.000	167	1
A.S.	1.7	1.000		1
	4.2	3.450		1
	6.2	1.725		1
	8.3*	1.000		1
	14.6	1.000		1

*Reactor volume was 8.5 liters.

Table A.5. HMW and LMW Peak Elution Vaolumes
(Simultaneous runs are grouped together)

System	Sludge Age (days)	Elution Volume (ml)	
		Peak One	Peak Two
A/0	3.3	4.5-9	18-22.5
A.S.	4.2	4.5-9	18-22.5
A/0	6.6	5.5-11	16.5-22
A.S.	6.2	5.5-11	16.5-22
A/0	11.9	5-10	20-25
A.S.	1.7	5-10	20-25
A/0	2.9	5-10	20-25
A/0	14.6	5-10	20-25
A.S.	8.3	5-10	25-30

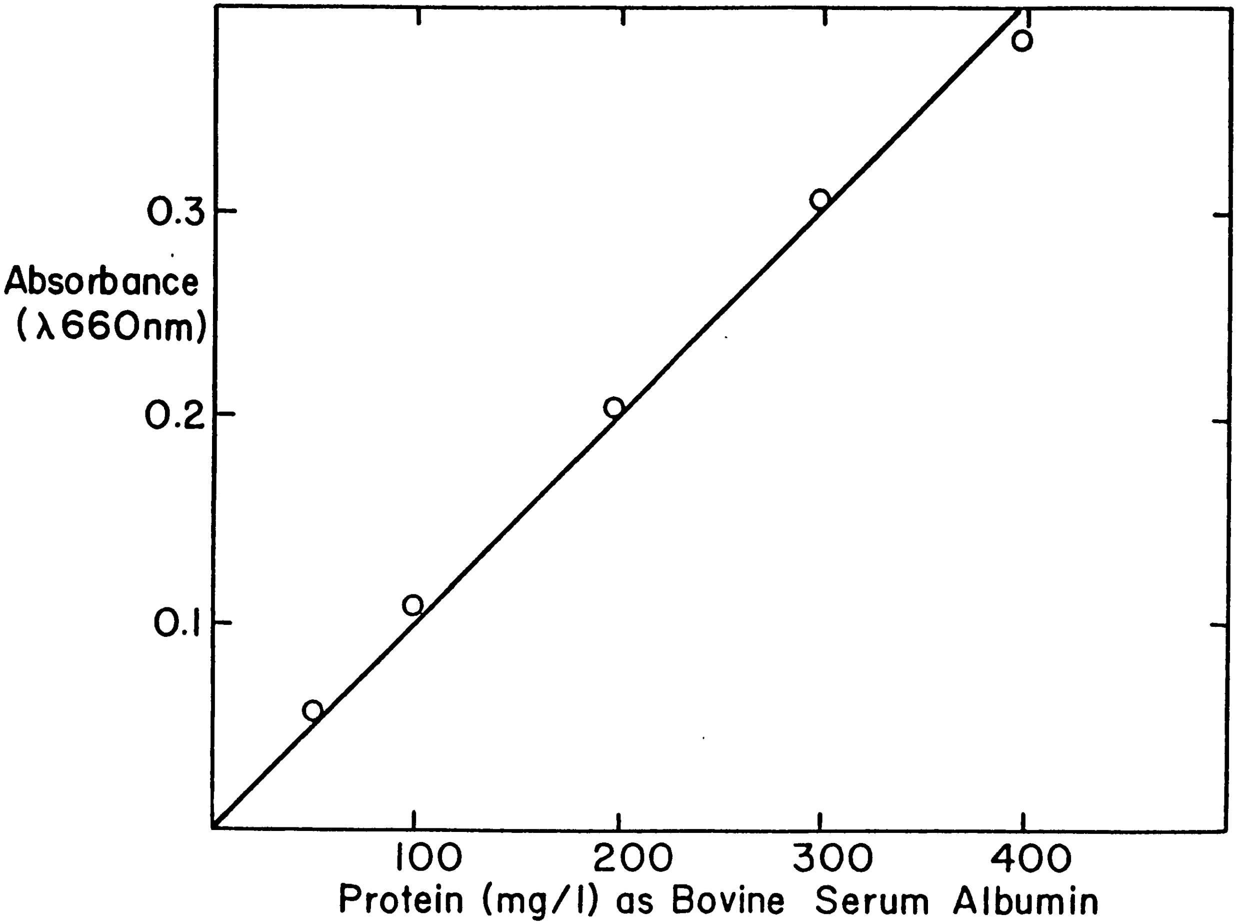

Figure A.1 Standard Curve For Protein Deteimination

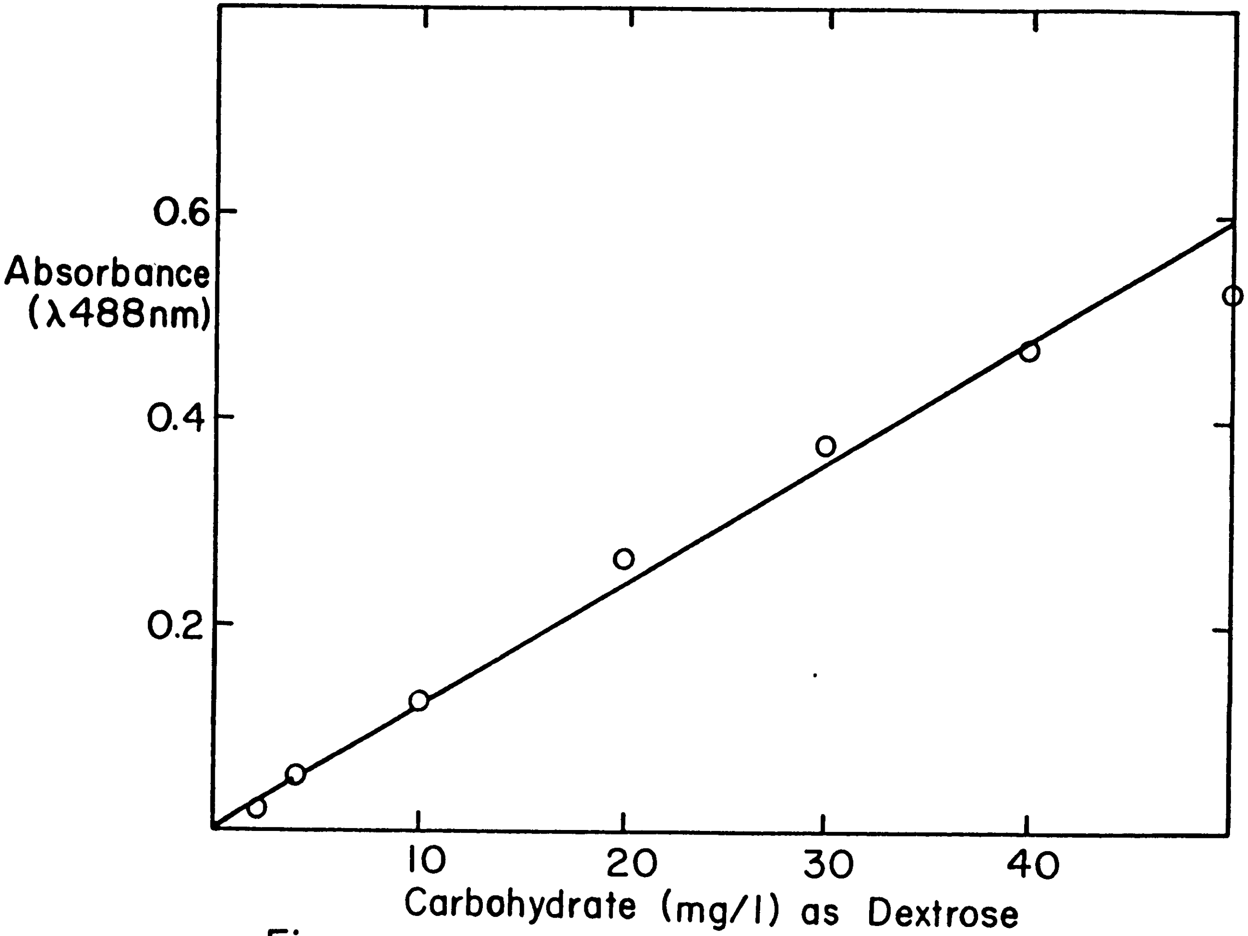

Figure A.2 Standard Curve For Carbohydrate Deteimination

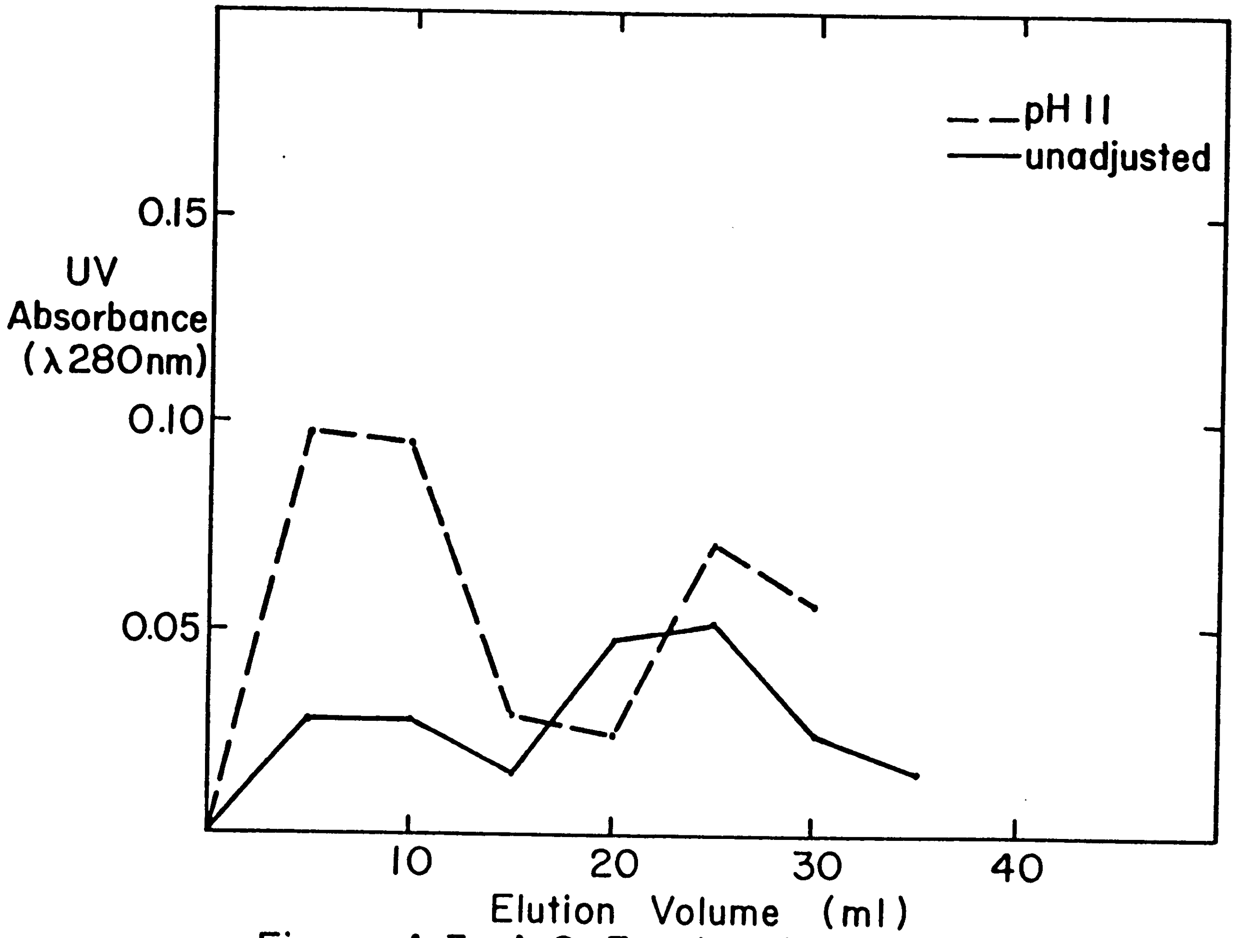

Figure A.3 A.S. Fractionation Data, Sludge Age 1.7 days

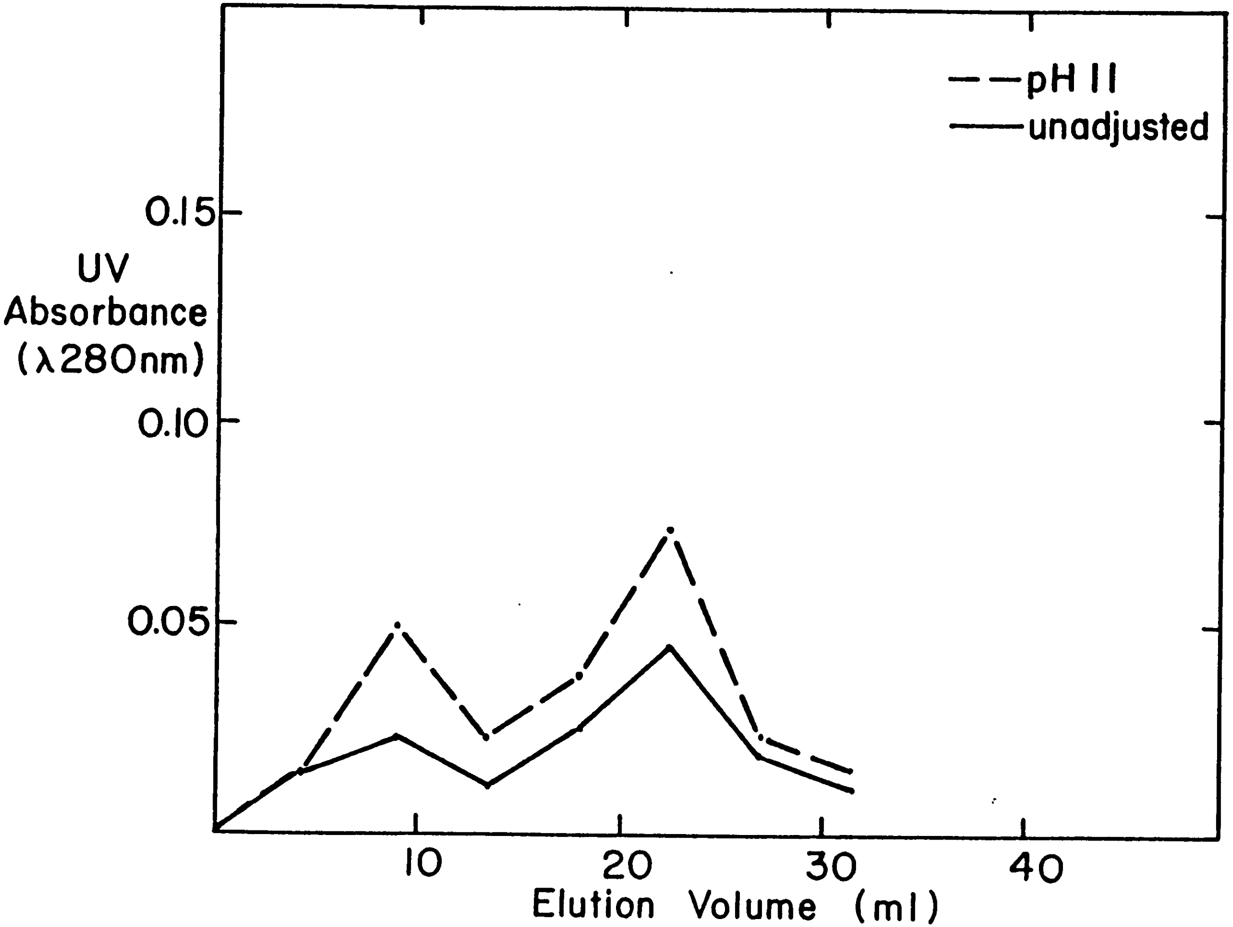

Figure A.4 A.S. Fractionation Data, Sludge Age 4.2 days

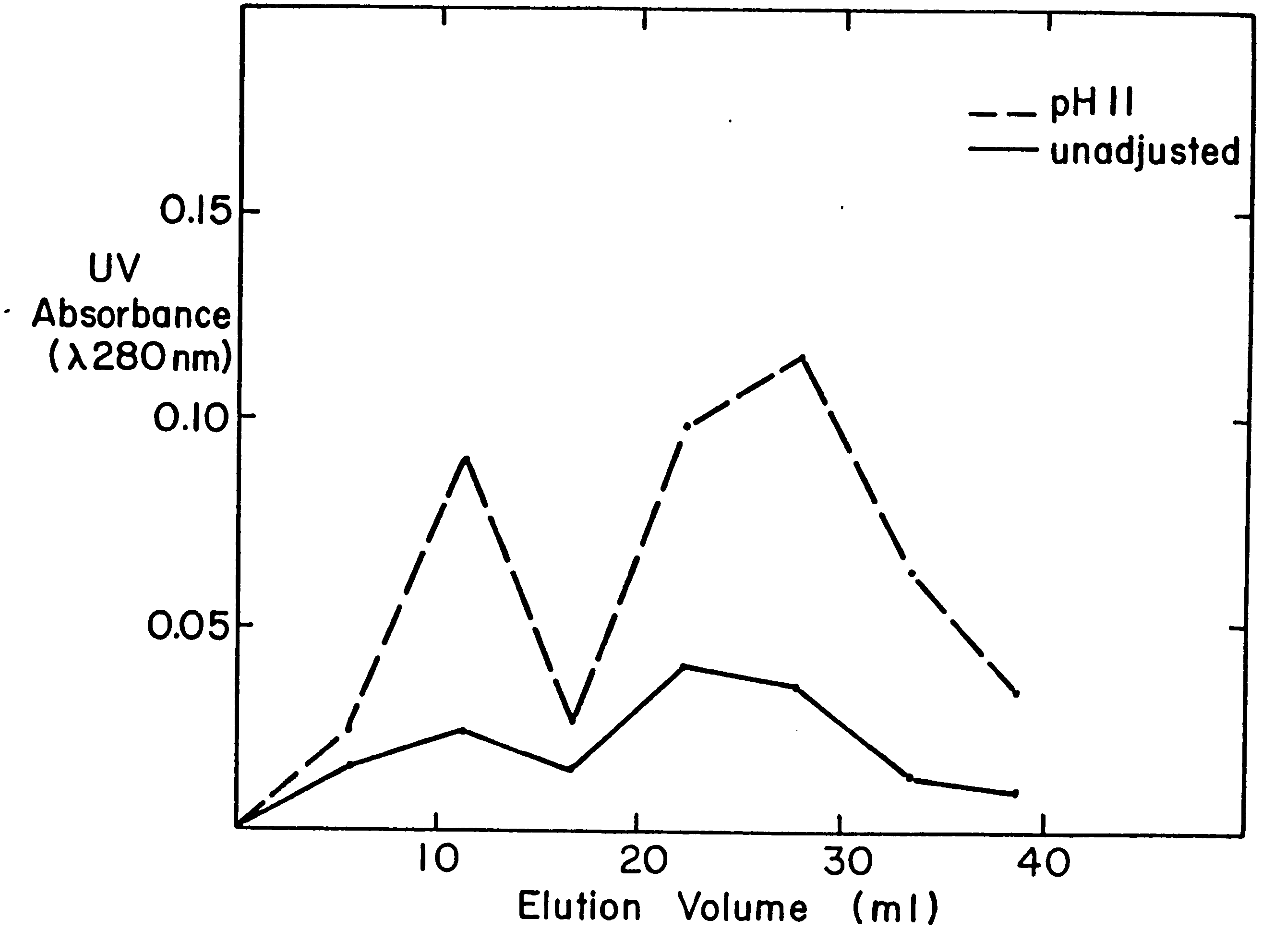

Figure A.5 A.S. Fractionation Data, Sludge Age 6.2 days

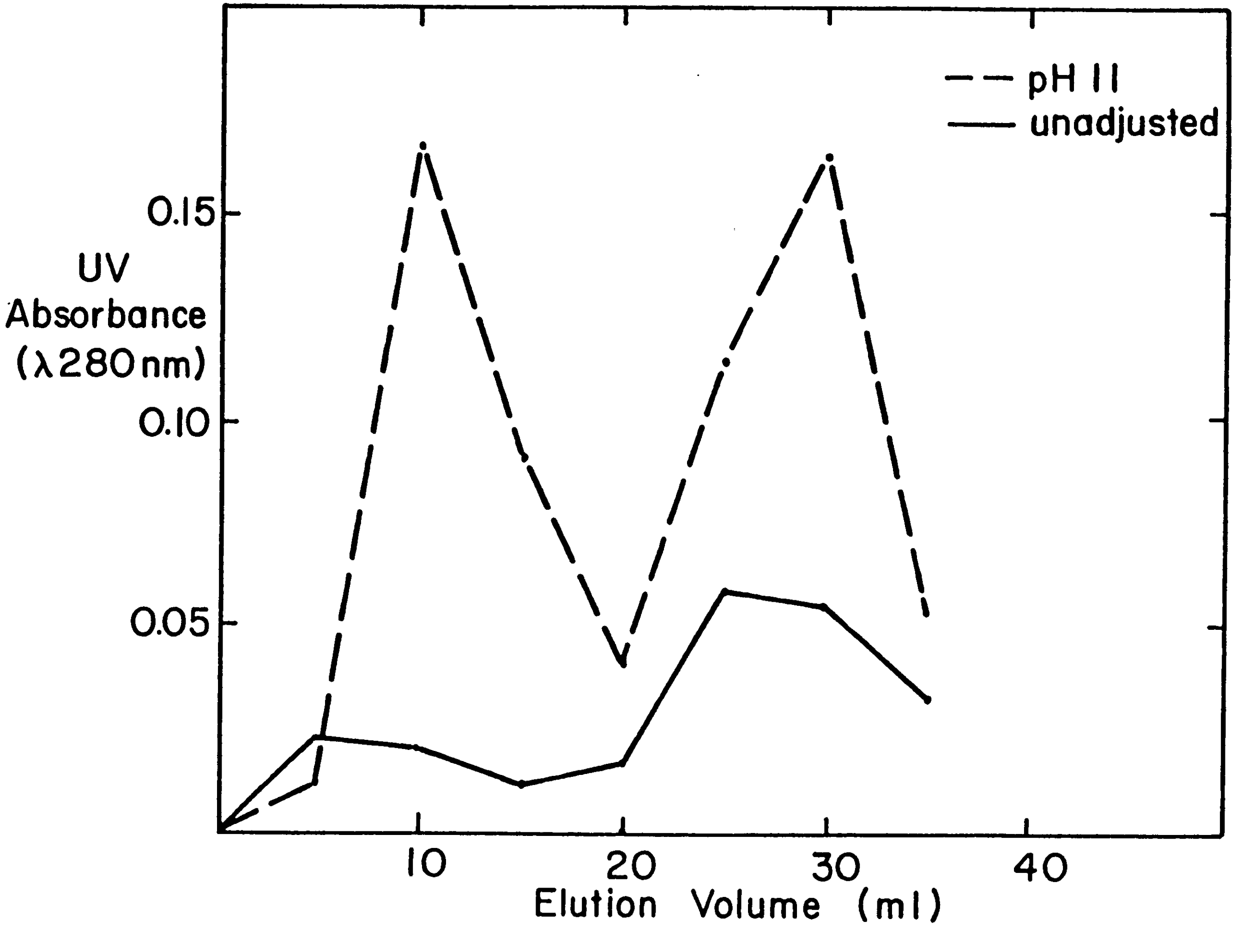

Figure A.6 A.S. Fractionation Data, Sludge Age 8.3 days

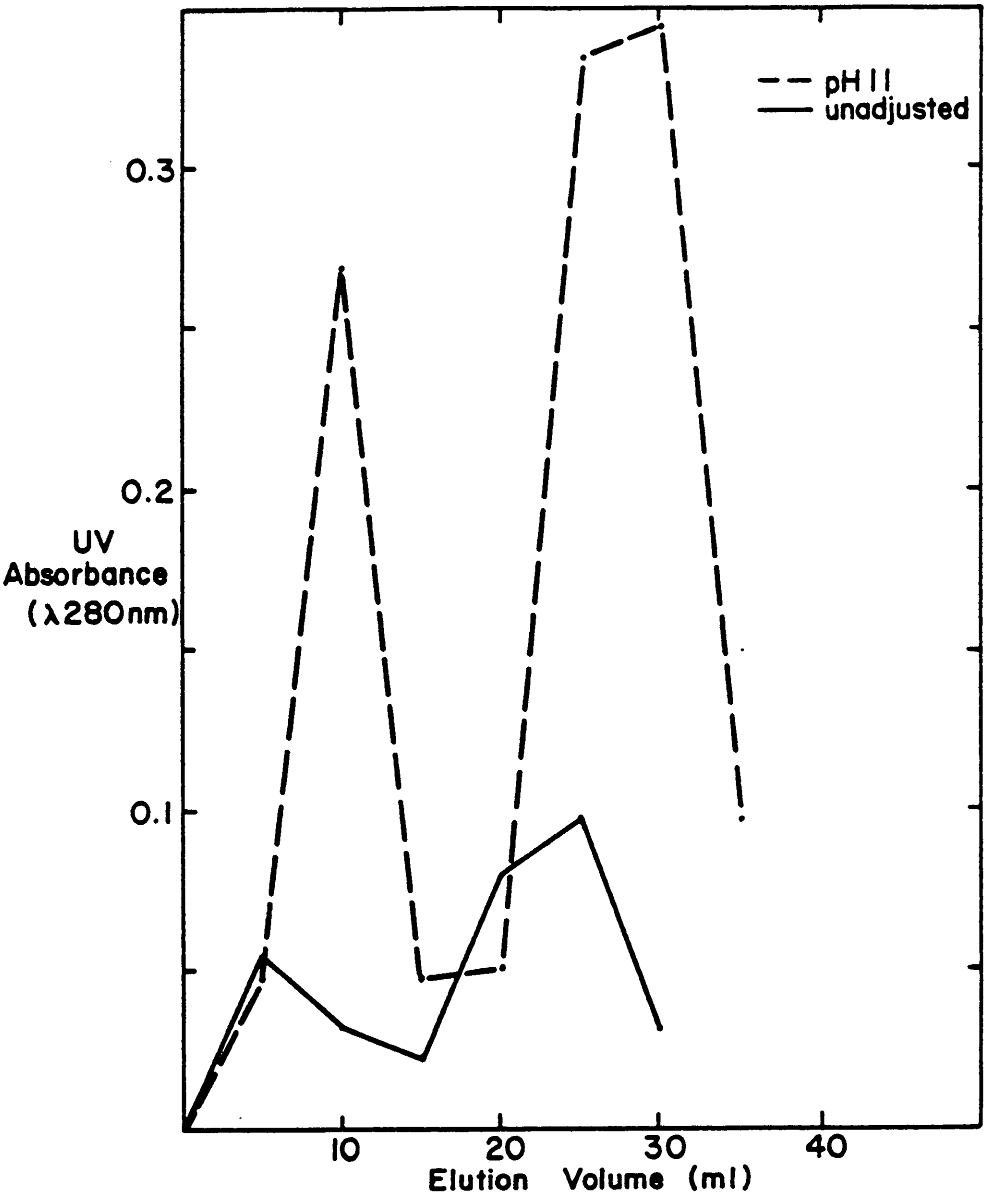

Figure A.7 A.S. Fractionation Data, Sludge Age 14.6 days

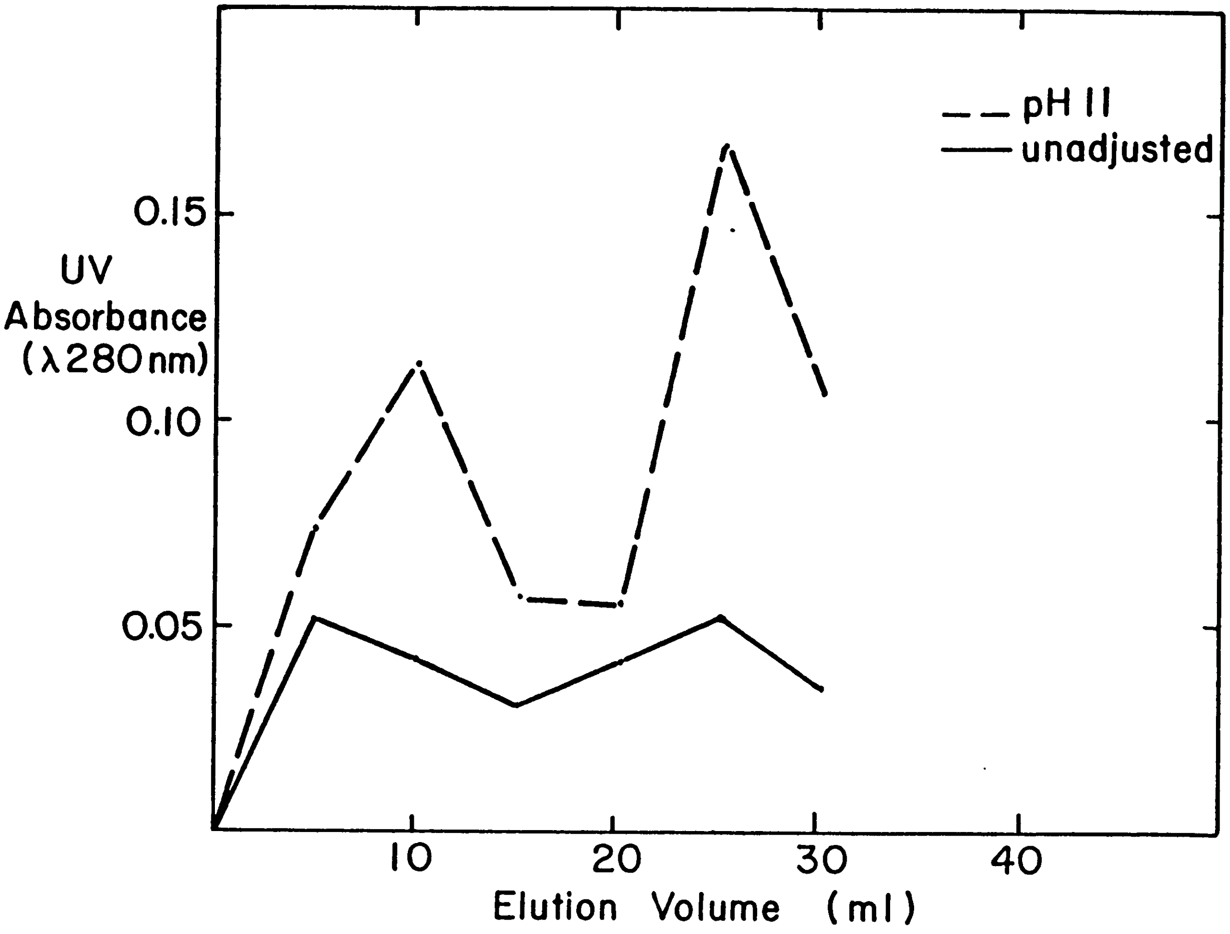

Figure A.8 A/O Fractionation Data, Sludge Age 2.9 days

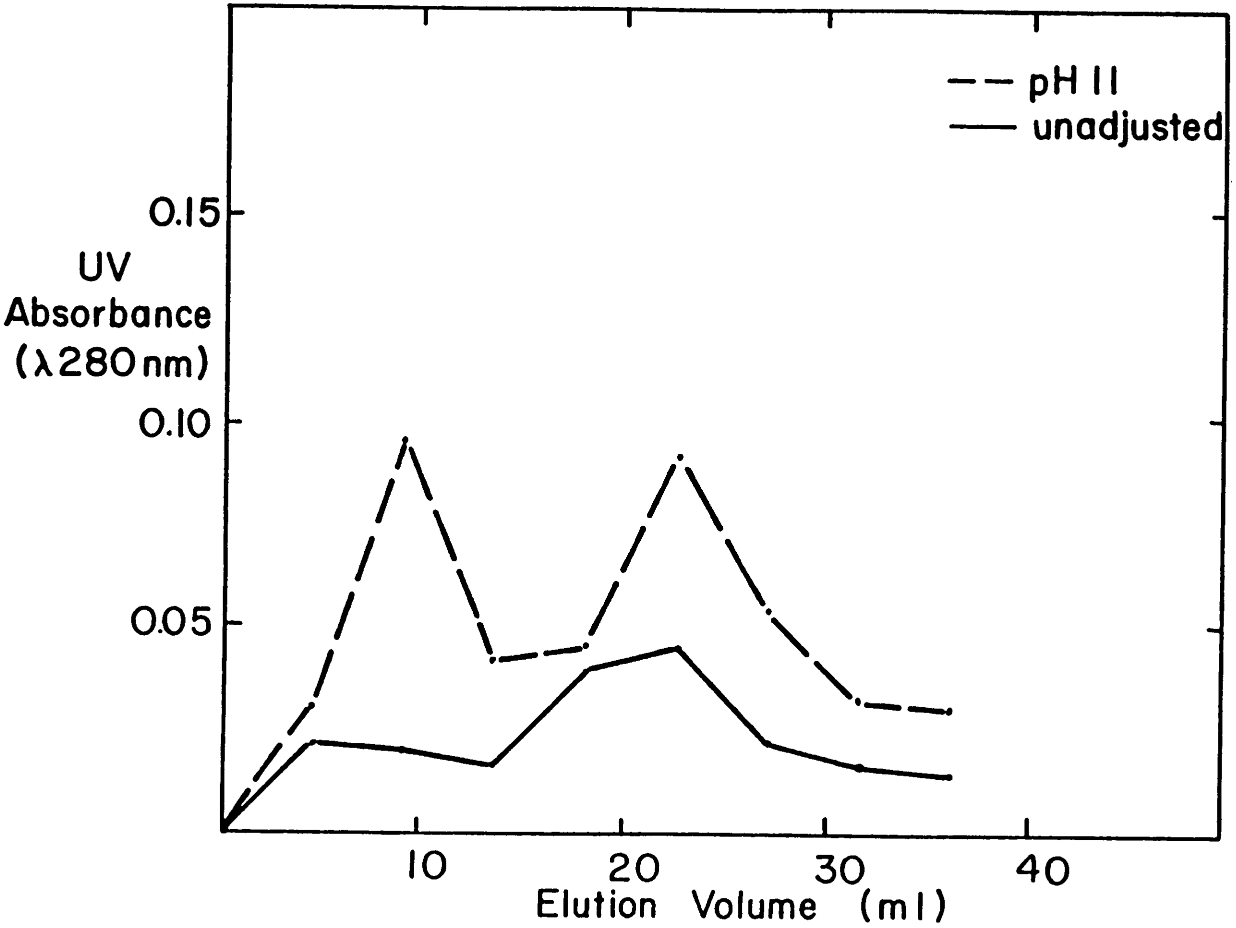

Figure A.9 A/O Fractionation Data, Sludge Age 3.3 days

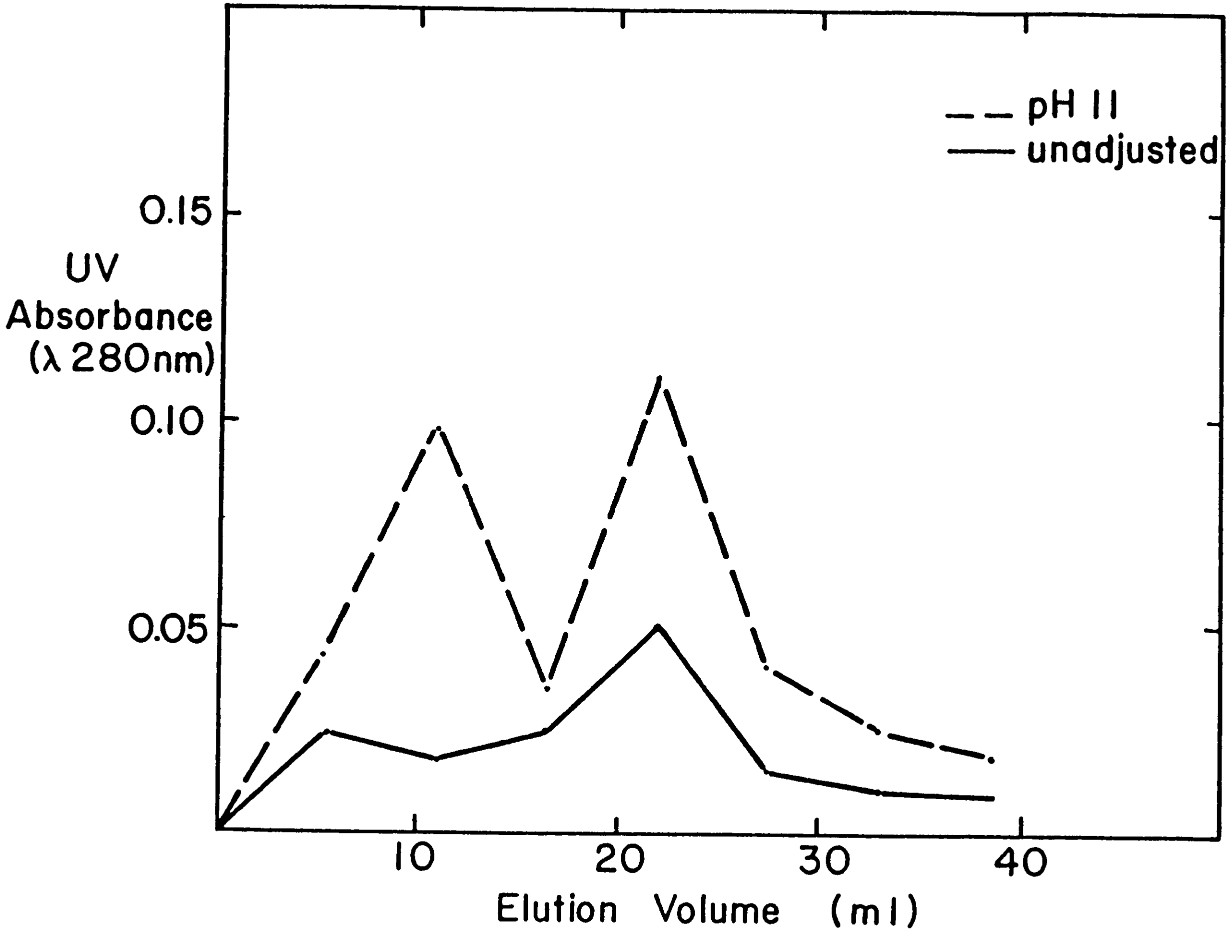

Figure A.10 A/O Fractionation Data, Sludge Age 6.6 days

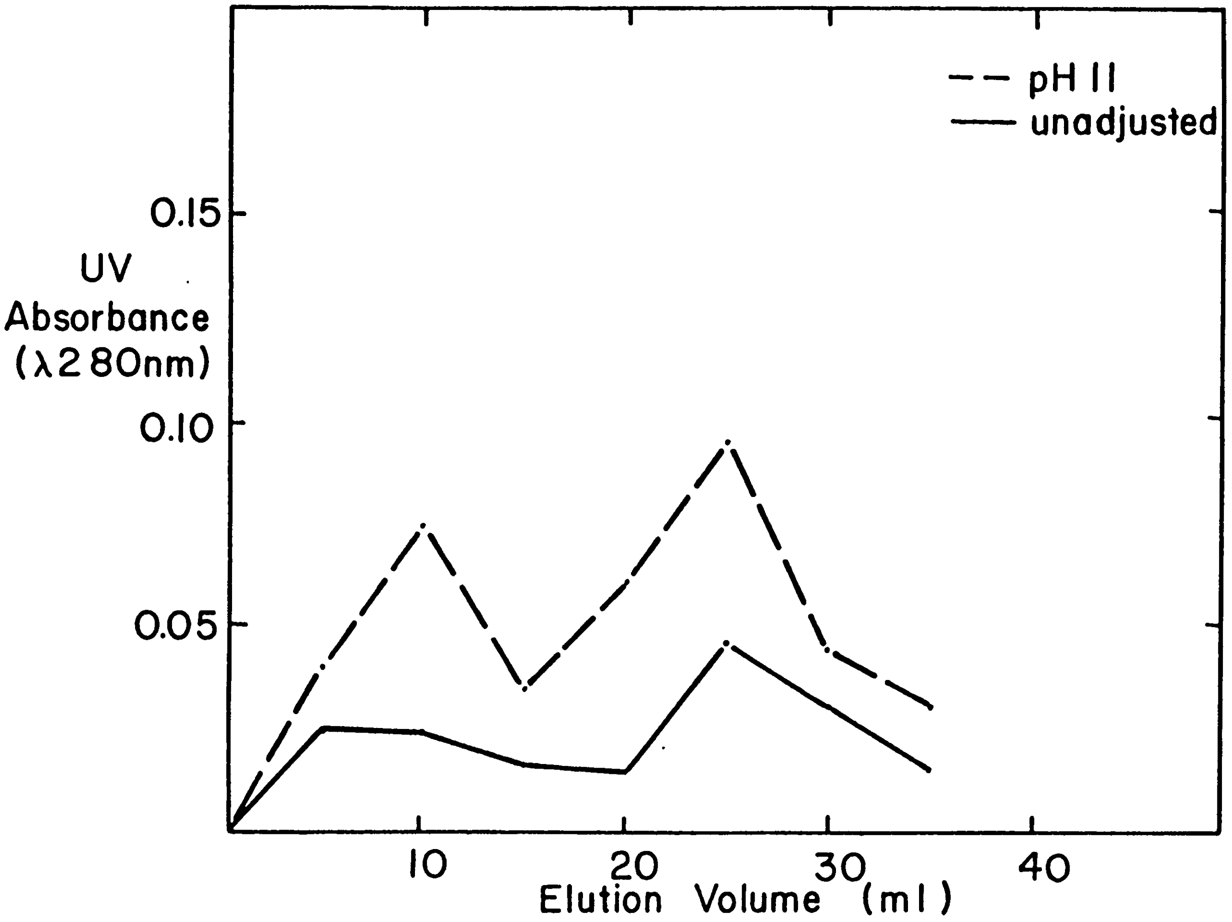

Figure A.11 A/O Fractionation Data, Sludge Age 11.9 days

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