MECHANISMS OF CONTACT STABILIZATION

SUBSTRATE REMOVAL

by

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CHAPTER I

INTRODUCTION

The efficacy of the contact stabilization process has been a focal point of controversy for many years. Since its origin in the 1950's, the process has been used to treat both domestic and industrial wastewaters successfully and, many times, unsuccessfully. This unreliability in the performance of a contact stabilization system has constituted one of the major criticisms of the process.

The contact stabilization modification has been historically used for treatment of wastewaters high in colloidal organics. From observation it can be seen that the process accomplishes rapid removal of the colloids from the influent wastewater by mixing the wastewater with activated sludge for a relatively short period of time (contact phase). It has been postulated that the colloids are adsorbed and/or enmeshed to the activated sludge flocs. Then, the colloid-sludge mixture is settled and pumped to another tank and aerated to accomplish complete utilization of the organic colloids (stabilization phase).

The validity of the above scenario has been questioned by several investigators. One of the best documented arguments has been that the adsorption or enmeshment phase does not occur for all types of colloids. Inspection of the process clearly reveals that if no physical removal of the organic colloids occurs, a contact stabilization system would surely fail. Therefore, a principal thrust of this investigation was to study the relationship between the nature

of the colloidal substrate and its amenability to treatment by contact stabilization, i.e., sorption.

Further attention has been focused upon the manner of colloidal substrate utilization by activated sludge. The original supposition was that a rapid physical removal of the organic colloids occurred followed by an increase of measurable soluble substrate as utilization began. This increase has been attributed to enzyme hydrolysis of the colloidal organics. The original design of the contact stabilization system was based on this substrate "uptake and release" phenomenon; however its existence has also been disputed. Additional information concerning this dispute was also obtained in this study.

Finally, contact stabilization has boasted the advantage of a better settling activated sludge and superior effluent quality. This has been widely reported throughout the literature and in the field. The most common explanation for this phenomenon has been that the activated sludge "conditions" itself in the stabilization basin by producing exocellular polymers. These biopolymer help agglomerate the dispersed bacteria in the activated sludge into larger settling masses thereby bettering its settling properties. Another possible explanation was investigated in this study dealing with the "conditioning" of the sludge by the organic colloids.

With this introduction in mind, the direction this research took was to investigate the fundamental reactions taking place for a variety of substrates when mixed with activated sludge. The

reactions monitored were both of a physical and metabolic nature. Specific intentions of this investigation were:

1) To evaluate the metabolic responses of activated sludge cultures when fed chemically and physically different colloidal organics.
2) To determine if the addition of small amounts of soluble substrate would affect the metabolic utilization of the organic colloids.
3) To qualify, as well as, quantify the adsorption or enmeshment of colloidal substrates occurring in activated sludge.
4) To evaluate conditions that may affect the physical removal of the organic colloids.
5) To suggest possible guidelines for the use of the contact stabilization design in practice, i.e. what substrate characteristics warrant the use of this activated sludge modification.

CHAPTER II

CONTACT-STABILIZATION PROCESS

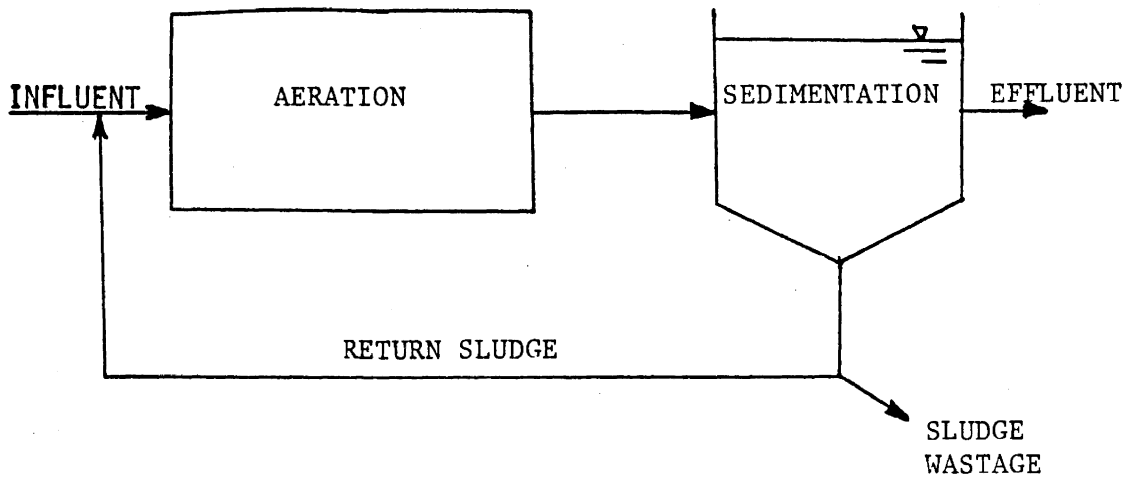
Description and Origin of Contact-Stabilization Process

The contact-stabilization process is one of the many variations of the activated sludge process. Commonly, a typical activated sludge system will involve a single aeration step whereas the contact-stabilization process uses a two-step approach. Figure II-1 illustrates this sequential arrangement.

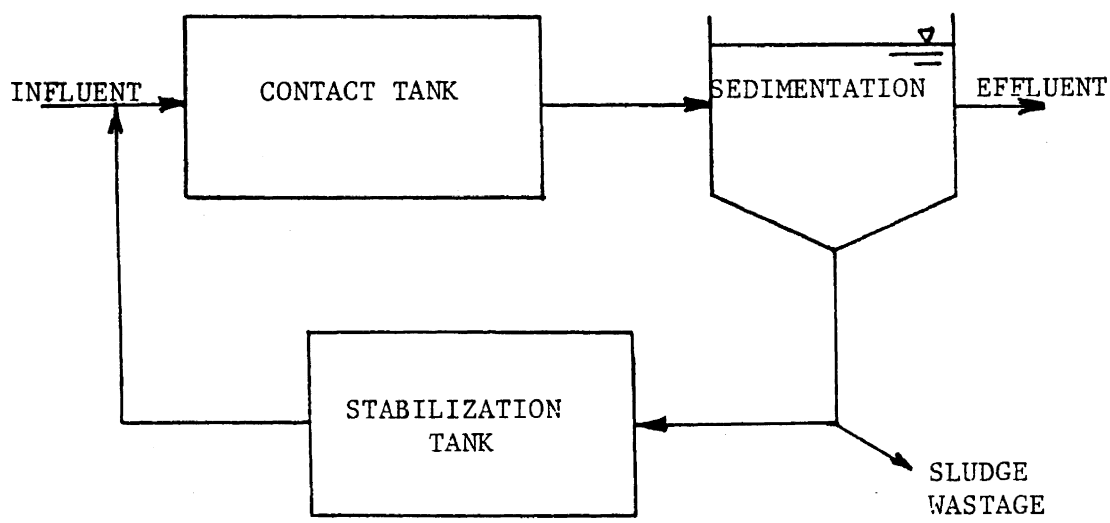
The contact tank contains mixed liquor concentrations of 1,000 to 3,000 mg/l, but, more importantly, has a detention time of 30 to 90 minutes. The stabilization tank receives the settled activated sludge from the secondary clarifier in concentrations of 6,000 to 10,000 mg/l and aerates it for approximately 3 to 6 hours. At the end of this period, the sludge is recycled back to the head of the contact basin to mix with incoming substrate.

This sludge reaeration scheme finds its origin in the work of Ullrich and Smith [1] and Eckenfelder [2]. Ullrich and Smith [1] took advantage of the highly sorptive nature of activated sludge to increase the load treated by the Austin Sewage Treatment Plant in Texas. This 16 MGD contact stabilization plant was modified from 6 MGD conventional activated sludge mode without any reduction in BOD or suspended solids removals.

Eckenfelder [2], working independently of Ullrich and Smith, developed his version of the contact stabilization process in studies on cannery wastes (tomato and apple processing wastes). The tomato



COMPLETELY MIXED PROCESS



CONTACT STABILIZATION PROCESS

Figure II-1: Schematic Flow Diagrams of the Completely Mixed and Contact Stabilization Activated Sludge Process

waste required detention times of 25 and 110 minutes in the contact and stabilization tanks, respectively, the apple waste needed longer contact times to maintain high process efficiencies.

Proposed Mechanism

Again, the basis for use of the contact-stabilization modification is the high sorptive nature of the activated sludge bacteria. However, there is great controversy on exactly what is happening in the two respective basins. The most common explanation found in the literature states there is a rapid removal of the soluble organics present (biosorption) and an adsorption or enmeshment of the colloidal organic fraction on the activated sludge flocs in the contact basin. The sludge - colloid mixture is then settled in the clarifier and pumped to the stabilization basin where the colloids are utilized by the bacteria. There are other theories proposed for substrate utilization in the contact-stabilization process that will be presented later.

The limiting factor in the contact-stabilization process appears to be the amount of soluble substrate the bacteria are able to utilize in the short contact tank detention period. Therefore, the process is thought to work best for wastes low in soluble organics. Most wastewaters contain significant amounts of insoluble material ranging in sizes from 10^{-6} mm to 1 mm. Solids found in a typical domestic wastewater are broken down in Table II-1 [3] into dissolved, colloidal, supracolloidal, and settleable forms. Clearly the insoluble forms

Table II-1

Fractionation of Solids in

Domestic Wastewater [3]

Fraction	Size Range (Microns)	Raw Wastewater		Secondary Effluent	
		Total Solids (mg/l)	Volatile Matter (mg/l)	Total Solids (mg/l)	Volatile Matter (mg/l)
Soluble	<0.001	351	116	312	62
Colloidal	0.001-1	31	23	8	6
Supra-Colloidal	0-100	57	43	28	24
Settleable	>100	74	59	0	0

contribute approximately 50% of the organics (volatile) fraction. Higher fractions have been reported not only for domestic wastewater but also pulp and paper, dairy, and textile industrial wastewaters.

Advantages of Contact-Stabilization

The most obvious advantage in using contact stabilization is high volumetric loading capacities that are allowed without decreasing BOD removal efficiencies. This results in a significant capital cost savings when compared to a complete mix activated sludge system. Jones [4] determined that capital cost savings could be as much as 60% or more.

The contact stabilization process appears to be quite resistant to shock loadings (organic, hydraulic, and toxic). This is attributed to the fact that only the sludge in the contact basin (10-25% of total sludge) is exposed to the transients, whereas in the conventional systems all the sludge is affected [5].

Finally, the sludge exiting the contact basin in a contact stabilization process possesses superior settling properties than that in a conventional process. Figure II-2 from the work of Jenkins and Orhon [6] clearly describes this phenomena. Both SVI and effluent suspended solids of the contact stabilization process are lower than those values for its conventional counterpart. It is postulated that the stabilization basin acts as a conditioner for the sludge. This conditioning phenomena finds its basis in the work of Pavoni,

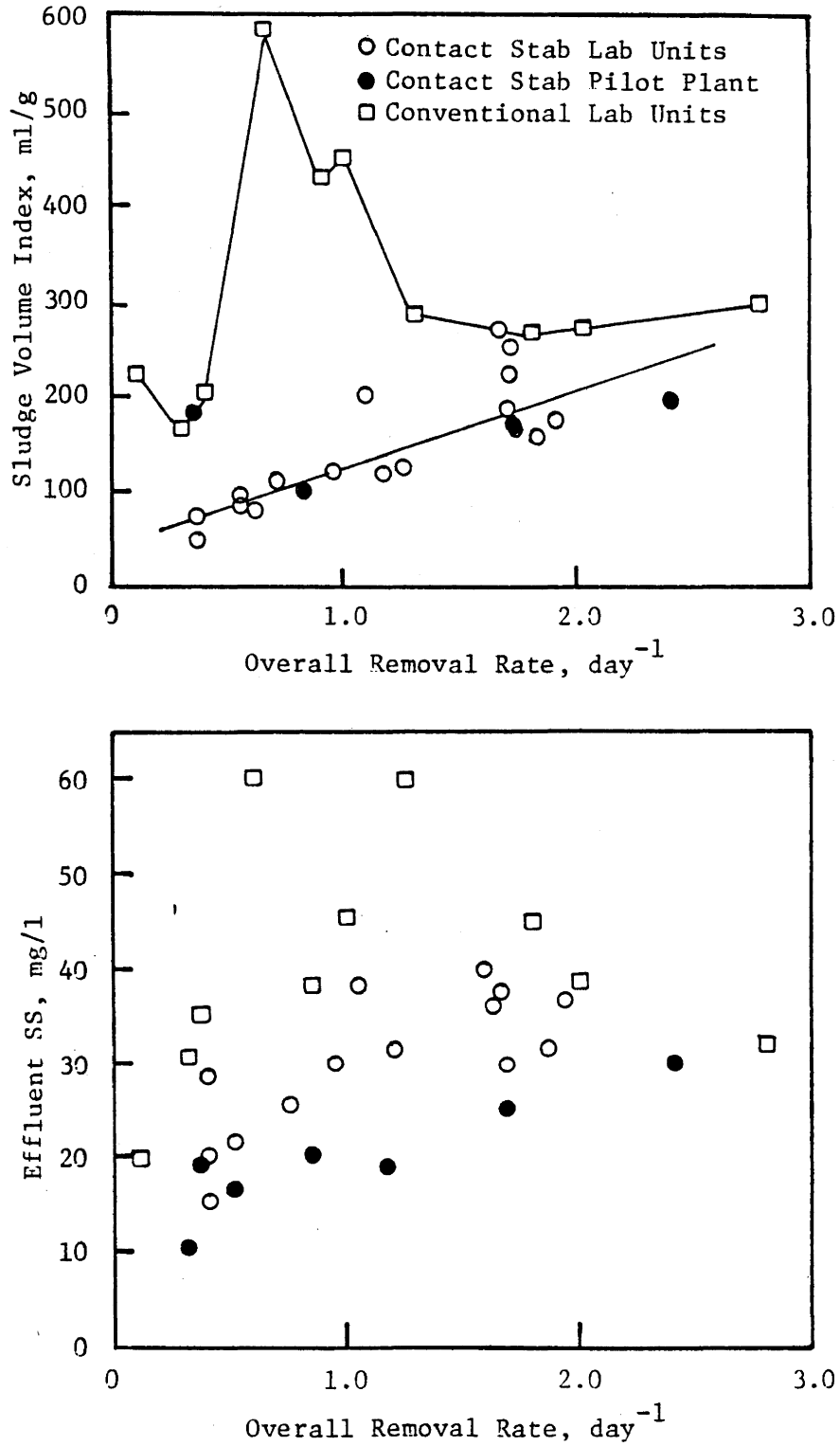


Figure II-2: Comparison of the Settling Characteristics of Activated Sludges from Completely Mixed and Contact Stabilization Processes [after Jenkins and Orhon, 6]

et. al. and others [7,8,9]. The activated sludge bacteria produce exocellular polymers in the declining or endogenous growth phase which promote bioflocculation of the sludge.

Organic Substrate Uptake

The majority of, if not all, types of wastewaters, both domestic and industrial, contain a significant colloidal fraction of organic material. In the past [4,8], high ratios of colloidal to soluble organic content in a waste have been used as justification for use of the contact-stabilization process. However, this approach has not always been successful. Therefore this section will briefly present the mechanisms expounded for both soluble and colloidal uptake by activated sludge cultures.

Soluble Substrate Utilization

From the standpoint of wastewater treatment, the disappearance rate of substrate from the bulk liquid is the primary key to sizing the biological unit processes. Therefore, the transport of the substrate across bacterial cell membranes establishes the kinetics of the system. This transfer of molecules may be classified into nonmediated and mediated (facilitated) processes.

Nonmediated transport is due only to physical diffusion across the membrane. Therefore, movement is dependent only on the concentration of solute, i.e. the solute travels down a concentration gradient. In nonmediated flux the solute molecule is neither chemically modified nor associated with another molecular species

in its passage through the membrane. Also, no energy is expended by the bacteria for this transfer. Except for water and some lipid soluble molecules, though, few compounds can enter the cell by this mode [9,10].

Mediated, or facilitated, diffusion is classified as either passive or active. It contrasts nonmediated diffusion by exhibiting:

a) the possibility of saturation of the transport system,

b) the specificity for the substance being transported, and

c) the susceptibility to inhibition.

Passive transport, like nonmediated diffusion, follows the concentration gradient. The exception lies in the fact that the solute combines reversibly with a specific carrier molecule in the membrane called a permease or porter. The permease-solute complex rotates or oscillates from the outer to the inner membrane (or vice versa) and releases (or binds) the solute on either side. Again, no metabolic energy is expended here since transport is in the direction of low concentration of solute [9,10].

Active transport of solutes involves movement of molecules from a zone of low concentration to a zone of high concentration. This requires expenditures of metabolic energy by the bacteria. This unidirectional movement has two mechanisms known as group translocation and active transport. In group translocation, the transported molecule undergoes a covalent change such that the reaction, itself, transports the molecule across the membrane. The active transport

mechanism pushes the solute molecule through the membrane without any type of alteration. Energy, though, is expended for this transfer [9,10].

In the activated sludge process, rapid, almost instantaneous, uptake of soluble substrate has been associated with activated sludge cultures. Two primary mechanisms are suggested for this phenomena: enzymatic complexing and surface phenomena.

Siddiqi, Engelbrecht, and Speece [11] investigated the role of enzymes in the rapid uptake simple soluble substrates. Figure II-3 represents a schematic model of the fate of a substrate molecule. Permease enzymes transport the simple solute molecules through the cell membrane to a free substrate pool. The rate of transfer is proportional to the external substrate concentration until saturation of the transport enzymes. Subsequent utilization of substrate from the pool by hydrolysis and/or synthetic and respiratory enzymes occurs thereby allowing continued substrate permeation (favorable concentration gradient).

Ford and Eckenfelder [12] supported the enzymatic hypothesis by presenting corroborative data from a brewery waste. Figure II-4 shows comparative data from both studies. The COD removal per unit of MLVSS after 15 minutes of contact reaches a saturation value for a simple (glucose) and complex (brewery) substrate alike as the substrate loading is increased.

Stumm and Stumm-Zollinger [13] challenged those results of Siddiqi et. al. They presented kinetic data for simple substrates

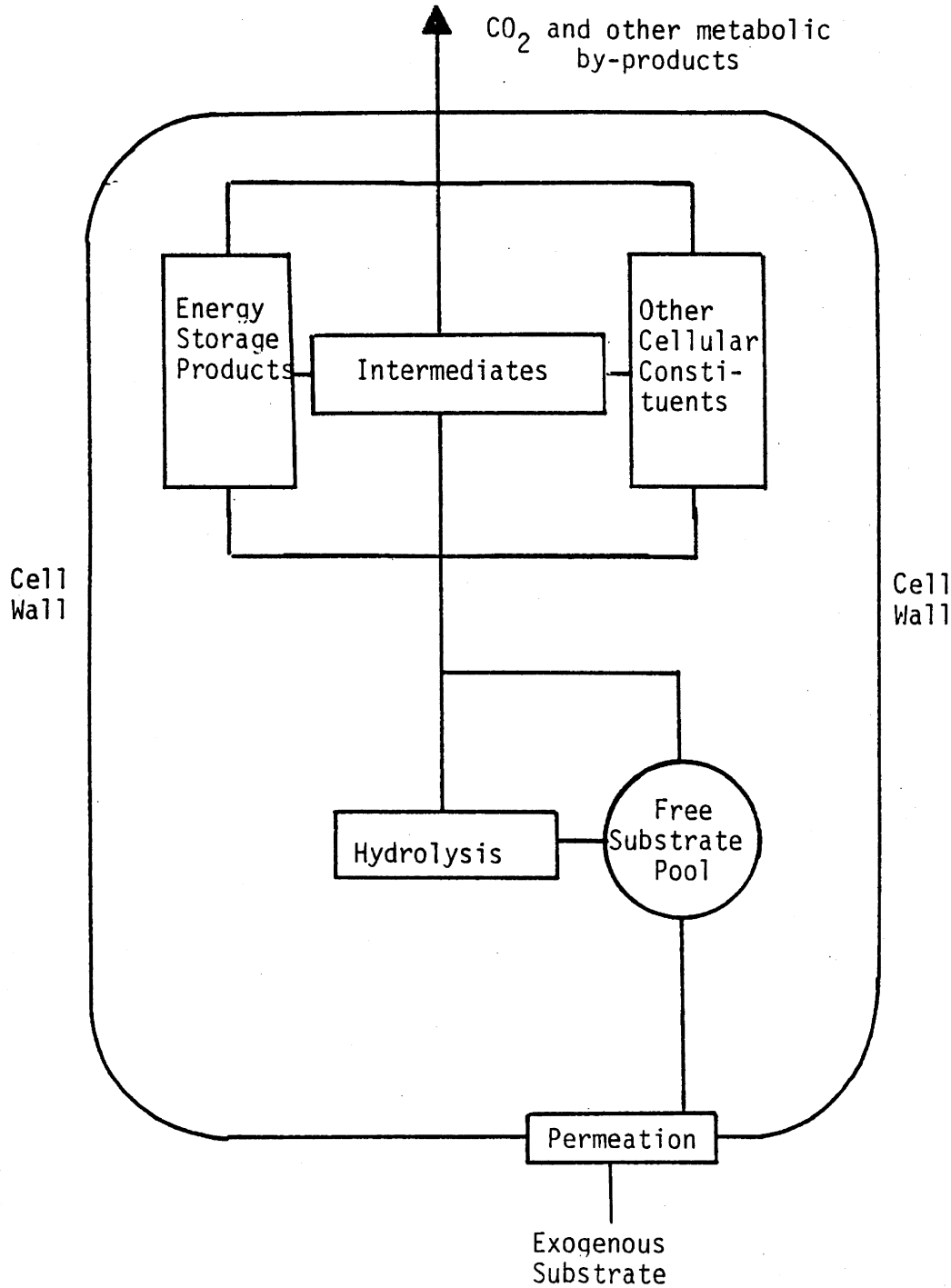


Figure II-3: Schematic Representation of a Bacterial Cell and Its Biochemical Activities [after Siddiqi, et.al., 11]

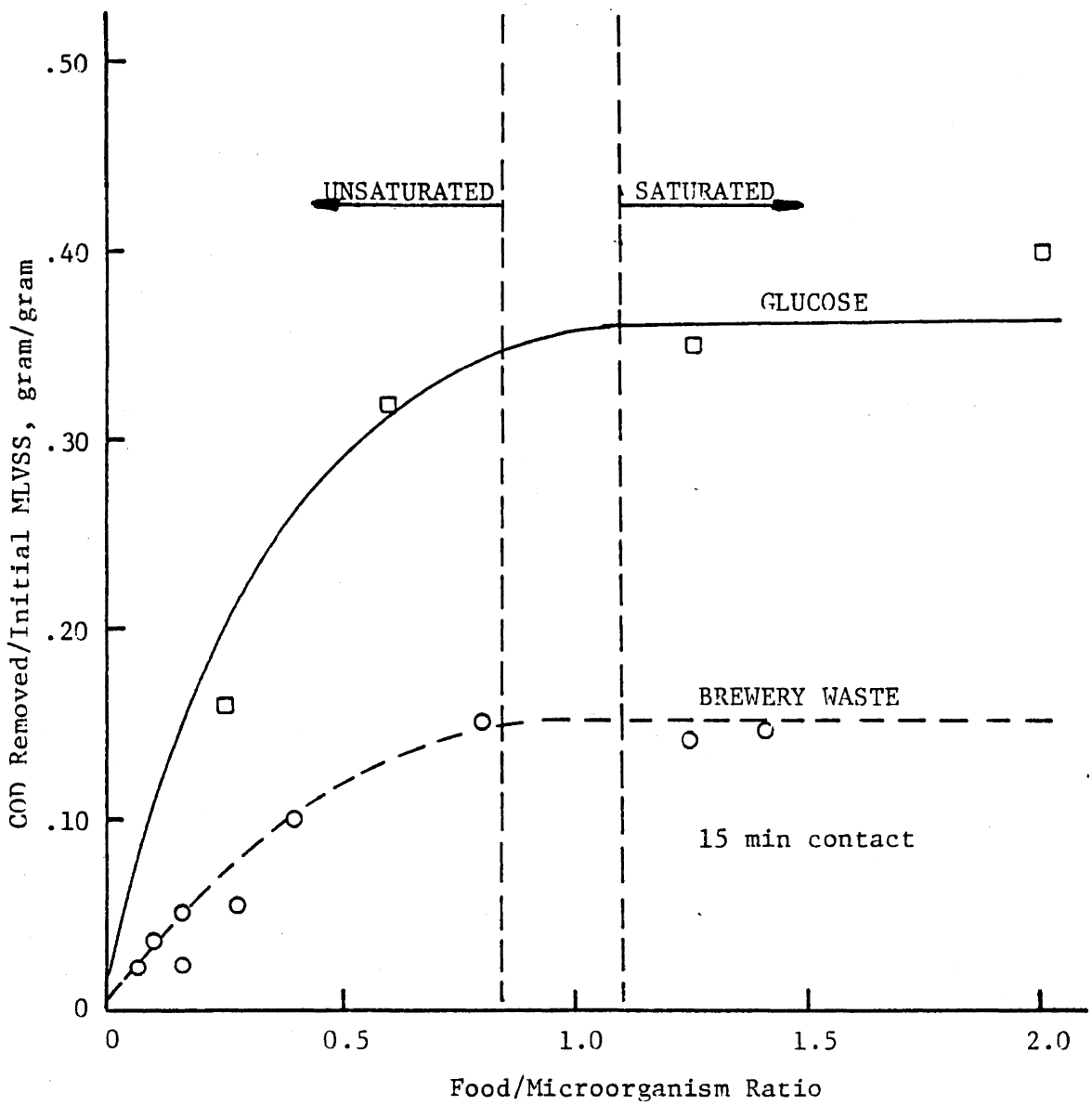


Figure II-4: COD Removals for Various Loadings of Glucose and Brewery Waste Substrates [after Ford and Eckenfelder, 13]

(glucose and phenylalanine) that showed no initial rapid uptake prior to the established utilization rate. They further stated rapid sorption for simple substrates does not occur, although this phenomena is quite commonly observed with complex wastes. Three explanations were presented to interpret this feature:

1. "In a multi-substrate medium, the overall carbon removal, as a first approximation, can be interpreted to occur as a superimposition of individual substrate elimination rates, each one being catalyzed by a different enzyme. The initial reaction rate, if measured COD or BOD, is governed partially by the most readily assimilable substrate.
2. "Surface active substrates, certain macromolecules, and hydrophilic colloids are sorbed at the bacteria-solution interface. Sorption of simple substrates of low molecular weight or low surface activity... is not relevant.
3. "In activated sludge, microorganisms excrete anionic polyelectrolytes that act as effective flocculants. These materials... cause almost instantaneous flocculation of colloidal and macromolecular substances."

In hypothesis 2 and 3 above lies the basis for the adsorption theory to rapid sorption of soluble organics. However, this also provides a basis for colloidal or suspended substrate removal in activated sludge.

Colloidal and Suspended Substrate Utilization

Two primary theories exist as to the utilization of large macromolecular colloidal molecules by microorganisms. One is phagocytosis (or pinocytosis) which refers to the engulfment or direct passage of the large molecules into the cell. This is the mechanism by which leucocytes (white blood cells) protect the body from foreign matter. The second hypothesis states the microorganism secretes extracellular enzymes which break down the large molecules to sufficiently small sizes that can be easily transported into the cell. This latter mechanism predominates in activated sludge bacteria due to the rigid cell walls of the bacteria involved. Therefore, since the breakdown of colloidal and suspended matter is mediated by the action of exocellular enzymes, questions arise as to the rate controlling step in the assimilation of the large molecules. Maier [14] presents a sequence of steps for the complete assimilation pointing out that any one could be rate controlling. Morris and Stumm [15] reiterated these concepts and provided a diagram shown in Figure II-5. The four steps are:

1) Mass transfer of substrate from the bulk medium to the cell surface or active enzyme site
2) Degradation or hydrolysis of the large molecules into smaller fragments that can be transported into the cell
3) Transfer of diffusion of substrate into the cell

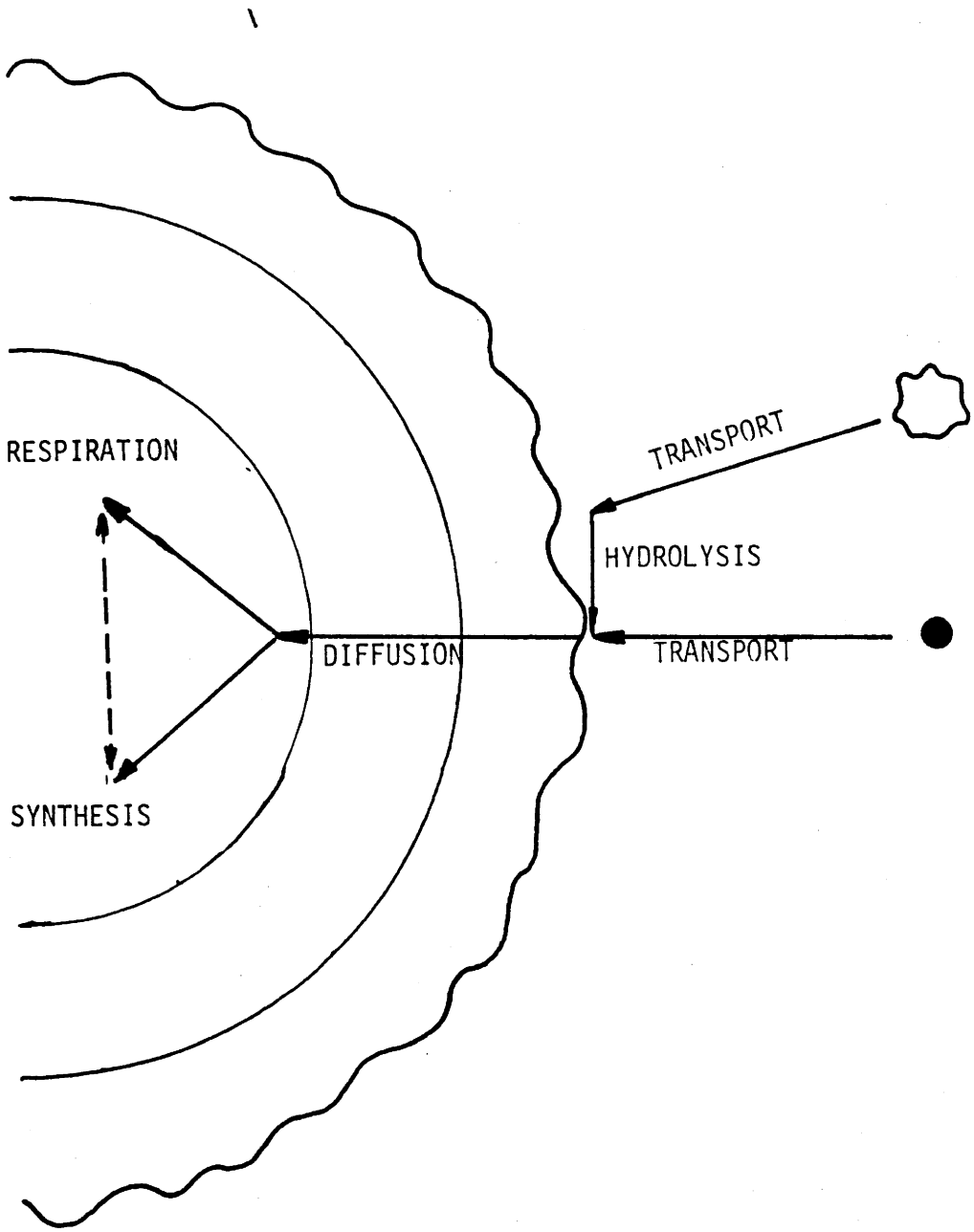


Figure II-5: Schematic Diagram of Substrate Transfer and Diffusion into a Bacterial Cell [after Morris and Stumm, 15]

4) Metabolism of the substrate with the cells for energy and growth.

With regard to mass transfer limitation, Maier [14] utilized starch as a colloidal substrate to determine rate of mass transfer in a laminar-film flow reactor (simulating trickling filter conditions) and in a well mixed reaction vessel (simulating activated sludge conditions). Mass transfer of substrate was found to be limiting in the laminar flow reactor while high rates of transfer were obtained in the other due to eddy current transport.

Once the colloid has been transported to the cell surface, it is unclear as to whether it is held there by adsorptive forces (Van der Waals forces and alike), chemical bonding, physical entrapment, or is the colloid released back into solution after partial hydrolysis by enzymes on the cell surface (cell bound enzymes). Another explanation follows that the hydrolytic enzymes are free in solution, rather than cell bound. Therefore, only simple hydrolyzed molecules are transported to cell surface and within, while the large macromolecules remain suspended in solution due to electrostatic repulsion between the bacteria and each other. This is plausible due to the negatively charged nature of both bacteria and colloids commonly found in activated sludge processes.

The bacteria in activated sludge possess a highly complex cell surface. Innate and necessary to the activated sludge process is the ability of the bacteria to separate themselves from solution in quiescent conditions. This efficiency determining step is induced by

presence of exocellular polymers extending from the cell surface which aids formation of the readily settleable biological flocs.

Many investigators [7,16,17] have studied these polymers composed primarily of polysaccharide and polyamino acids. Bacteria also appear to produce these polymers in the declining and endogenous growth phases. Novak and Haugan [18,19] in a series of excellent studies proposed a model for the activated sludge floc seen in Figure II-6, and related it to sludge dewatering properties. They, also, presented a proposed mechanism for bioflocculation shown in Figure II-7. Here organic colloids, bacterial or otherwise, are enmeshed in a biopolymer matrix in which cations (such as Mg^{+2} and Ca^{+2}) play an important role.

Eckenfelder [20] stated that suspended and colloidal BOD removal from a waste occurs by enmeshment into the biological floc and by physiochemical adsorption onto the floc, respectively. Smallwood [21] used a Chorella algal culture grown of radioactive carbon dioxide, $C^{14}O_2$, as a model colloid to determine a mechanism of removal. He concluded that the balance of the radioactivity had been adsorbed onto the biological floc.

Banerji, et. al. [22] commenting on Samllwood's studies suggested that the colloidal waste chosen was not representative, and there was a good possibility that the radioactivity assumed to be adsorbed could have been incorporated into the cell protoplasm. Using potato starch as a colloidal substrate, they conducted batch studies on starch removal in activated sludge at various loadings. They observed in all runs that a portion of starch was immediately adsorbed onto or

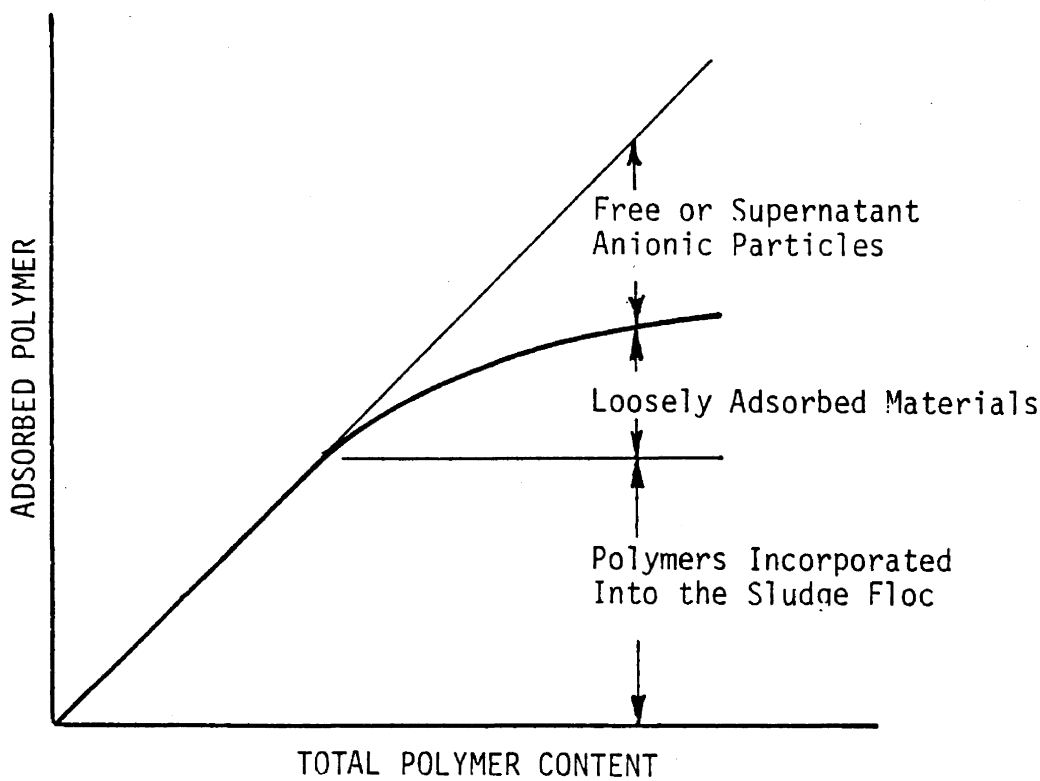


Figure II-6: Proposed Adsorption Model for Activated Sludge Flocs [after Novak and Haugan, 18]

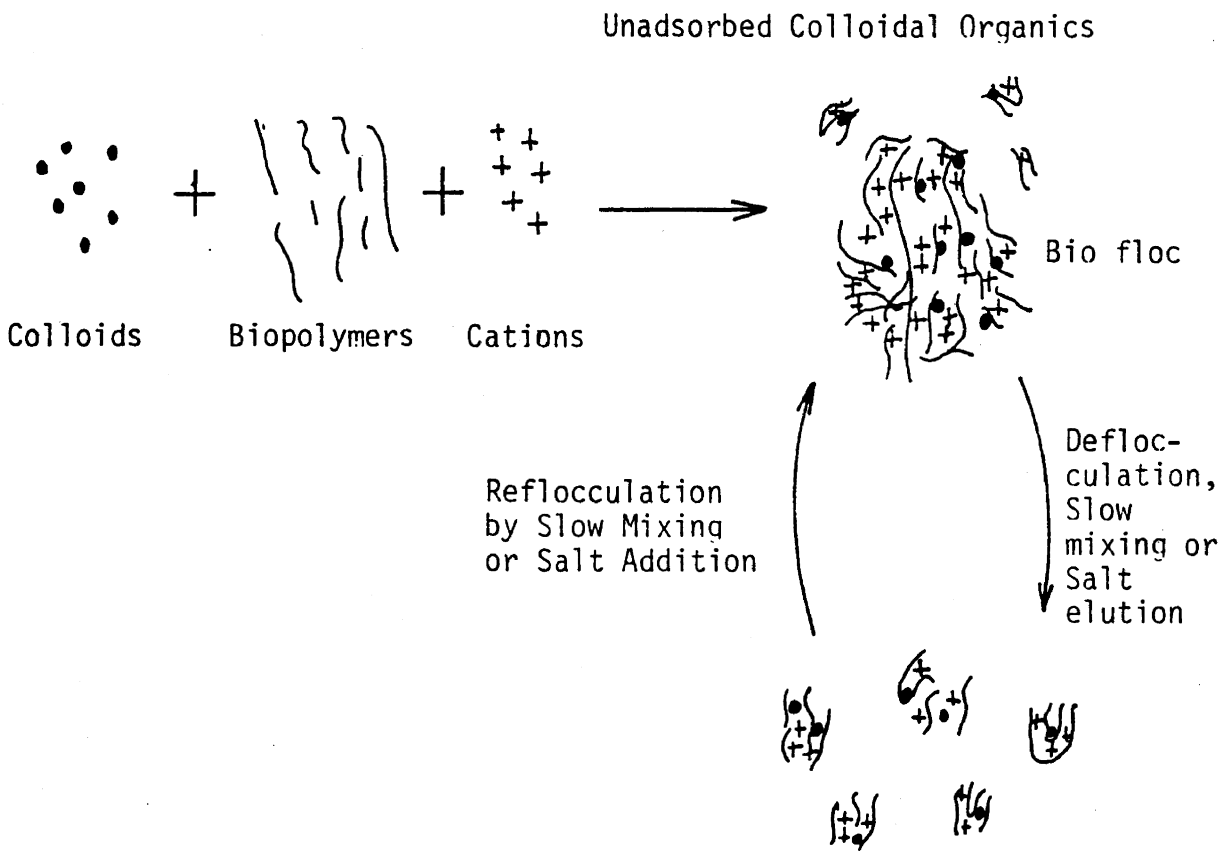


Figure II-7: Proposed Mechanism for Bioflocculation of Activated Sludge [after Novak and Haugan, 18]

enmeshed in the biological floc. A Freundlich isotherm plot was unsuccessfully attempted to describe the data. Nonetheless, Benedek and Farkas [23], while investigating temperature dependence of substrate removal, stated that the removal of starch from solution was purely an adsorptive process.

Contrary to the adsorptive or enmeshment theory, Kharajian and Sherrard [24] presented data on yogurt (casein) fed activated sludge batch systems. The percent colloidal matter was varied from zero to 54% over a wide range of F/M ratios. No apparent substrate adsorption appeared to occur.

Most authors, therefore, seem to agree that adsorption or enmeshment is the primary mechanism for colloid or suspended substrate removal. In lieu of Kharajian and Sherrard's results though this should not be taken as a blanket statement.

Whether transfer or diffusion of substrate into the cell is rate limiting is another much disputed point in the literature, especially with regard to colloidal substrates. Two mechanisms with regard to transfer rate versus hydrolysis of colloidal substrate appear to exist. One is that the rate of transport of the hydrolyzed substrate into the cell is faster than the rate of hydrolysis itself. The second is the reverse, that is, the rate of hydrolysis is greater than the rate of transport. If the latter is true, there would be a net increase in the soluble organic fraction of substrate in the bulk medium metabolism. Herein lies another controversy associated with the contact stabilization process.

McKinney [25] stated that early researchers, notably Eckenfelder [2] and Smith [1], observed an uptake and release phenomena upon mixing raw sewage and activated sludge together in an aeration vessel. This is demonstrated in Figure II-8. The suggestion here is that soluble organics are rapidly sorbed, preferentially or otherwise, while colloidal and suspended substrate matter remains adsorbed or enmeshed in the sludge flocs. Hydrolysis of these large particles causes a "release" of soluble substrate back into the bulk medium. Eventually, all the colloidal and suspended substrate is hydrolyzed and then utilized as described by the second and final decrease. This supernatant BOD response, therefore, initiated the contact stabilization modification. The short hydraulic detention time in the contact tank was designed to take advantage of rapid sorption of both soluble and particulate organics; whereas the stabilization tank was to act to provide aeration for particulate substrate hydrolysis and subsequent utilization.

Many investigators, though, seriously challenge this "uptake and release" pehnomenon. They suggest the hydrolysis of the colloidal or suspended matter proceeds at a slower rate than transport across the cell membrane. Takahashi, et. al. [26] investigated the metabolism of suspended matter using ^{14}C labelled substrates. Rates of substrate removal were found to decrease as particle size increased. More importantly, though, no soluble radioactivity was detected in the mixed liquor leading to the conclusion that no soluble substrate was released to the bulk solution from particulate hydrolysis.

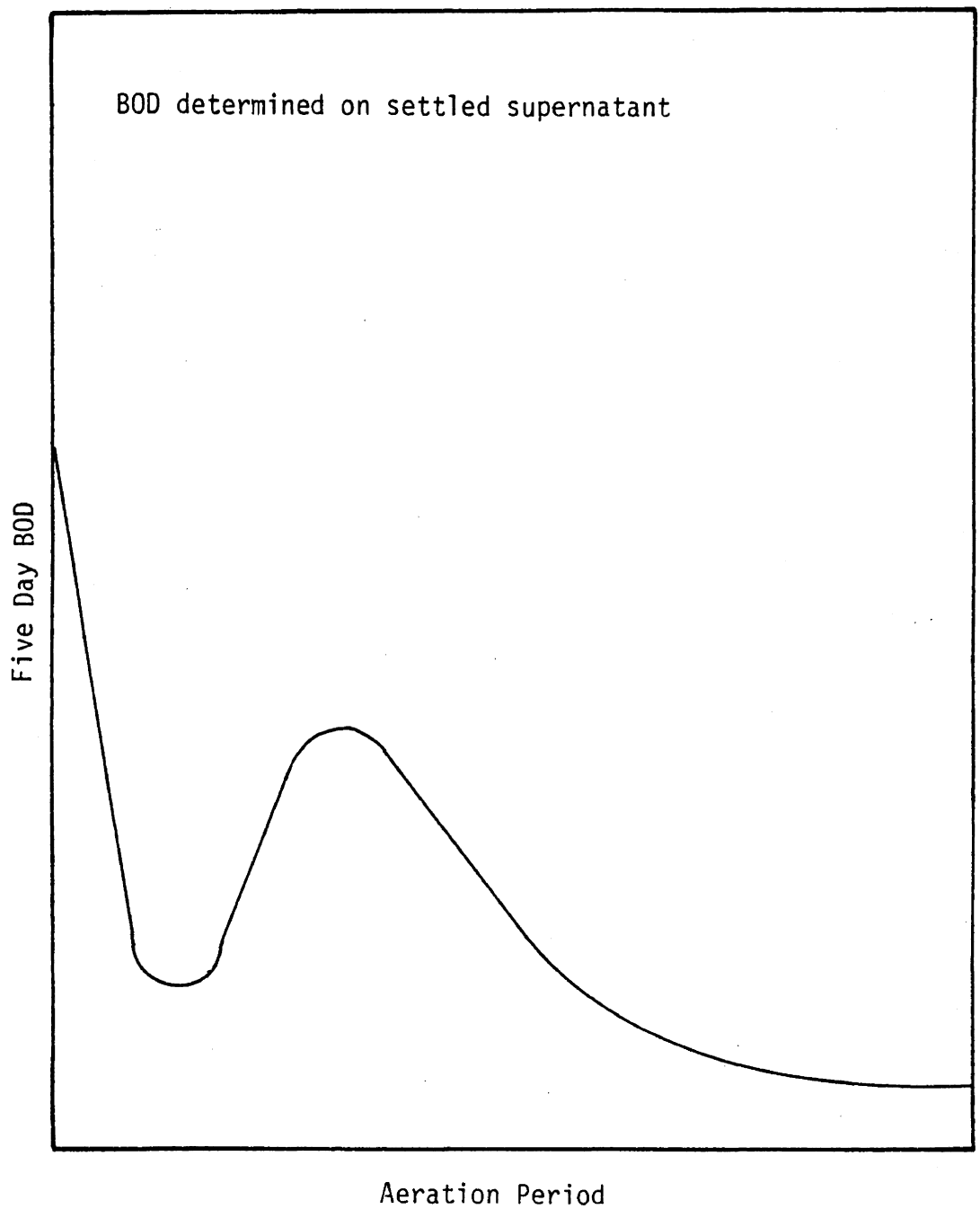


Figure II-8: Variation in Five Day BOD of a Raw Sewage-Activated Sludge Mixture with Aeration Period [25]

McKinney [27] commented that no soluble release was due to the responsible hydrolytic enzymes being cell bound rather than free in solution. He suggests that this prevents any loss of hydrolyzed products. Khararjian and Sherrard [24], also, concluded no "uptake and release" phenomenon occurred in studies with colloidal casein. Armentrout [28] and Hearne [29] each performed batch studies on various types of colloidal wastewaters-synthetic, domestic and industrial in nature. For the most part, no adsorption and solubilization of the substrate was found.

With regard to the final rate limiting mechanism, metabolism of the intracellular substrate, only a few subjects of importance to the contact stabilization process will be addressed and will apply to both particulate and soluble substrates alike.

During sludge reaeration, the substrate removal capacity of activated sludges has been found to increase. However, this activity was also shown to decrease with prolonged aeration or stabilization. Several ideas have been presented to explain these effects. Ford and Eckenfelder [12] maintain the increase in activity during stabilization is attributed to solubilization of entrapped colloidal or suspended matter and/or assimilation of intracellular stored substrate which makes the sludge again capable of removing additional organic matter. Takahashi, et. al. [26] showed adsorption activity of the sludge increased as metabolism of sorbed colloids proceeded. Walters, et. al. [30] presented Figure II-9 and concluded that the levels of stored metabolites, polyhydroxybutyric acid (PHB) and glycogen, have a major effect on substrate removal rate. Siddiqi, et. al. [11],

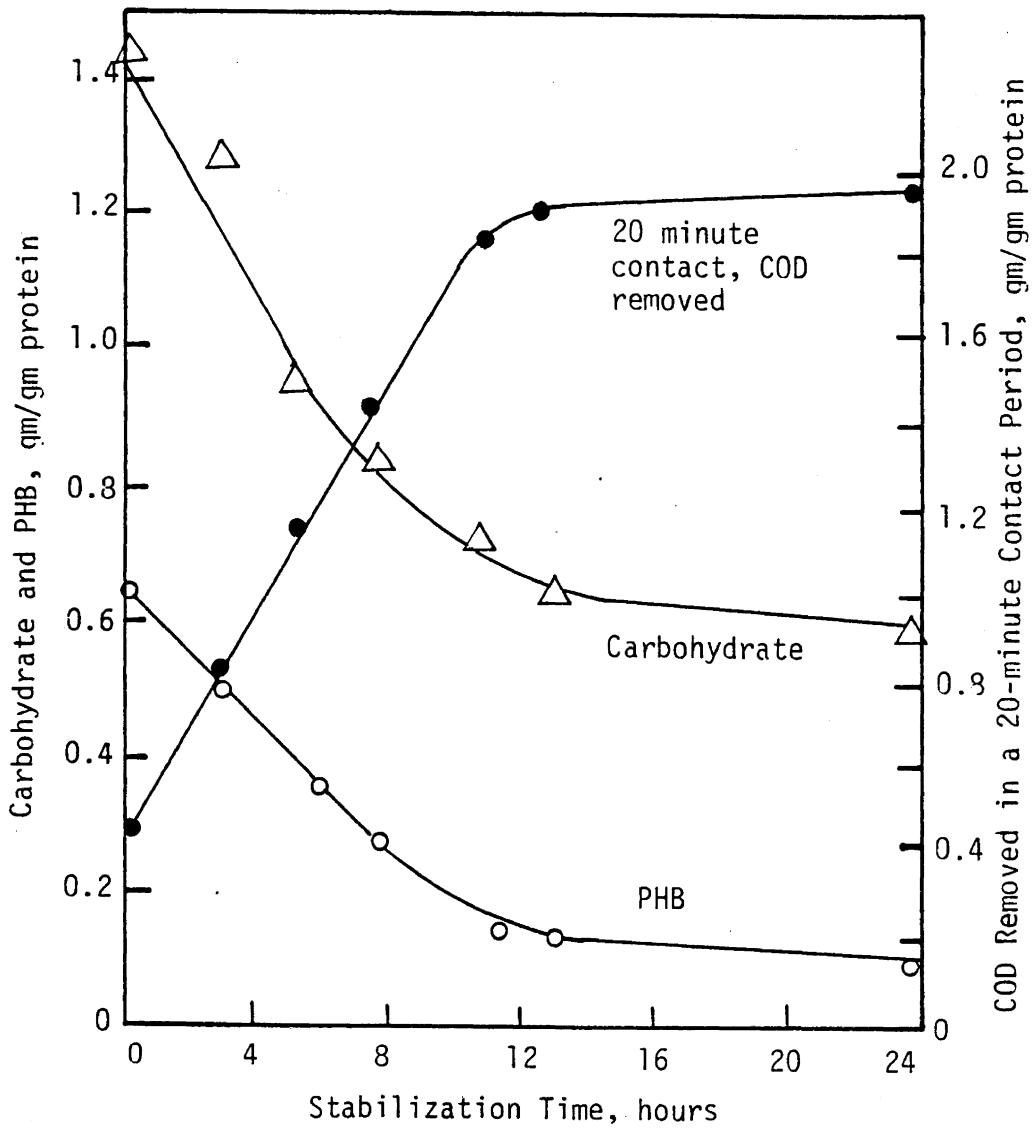


Figure II-9: Relationship Between Cellular Carbohydrate and Cellular PHB Levels and the Rate of Substrate Removal [after Walters, et.al., 30]

however, single out the production of intracellular synthesis and respiration enzymes as the key to the rapid substrate removal theory. The activity of these enzymes produces a more favorable concentration gradient for the metabolic processes described in Figure II-3.

With regard to the reduction in activity after prolonged aeration times, all sides seem to agree. Inducible enzymes are those enzymes which require a substrate (inducer) to be present before they are formed. Once the inducer is removed or metabolized, the inducible enzyme will become "inactivated". This is believed to occur during extended stabilization periods causing the reduction in activity.

Contact Stabilization Process in Theory and Practice

Models

Earlier, a proposed and widely accepted mechanism on the substrate utilization in contact stabilization was presented. The substrate, primarily colloidal in nature, enters the contact tank where the soluble organic fraction is metabolized and the colloidal organic fraction is physically adsorbed or enmeshed. The sludge-colloid mixture is then settled in a secondary clarifier and pumped to the stabilization basin. Here, the colloids are broken down by extracellular enzymes and utilized by the bacteria. Once this is accomplished the sludge is recycled back to the contact basin. Some kinetic models that have been developed based on this scenario and others will be presented here.

Bhatla et. al. [31] in an attempt to provide a method for evaluating substate removal kinetics on a batch system developed a justification for use of the contact stabilization modification for treatment of wastewaters. For easily metabolized wastes, organic substrate removal rate is commonly paralleled by the rate of oxygen uptake. Therefore, when removal of the organics is complete, the oxygen demand is close to or at the original level prior to substrate introduction. However, if the substrate removal is essentially complete considerably prior to the return of the oxygen demand to base levels, contact stabilization may be a justifiable alternative for treatment. Figure II-10 describes these two cases. Obviously, oxygen uptake is the key parameter here. Even though substrate removal is perceived complete, the biomass is still actively metabolizing either stored metabolites or enmeshed/adsorbed particulates as shown by the oxygen demand. Therefore, the authors suggest the contact basin be designed of the removal of substrate and the stabilization tank on the satisfaction of the oxygen demand.

This compartmentalization concept of soluble and colloidal metabolism is used in the model developed by Benefield and Randall [32]. They provided design equations based on Lawrence and McCarty [33] kinetics. First order removal kinetics were assumed for soluble and nonsettleable particulate substrate alike, however, soluble removal is assumed to occur only in the contact tank. Material balances were performed for both solids and substrate in the system (assumed to be at steady-state) yielding design formulations.

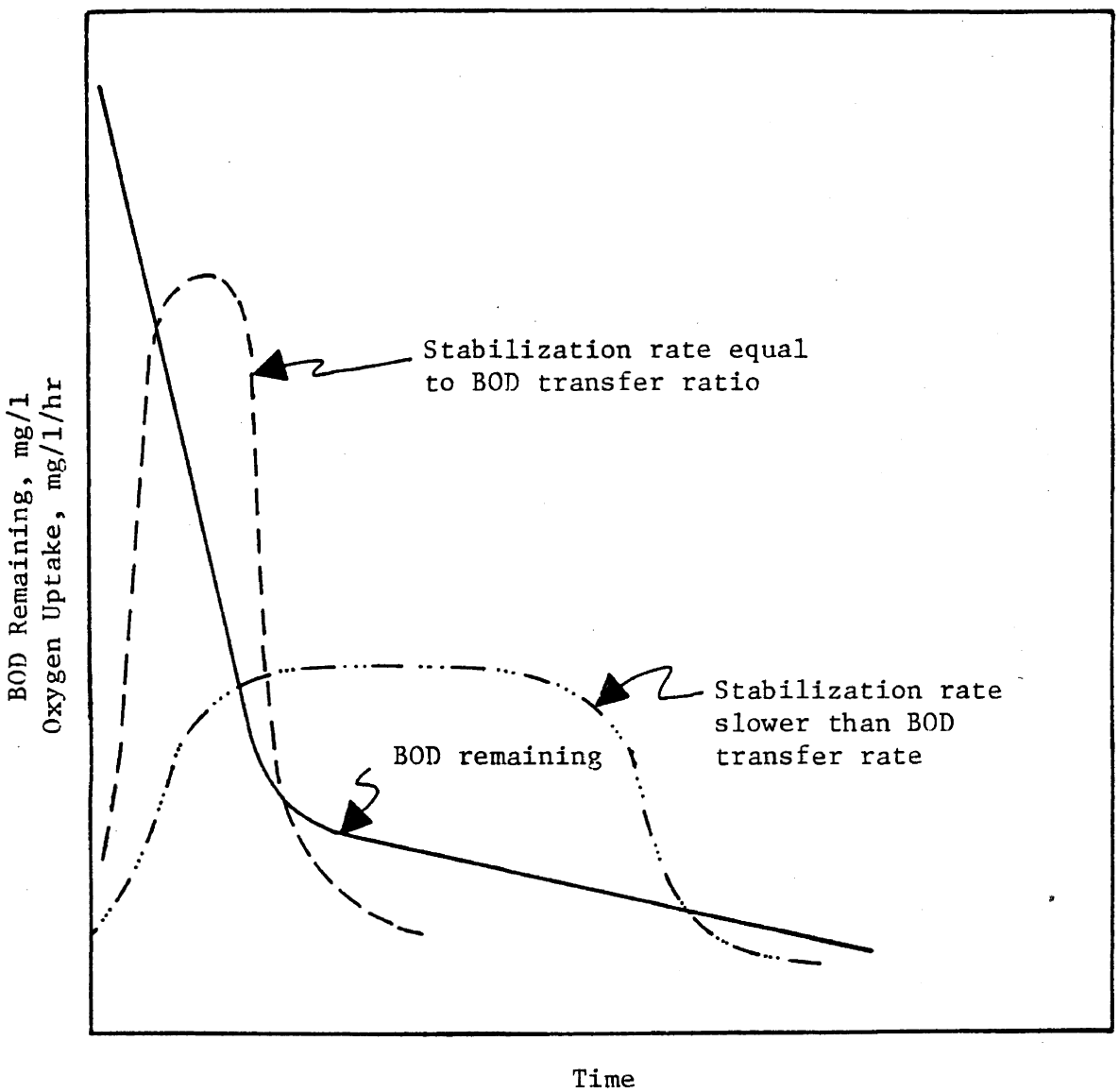


Figure II-10: Relationship Between Batch Kinetics of BOD Transfer and Stabilization Reactions [after Bhatla, et.al., 31]

McKinney [34] proposed in 1965 that the contact stabilization process did not operate as previously theorized. Instead of colloidal matter being sorbed in the contact tank and metabolized in the stabilization tank, he suggested that all substrate is utilized in the contact tank. The stabilization basin simply provides conditions for endogenous respiration. Jenkins and Orhon [35] furthered this theory by introducing a growth and death model for the contact stabilization process. They proposed that during contact a rapid growth of microorganisms leads to an increase in the activated sludge viable fraction, followed by a death phase in the stabilization basin which decreases the viable fraction. Observations in the study included:

a) Stabilization decay rates increased with increases in contact tank substrate removal rates.

b) Sludge growth rates were reduced and nitrification occurred at higher loadings rates due to the phenomenon in a).

c) The fractional distribution of sludge in the contact and stabilization basins appeared to be of great operational importance.

Gujer and Jenkins [35,36] described contact stabilization using four independent parameters: process loading, temperature, recycle ratio, and sludge distribution between reactors. Process loading and temperature had marked effects upon sludge production, oxygen consumption, COD removal efficiency, effluent solids, and nitrification.

Recycle ratio, though, was found to be the most important parameter since it directly affects the residence time distribution. For high rates of recycle, the system approached a complete mix system whereas at low rates, most of the waste flows only through the contact reactor. Recycle ratio was found to have little effect on particulate substrates that are removed quickly by physical sorption. Finally, the sludge distribution between reactors only affected nitrification in this study and did not significantly influence sludge production as suggested by Jenkins and Orhon [6].

Jones and Brown [8] introduced the concept of solubility index for treatment of wastewaters. The index is defined as the ratio of soluble organic carbon to the total organic carbon (the organic carbon can be expressed as TOC, BOD, COD, etc.). They found as this index increases (waste becomes more soluble), the organic removal efficiency decreases in a contact stabilization process. Later, Jones [4] attempted to model the contact stabilization process based on a batch system (plug flow) response. He proposed the absorption of soluble organics and the adsorption and/or physical entrapment of particulate matter as the primary mechanisms of removal. The model developed simply curve fits a number of organic substrate concentration responses as partially described in Figure II-11.

Performance of the Contact Stabilization Process

Many advantages are cited for the use of contact stabilization. Table II-2 lists these advantages and the literature references in

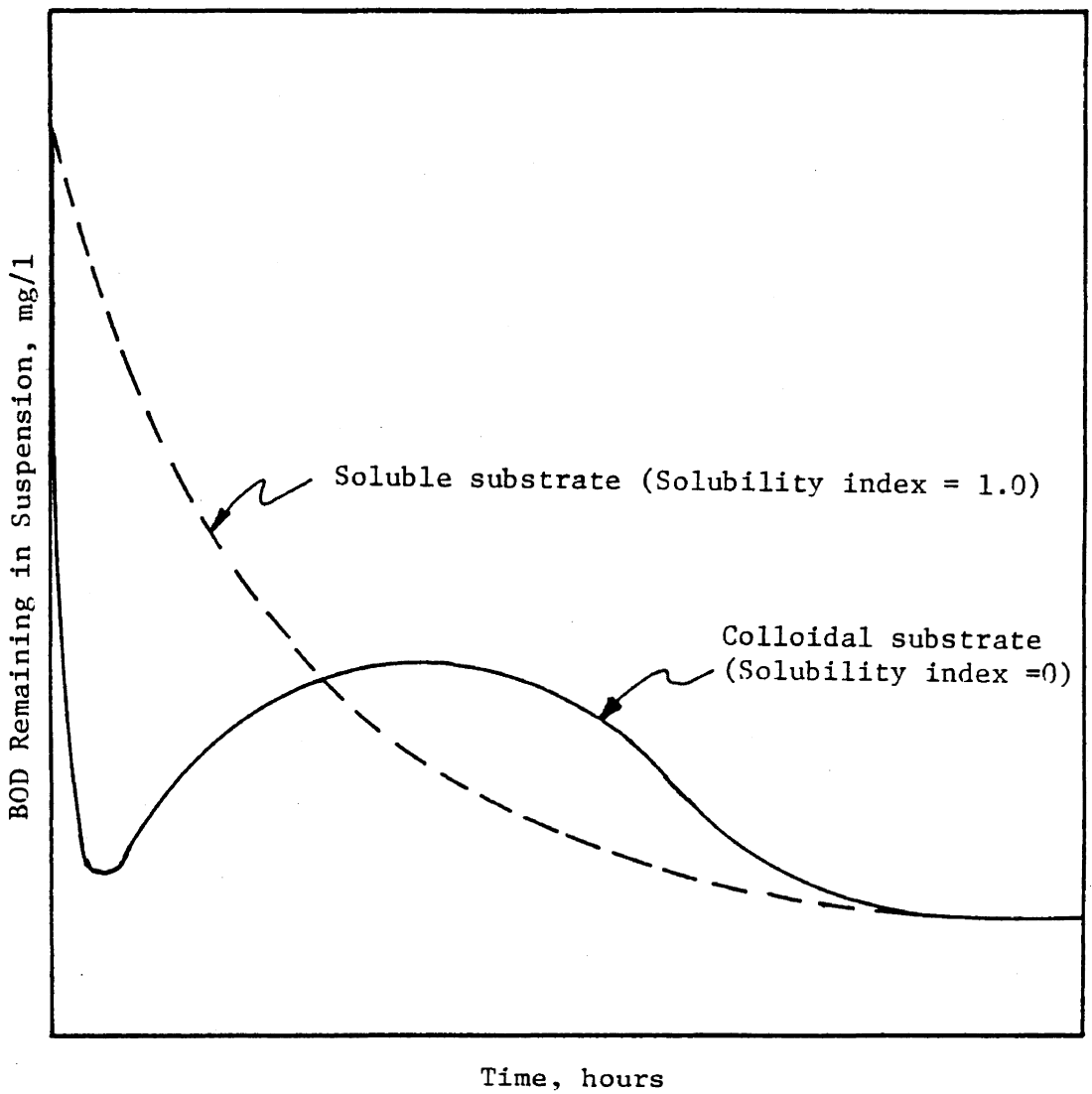


Figure II-11: BOD Removal in Batch Tests with Wastewater Containing Primarily Soluble and Particulate Substrates [after Jones, 4]

Table II-2

Advantages of Contact Stabilization Process

Advantages	Literature Reference
Capital Cost Savings	[1], [5], [37], [39]
Improved Settling Characteristics	[6], [38], [39]
Resistance to Shock Loadings	[5], [39]
High Organic Removal Efficiencies	[2], [6], [40], [42]
Reduction in Frothing	[37]

which they are cited. Most of these positive attributes have been discussed earlier; therefore, this section will present concepts from studies that downplay the contact stabilization modification.

Dague et. al. [41] studied two contact stabilization package plants. High F/M ratios in the contact basin resulted in poor solids separation in the clarifiers thereby decreasing the sludge concentration in the return. Overall, the authors felt the process was unstable for small systems and suggested employment of step-aeration or complete mix flow patterns.

Khararjian and Sherrard [42] compared the contact stabilization and complete mix modification of activated sludge. Laboratory prototypes were utilized and fed a casein-beef extract substrate (45 $\pm$ 3% colloidal COD). Based on data obtained for both systems at various mean cell residence times, it was concluded that both processes are similar in nature on the basis of the equivalent COD removal efficiencies and yield and maintenance coefficients obtained. No differences in process performances were found other than the obvious hydraulic configuration.

Thirumurthi [43] also performed laboratory studies comparing contact stabilization to the conventional and high-rate activated sludge processes. Both synthetic (milk powder and dog chow) and settled domestic wastes were used as substrates in a series of five simulation studies. Loadings remained constant on four tests while the fifth involved imposing a hydrograph flow variation. The final

conclusion given was that contact stabilization does not always enhance BOD or COD removal. This stemmed from the fact that in all tests COD and BOD removal efficiencies for contact stabilization were equivalent or lower to those of the conventional and high rate systems. Thirumurthi's contact stabilization prototype, though, represents a serious flaw in the study. The contact and stabilization detention times were approximately two hours and one-half to one hour, respectively. This distribution is likened more to simple sludge reaeration rather than contact stabilization.

CHAPTER III

PHYSICAL CHARACTERISTICS OF ACTIVATED SLUDGE

Introduction

The ability of activated sludge to physically adsorb or enmesh colloidal and particulate organics is very important in the contact stabilization process. All too often, the activated sludge process is viewed as strictly a biological and biochemical phenomenon. In fact, the physical-chemical functions taking place are an integral part of the waste treatment and cannot realistically be ignored. This chapter will draw light on some of the physical-chemical attributes of activated sludge and the important roles played by these forces.

Prior to discussion of specific physical characteristics of activated sludge, a more general discussion of colloids and their chemistry is in order since the bacteria, as well as the waste organics, can be classified as colloidal in nature.

Colloids

The word "colloid" is a Greek derivative meaning "glue-like" originating from work done on gelatin and gum arabic. Colloids, today, refer to any particle in solution within a size range of 10^{-3} -100 μ. Two general classes of colloids exist whose general behavior is quite different: lyophobic and lyophilic.

Lyophobic colloids exhibit little affinity for the medium in which they are suspended (solvent hating). Commonly, they are

regarded as suspensions and are susceptible to flocculation and coagulation by addition of electrolytes. Clay and metal oxide particles in water are examples of lyophobic colloidal suspensions. Lyophilic colloids on the other hand, are distinguished by the inherent attraction to the bulk medium, i.e. they are solvent loving. High molecular weight polymers or aggregates of smaller molecules, e.g. micelles, are classified as lyophilic colloids. Unlike its lyophobic counterpart, this colloid is quite stable and, therefore, relatively unaffected by salt addition. Of great interest is the stability of both colloidal types, which in most cases is a function of the charge sphere around the colloid. The physical and electrostatic nature of both a lyophobic and lyophilic colloid in solution is conceptualized in Figure III-1.

The lyophobic colloid is composed of a layer of fixed counter or gegen-ions (Stern layer) and a diffuse secondary layer of simil-ions and gegen-ions. Moving away from the surface, the simil-ions in the diffuse layer increase whereas the gegen-ion concentration decreases to levels consistent with the bulk solution. This distribution of ions on and around the colloid is referred to as the electrical double layer and is due to Coloumbic interaction. This is the inherent source or stability (or instability) of these colloidal systems.

Methods for destabilizing systems of lyophobic colloids have been discussed time and again [44,45]. These modes of destabilization and their characteristics are described in Table III-1 [45]. Fundamental to the double layer compression and the adsorption-charge

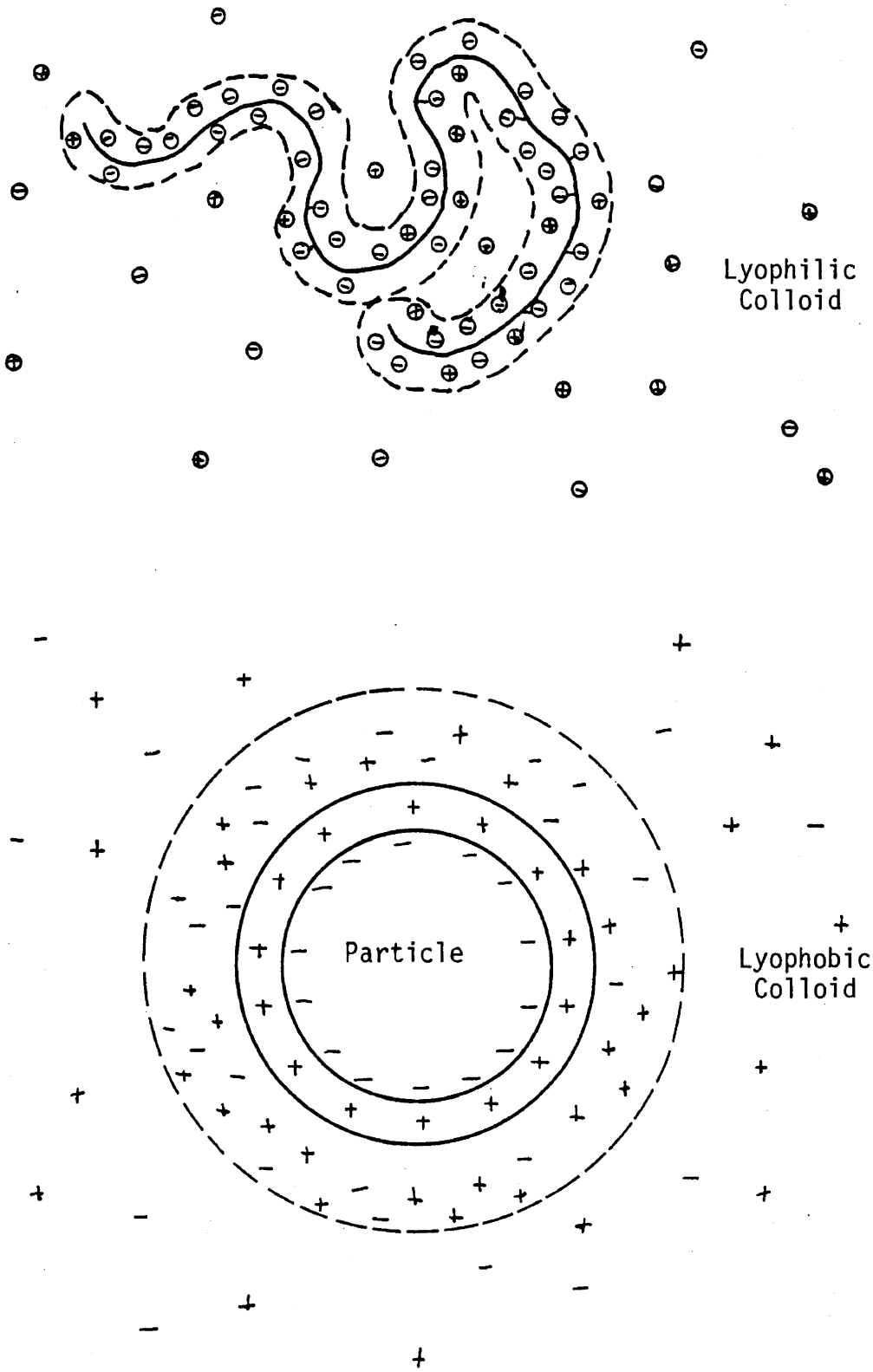


Figure III-1: Physical and Electrostatic Nature of a Lyophilic and a Lyophobic Colloid in Solution

Table III-1

Modes of Destabilization and Their Characteristics [45]

	Physical Double-Layer Theory (coagulation)	Chemical Bridging Model (flocculation)	Aggregation by Adsorbable Species (adsorption coagulation)
Electrostatic Interaction	Predominant	Subordinate	Important
Chemical Interactions, and Adsorption	Absent	Predominant	Important
Zeta Potential for Optimum Aggregation	Near Zero	Usually not zero	Not necessarily zero
Addition of an Excess of Destabilizing Species	No effect	Restabilization due to complete surface coverage	Restabilization usually accompanied by charge reversal
Relationship Between Optimum Dosage of Destabilizing Species and the Concentration of Colloid (or concentration of colloidal surface)	CCC* Virtually independent of colloid concentration	Stoichiometry, a linear relationship between flocculant dose and surface area	Stoichiometry possible but does not always occur
Physical Properties of the Aggregates Which are Produced	Dense, greater shear strength but poor filtrability in cake filtration	Flocs of three-dimensional structure; low shear strength but excellent filtrability in cake filtration	Flocs of widely varying shear strength and density

*Critical Coagulation Concentration

neutralization methods is the presence of counter ions. Both the type and concentration of these ions are important. Shultz-Hardy rule states that the critical coagulation concentration of a cation is inversely related to charge of the counter-ion raised to the sixth power, e.g. it would require $1/1^6$ and $1/3^6$ units of sodium and aluminum ions respectively to effect the same degree of coagulation. This is only true if the double layer compression mechanism is dominant. In adsorption and charge neutralization, not only do cations destabilize the charge by adsorbing onto the colloid and neutralizing, but they may also create a reversal in the charge by excessive adsorption. This can be measured in the laboratory by the electrophoretic mobility and/or zeta potential of the colloids. These parameters theoretically determine the potential difference between the Stern layer and the diffuse layer and are obtained by monitoring the movement of the charged colloids in an applied potential gradient, through a stationary soltuion. Cations may also play a large role in acting as a "bridge" between polymers and colloids of like negative charge. However, this bridging mechanism more often involves oppositely charged polymer and colloidal systems. Finally, the enmeshment mechanism is strictly a physical phenomena and may be the primary mechanism involved in removing most colloidal organics in the activated sludge system.

Lyophilic colloids found in wastewater include compounds such as gelatin, starch, proteins, and germs. Commonly a charge is

associated with colloid and often originates from charged functional groups in the colloid structure. These groups could be carboxyl, amino, and sulfhydryl, or others. The stability of lyophilic colloids is influenced by salt addition similar to hydrophobic systems. That is, the presence of excessive ionic species help to lessen the mutual electrostatic attraction of the charged groups. Free ions in solution may also interact with the charged groups to neutralize or reverse the charge on the colloid. However, lyophilic systems are greatly affected by the pH of solution. Figure III-2 provides an example of how the addition or removal of H^+ ions affects the overall charge of functional groups. In the past, this principal has been utilized for separation of proteins in solution [9]. This procedure involves the adjustment of pH to the zero point of charge (the pH at which there is a zero overall charge on the molecule), at which time the protein will precipitate out of solution due to its more lyophobic nature.

Physical Characteristics of Activated Sludge

Surface characteristics of cells have been studied quite intensively - both of the eucaryotic and procaryotic forms. These characteristics can vary greatly from one cell type to another; yet in this section, those extracellular features of the procaryotic cell so important to the activated sludge performance will be discussed. More specifically, this section will provide information on the exocellular polymers found in activated sludge cultures which play

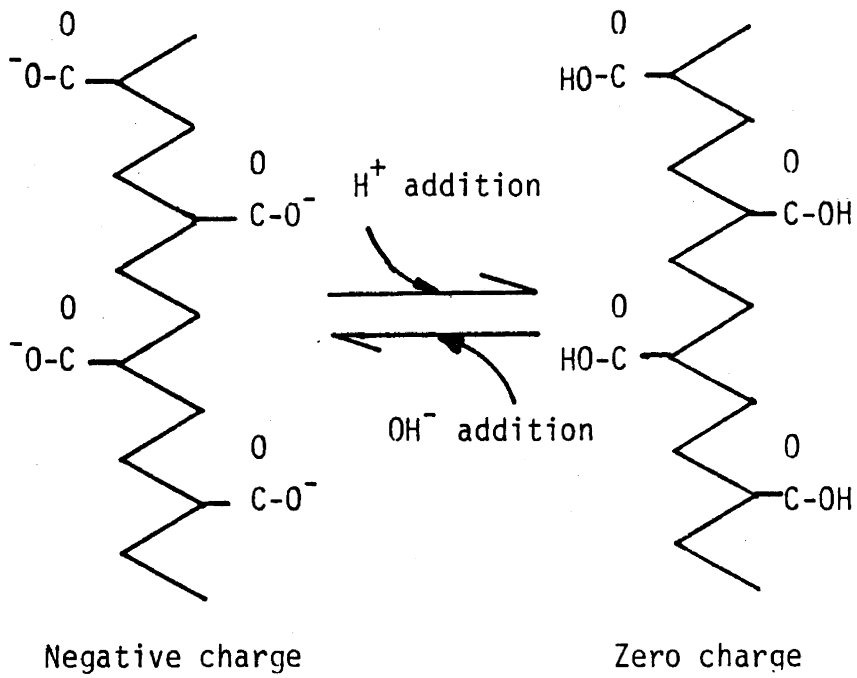


Figure III-2: Effect of Hydrogen Concentration on the Charge of Lyophilic Molecules

the major role in the agglomeration or "bioflocculation" of the bacteria.

Bioflocculation

The activated sludge process may be separated into two distinct steps or phases. First, organic matter is utilized by the heterogenous activated sludge culture for synthesis (protoplasm production and binary fission) and for maintenance energy. The second step involves the coalescing of the bacteria into readily settleable masses enabling their separation from the solution supernate under quiescent conditions. This phenomena is known as bioflocculation and is the efficiency determining step in the process scheme.

Many theories have been proposed for the flocculation mechanism in bacteria. Suggestions of flagella entanglement [46], protozoal presence [47], and simple physical attraction [48] have been suggested as the predominant mechanisms; but the most plausible theory has to do with the existence of exocellular polymeric molecules. The bacteria in the activated sludge process are believed to excrete high-molecular-weight, long-chain polyelectrolytes that serve to aggregate the individual cells.

In a literature review of bacterial exocellular polymer roles in metal removal, Brown and Lester [49] compiled data from fifteen references on the composition of these polymers. In all cases, carbohydrate units, such as glucose, galactose, fucose, etc., were detected. Some studies also measured significant concentration of

proteins and nucleic acids, the latter suggesting that autolysis of the bacterial cell is a contributor to polymer presence rather than just pure synthetic reactions. Also, the overall negative charge activated sludge bacteria possess (Isoelectric pH=2-3) is due in part to the anionic nature of these polymers.

The production of these polymers has been related to the physiological state of the bacteria [7,16], the presence of excess carbohydrate, and the carbon: nutrient ratio. Most work [7,16] points to the primary production of the polymer in the declining or endogenous phase of growth. Other investigators [50,51] have demonstrated that certain strains of bacteria in the presence of excess carbohydrate substances tend to accumulate polysaccharides. Varying nitrogen, phosphorus, and sulphur concentrations in the presence of excess carbon tended to increase or decrease polymer production of various species while the presence of magnesium, potassium, and calcium ions tended to stimulate production [52,53].

A mechanism or model for bioflocculation has been presented by Novak and Haugan [18]. In some well devised studies, they demonstrated that enmeshment of colloids in flocs comprised of the biopolymers and cations is the major mechanism of bioflocculation with particle bridging playing a secondary role. The cations appear to act simply as binding agents between polymers. The adsorption model shown in Figure III-3 describes the salt effects on the amount of polymer adsorbed. This lower binding capacity, exhibited by the

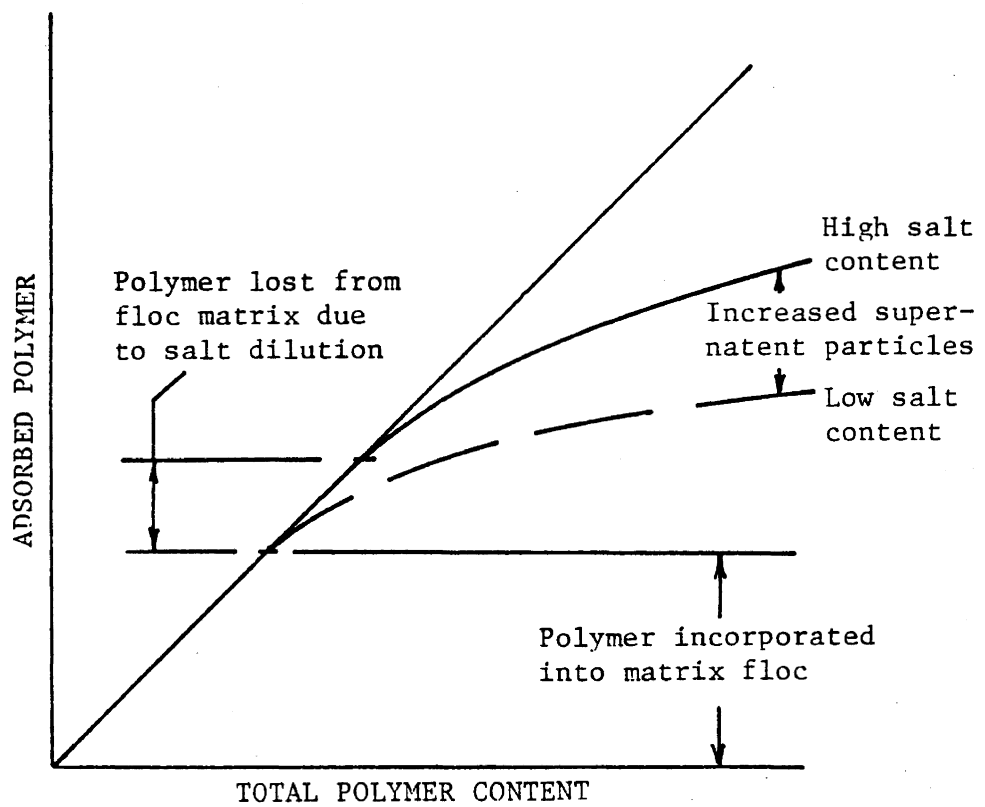


Figure III-3: Adsorption Model for Activated Sludge Flocs Modified to Show Salt Effects [after Novak and Haugan, 18]

lower cation concentration, results in greater floc breakup and, therefore higher supernatant particle concentration.

Conditions Affecting Bioflocculation

The surface charge of bacteria is commonly negative and this is true for activated sludge cultures. The negative charge exhibited by the bacteria is thought to originate from ionized functional groups, primarily carboxyl groups, in the exocellular polymers extending from the surface. Certain physical conditions have also been found to vary the magnitude of this charge and therefore the disposition of the bacterial floc. Logically, then, this alteration of surface charge may have implications on colloidal substrate-bacterial floc interactions so important in the contact stabilization process.

By use of electrophoretic mobility measurements, Forster [54,55,56] has placed the isoelectric point for activated sludge between the pH values of 1 and 3. He further measured the electrophoretic mobilities of various cultures in pH ranges of 1 to 12. The mobilities appeared to change quite rapidly below a pH of five. For values of pH greater than five, the electrophoretic mobility increased slowly or tended to level off. The surface charge was also found to vary inversely with the ionic strength of the solution at constant pH.

The ionic character of the supporting medium as well as the ionic strength affects the physical condition of the sludge. Presence of cations has been shown by Novak and Haugen [18] to improve dewatering of activated sludge. Divalent cations

(Ca^{+2} and Mg^{+2}) appeared to have a greater impact on the filtration rate than did the monovalent ions (Na^{+} and K^{+}), yet the differences were not large. Endo, et. al. [57] found that pure cultures of Flavobacterium isolated from activated sludge did not flocculate well when grown in mediums containing less than 0.1 mM calcium. Good flocculation was achieved in cultures grown at concentrations of 0.3 mM calcium or greater.

CHAPTER IV

EXPERIMENTAL APPROACH

Introduction

The investigations performed in this study were divided into two major sections. The first dealt with the biochemical and kinetic response of batch activated sludge cultures to different types of colloidal substrates. The second portion of the study attempted to evaluate the physical response of activated sludge to colloidal substrate addition.

Metabolic Studies

Substrates

Potato Starch

Starch is a naturally occurring ubiquitous compound found in all domestic wastewaters. The starch molecule, itself, consists of a series of glucose molecules linked together in one of two ways. Depending upon this type of linkage, starch is separated into two fractions: amylose and amylopectin. Amylose is a flexible linear chain molecule of 500 or more glucose units joined together by α-1, 4-glucosidic linkages. Figure IV-1 describes this repeating molecule whose molecular weight commonly ranges between 69,000 to 82,600. It prefers a coiled cylindrical geometric structure in solution. Also described in Figure IV-2, amylopectin is a glucose polymer with a configuration consisting of linear chains of 20-30 glucose units connected at branch points. The binding at these

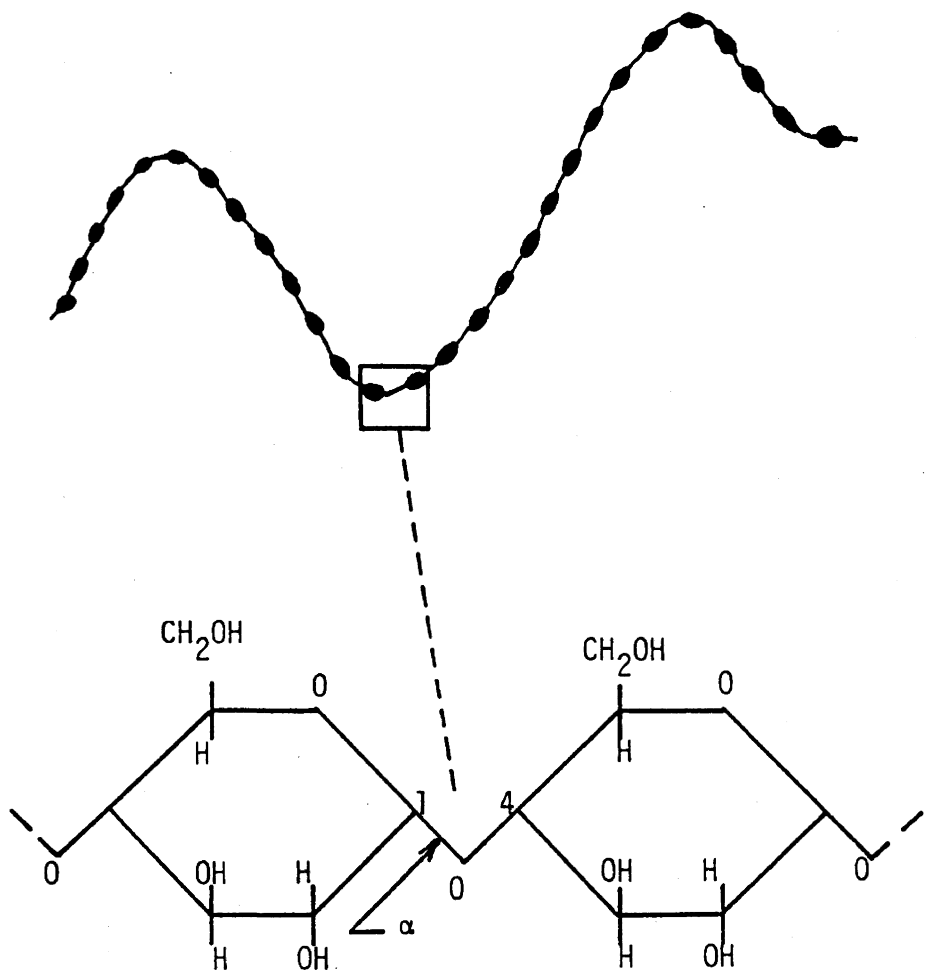


Figure IV-1: Structure of the Amylose Starch Molecule

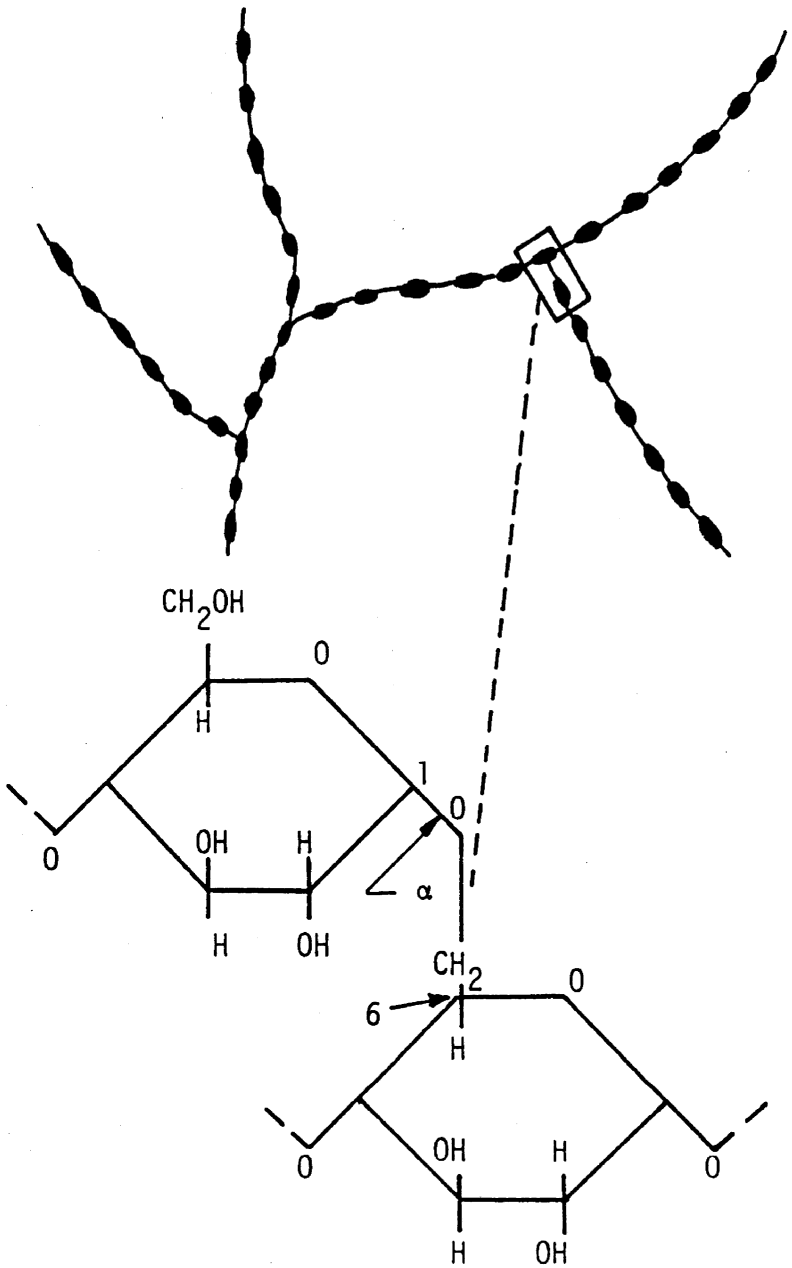


Figure IV-2; Structure of the Amylopectin Starch Molecule

branch points is characterized by formation of α-1,6-glucosidic linkages. The exact structure of this molecule is not known partly due to its high molecular weight polymeric structure (molecular weights exceeding one million have been measured).

The specific starch used in this study was potato starch. Lyophilic and highly adsorptive nature of this colloidal substrate were of great interest in this study. Also, the ease of bacterial acclimation and the availability of published metabolic data with activated sludge were two other reasons for its choice.

Egg Albumin (Ovalbumin)

Egg albumin or ovalbumin is a major protein constituent of egg white. A molecular weight of approximately 45,000 has been assigned to this polymer which contains almost all of the naturally occurring amino acids and a small carbohydrate fraction (approximately 2-4% by weight) [58,59]. An isoelectric point of 4.58 has been determined for this protein molecule. Ovalbumin is also quite surface active due to its relatively hydrophobic nature. Therefore, the molecule tends to concentrate at interfaces such as an air-water or a lipid-water interface. The latter example suggests a possible interaction between this substrate and a bacterial cell surface. This characteristic and the predominant protein nature were two facts leading to the choice of this colloidal compound as a substrate. Ovalbumin used in this study was purchased from Sigma Chemical Company.

Jack Bean Meal

The jack bean (Canavalia ensiformis) is indigenous to the West Indies, Mexico, Peru, and Brazil [61]. The jack bean meal was chosen due to its chemical make-up, i.e., it is composed of both protein and carbohydrate material. The exact composition of the jack meal is described in Table IV-1 [62]. The substrate was also purchased from Sigma Chemical Company.

Batch Kinetic Studies

Acclimation

Activated sludge was obtained from a motor vehicle rest stop on Interstate 81 near Radford, Virginia. Acclimation of the sludge took place in three liter cylindrical batch reactors under controlled temperature conditions (20 $\pm$ 1°C) as did all the studies performed in this work. Three aerated reactors containing activated sludge were each fed a combination of settled domestic sewage and one of the aforementioned substrates. The acclimation period consisted of feeding an influent with increasing ratios of synthetic substrate to domestic sewage over a three week period. A wasting rate equal to one tenth of the reactor volume was maintained during acclimation and after.

Daily Protocol

Each day, as mentioned, one tenth of each batch reactor was initially wasted while the sludge was still mixing. Aeration stones were then removed and the sludge allowed to settle for approximately fifteen to thirty minutes at which time the supernatant

Table IV-1

Composition of Jack Bean (Canavalia ensiformis)

Constituent	Quality (grams)*
Moisture	88.6
Protein	2.7
Fat	0.2
Carbohydrate	7.9
Ash	0.6

*The above analysis is of 100 grams of raw, immature pods.

was siphoned off. Tap water was used to restore the cultures to the original three liter volume. Substrate (~500 mg/l COD) and essential nutrients were then added and aeration begun.

Substrate were prepared daily. The preparation of albumin simply involved adding the albumin to tap water and mixing vigorously using a magnetic stirrer. The starch and jack bean meal, though, required mixing in tap water and heating to a boil. One other preliminary step for the jack bean meal involved the sieving of the powdered substrate to remove large insoluble and easily settleable particles. The particles passing a No. 100 sieve (.149 mm diameter) were used for the substrate.

Nitrogen, phosphorus, and other essential nutrients were added to supplement the substrates. Table IV-2 lists the nutrient components and the relative amounts added per unit of substrate COD. Note the phosphorous not only functions as a nutrient but also as a pH buffer. The pH of the buffer solution was approximately seven.

Batch Studies

Batch kinetic investigations were performed on each of the albumin, starch, and jack bean meal acclimated sludges. In these barch studies, the biomass concentration, measured by mixed liquor suspended solids (MLSS), was adjusted to approximately 1500 mg/l. The appropriate substrate and nutrients were then added to the culture and timing begun. The run was ended when the culture was believed to be close to its original state prior to substrate addition.

Table IV-2

SUBSTRATE COMPONENT RATIOS

Substrate Component	Ratio
Nitrogen (NH_4Cl)	10/1 as COD/N
Manganese ($MnSO_4 \cdot H_2O$)	250/1 as COD/Mn
Magnesium ($MgSO_4 \cdot 7H_2O$)	250/1 as COD/Mg
Iron ($FeCl_3 \cdot 6H_2O$)	250/1 as COD/Fe
Calcium ($CaCl_2$)	250/1 as COD/Ca
Phosphorus*	

*A phosphate buffer was utilized for both nutrient requirements and pH control by adding 10 mls of the stock buffer solution (80 grams KH_2PO_4 + 160 grams K_2HPO_4 in 1 liter of distilled water) in every liter of feed solution.

More specifically, nine separate runs were performed on each activated sludge culture. Pure substrate was added at loadings of 0.25, 0.50, and 0.75 pounds of substrate COD per pound of MLSS. These loadings were then repeated with 20% and 40% of the substrate COD supplemented with glucose. The glucose addition was utilized in order to provide an easily metabolized soluble substrate to the bacteria. Glucose is also a known catabolic repressor [10], i.e. it represses the synthesis of inducible and constitutive enzymes. This enzyme repressing ability was thought, a priori, to have an effect in the "uptake and release" phenomenon of colloidal substrates by possibly retarding the synthesis of extracellular hydrolytic enzymes.

During the tube run, samples of mixed liquor were withdrawn at 5, 10, 20, 30, 45, 60, 90, 120, 180, and 240 minutes and every two to four hours thereafter until completion. Parameters monitored were filterable and settleable COD, oxygen uptake, and MLSS concentrations. The filterable COD was measured from a sample of mixed liquor passed through a No. 1 Whatman filter; settleable COD values were obtained by allowing an aliquot of mixed liquor to settle for 30 minutes and measuring the COD of the supernatant. The COD procedure followed is found in Standard Methods Section 508 [63]. MLSS measurements were made in accordance with Standard Methods Section 208D using Reeve Angel glass fiber filters (pore size = 0.45μ). The oxygen uptake procedure was followed from Standard Methods Section

213B. When both the COD values and oxygen uptake rates approximately equaled their values prior to substrate addition, the batch study was terminated.

Physical Studies

The physical interaction studies between the colloidal substrates and the biomass were separated into two sections. Initially, electrophoretic mobility measurements were made to determine stability of both the colloids and biomass under various conditions. Secondly, adsorption studies were performed under conditions similar to those imposed in the electrophoretic mobility studies. Detailed information on these two interrelated investigations will follow.

Electrophoretic Mobility

Environmental Conditions

Discussed in the literature review were the factors affecting both lyophobic and lyophilic colloidal systems. Three of these factors - pH, ionic strength, and ionic mecium - were varied in these studies. Both the substrates and acclimated cultures were subjected to the electrophoretic mobility measurements at pH and ionic strengths comparable to those found in wastewater treatment systems. Listed below are the conditions imposed during the study:

a) Five pH values (5,6,7,8,9)

b) Two ionic strengths (I = .001 and .01)

c) Two ionic mediums (NaCl and $CaCl_2$)

The pH adjustments were made using buffer solutions and 0.1N NaOH and 0.1N H_2SO_4. The type and preparation of buffer solutions can be found in Appendix A.

Substrate and Sludge Preparation

The substrates were mixed in concentrations of one gram per liter for measurement of the electrophoretic mobility. Preparation methods for these colloidal solutions were identical to those in the daily protocol except that known ionic strength solutions of NaCl or $CaCl_2$ were used instead of tap water. Measurements on two of the three substrates were made with little difficulty; no values could be obtained for starch.

Activated sludge mobility measurements were made by first withdrawing an aliquot (250 mls) of mixed liquor from the reactors. These measurements were made to determine if a link existed between adsorption of colloidal substrates to activated sludge and the surface charge on the bacterial flocs. The cultures were allowed to aerate at least 24 to 36 hours after feeding to ensure no colloidal substrate remained which might affect the results. The portion of mixed liquor was then centrifuged at 1500 rpm, the supernatant was decanted, and the remaining sludge was brought back to its original volume with distilled water. This procedure was repeated three more times. After the final elution, a portion of this sludge was suspended in a prespecified ionic solution for measurements.

Concentrations of activated sludge used for electrophoretic mobility measurements ranged from 40 to 70 mg/l.

Electrophoretic Mobility Measurements

The instrumentation used to measure the electrokinetic potentials of the substrates and sludges was the Zeta Meter. Basically, the procedure involved pumping a sample into a calibrated viewing cell. A known voltage was then applied across this cell and the rate of migration of the colloids was measured. The colloids were viewed through a microscope with calibrated tracking line etched in the eyepiece; and by using the supplied timer, the velocity of the colloids could be determined.

More specifically, thirty colloids were tracked for each sample and an average time taken. This value along with the applied voltage and distance traveled by the colloid was entered into the equation below for calculation of the electrophoretic mobility:

$$EM = \frac{D}{tV}$$

where EM = electrophoretic mobility, μm/sec/volt/cm

D = distance traveled by colloid, μm

t = time to travel distance, D, sec

V = voltage strength, volts/cm

Also, the temperature of the sample was taken before and after the tracking since the viscosity of water changes with temperature which,

in turn, adversely affects the colloid mobility. The average temperature was then used to determine viscosity.

Adsorption and Interaction Studies

The purpose of these studies was to obtain information on the physical interaction between the colloidal substrates and the activated sludge cultures and the factors that may affect this interaction. Yet, for a purely physical interaction to occur, the metabolic uptake of substrates by the bacteria had to be prevented. Therefore, a short study on the use of mercury to metabolically inactivate activated sludge was conducted.

Mercury Poisoning in Activated Sludge

The mercuric ion is a known bacteriacide [10] which has the ability to inactivate certain bacterial enzymes. Therefore, mercuric chloride was chosen to prevent or retard metabolic uptake of the colloidal substrates by the bacteria. A series of studies were undertaken to evaluate the effect of the mercuric ion on the sludge both metabolically and physically.

Optimum dosages of mercuric chloride were obtained by adding varying amounts of the inhibitor to a sample of sludge. The mixture was agitated for approximately fifteen minutes, after which, oxygen uptake measurements were performed. From these data, an optimum mercury to MLSS ratio was chosen for use in the adsorption studies. Figures IV-3, IV-4, and IV-5 show the effect of the mercuric ion on the specific oxygen uptake rate of the activated sludge. A common

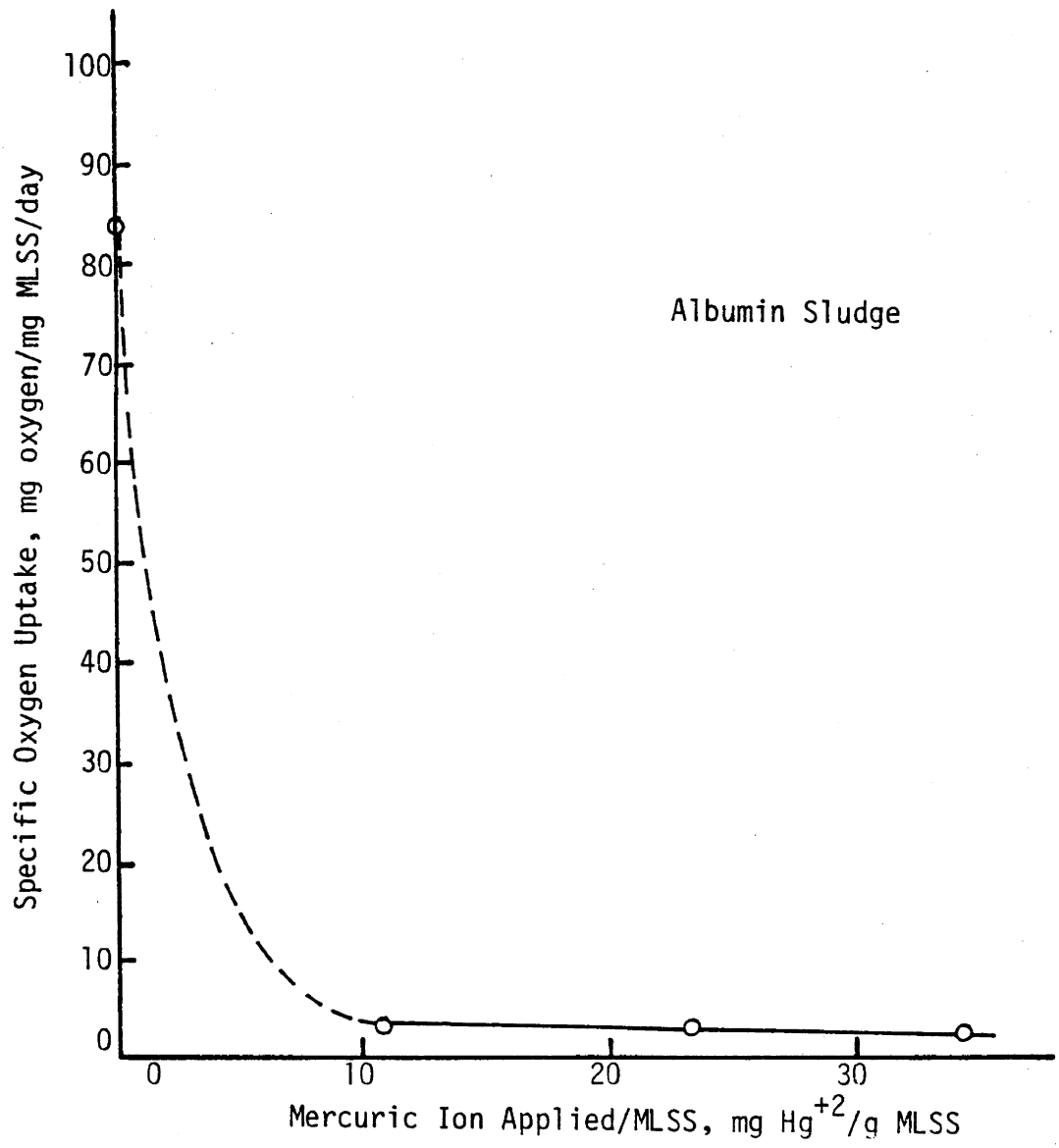


Figure IV-3: Relationship Between Specific Oxygen Uptake Rate of Albumin Acclimated Activated Sludge and the Amount of Mercuric Ion Applied

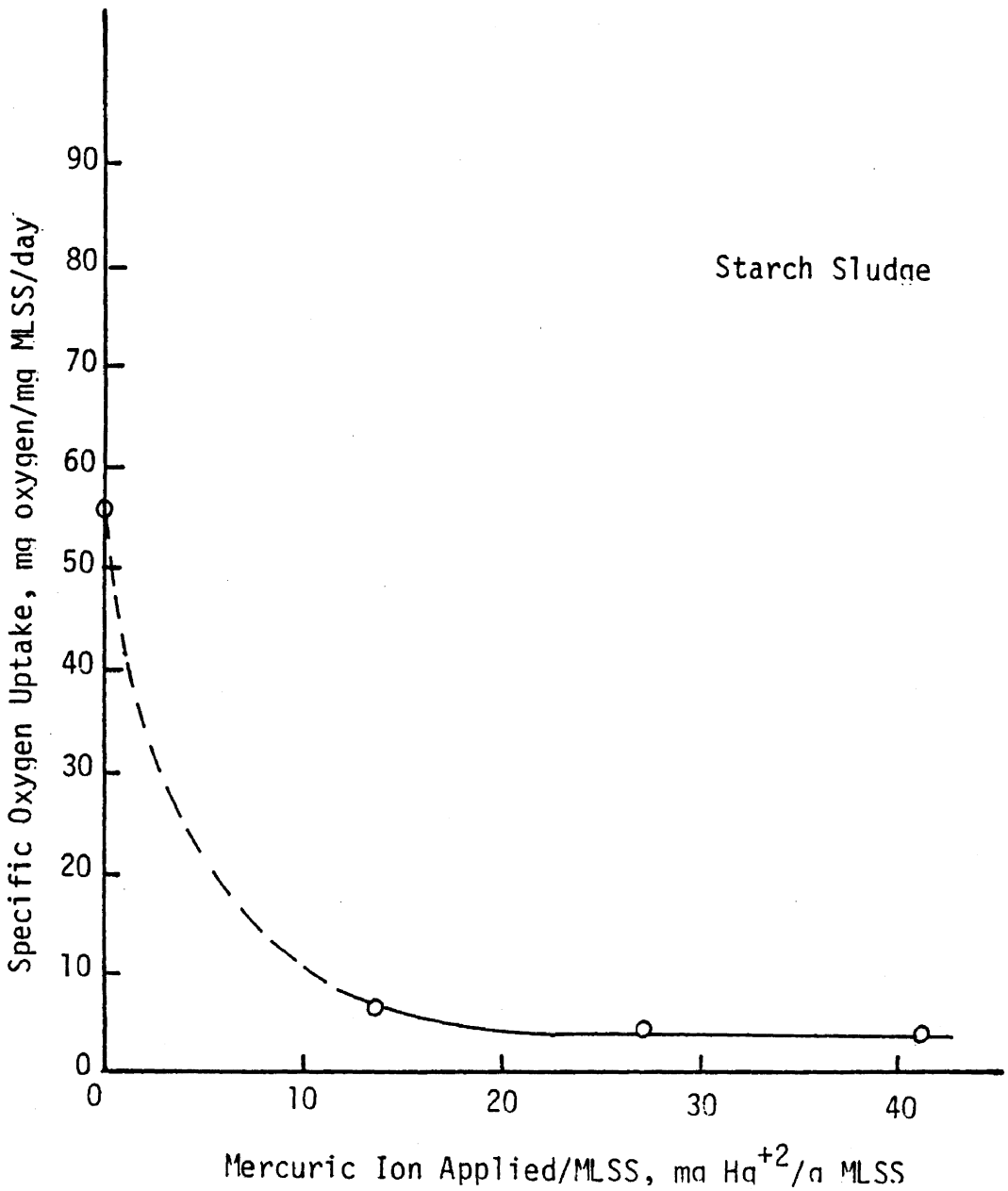


Figure IV-4: Relationship Between Specific Oxygen Uptake Rate of Starch Acclimated Activated Sludge and the Amount of Mercuric Ion Applied

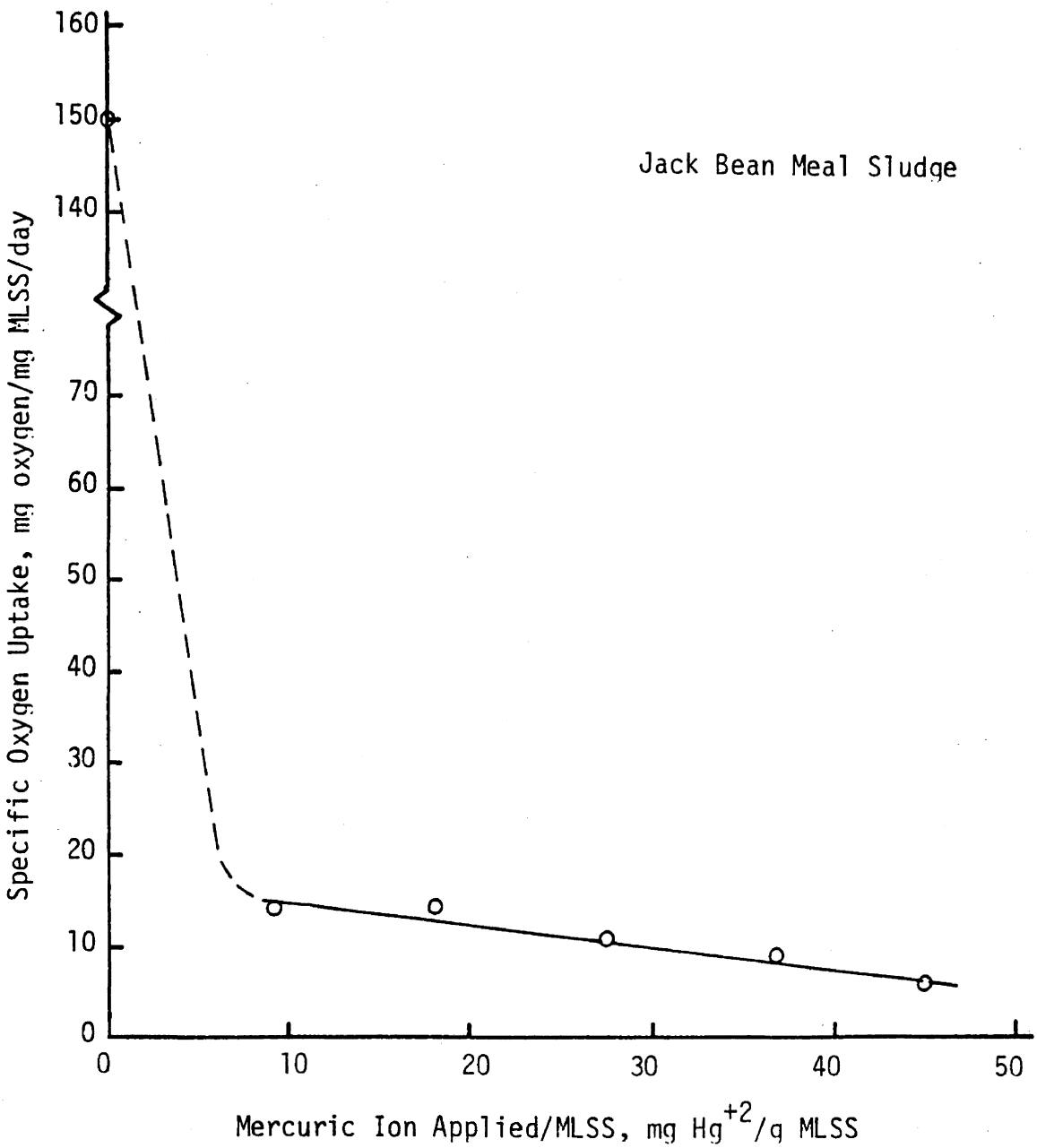


Figure IV-5: Relationship Between Specific Oxygen Uptake Rate of Jack Bean Meal Acclimated Activated Sludge and the Amount of Mercuric Ion Applied

dosage of 20 mg Hg^{+2} per g of MLSS was chosen for all three sludges. Since no data were obtained for low mercury concentrations as denoted by the dotted lines, this dosage was thought to represent a very conservative choice.

Another series of investigations was performed based on the mercury:sludge ratio chosen. After each sludge culture was poisoned with the "optimum" dosage of mercury, it was washed with a $CaCl_2$ solution (I = .01). This procedure involved centrifuging the sludge for 10 minutes at 1500 RPM, decanting the supernatant, and restoring the sample to the original volume with the $CaCl_2$ solution. This was repeated three times. Oxygen uptake rates were then measured on the washed sludge. Approximately 11.5 minutes after monitoring the oxygen drop, substrate was introduced into the bottle in which the respiration measurements were being made. Substrate COD concentrations between 225 and 280 mg/l were established. As seen in Figure IV-6, no deviation resulted in the oxygen utilization of the starch and jack bean meal culture. This provides strong evidence that metabolism of the substrate was effectively blocked. However, the addition of albumin caused an immediate drop in the oxygen concentration which subsequently leveled off. This was probably due to the surface activity of the albumin, which would produce a change in the diffusion patterns of gases into and out of the liquid, and was probably not due to bacterial metabolism.

Finally, the physical effects of the mercuric ion addition were checked. Settling tests and electrophoretic mobility tests

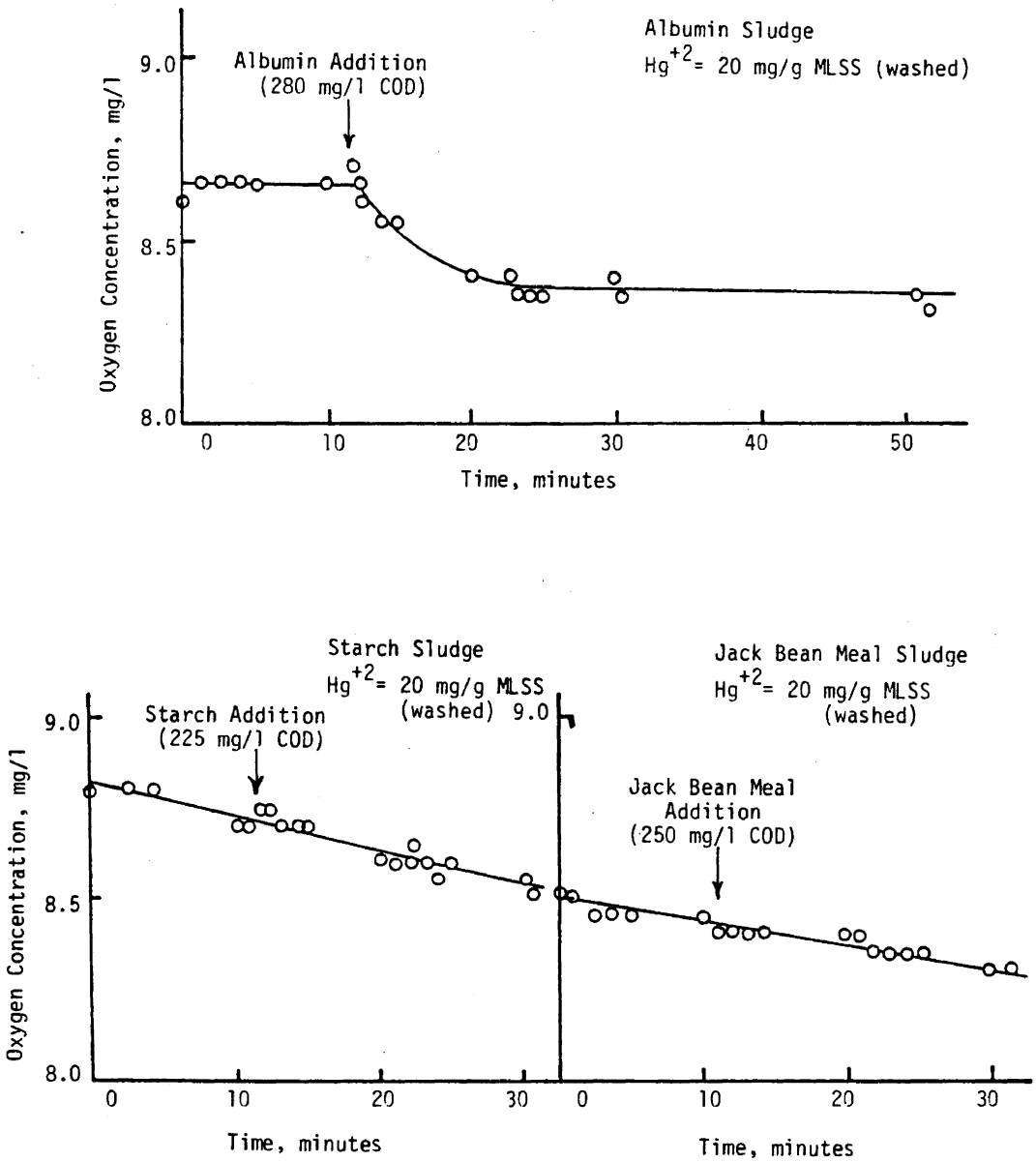


Figure IV-6: Oxygen Utilization of Three Mercury Poisoned and Washed Activated Sludge Cultures

were used to evaluate the physical stability of the sludge. From Figures IV-7, IV-8, and IV-9, there appeared to be no significant differences in the settling rates of all three sludges due to the presence of mercury. The results of the studies described in Table IV-3 showed no appreciable effects on the surface charge of the bacteria. The three sludges in the electrophoretic mobility study were exposed to the optimum dosage, washed, and then suspended in NaCl and $CaCl_2$ ionic mediums at pH 7.

Adsorption Studies

This portion of the investigation involved mixing varying amounts of the metabolically inactive sludge and substrate under controlled conditions. Various parameters were then measured to determine the extent of the interaction.

Initially, a quantity of sludge was removed from a batch reactor vessel and the required dosage of mercuric chloride applied. Poisoned mixture was agitated for 15 minutes and then centrifuged at 1500 RPM for 10 minutes using 250 ml centrifuge tubes. Supernate was then decanted and replaced with distilled water and remixed. This procedure was repeated three more times. The concentration of the sludge was further diluted to a concentration between 1000 and 3000 mg/l. Meanwhile, concentrations of substrate ranging from 100 to 4000 mg/l were prepared for mixing with the sludge.

The pH and ionic strength of the sludge and substrate mixtures were then adjusted in a manner identical to that described in the

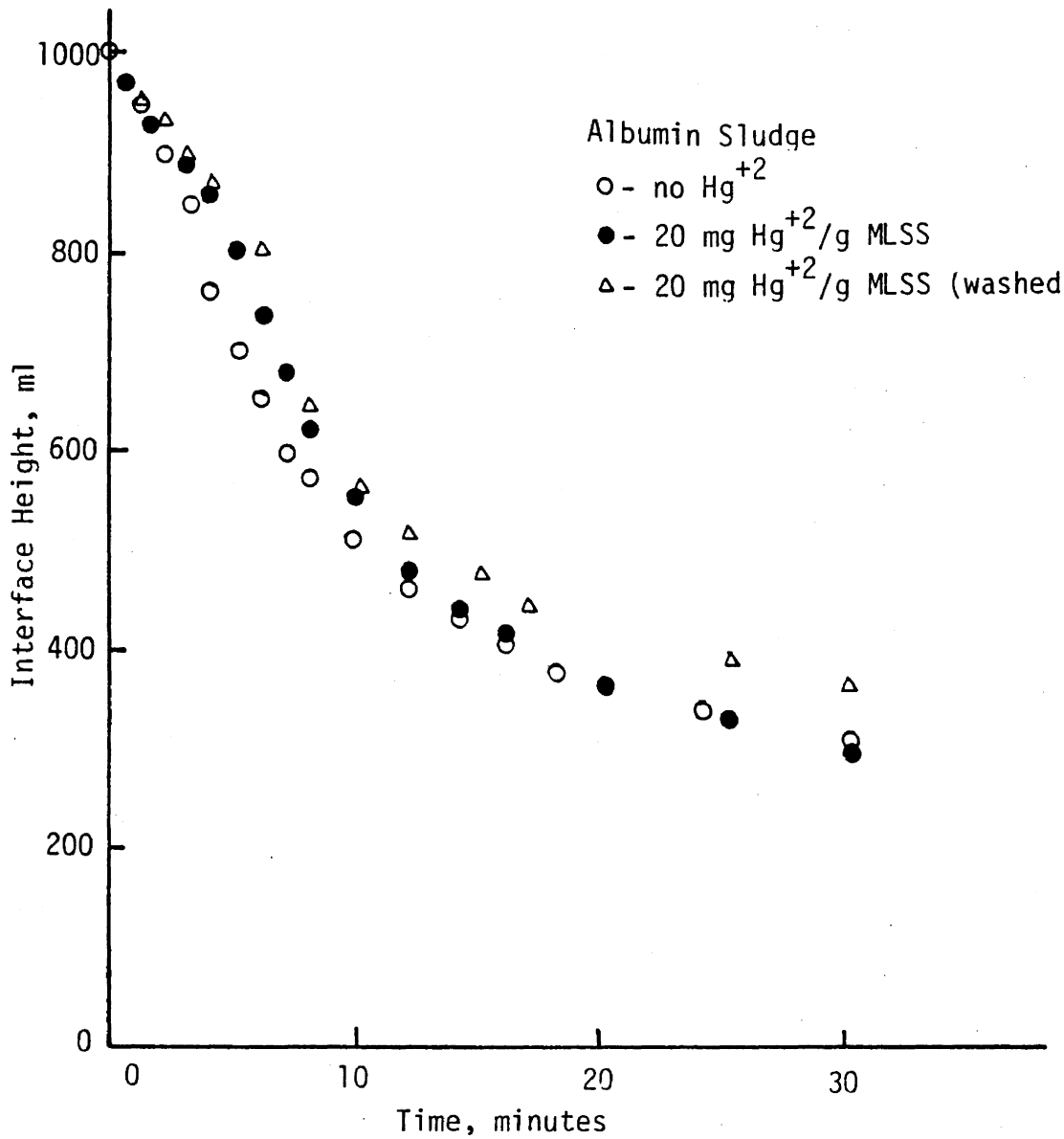


Figure IV-7: Settling Curves for an Albumin Acclimated Activated Sludge Culture when Metabolically Active, Mercury Poisoned, and Mercury Poisoned and Washed

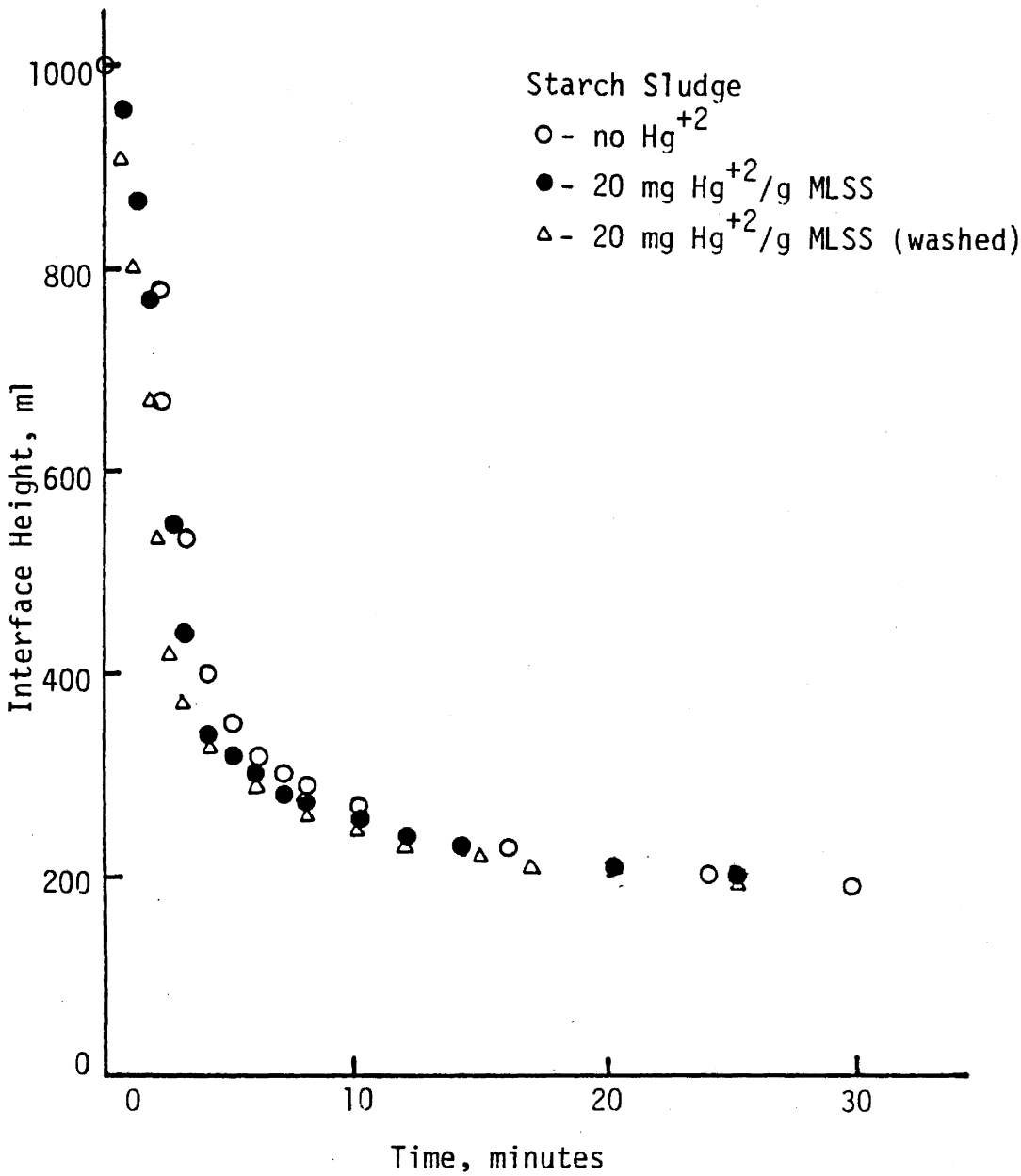


Figure IV-8: Settling Curves for a Starch Acclimated Activated Sludge Culture when Metabolically Active, Mercury Poisoned, and Mercury Poisoned and Washed

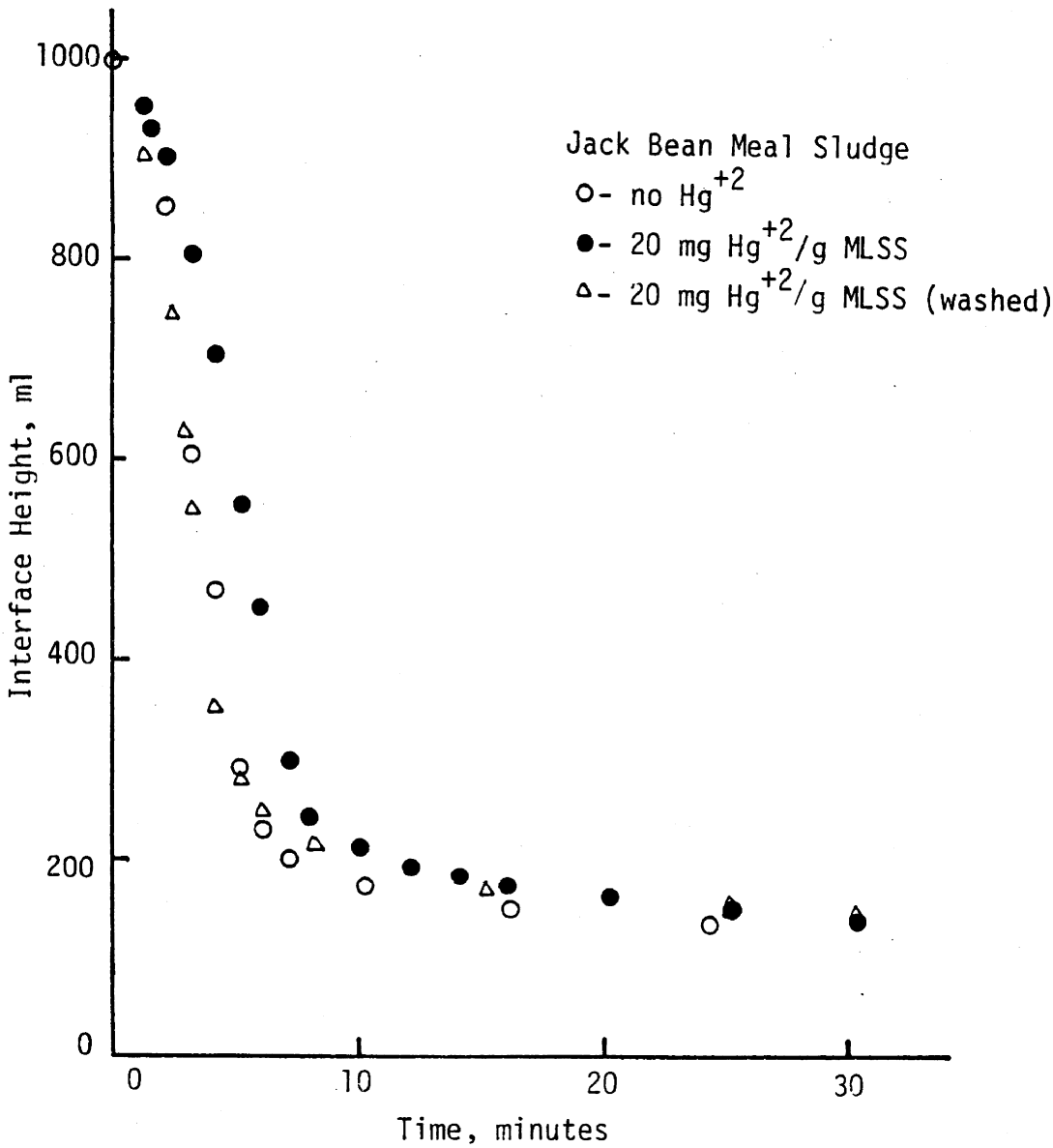


Figure IV-9: Settling Curves for a Jack Bean Meal Acclimated Activated Sludge Culture when Metabolically Active, Mercury Poisoned, and Mercury Poisoned and Washed

Table IV-3

Type of Activated Sludge	Ionic Medium/Strength	Electrophoretic Mobility cm/sec/volt/cm	
		No Mercury Addition	Mercury Addition
Albumin Acclimated	NaCl/.001	5.15	4.90
	$CaCl_2$/.01	1.50	1.60
Starch Acclimated	NaCl/.001	2.90	3.30
	$CaCl_2$/.01	0.90	1.00
Jack Bean Meal Acclimated	NaCl/.001	3.90	3.90
	$CaCl_2$/.01	1.10	1.00

electrophoretic mobility study. The matrix of conditions, though, was reduced by eliminating the pH values of 5 and 9 limiting the number pH values tested to three. The conditions are summarized below:

a) Three pH values (~6,7,8)

b) Two ionic strengths (I = .001 and .01)

c) Two ionic mediums (NaCl, $CaCl_2$)

One other variable tested in this study was the effect of different concentrations of MLSS. By using two MLSS values between 1000 and 3000 mg/l, it was believed some insight into the mechanism of interaction, i.e. adsorption or enmeshment, could be obtained. If adsorption played the predominant role, isotherm plots for the two MLSS values would overlay one another. In contrast, enmeshment of substrate would probably result in two different isotherms. However, these a priori assumptions were not validated as will be shown in the discussion of results.

The protocol of the adsorption study follows below. For a given set of conditions, i.e. one pH, one ionic strength, one ionic medium and MLSS concentration, six 300 ml erlenmeyer flasks were used. Into five of these flasks, varying concentrations of substrate were added in 100 ml aliquots. The sixth flask was a blank to which 100 mls of "conditioned" water was placed. Then, 100 mls of sludge was poured into each flask. Once mixed, the final concentrations of substrate and sludge were, again, between 100 to 4000 mg/l and 1000 to 3000 mg/l, respectively. These mixtures were subsequently placed on a rotary shaker and agitated for five minutes. The flasks

were then removed and allowed to settle for 30 minutes at which time approximately 40 mls of supernate was removed.

The schematic in Figure IV-10 describes the measurements performed on the supernatant and sludge sludge fractions. COD concentration on the supernate were monitored and used primarily for construction of isotherm plots. Turbidity measurements were also marked on the supernatant to obtain a gross measure of the particles remaining in solution. A Hach 2100A Turbidimeter was used for this purpose.

Particle counts and size distributions were determined on both the supernatant and sludge-supernatant fractions. Particle counts were obtained using a HIAC Particle Size Analyzer Model PC-320 counter; a description of this counter and how it operates can be found in Appendix B. This particular counter was equipped with two counting sensors--one measuring particles between 1 and 60 microns and the other sensing particles from 5 to 300 microns. Supernatant samples were carefully diluted (1/250 to 1/50) then particle counts were measured using the 1 to 60 micron sensor. Particle size distributions as well as particle count values were acquired from this data. For the sludge-supernatant samples, only shifts in particle size distribution were monitored therefore no measured dilutions were made. Also, the 5 to 300 micron sensor was used due to the relatively large floc particles.

All three organic substrates were tested in the same manner described above yet without any sludge addition. The only change

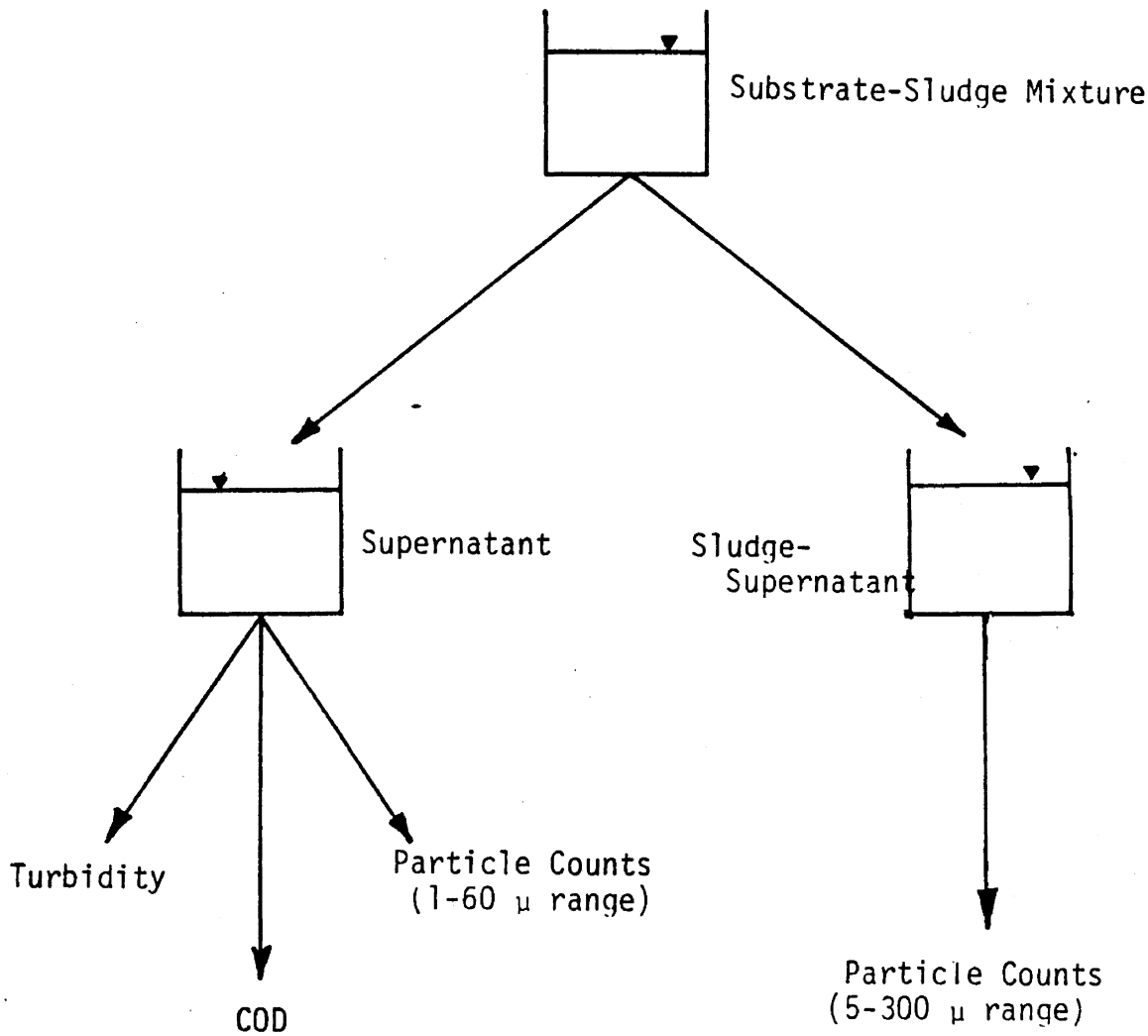


Figure IV-10: Schematic Representation of the Analysis Performed on the Substrate-Activated Sludge Mixtures

made in the protocol was that the substrate mixture was not allowed to settle the allotted thirty minutes. Immediately after the five minute mixing period, the samples were removed and tested. The thirty minute settling was tried for one series of studies, but the results were quite unreasonable for the supernatant fraction. Since the aliquots were extracted by use of a pipette, the problem was in all probability caused by the nonuniform depth at which the sample removals were taken.

CHAPTER V

RESULTS

Metabolic Studies

Introduction

These investigations were performed in an attempt to describe and explain metabolic responses of activated sludge to various types of colloidal-soluble substrate mixtures. A protein (albumin), a carbohydrate (potato starch), and a protein-carbohydrate (jack bean meal) substrate were separately studied to determine if substrate composition is important for the substrate removal mechanisms that favor contact stabilization over other activated sludge process variations. From the standpoint of previously reported studies, it was important to monitor and establish the existence of an "uptake and release" phenomenon, and the variation of oxygen uptake in relation to substrate uptake was closely scrutinized. Both of these responses would indicate the manner of colloidal substrate usage. Finally, the activated sludge yield, the specific oxygen utilization and substrate adsorption characteristics were measured to help evaluate the effects of glucose supplementation on storage and adsorption capacity.

Albumin-fed Culture

Metabolic Response

The albumin-fed culture gave some very interesting insights into colloidal substrate utilization. Figures V-1 to V-8 describe

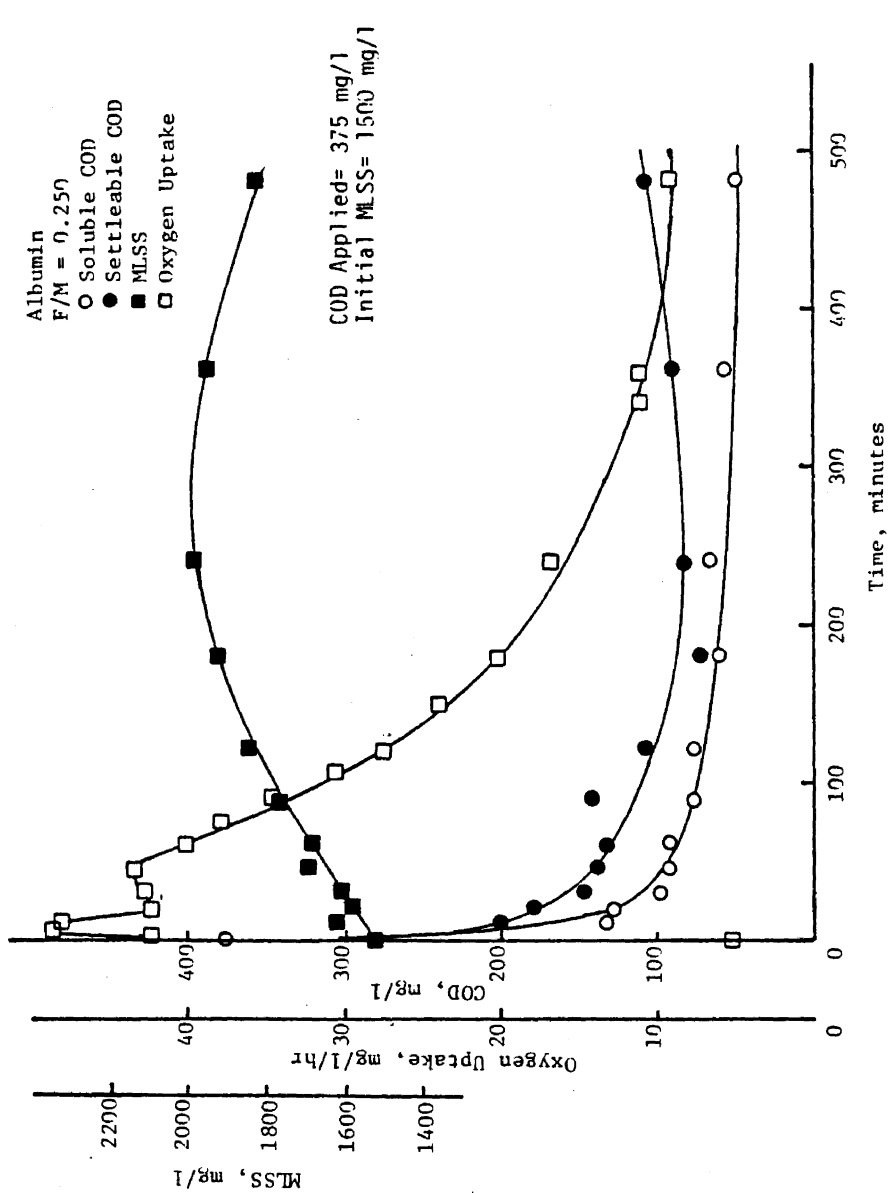


Figure V-1: Results of a Batch Study on an Albumin Acclimated Activated Sludge Culture Fed Only Albumin

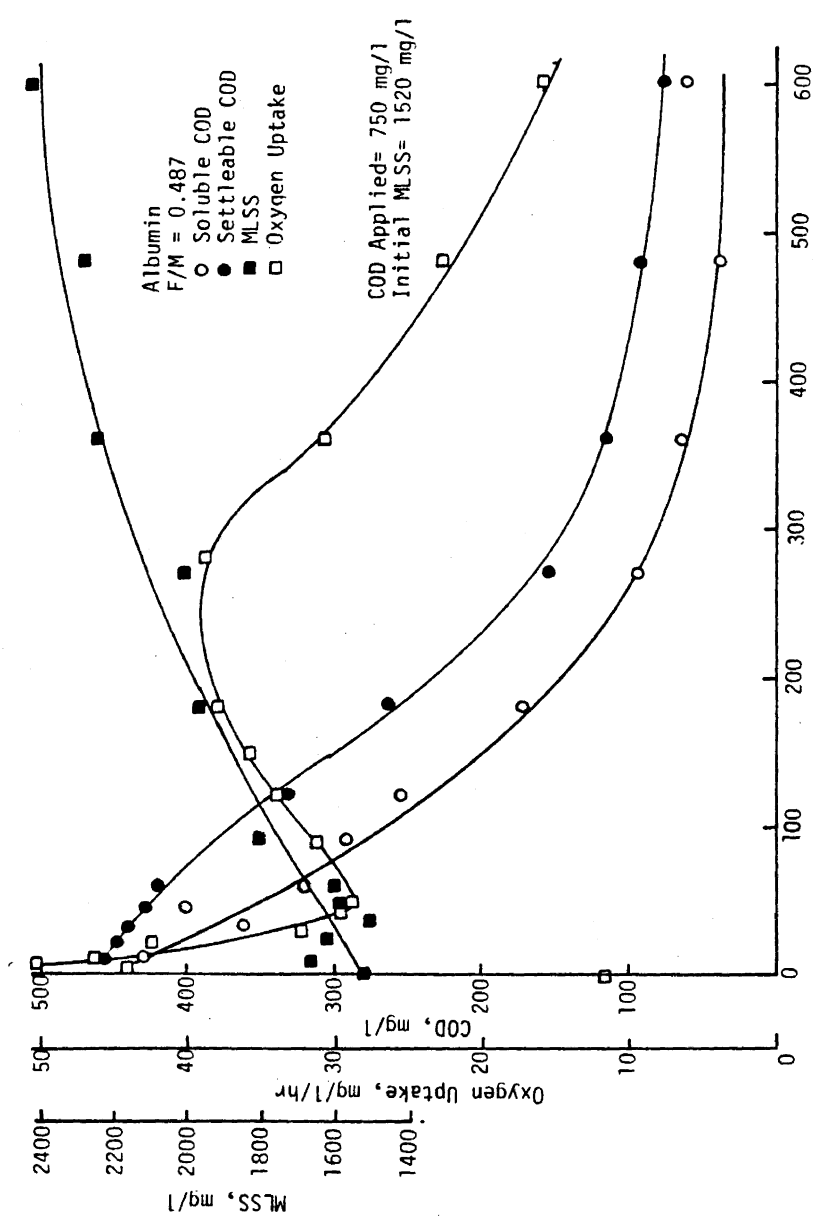


Figure V-2: Results of a Batch Study on an Acclimated Activated Sludge Culture Fed Only Albumin

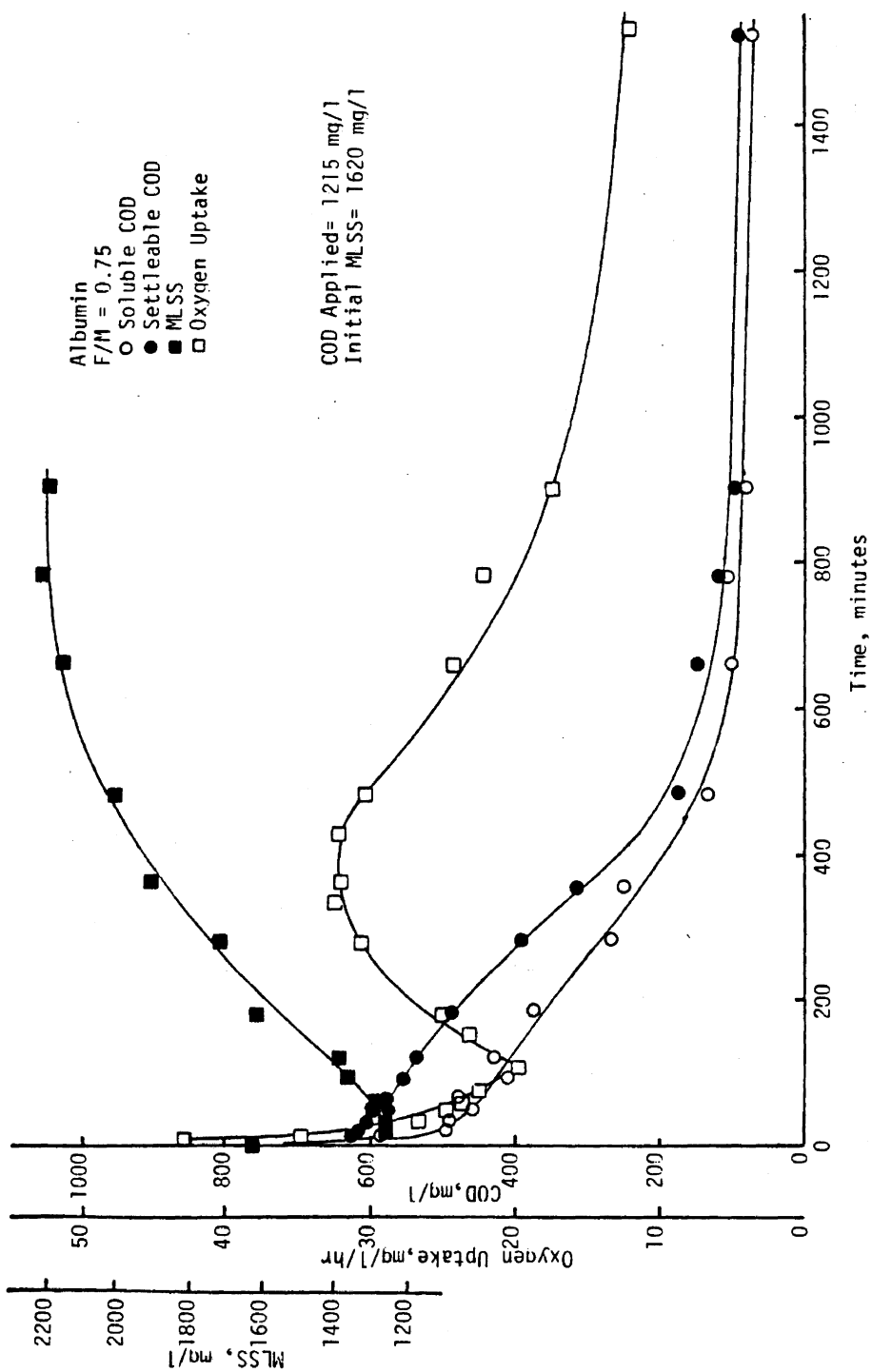


Figure V-3: Results of a Batch Study on an Albumin Acclimated Activated Sludge Culture Fed Only Albumin

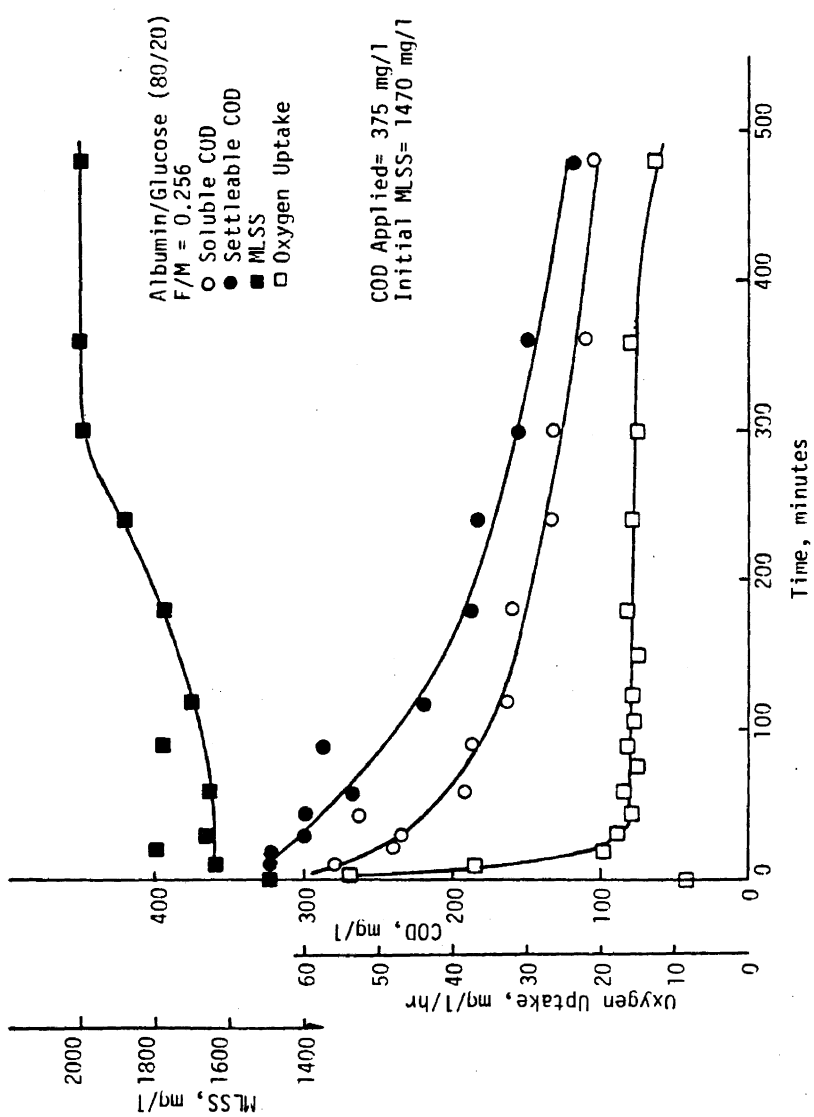


Figure V-4: Results of a Batch Study on an Albumin Acclimated Activated Sludge Culture Fed Eighty Percent Albumin and Twenty Percent Glucose

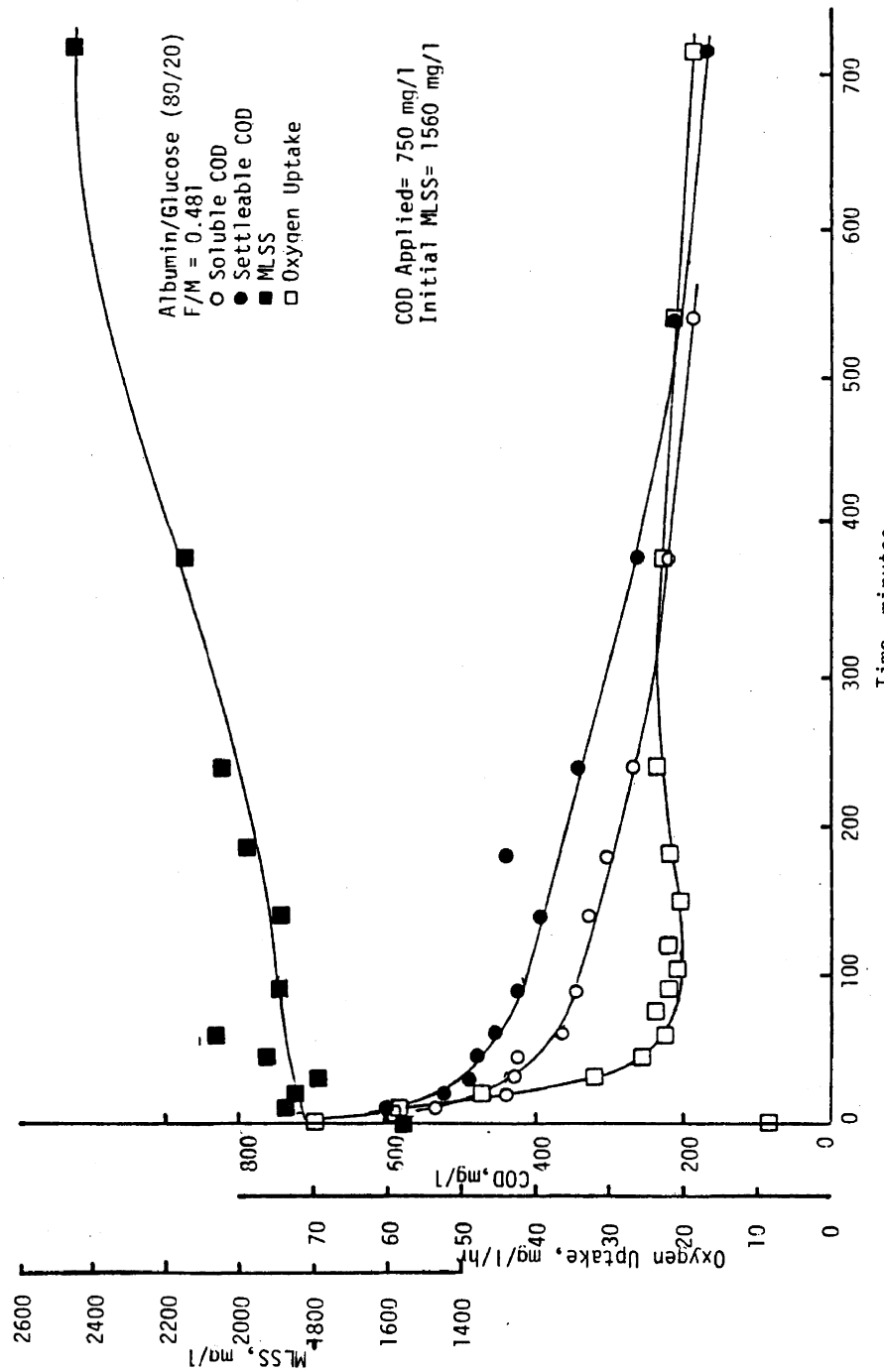


Figure V-5: Results of a Batch Study on an Albumin Acclimated Activated Sludge Culture Fed Eighty Percent Albumin and Twenty Percent Glucose

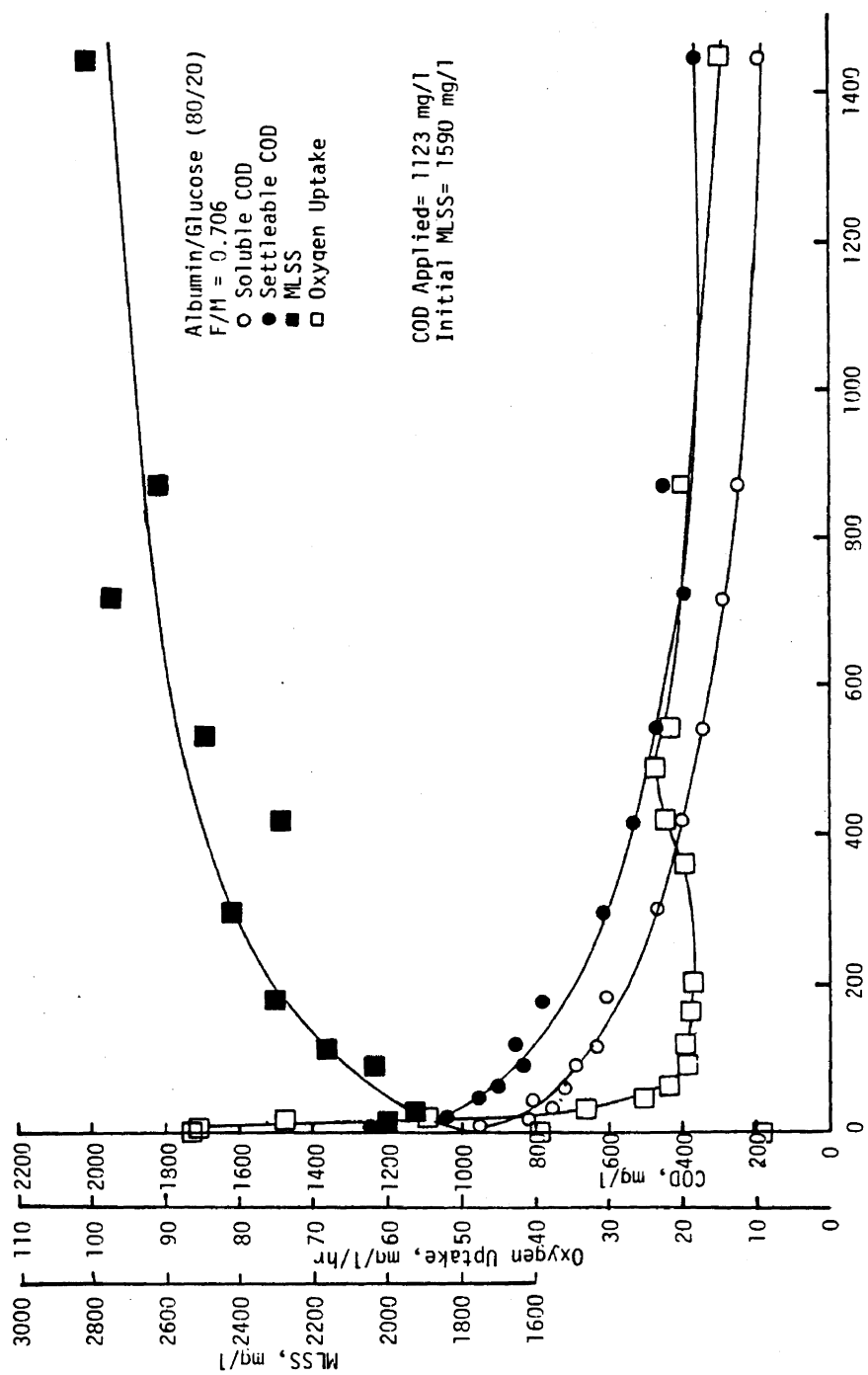


Figure V-6: Results of a Batch Study on an Albumin Acclimated Activated Sludge Culture Fed Eighty Percent Albumin and Twenty Percent Glucose

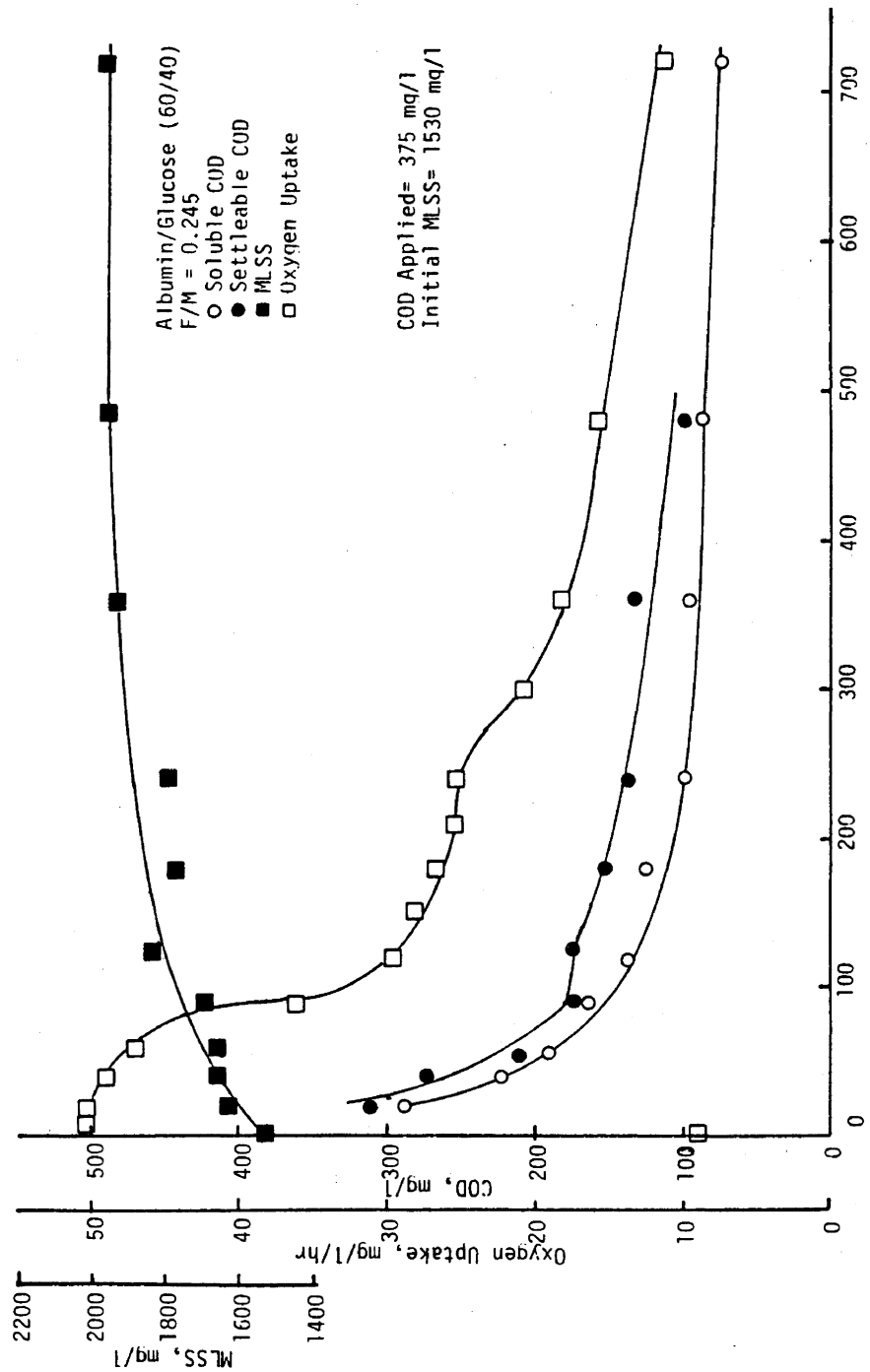


Figure V-7: Results of a Batch Study on an Albumin Acclimated Activated Culture Fed Sixty Percent Albumin and Forty Percent Glucose

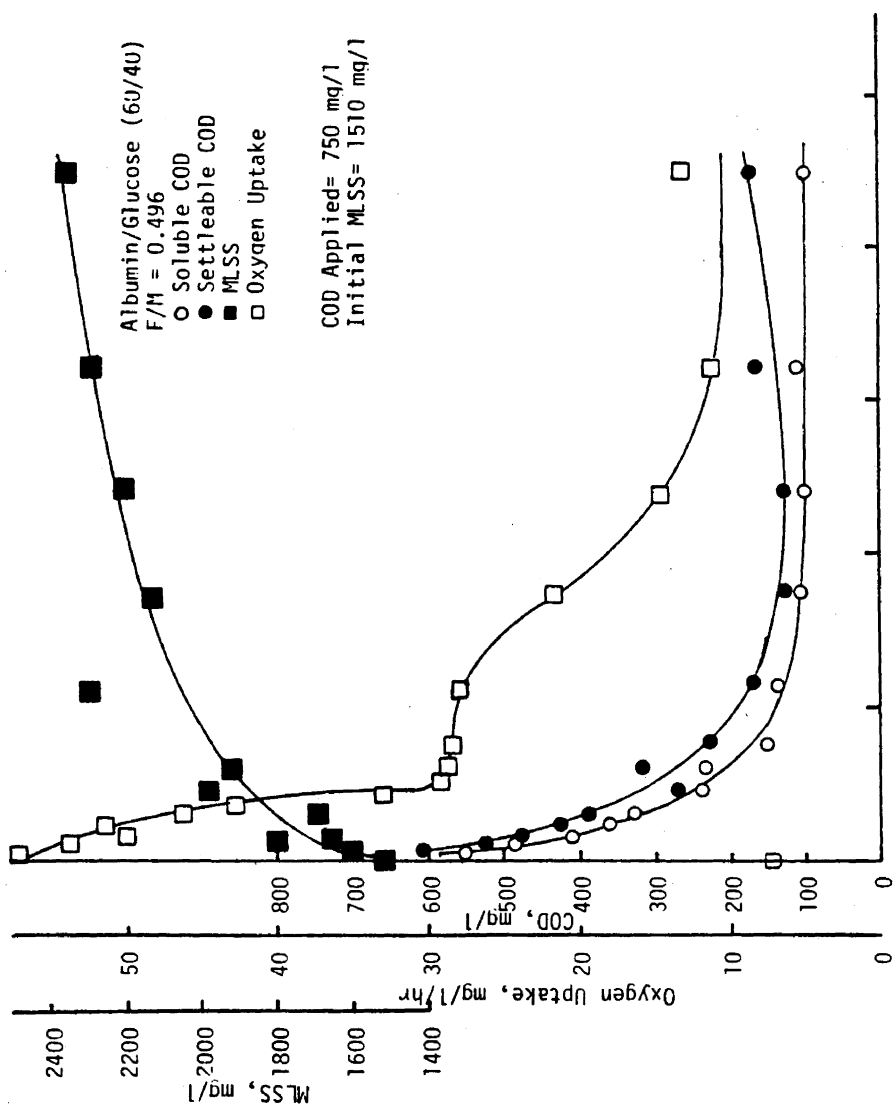


Figure V-8: Results of a Batch Study on an Albumin Acclimated Activated Sludge Culture Fed Sixty Percent Albumin and Forty Percent Glucose

the series of batch experiments performed. Figures V-1 to V-3 show the response of albumin activated sludge culture to varying concentrations of albumin with no supplemental glucose. There was no uptake and release phenomenon apparent but the oxygen uptake curve responded in a very curious manner. In each of these three studies, the oxygen uptake, as expected, increased to a high rate upon the introduction of the substrate then began to decrease. Unexpectedly, though, the respiration rate then noticeably increased for a period of time before making a second and final descent. This phenomenon can be explained with the premise that the utilization of the albumin substrate is a two step process. Energy is required to produce enzymes capable of hydrolyzing the protein molecule such that it can be passed through the cell membrane into the cell. Then, additional energy is required to metabolize the hydrolyzed molecules. Therefore, the two peaks on the oxygen uptake curve probably correspond to hydrolysis and intercellular utilization of the substrate, respectively.

In the studies shown in Figure V-4 through V-6, glucose was supplemented for twenty percent of the total COD. Again, substrate release was not noticed and even the oxygen uptake response was dampened. A slight increase in the respiration rate could be seen in the two higher loadings (Figure V-5 and V-6). The rapid decrease of the oxygen uptake rate denoted the albumin-glucose substrate mixture was utilized sequentially. The glucose was metabolized first followed by the albumin. This is clearly confirmed by the

final two studies wherein glucose was added as forty percent of the total COD supplied to the culture. The rate of respiration shifts significantly describing this sequential nature. Here, though, the oxygen uptake proceeded to decrease significantly following glucose utilization, whereas it varied slightly in the series of studies where twenty percent glucose was supplemented.

Kinetics

Due to the sequential nature of the utilization of the albumin-glucose substrate mixtures, rates of soluble COD removal were assumed to be zero order. For a predictive model, this assumption would be ludicrous; however, since only comparative rates of removal are of interest here, the zero order assumption will suffice. Simply, a line was drawn for the initial COD value to a point judged to be the end of detectable metabolic functions upon the albumin. An example is shown in Figure V-9.

The rates of removal are listed in Table V-1 for all the substrate combinations tested. The introduction of glucose appeared to slightly reduce or retard the consumption of albumin. As larger amounts of glucose were supplemented though, the rate was augmented due to the easily assimilated glucose substrate.

Yield and Oxygen Utilization Coefficients

Yield for the albumin acclimated culture were obtained for each substrate combination. Values for the amount of biomass generated

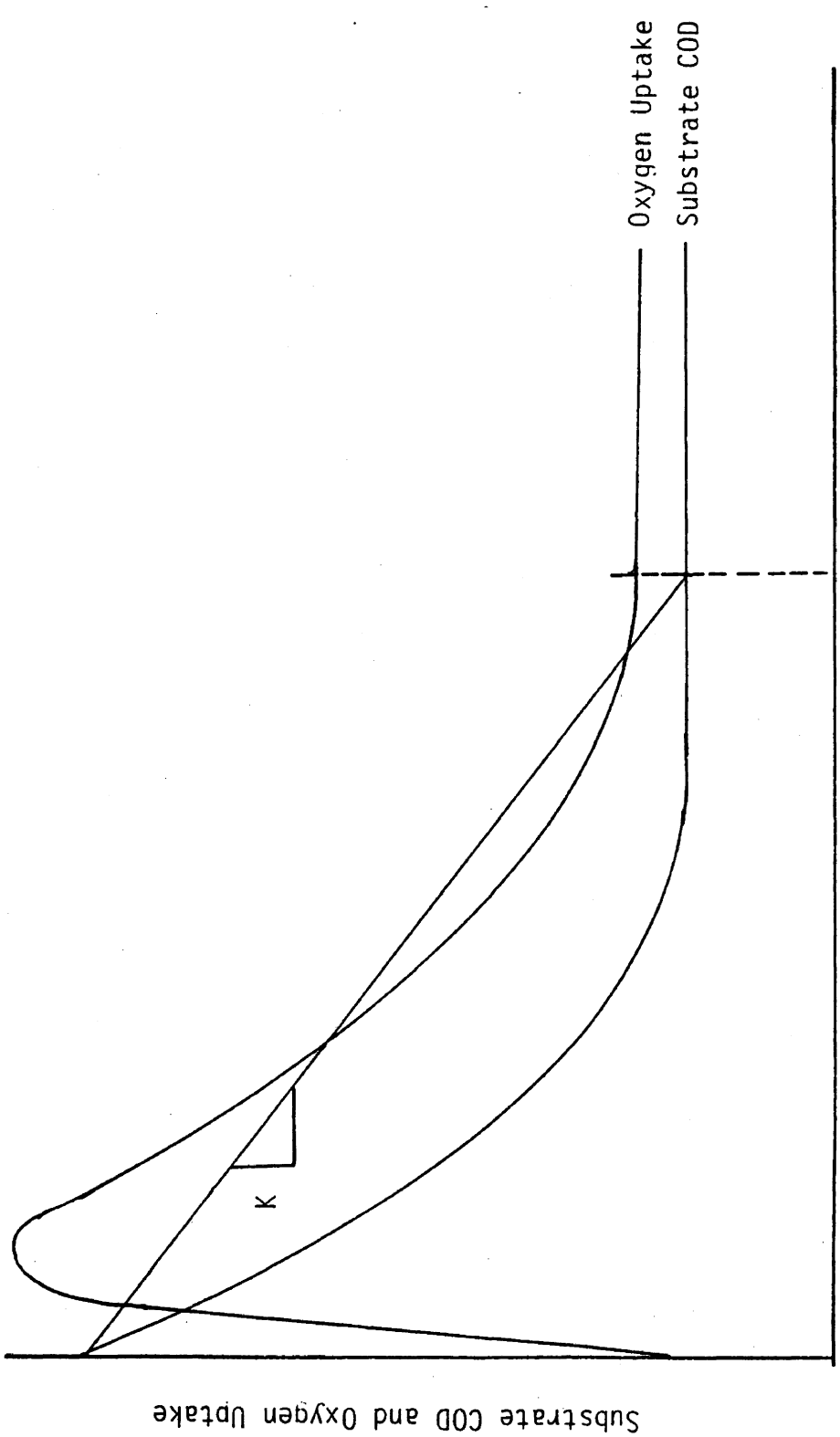


Figure V-9: Description of the Kinetic Rate Constant, K, Determination

Table V-1

Albumin Removal Rates

Substrate	F/M (mg/mg-day)	Removal Rate (mg COD/min)
Albumin	0.250	0.812
	0.487	1.150
	0.750	1.150
Albumin/Glucose 80/20	0.256	0.688
	0.481	0.806
	0.706	0.979
Albumin/Glucose 60/40	0.245	0.990
	0.496	1.020

and the amount of substrate utilized were obtained by measuring the differences in biomass and substrate levels at the beginning and end of a batch run. These data were plotted resulting in the three lines shown in Figure V-10. The slope of each line represents the yield coefficient. The three values obtained ranged from 0.765 to 0.989 gram of biomass generated per gram of substrate utilized.

The question whether these yield coefficients were different should have been answered through a statistical approach; however, this avenue was not chosen since the statistical results would be based on a weak foundation due to the small number of points plotted per line. Therefore, complimentary data were evaluated to detect if differences in yield coefficients were real. The oxygen utilization of the activated sludge was the major variable used for this purpose. Since substrate is required for both synthesis of essential macromolecules and respiration for energy, the yield and oxygen utilization coefficients should be inversely proportional to one another.

The oxygen utilization coefficients for the albumin studies suggest that the yield coefficients are equal. Shown in Figure V-11 is a plot of the amount of oxygen used versus the corresponding amount of substrate used during the batch study. The total oxygen utilized was determined by measuring the area under the oxygen uptake curve in Figures V-1 through V-8. Clearly, the amount of oxygen consumed per unit of substrate utilized (slope of the line) did not vary significantly for any substrate combination.

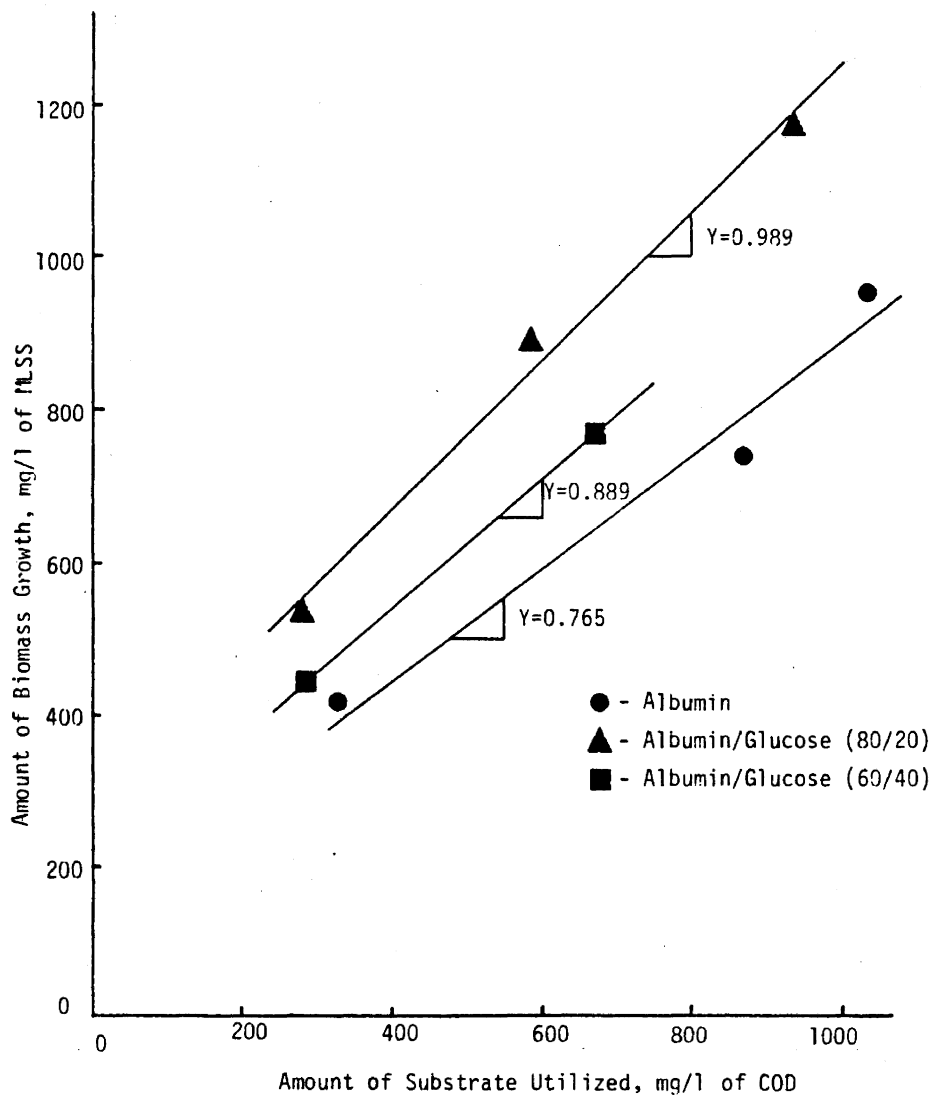


Figure V-10: Determination fo the Yield Coefficients for the Albumin Acclimated Activated Sludge Culture Fed Various Substrate Combinations

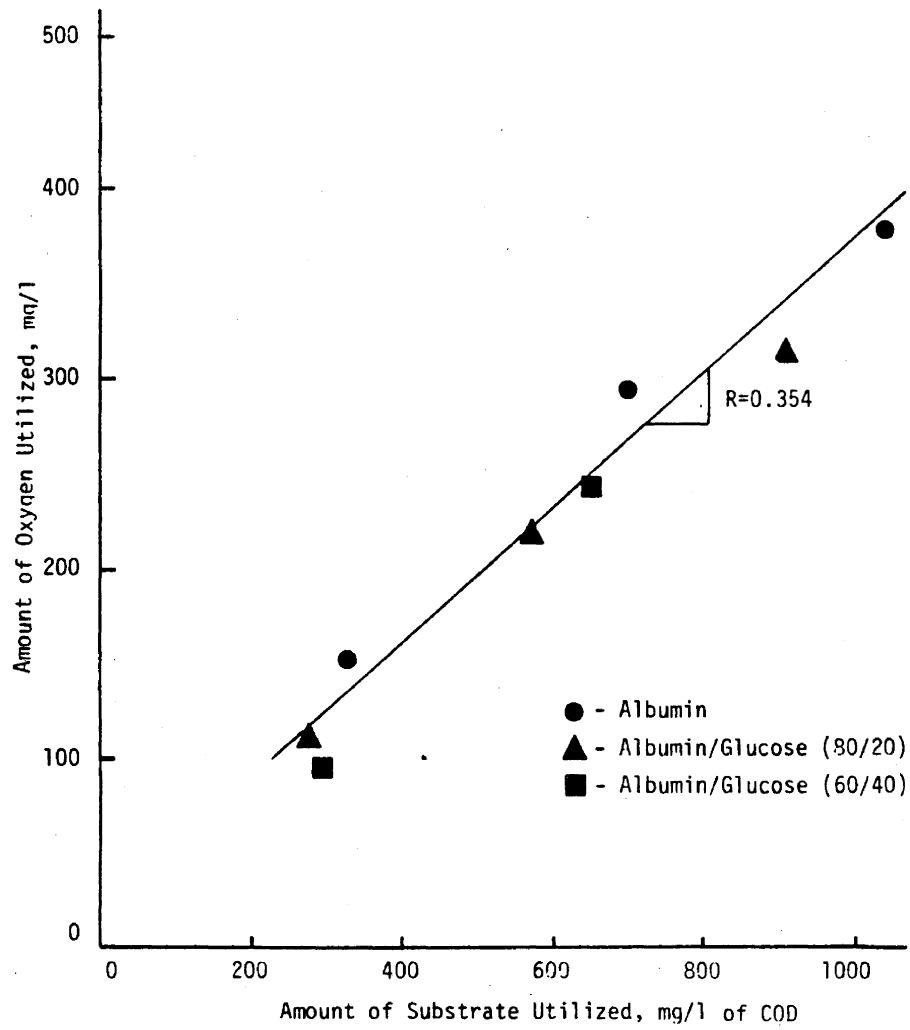


Figure V-11: Determination of the Oxygen Utilization Coefficient for the Albumin Acclimated Activated Sludge Culture Fed Various Substrate Combinations

Adsorption Capacity

The adsorption ability and capacity was of great interest in this study because of its importance in the contact stabilization process. Figure V-12 describes adsorption isotherms obtained in the batch studies. The difference between the amount of substrate added and the settleable amount remaining in solution after five minutes of contact with the sludge was used for the amount of substrate adsorbed. This value was then divided by the initial MLSS measured. The equilibrium concentration was the total amount of settleable substrate remaining after the contact period. It is extremely interesting to note the reduction in adsorption capacity when glucose was added to the substrate. Also, the adsorption isotherm for pure albumin increased markedly as the concentration increased. Discussions of these two phenomena will be taken up in the next chapter.

Starch-fed Culture

Metabolic Response

The starch substrate provided the "adsorption-release" phenomena cited in the literature. Figures V-13 to V-21 describe the series of metabolic studies for starch. Potato starch required extensive hydrolyzation prior to utilization by the bacteria. Large increases in soluble COD resulted from the hydrolysis. Only for the three studies on pure starch did "adsorption and release" for settleable COD occur and only in the highest loading was this greatly apparent.

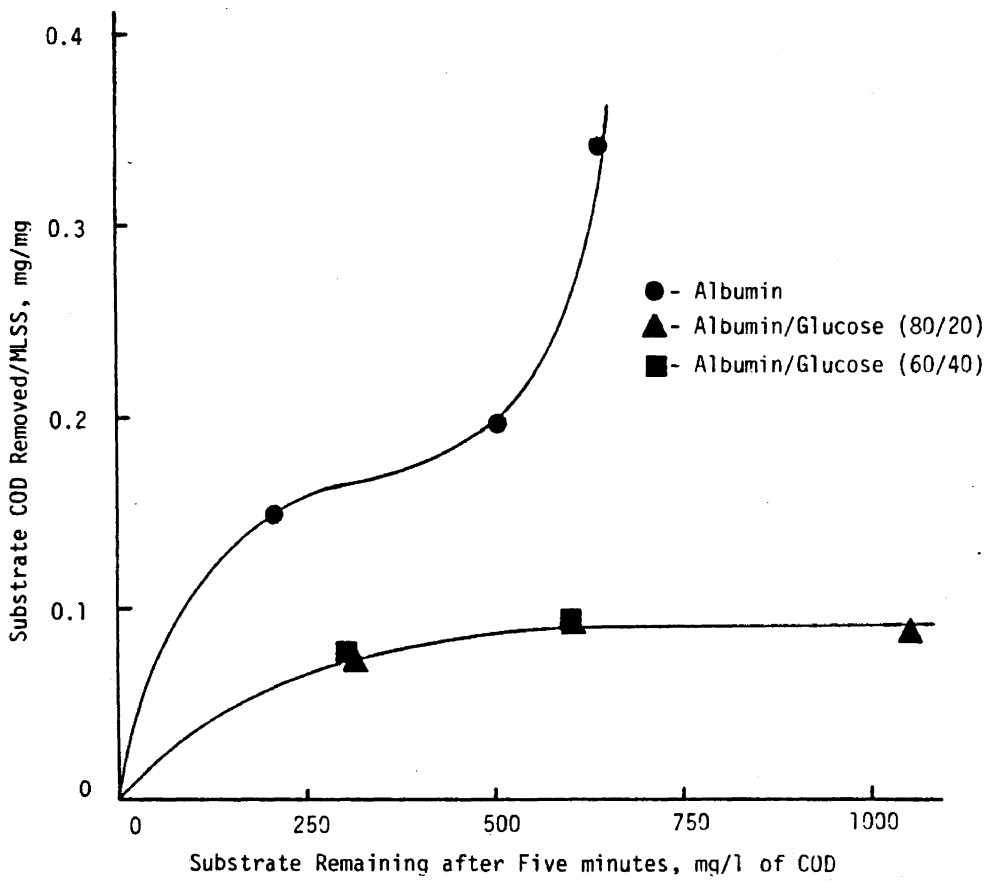


Figure V-12: Isotherm Plots for Albumin Acclimated Activated Sludge Fed Various Substrate Combinations

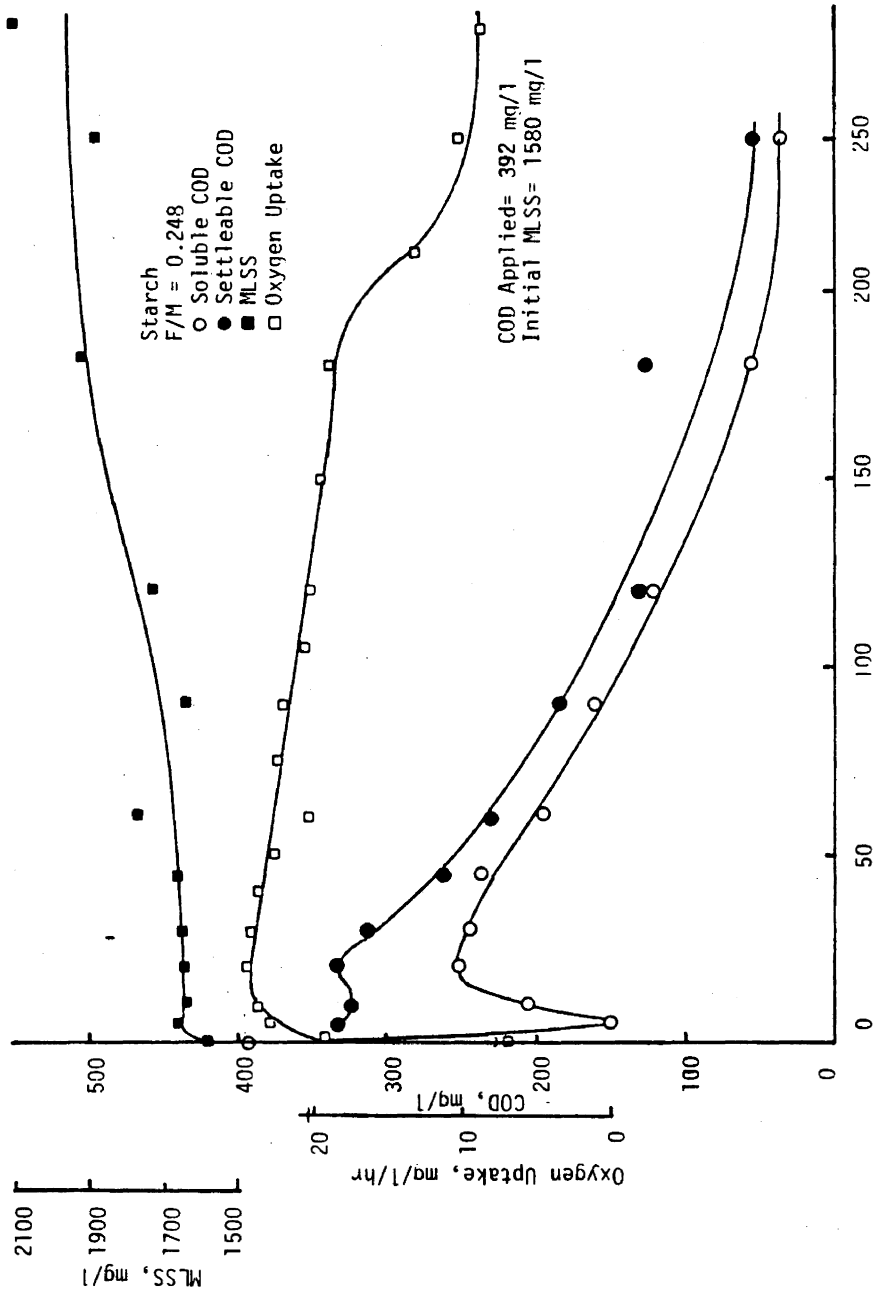


Figure V-13: Results of a Batch Study on a Starch Acclimated Activated Sludge Fed Only Starch

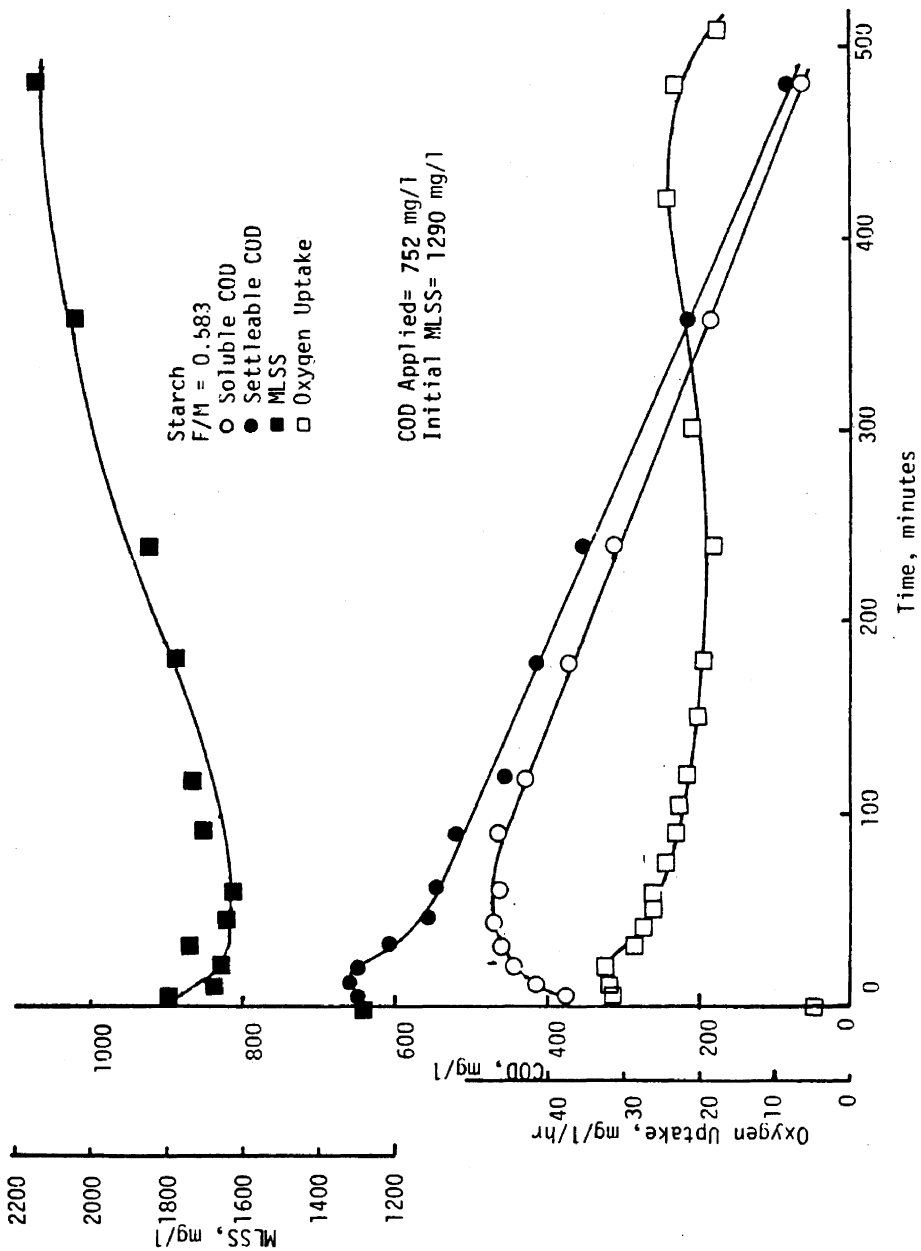


Figure V-14: Results of a Batch Study on a Starch Acclimated Activated Sludge Culture Fed Only Starch

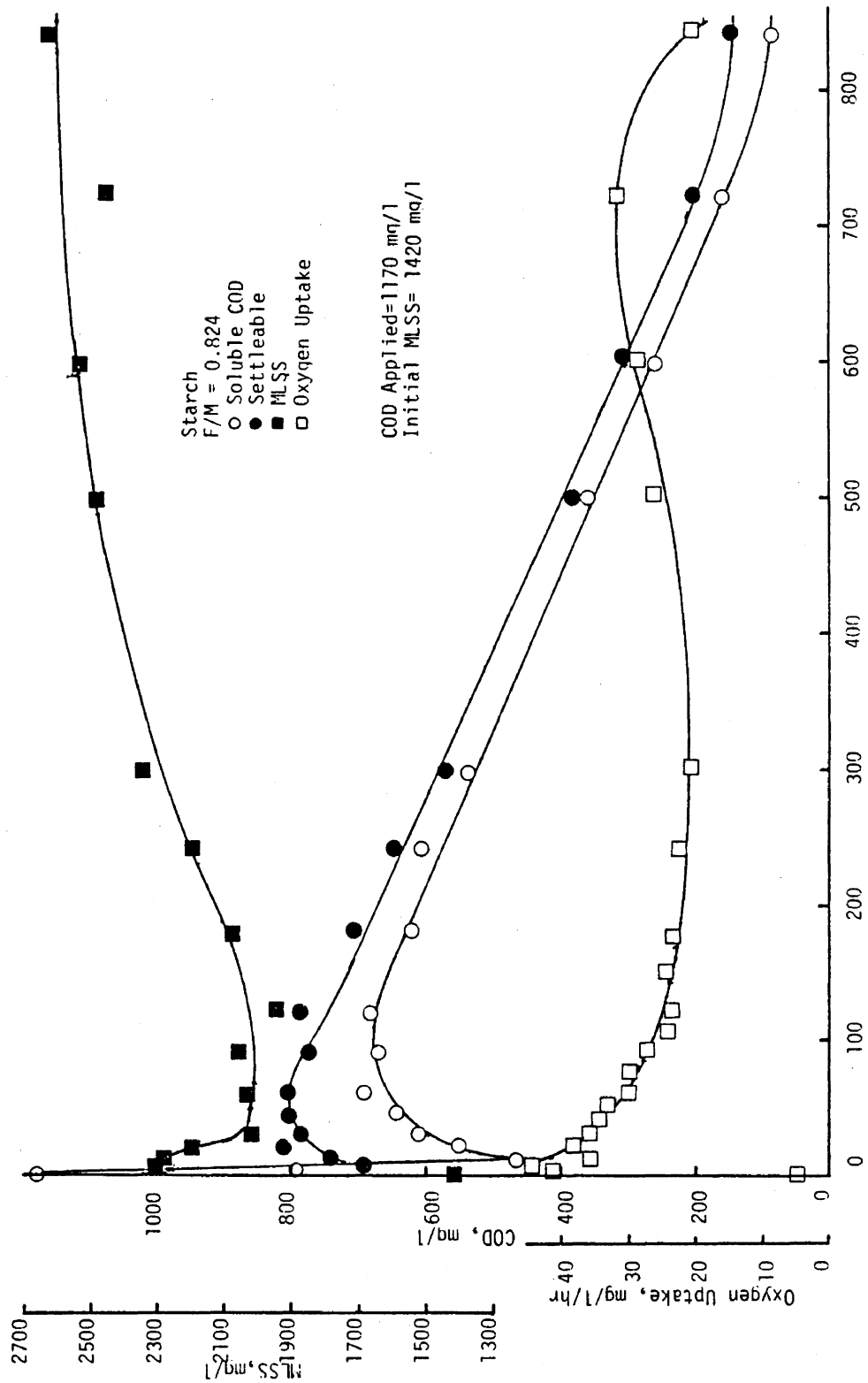


Figure V-18: Results of a Batch Study on a Starch Acclimated Activated Sludge Fed Only Starch

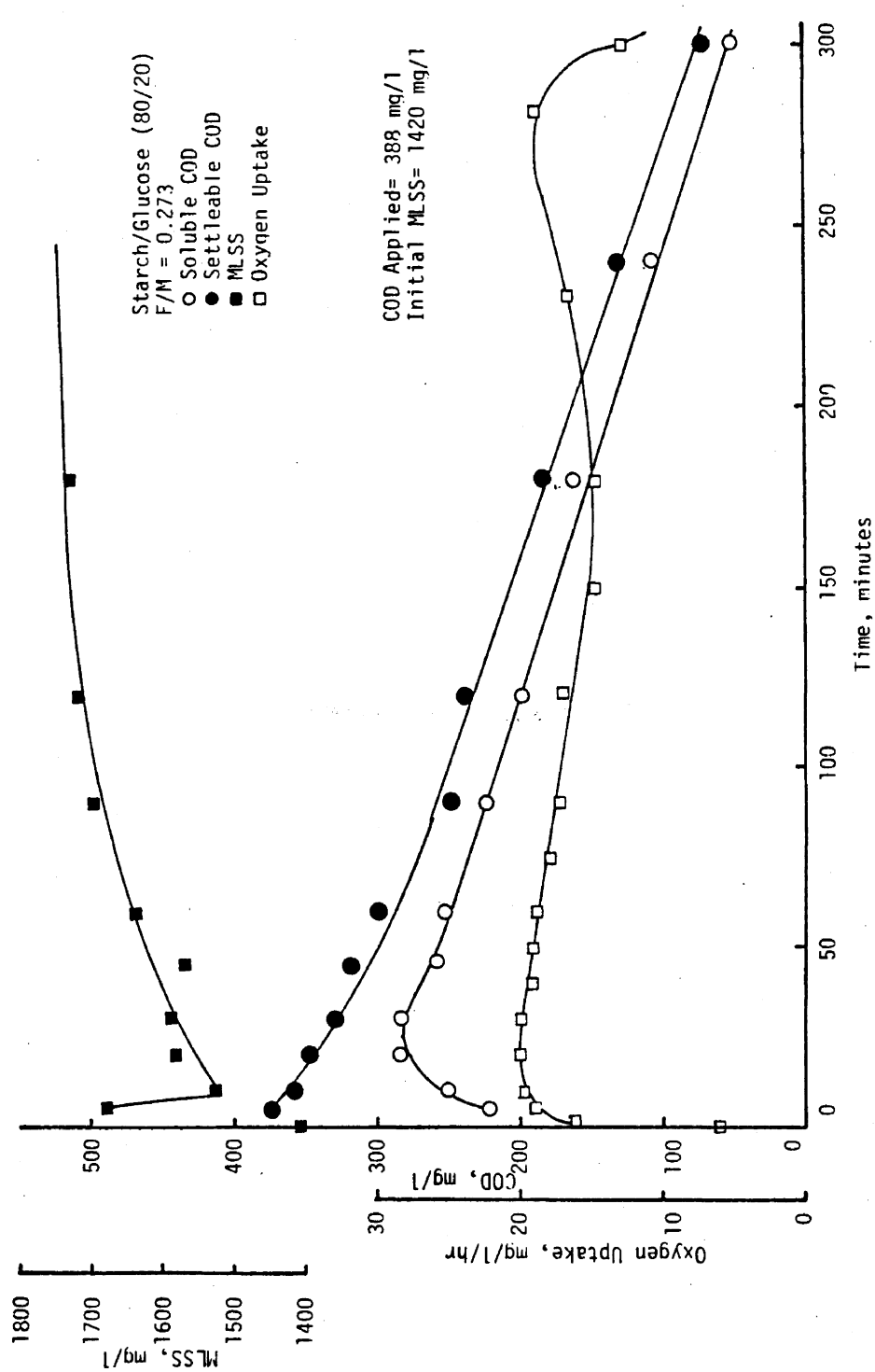


Figure V-16: Results of a Batch Study on a Starch Acclimated Activated Culture Fed Eighty Percent Starch and Twenty Percent Glucose

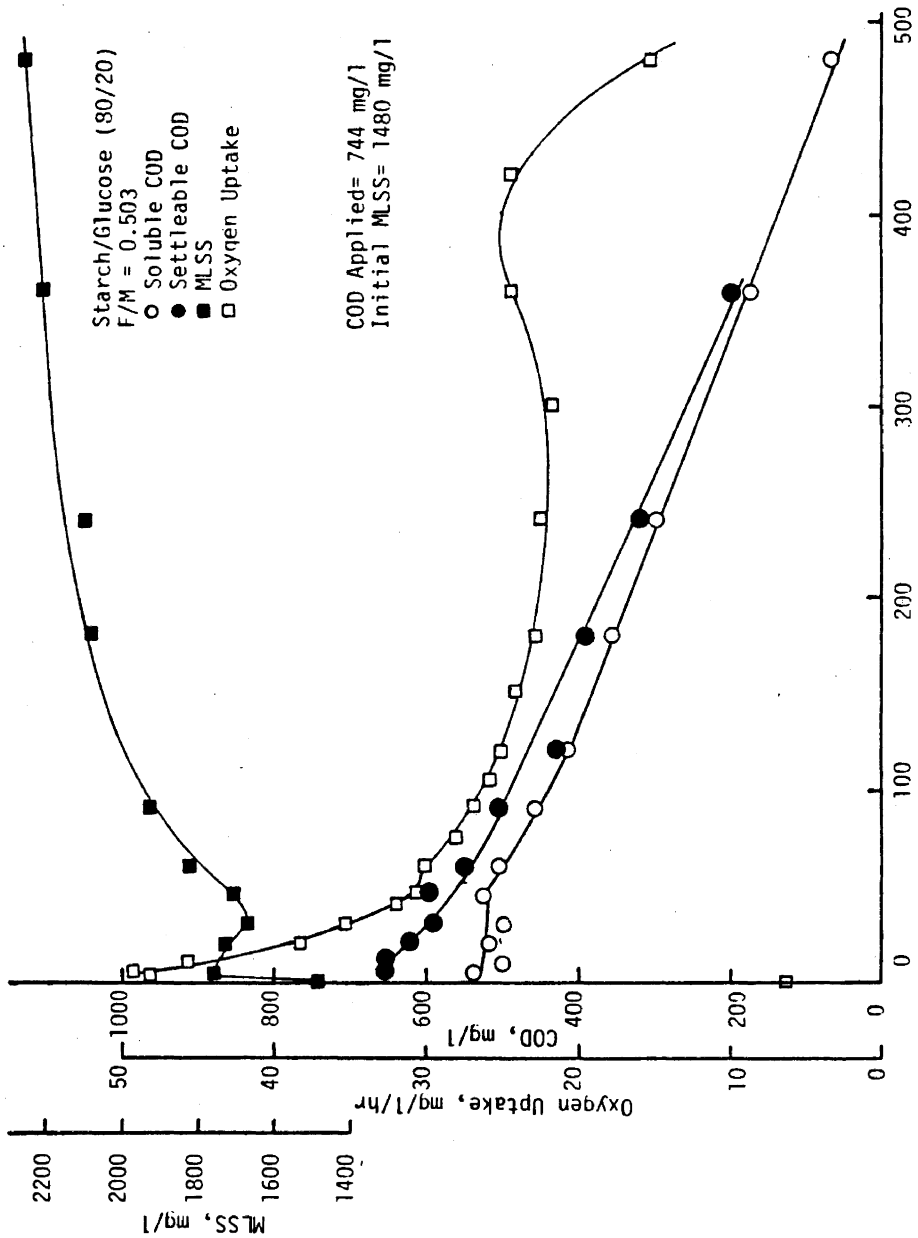


Figure V-17: Results of a Batch Study on a Starch Acclimated Activated Sludge Culture Fed Eighty Percent Starch and Twenty Percent Glucose

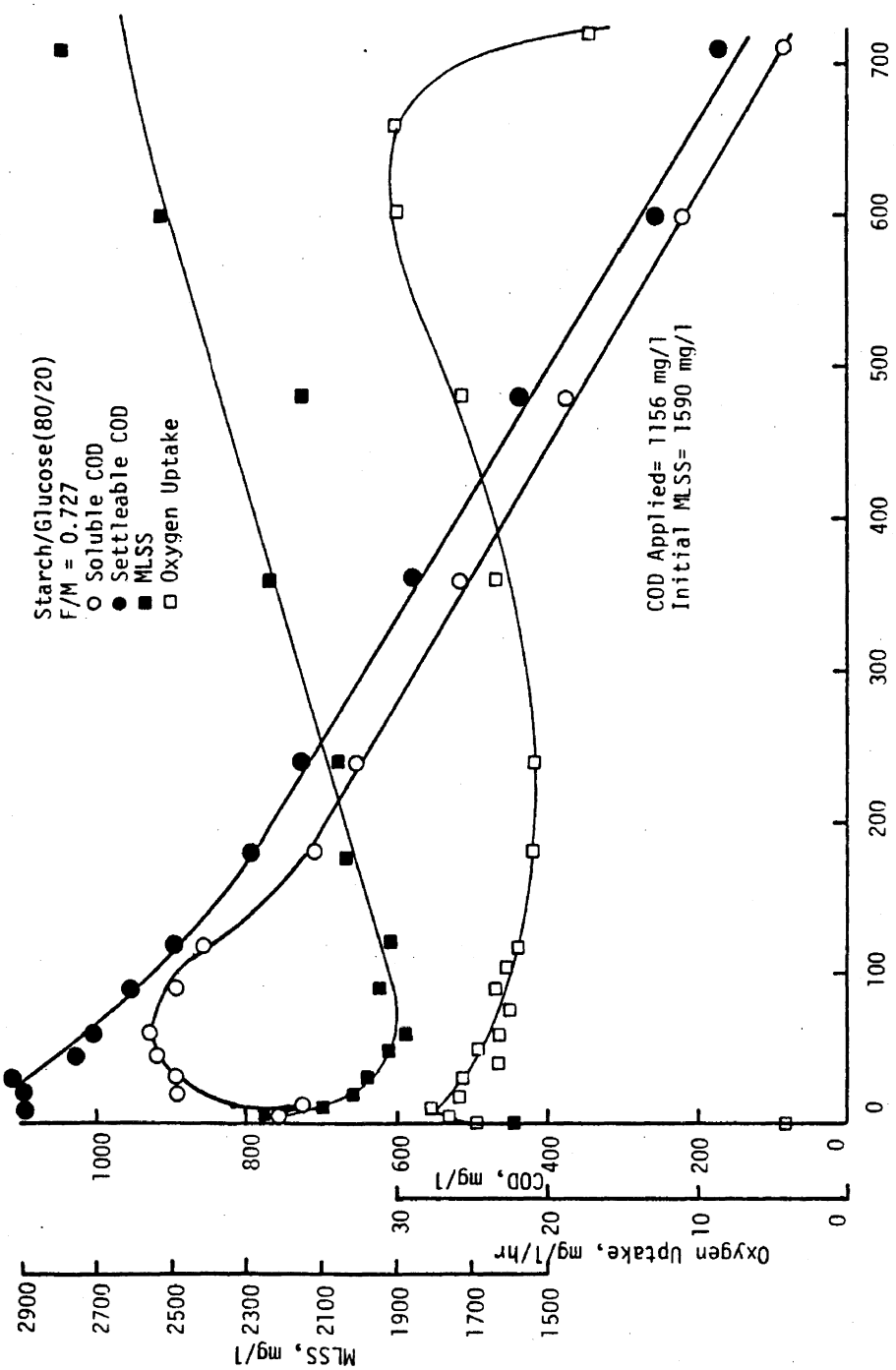


Figure V-18: Results of a Batch Study on a Starch Acclimated Activated Sludge Culture Fed Eighty Percent Starch and Twenty Percent Glucose

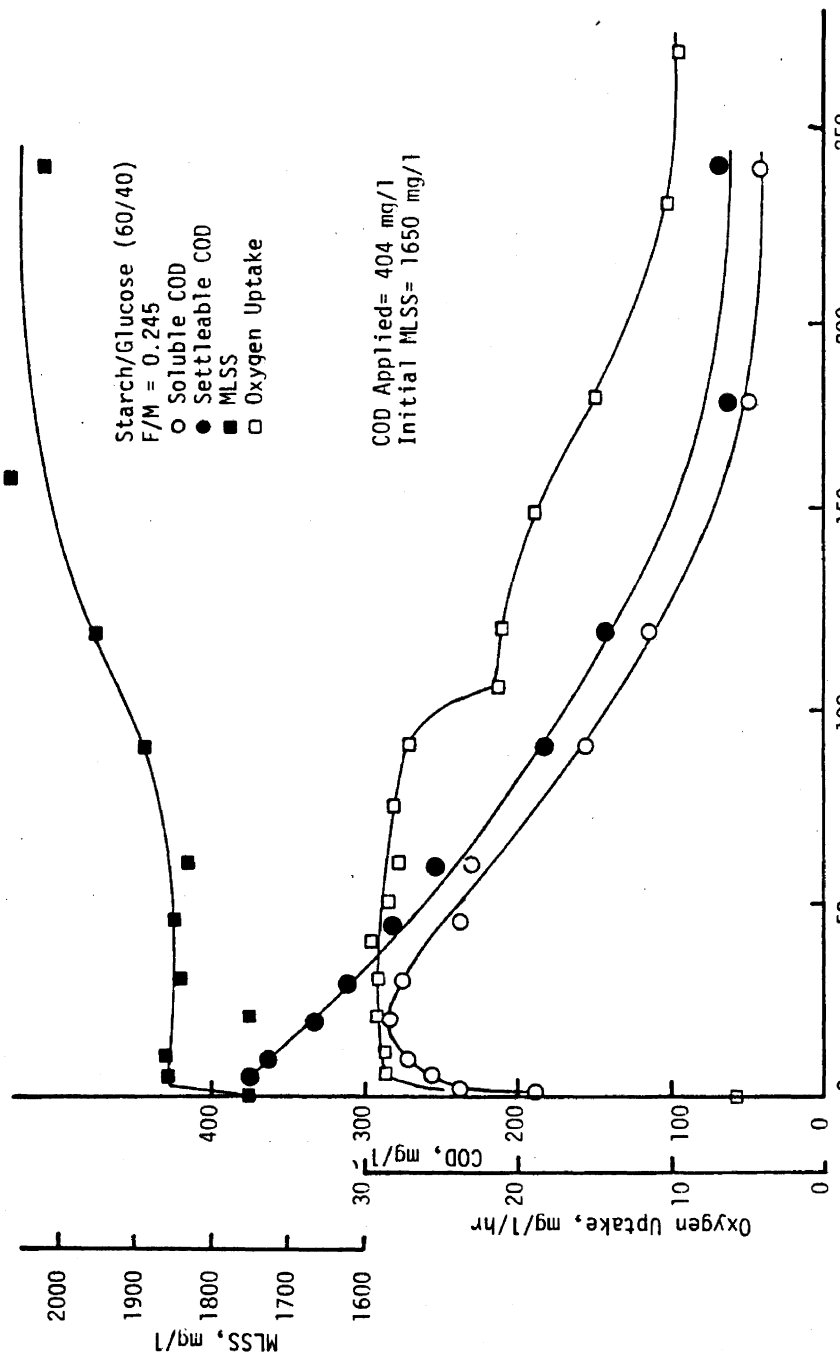


Figure V-19: Results of a Batch Study on a Starch Acclimated Activated Sludge Culture Fed Sixty Percent Starch and Forty Percent Glucose

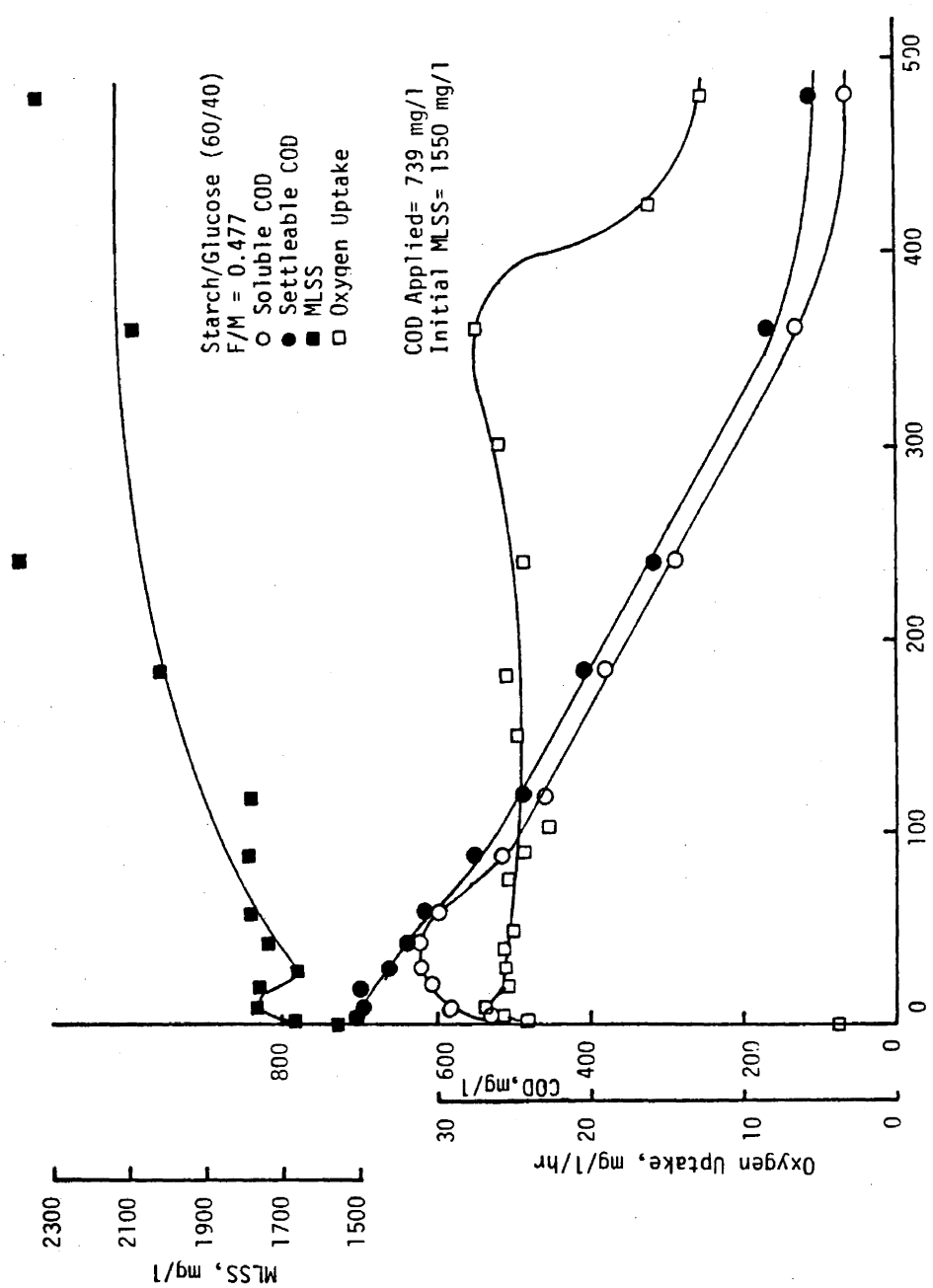


Figure V-20: Results of a Batch Study on a Starch Acclimated Activated Sludge Culture Fed Sixty Percent Starch and Forty Percent Glucose

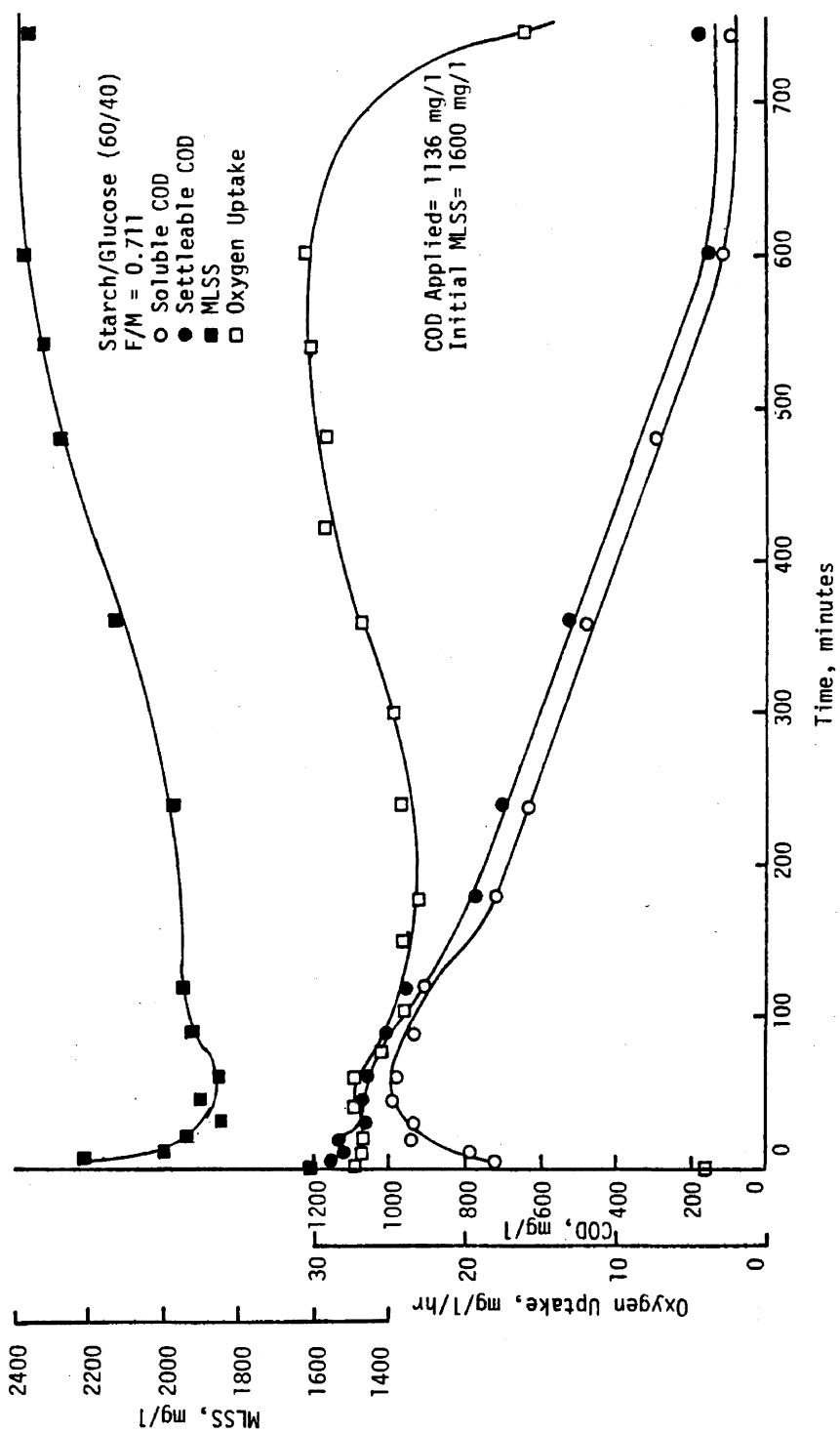


Figure V-21: Results of a Batch Study on a Starch Acclimated Activated Sludge Culture Fed Sixty Percent Starch and Forty Percent Glucose

Oxygen uptake curve in most cases decreased steadily for a period of time and then increased near the end of the run before finishing with a rather abrupt drop. As in the albumin studies, this was because of the subsequent hydrolyzation and utilization of the substrate. Finally, there appears to have been no sequential uptake in the glucose supplemented studies with the exception of the 80-20 percent starch-glucose study seen in Figure V-17. Here, the oxygen uptake rate is the highest recorded for any of the starch studies and appears to decrease in a two step fashion.

Kinetics

The soluble COD removal rate coefficients are listed in Table V-2. They were obtained in the same manner as the albumin removal rates. Glucose addition appeared to augment the rates of removal.

Yield and Oxygen Utilization Coefficients

Shown in Figure V-22 is the plot for determination of the yield coefficients for the starch substrate mixtures. The yield coefficients significantly decreased as the glucose ratio increased. However, the oxygen utilization coefficients shown in Figure V-23 for these three starch mixtures varied little, if any. This tends to support the conclusion that:

1. the data plotted for yield determination were quite variable and of no statistical significance, or
2. there was significant storage occurring, either intracellular or extracellular.

Table V-2

Starch Removal Rates

Substrate	F/M (mg/mg-day)	Removal Rate (mg COD/min)
Starch	0.248	1.417
	0.583	1.400
	0.824	1.259
Starch/Glucose (80/20)	0.273	1.126
	0.503	1.425
	0.727	1.479
Starch/Glucose (60/40)	0.245	1.497
	0.477	1.532
	0.711	1.597

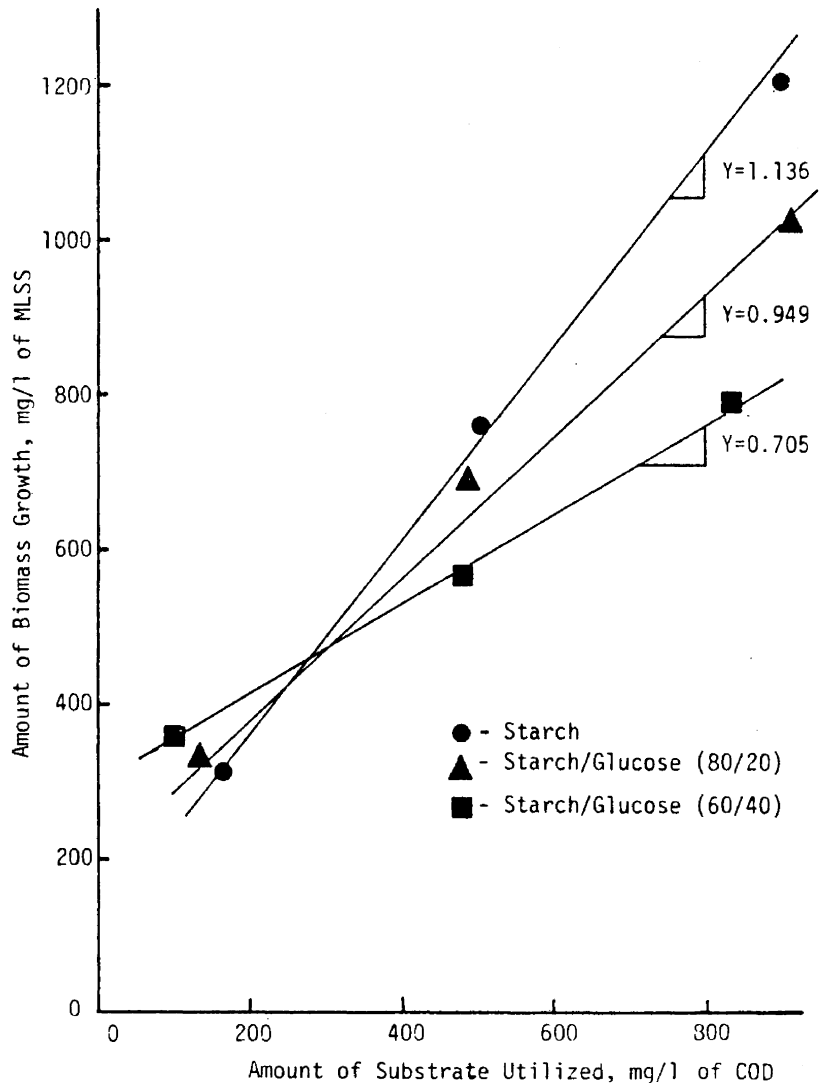


Figure V-22: Determination of the Yield Coefficients for Starch Acclimated Activated Sludge Culture Fed Various Substrate Combinations

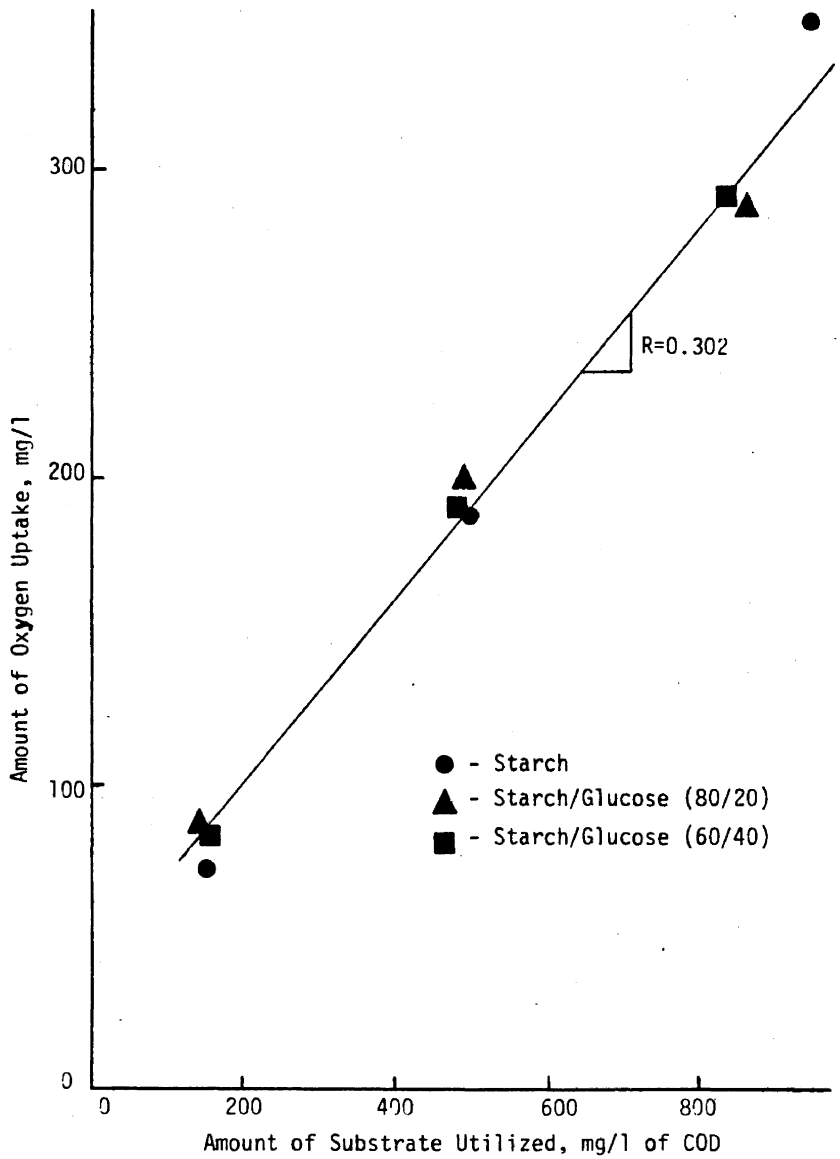


Figure V-23: Determination of the Oxygen Utilization Coefficient for the Starch Acclimated Activated Sludge Culture Fed Various Substrate Combinations

This will be addressed later in more detail.

Adsorption Capacity

The adsorption capacity of the starch sludge followed the same pattern as that found in the albumin studies, i.e. with the supplementation of glucose the adsorption capacity decreased. Figure V-24 illustrates the evidence for this statement.

However, attention should now be directed toward the isotherm for pure starch. There is one outlying point which corresponds to the batch study performed at the 0.824 F/M ratio. In fact, this particular study provided several peculiarities. The rate substrate removal was low in comparison to the other removal rates for starch, and also it was the only study that exhibited a marked "uptake and release" phenomena for the settleable COD. In reviewing procedural experimental notes, one anomaly was found which may provide an explanation. In the previous chapter, mention was made of maintaining a stock acclimated culture of activated sludge for each substrate. Quantities of sludge required for the batch studies were obtained from these stock cultures. Prior to performing the first batch study for starch, the stock was only receiving starch as a carbon source. The first study made was at the F/M ratio of 0.824. After this study but prior to the next one, a small amount of glucose (10% of total COD) was supplemented with starch for the stock culture substrate. It was believed that this would have no effect on the batch studies since the starch is a glucose polymer. However, if

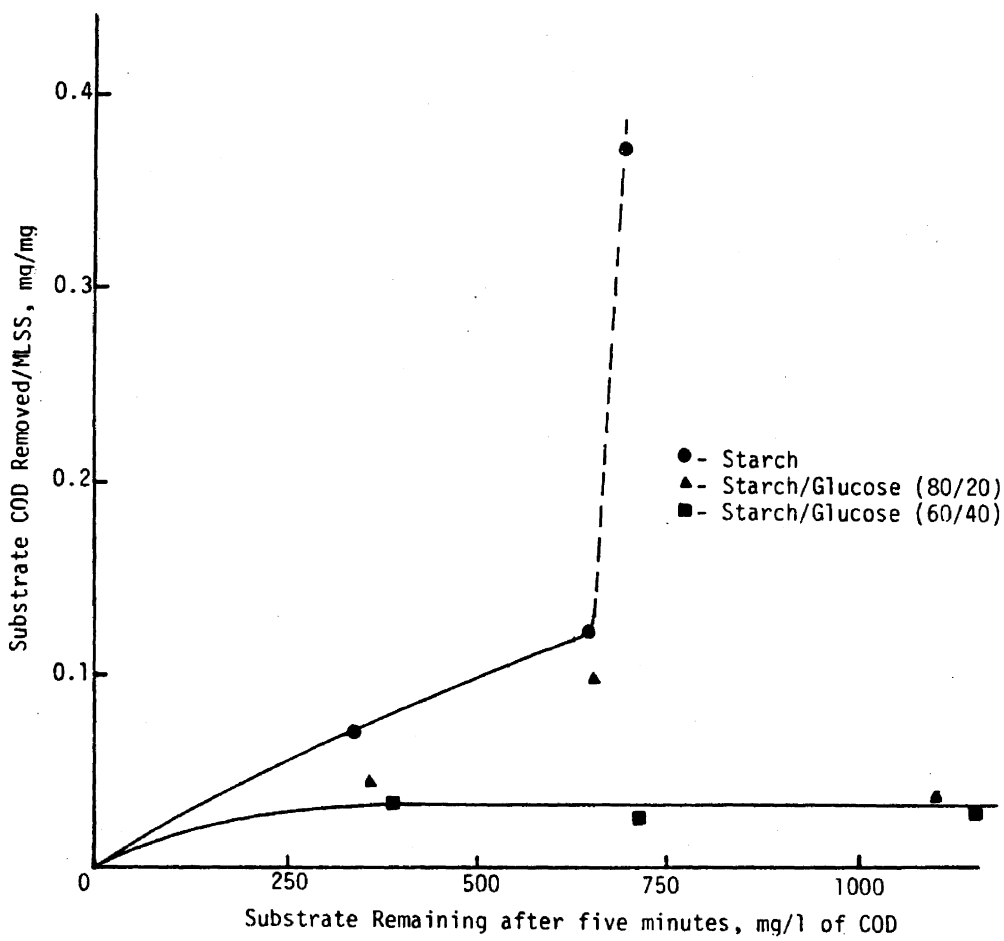


Figure V-24; Isotherm Plots for Starch Acclimated Activated Sludge Fed Various Substrate Combinations

a cause-effect relationship did exist, the glucose apparently altered the adsorption characteristics of the stock sludge directly or indirectly.

Jack Bean Meal-fed Culture

Metabolic Response

Unlike the starch and albumin substrates, the jack bean meal consisted of a large insoluble fraction of organic material. As described in the previous chapter, efforts were made to remove the larger material by sieves; however, there remained a portion of the substrate that would settle out within thirty minutes. This had a significant effect on some of the results obtained.

Figures V-25 to V-33 present the graphical data for the jack bean meal tube runs. In regard to the "adsorption and release" phenomenon, there appears to be a slight "hump" registered in the settleable COD data. However, the soluble COD shows no concurrent response. This could mean that either the "released" substrate particles were too large to pass the filter or there was an increase in the number of dispersed bacteria in the solution. In any case, the increase in the settleable COD, however small, was consistent. Even with glucose supplementation, the hump was still present. As in the case of albumin, the utilization of jack bean meal and glucose mixtures occurred in a sequential manner, with glucose again leading the way. However, it is interesting to find that the "adsorption-release"

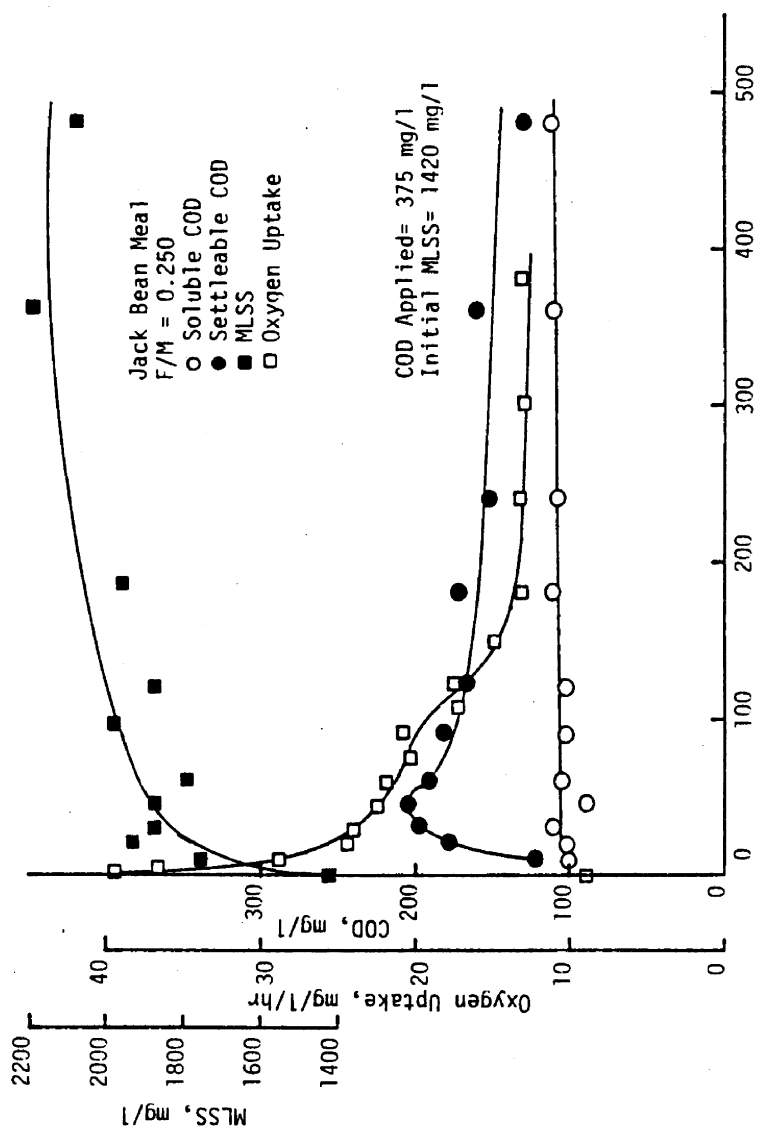


Figure V-25: Results of a Batch Study on a Jack Bean Meal Acclimated Sludge Culture Fed Only Jack Bean Meal

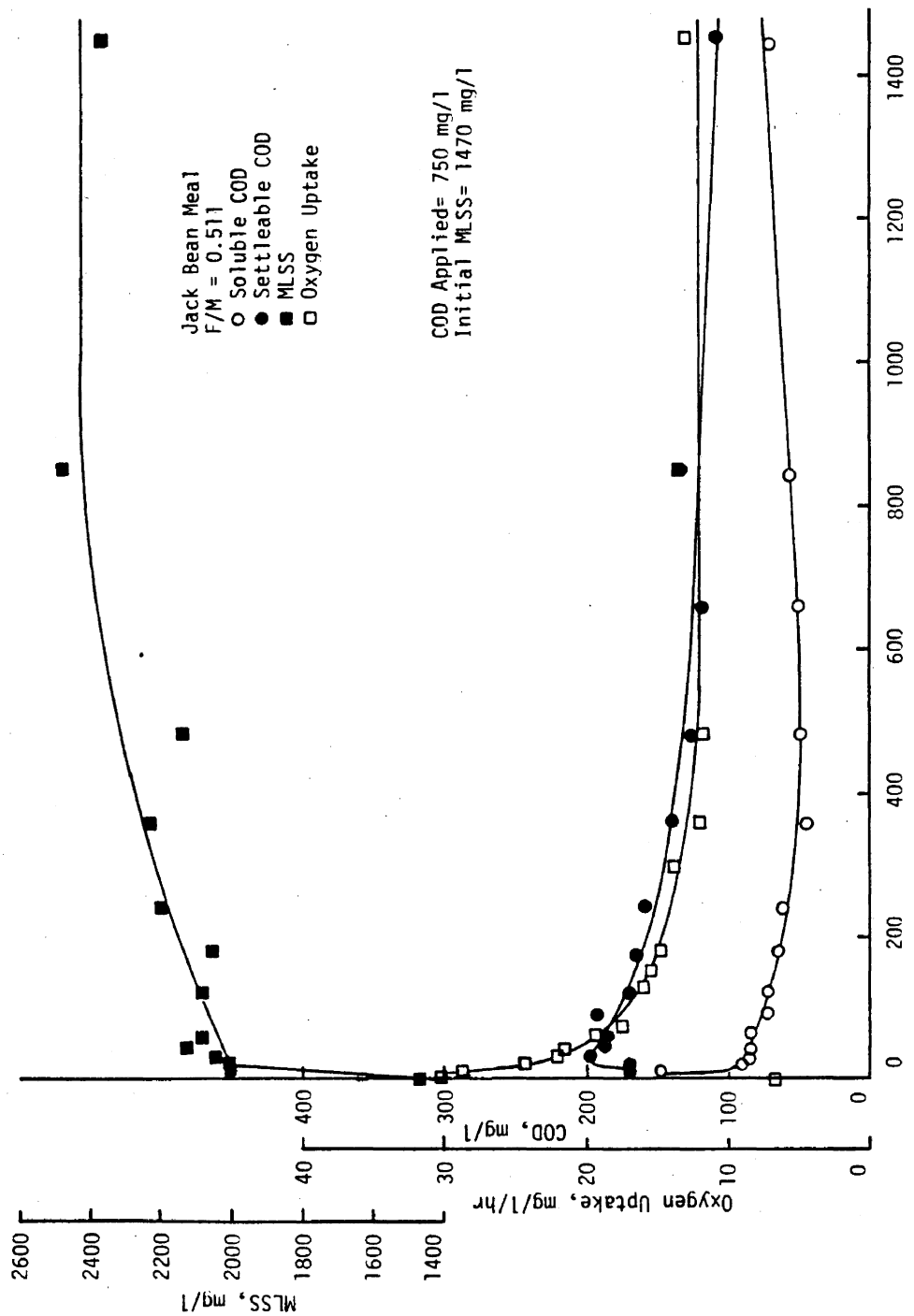


Figure V-26: Results of a Batch Study on a Jack Bean Meal Acclimated Sludge Culture Fed Only Jack Bean Meal

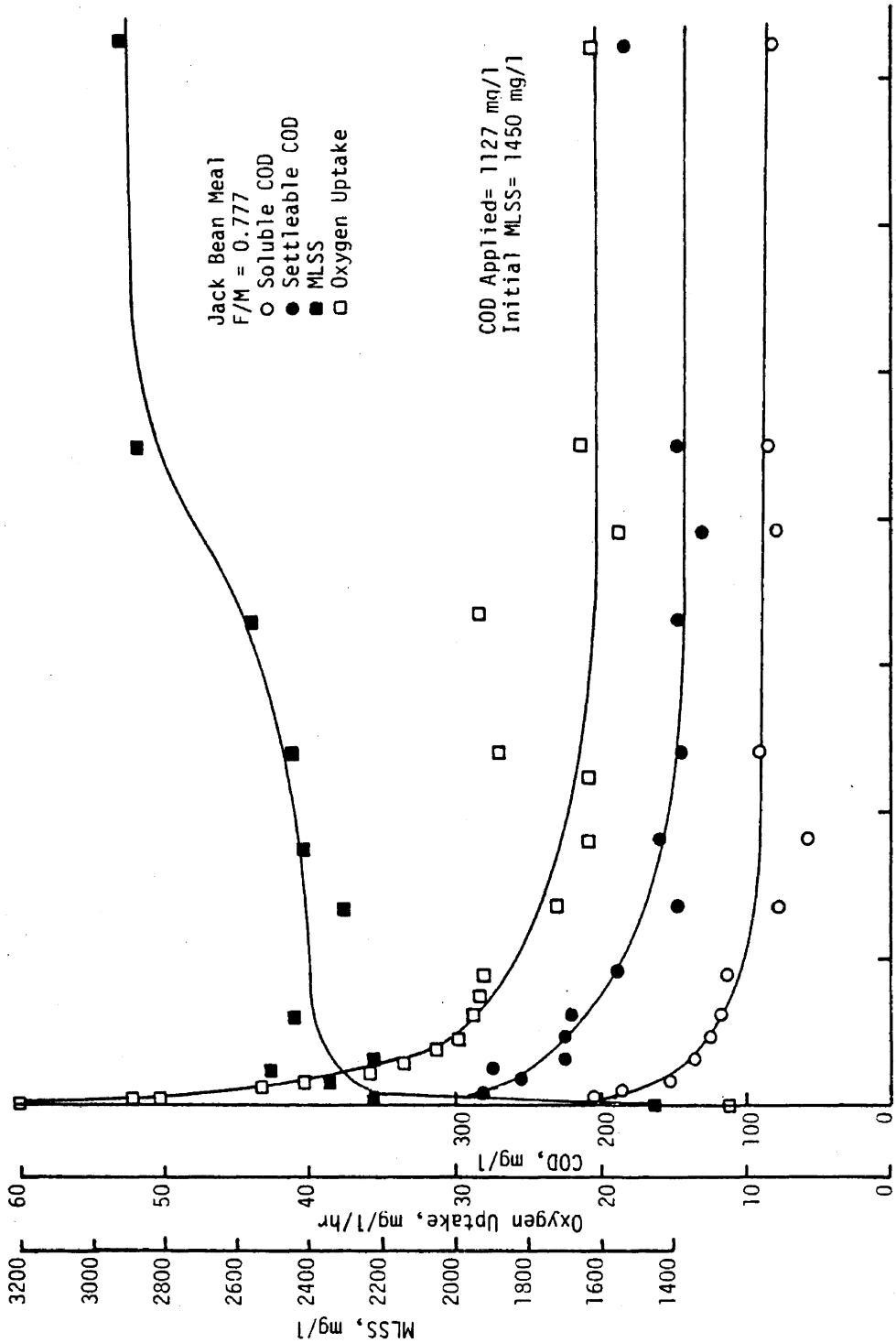


Figure V-27: Results of a Batch Study on a Jack Bean Meal Acclimated Sludge Culture Fed Only Jack Bean Meal

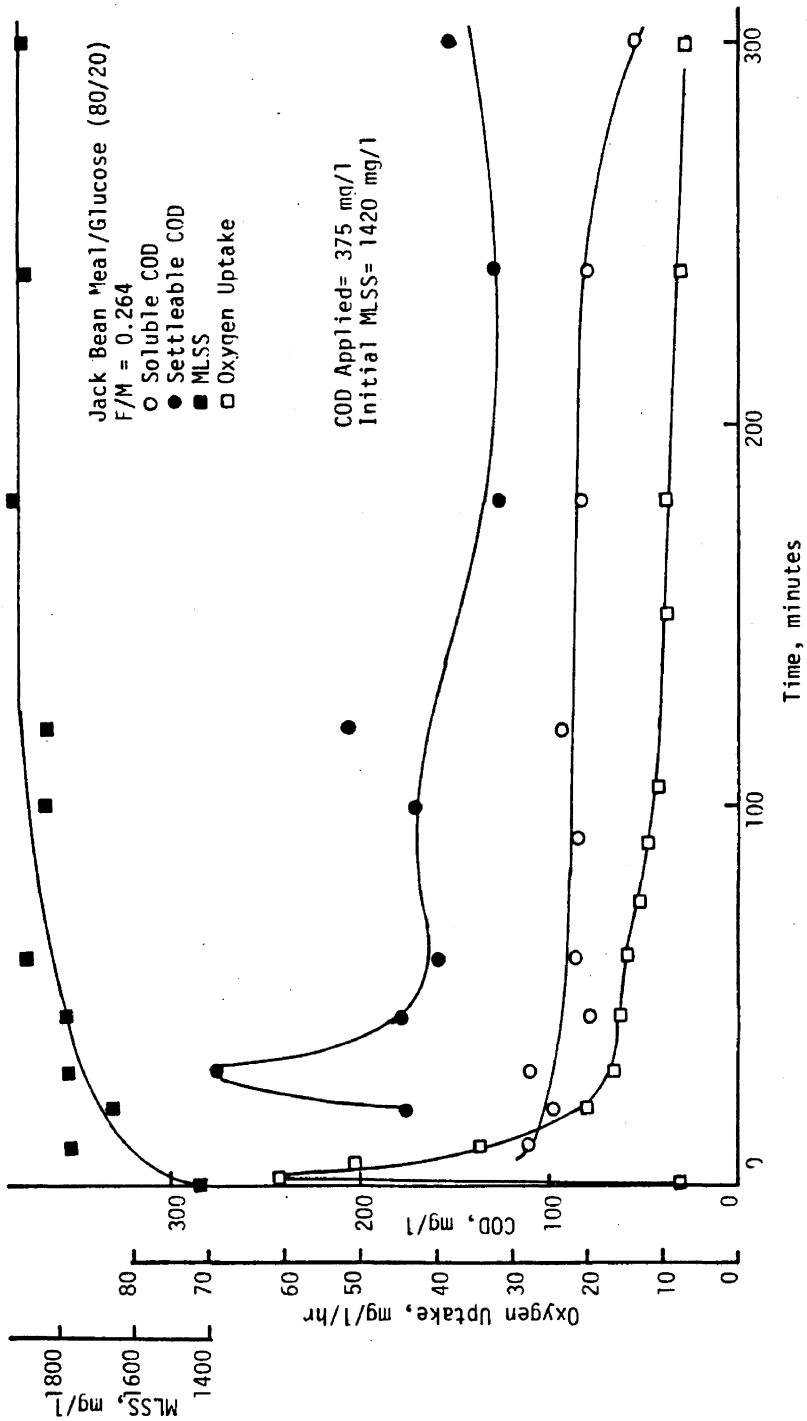


Figure V-28: Results of a Batch Study on a Jack Bean Meal Acclimated Sludge Culture Fed Only Jack Bean Meal

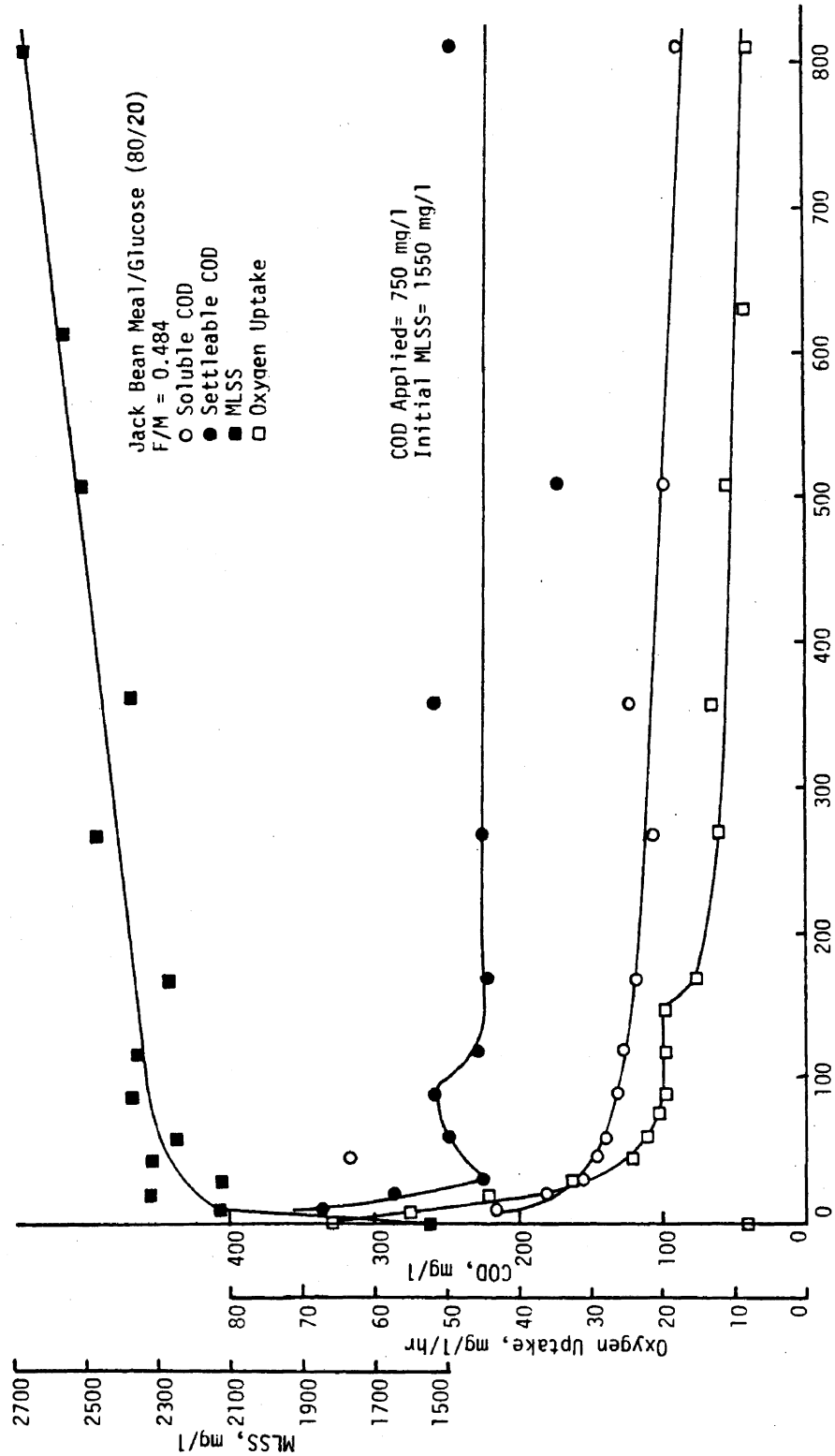


Figure V-29: Results of a Batch Study on a Jack Bean Meal Acclimated Activated Sludge Culture Fed Eighty Percent Jack Bean Meal and Twenty Percent Glucose

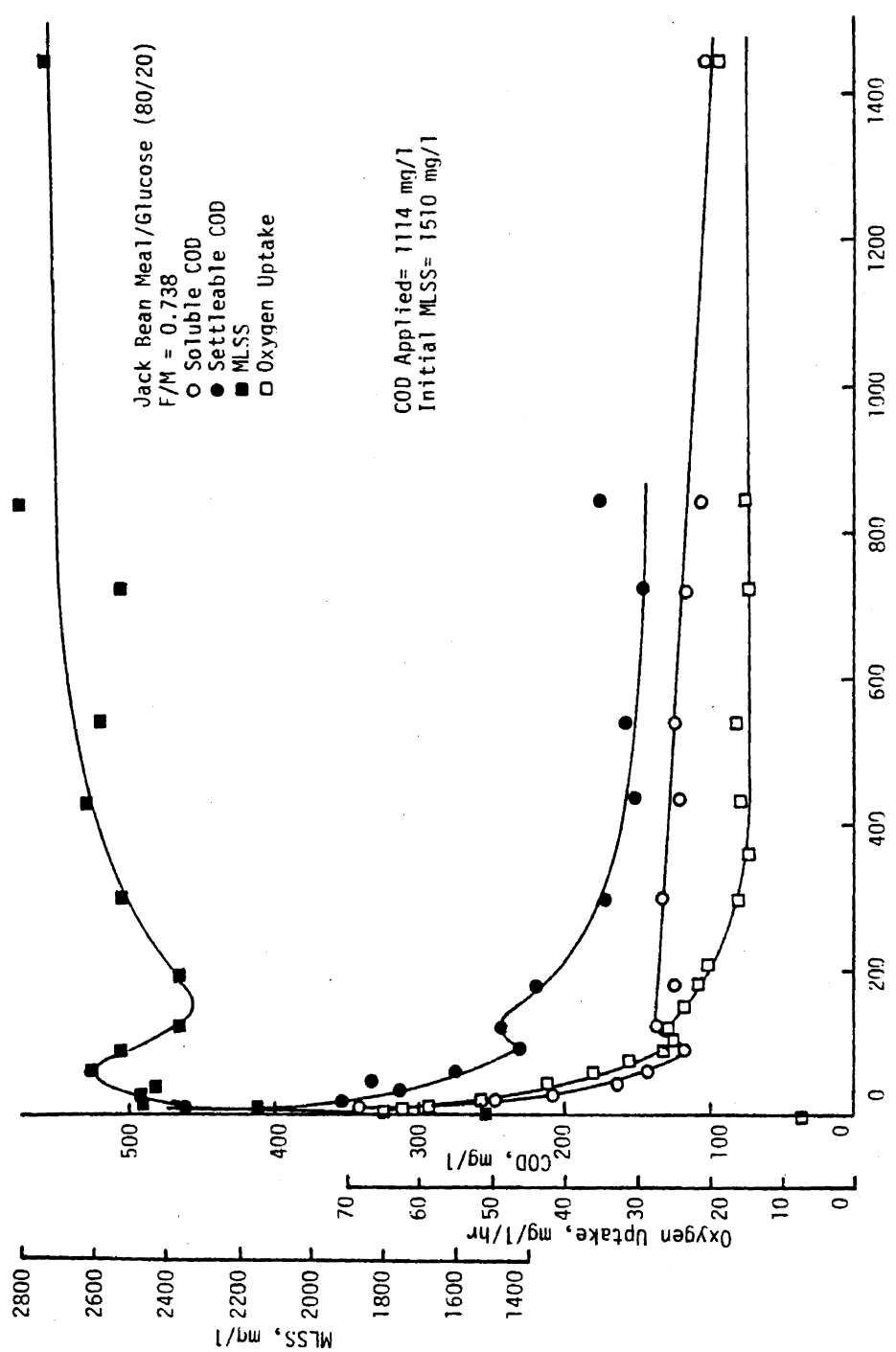


Figure V-30: Results of a Batch Study on a Jack Bean Meal Acclimated Activated Sludge Culture Fed Eighty Percent Jack Bean Meal and Twenty Percent Glucose

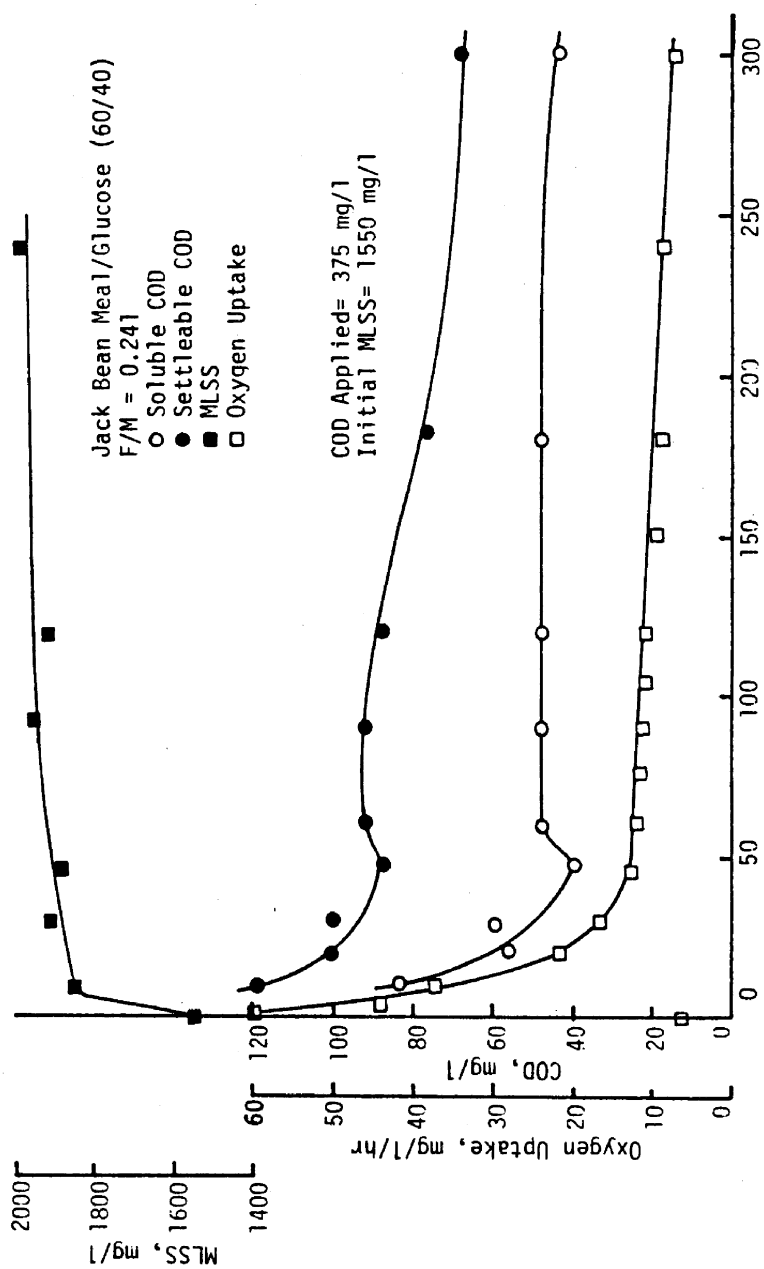


Figure V-31: Results of a Batch Study on a Jack Bean Meal Acclimated Activated Sludge Culture Fed Sixty Percent Jack Bean Meal and Forty Percent Glucose

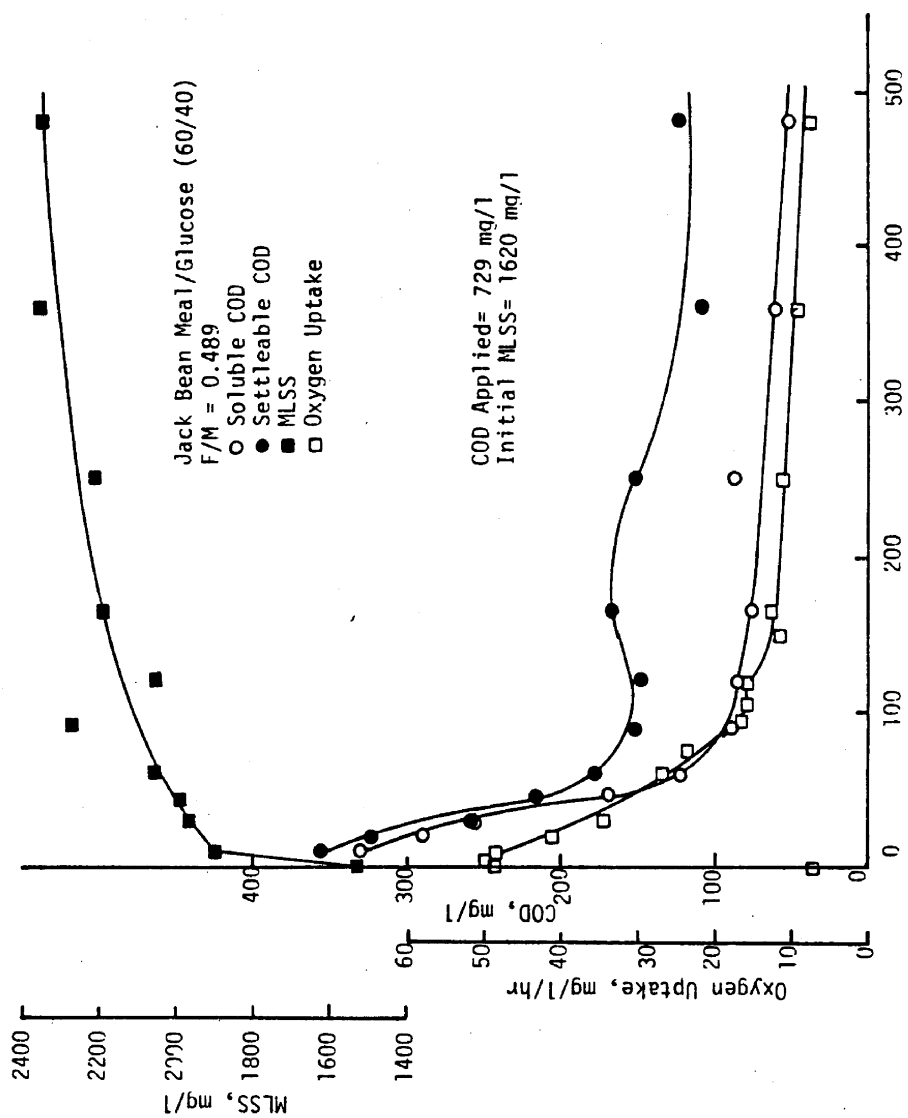


Figure V-32: Results of a Batch Study on a Jack Bean Meal Acclimated Activated Sludge Culture Fed Sixty Percent Jack Bean Meal and Forty Percent Glucose

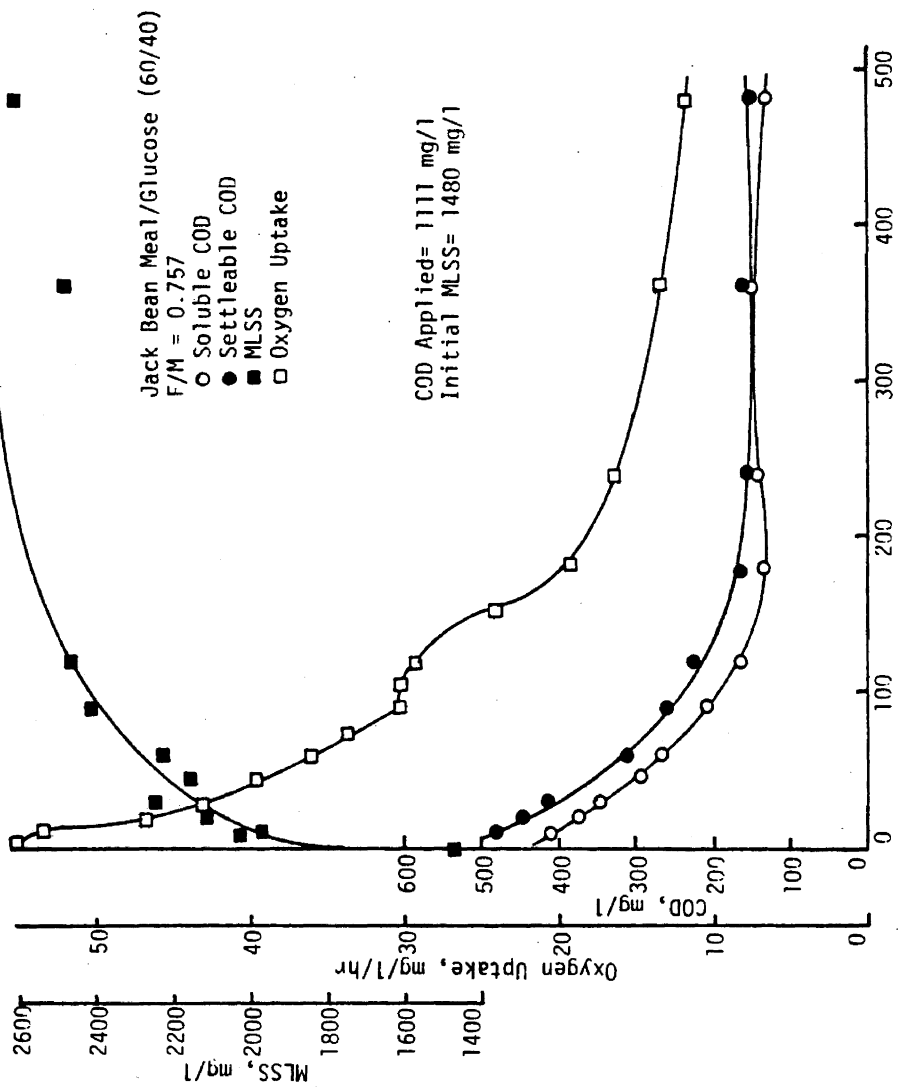


Figure V-33: Results of a Batch Study on a Jack Bean Meal Acclimated Activated Sludge Culture Fed Sixty Percent Jack Bean Meal and Forty Percent Glucose

phenomena occurs at the termination of the glucose uptake implying that hydrolysis of the colloids begins after glucose utilization.

Kinetics

A procedure exactly similar to that used in the albumin and starch was employed for determining rates of jack bean meal-glucose substrate utilization. Table V-3 lists the removal rates for the various substrate combinations and loadings. There was an overall increase in the rate of substrate disappearance as the glucose fraction was increased, implying that glucose addition did not inhibit the COD removal rate.

Yield and Oxygen Utilization Coefficients

Figure V-34 plots the amount of MLSS generated versus the amount of substrate utilized for the jack bean meal and glucose studies. The yield coefficients (the slope of each line) are not significantly different; but, the oxygen utilization plots in Figure V-35 suggest the yields are different. The oxygen utilization coefficients decreased with increasing glucose content indicating that more substrate was available for synthesis or storage. One explanation for this contradictory data originates from the statement made earlier about the insoluble character of the jack bean meal. Apparently, a portion of the jack bean meal remained unmetabolized at the end of the tube run and was measured as biomass solids, thereby masking any variations in the yield.

Table V-3

Jack Bean Meal Removal Rates

Substrate	F/M (mg/mg-day)	Removal Rate (mg COD/min)
Jack Bean Meal	0.250	0.883
	0.511	1.061
	0.777	0.867
Jack Bean Meal/Glucose 80/20	0.264	1.475
	0.484	1.290
	0.738	1.426
Jack Bean Meal/Glucose 60/40	0.241	1.635
	0.489	1.733
	0.757	2.063

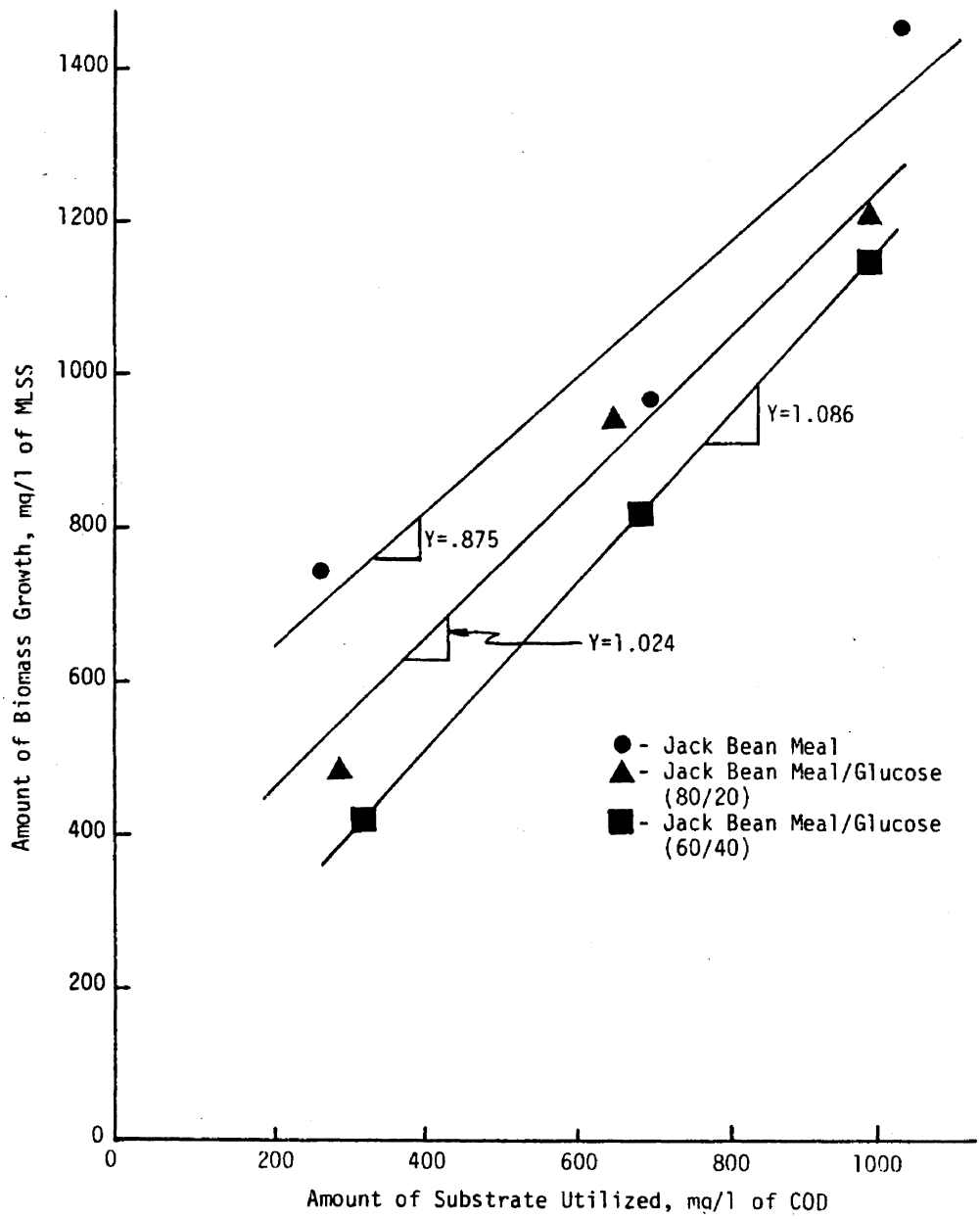


Figure V-34: Determination of the Yield Coefficients for the Jack Bean Meal Acclimated Activated Sludge Culture Fed Various Substrate Combinations

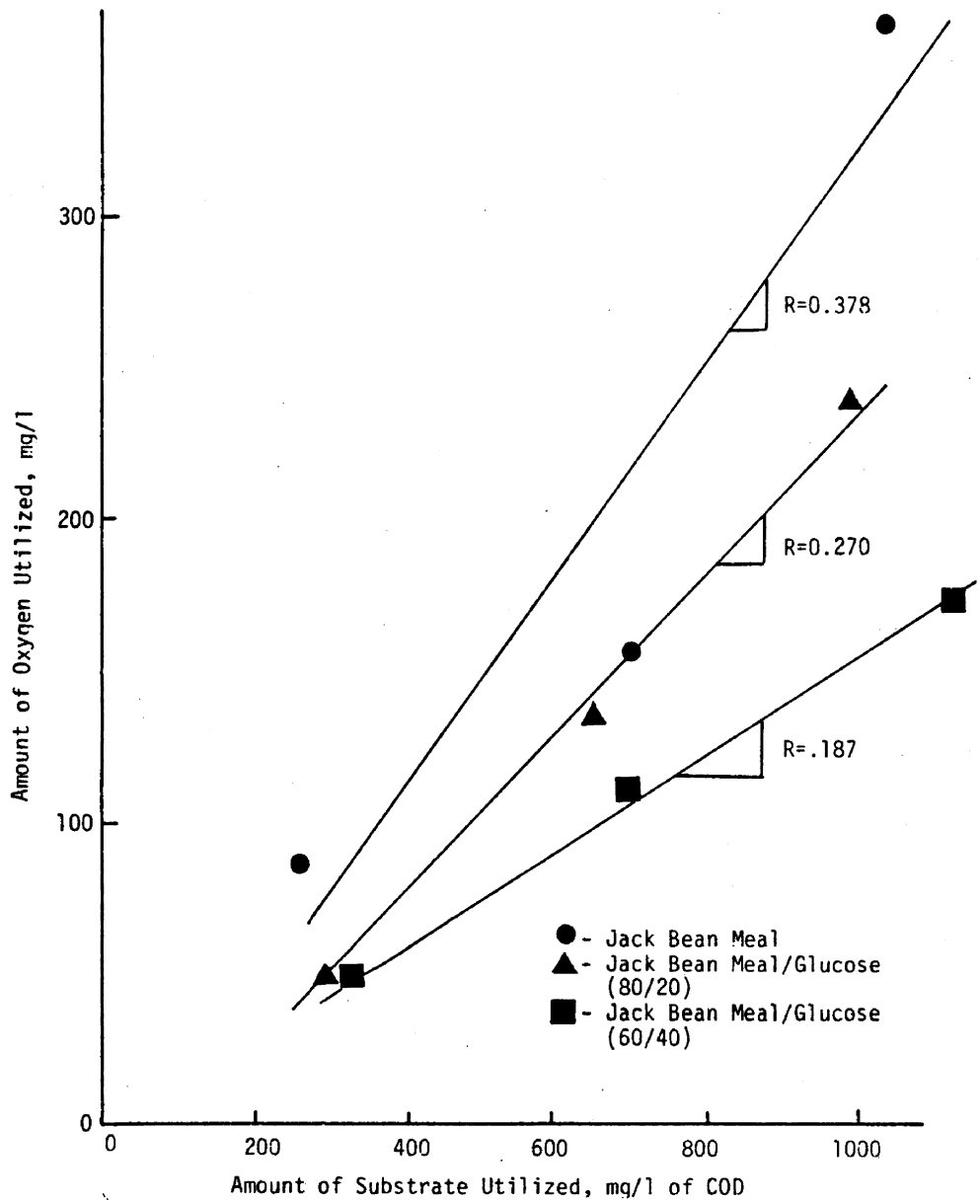


Figure V-35: Determination of the Oxygen Utilization Coefficients for the Jack Bean Meal Acclimated Activated Sludge Culture Fed Various Substrate Combinations

Finally, the yield coefficients determined for the jack bean meal acclimated sludge as well as for the starch and albumin sludges were unusually large. Common numbers reported in the literature are between 0.4 to 0.6. This inconsistency may be due to the possible presence of non-biodegradable organic material in the substrate being measured as biomass or to an increase in the production of metabolic storage products by the bacteria. In any case, there does exist some consistency in the yield values obtained throughout the investigation.

Adsorption Capacity

The jack bean meal sludge possessed the highest removal capacity via adsorption or enmeshment for all three sludges. This was in large part due to the settleability of the colloids themselves. Even so, as Figure V-36 shows, once again, glucose inhibited the initial removal of the substrate.

In all isotherms plotted, the total amount of COD substrate remaining after 5 minutes was used for the abscissa values. A question of why the total COD was used rather than the COD contributed by only jack bean meal should be answered. This is a valid question since the added glucose may contribute significantly to the total substrate COD remaining thereby falsely indicating a reduction in the absorption capacity for the colloidal substrate by the sludge. Therefore, the isotherms values for the colloidal substrate-glucose studies were recalculated to account for presence of

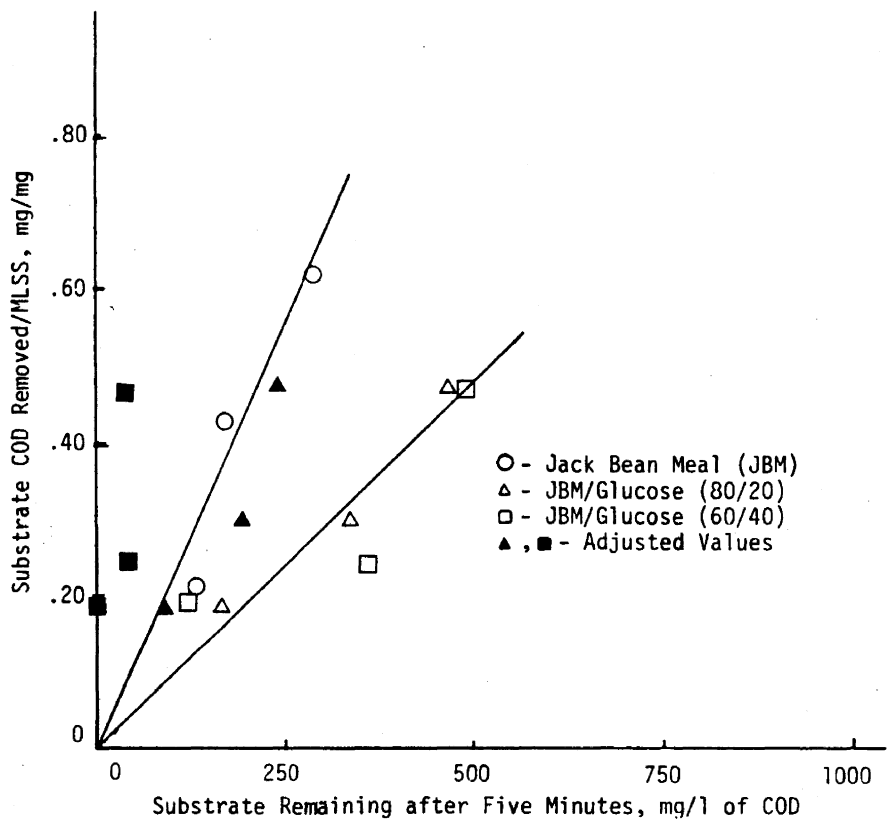


Figure V-36: Isotherm Plots for Jack Bean Meal Acclimated Activated Sludge Culture Fed Various Substrate Combinations

jack bean meal only. It was assumed no glucose had been removed in the initial five minutes of the study (a very liberal assumption), and the COD contributed by the glucose was simply subtracted from the total substrate COD remaining after five minutes. This procedure was done for all the colloidal substrate-glucose combinations. For albumin and starch, the general results were not affected, i.e. the presence of glucose reduced the adsorption capacity of the sludge. However, the jackbean meal glucose combination reacted differently. The solid black points in Figure V-36 represent the adjusted values. For the twenty percent glucose supplementation, there was no inhibition of adsorption by glucose; and for the forty percent glucose supplementation, there appeared to be an enhanced adsorptive capacity. However, this enhancement effect was based on a liberal data interpretation, i.e. there was no glucose utilization in the first five minutes of the batch study. Therefore, this enhancement of adsorption capacity of the sludge is questionable, at best.

Physical Studies

Introduction

Since the adsorption and/or enmeshment of organic colloids in activated sludge is so important in the contact stabilization process, a series of studies was formulated to investigate this interaction of colloid-activated sludge from a pure physical standpoint. Factors such as pH, ionic strength, and cation types were investigated in an

attempt to define their effects on the adsorption interaction. The substrates and the sludges were first studied separately under various physical environments, and then combined under the same conditions. Interpretation of the data was designed to answer questions such as "What substrate characteristics are of greatest significance to the contact stabilization mechanism?" and "What factors might enhance adsorption and/or enmeshment of organic colloids in a sludge?"

Substrates

Introduction

Several series of tests were conducted to characterize each substrate. Electrophoretic mobility measurements were performed to evaluate the colloid stabilities. Since this method has proved successful in evaluating the effectiveness of coagulation in water treatment, it was speculated there would be some correlation between the electrophoretic mobilities of the colloids and their susceptibility to physical removal by activated sludge. Turbidity and particle count studies were also performed to determine colloidal concentrations and size distribution. These properties were assumed to be very important in the colloid-sludge interactions. Finally, all studies were conducted under various conditions of pH, ionic strength, and ionic mediums. The theoretical basis for use of these three variables was established in Chapter III and will not be discussed further.

pH Effects

Figure V-37 and V-38 describe the electrophoretic mobilities of albumin and jack bean meal, respectively, under various

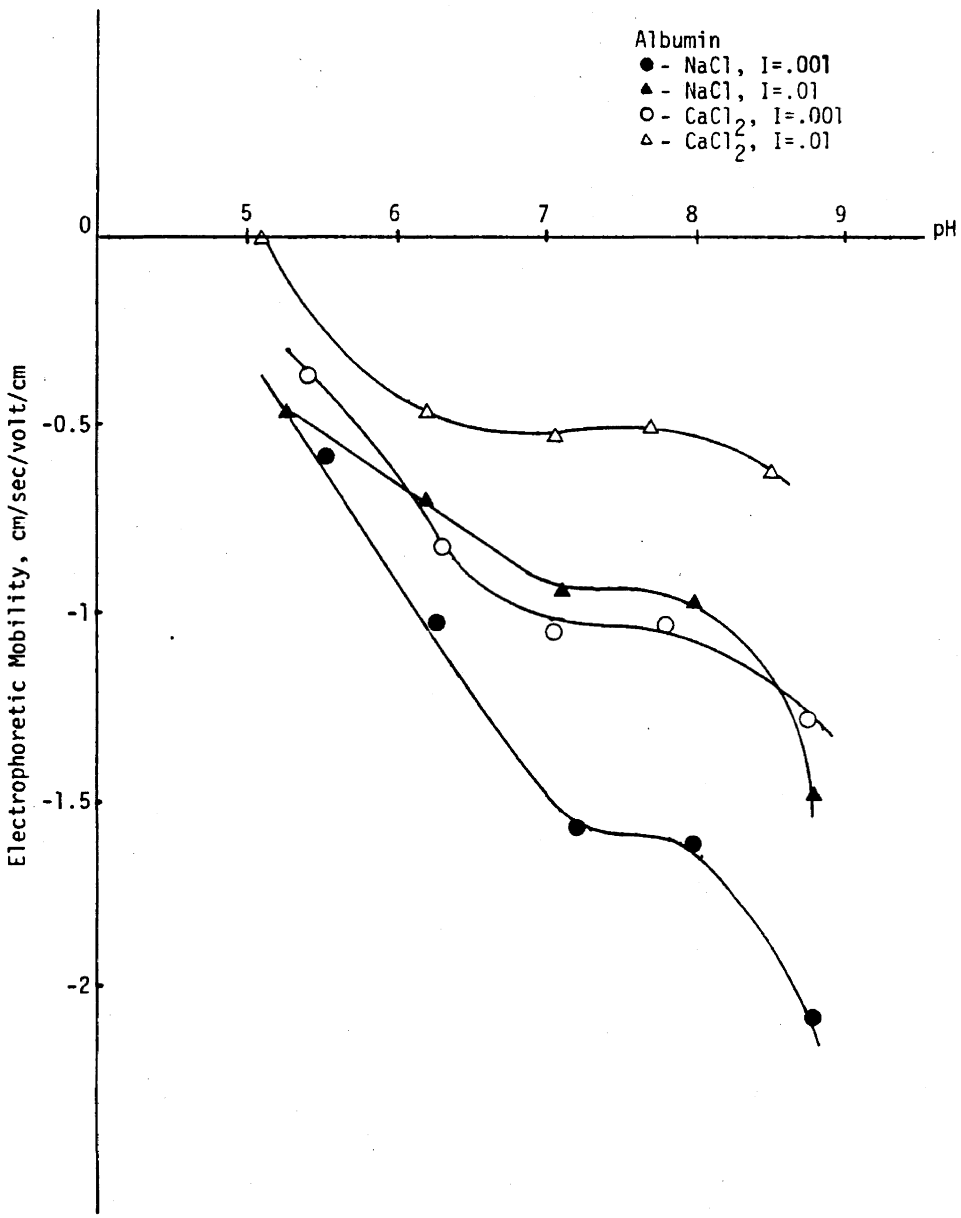


Figure V-37: Relationship Between Electrophoretic Mobilities of Albumin and pH in Two Ionic Mediums and Two Ionic Strengths

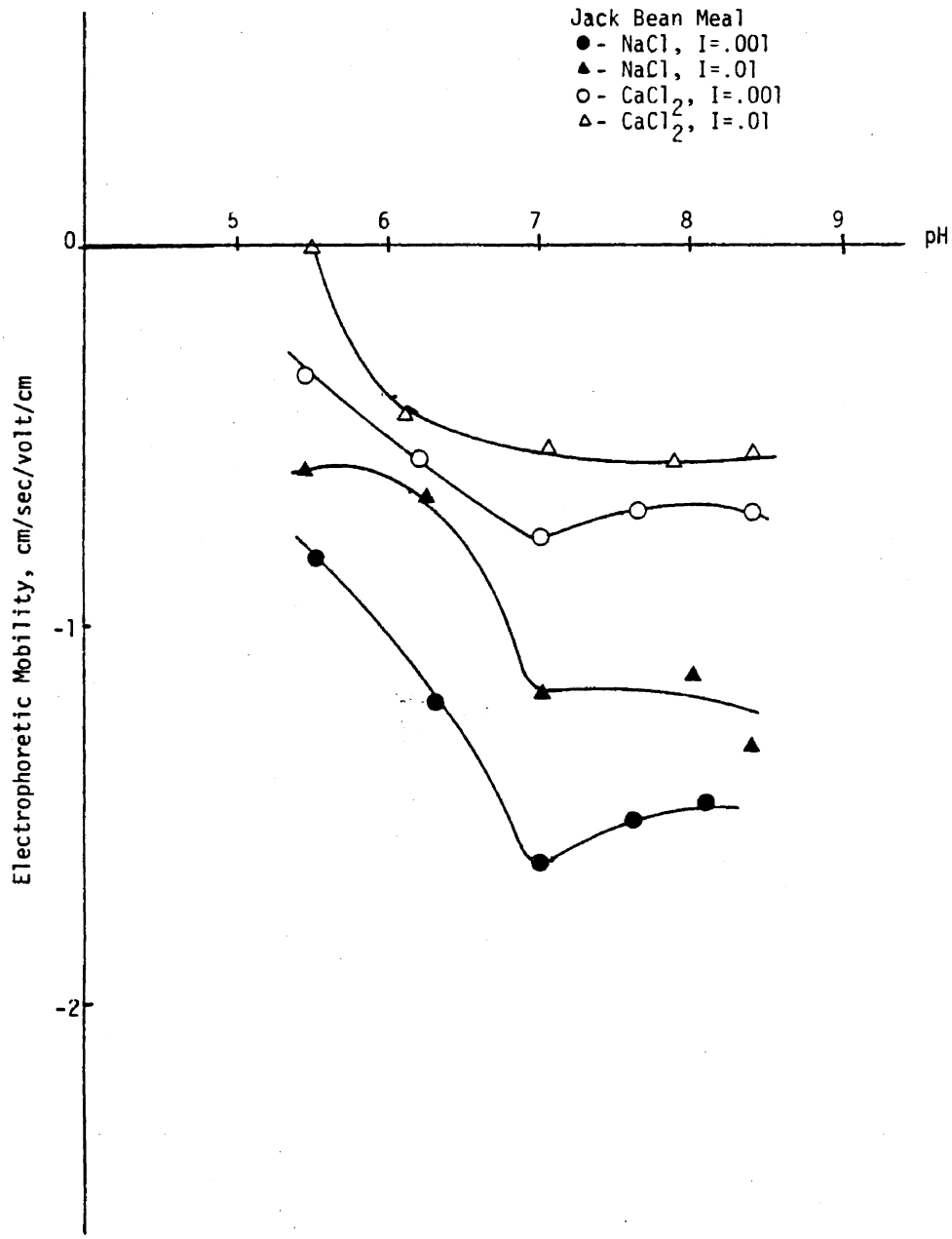


Figure V-38: Relationship Between Electrophoretic Mobilities of Jack Bean Meal and pH in Two Ionic Mediums and Two Ionic Strengths

environmental conditions. In both cases, as the pH dropped, the electrophoretic mobility slowed due to probable protonation of negatively charged functional groups. In the case of albumin, the trend under every condition tested was toward zero mobility at pH values below 5. This was expected since the isoelectric point of albumin is 4.6.

In the literature review, mention was made of precipitation of molecules at the isoelectric point. This occurred for albumin as shown in Figure V-39 wherein the number of measureable particles between 1 and 60 microns increased as the pH was lowered to six. Turbidity measurements (not shown) verified this change. With regard to the particle sizes listed in Table V-4, no statistically significant variations were found for albumin. Appendix C lists all the statistical studies.

The jack bean meal substrate showed very little variation under the conditions tested. pH appeared to have no effect on the particle sizes (Table V-4) and particle numbers (Figure V-40).

As previously mentioned, electrophoretic mobility measurements were not performed on the potato starch substrate. It is safe, though, to assume that pH variations would have some effect upon this substrate. Negatively charged phosphate ester groups are intimately related to the amylopectin in starch at neutral and alkaline pH values. However, from the particle counts and mean particle sizes in Figure V-41 and Table V-4, respectively, pH had very little effect on starch over the ranges tested. Particle count

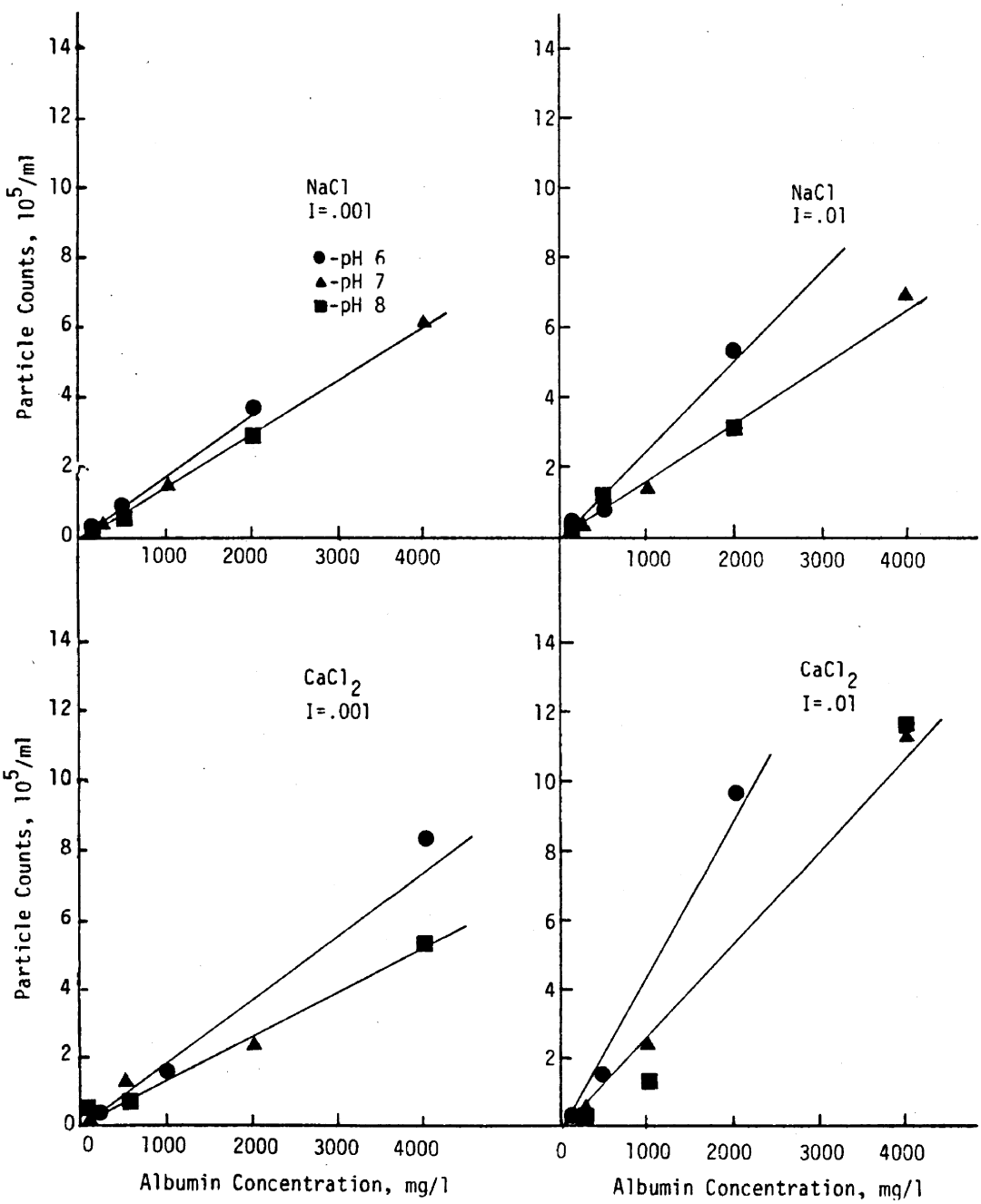


Figure V-39: Relationship Between Albumin Particle Counts (1-60 micron sensor) and Albumin Concentration in Two Ionic Mediums and Two Ionic Strengths

Table V-4

Mean Particle Sizes of Substrates*

Substrate	pH	Ionic Medium / Ionic Strength	NaCl .001	NaCl .01	$CaCl_2$ .001	$CaCl_2$ .01
Albumin	6		11.0	10.6	11.7	11.0
	6		11.4	11.6	11.7	11.0
	6		11.4	13.5	11.4	12.5
	7		11.2	11.1	11.4	11.2
	7		10.8	10.8	11.5	11.3
	7		10.9	11.8	10.4	11.9
	8		11.1	10.8	11.8	11.4
	8		10.8	11.5	11.1	11.3
	8		12.3	13.5	11.2	11.1
Starch	6		10.0	14.2	11.8	15.5
	6		10.2	13.2	11.4	14.4
	6		9.9	12.4	11.0	11.5
	7		10.9	14.0	11.3	14.8
	7		9.7	12.5	10.9	12.3
	7		9.8	13.3	11.2	11.9
	8		10.2	13.7	12.4	14.9
	8		9.6	12.8	11.0	13.0
	8		11.0	11.9	10.6	11.9
Jack Bean Meal	6		13.4	16.2	15.6	12.5
	6		11.3	15.4	15.7	12.0
	6		12.5	14.5	15.2	12.5
	7		12.9	16.2	15.2	12.2
	7		13.6	15.9	16.0	12.1
	7		13.6	14.6	15.7	11.6
	8		13.0	15.5	16.0	12.5
	8		13.2	14.3	16.3	12.1
	8		13.9	13.3	14.8	12.3

*Mean particle sizes are expressed in microns.

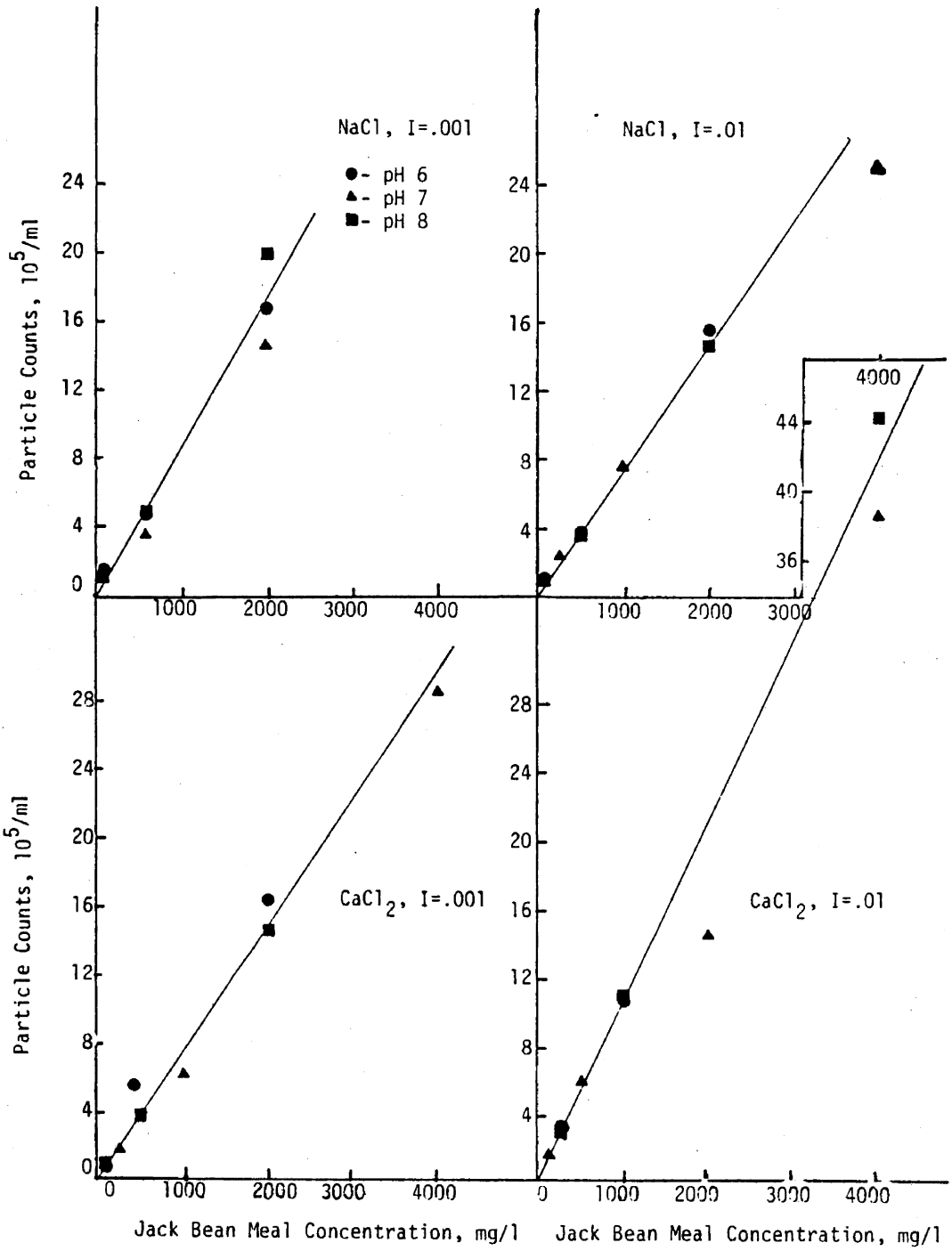
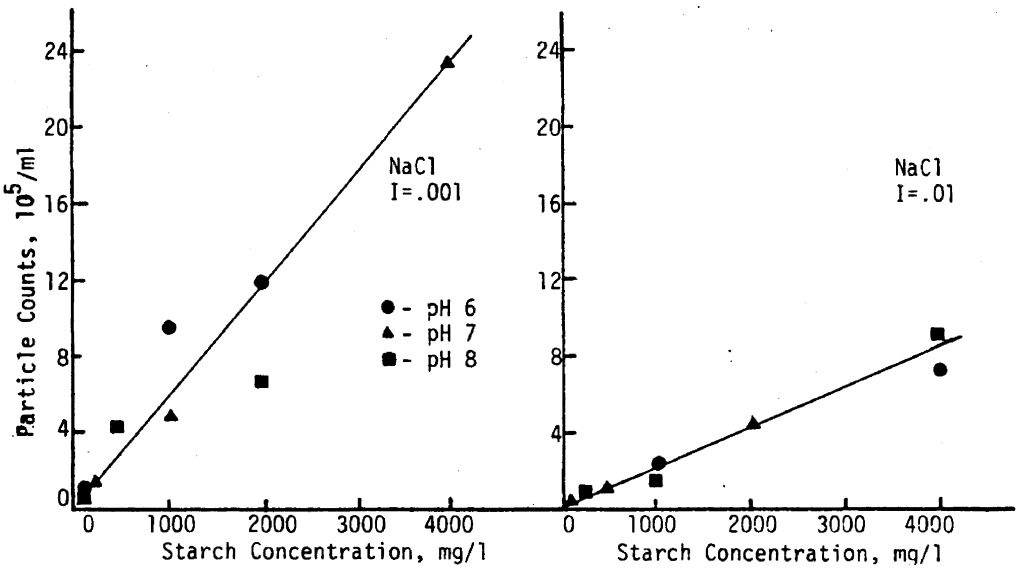


Figure V-40; Relationship Between Jack Bean Meal Particle Counts (1-60 micron sensor) and Jack Bean Meal Concentration in Various Ionic Mediums and Ionic Strengths



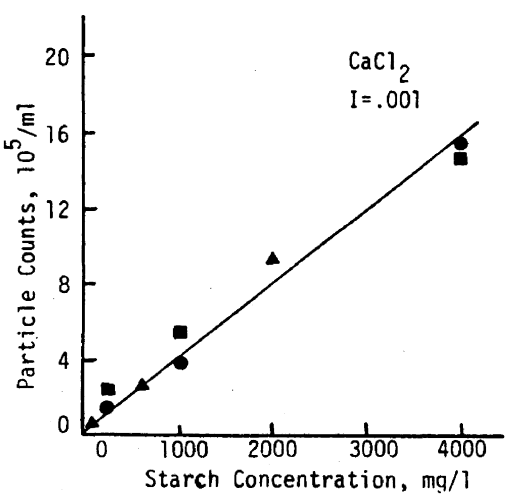


Figure V-41: Relationship Between Starch Particle Counts (1-60 micron sensor) and Starch Concentration in Various Ionic Mediums and Ionic Strengths

data for starch in .01 ionic strength $CaCl_2$ was not obtained. The starch at those conditions formed particles too large to pass through the 1-60 micron sensor.

Ionic Medium and Strength

As discussed in the literature review, both the type of ions (anionic or cationic, monovalent or divalent) and the number of ions in solution strongly affect the stability and character of a co-existing colloid. This was most evident in the electrophoretic mobility results in Figures V-37 and V-38. The charge on both albumin and jack bean meal colloids decreased as the ionic strength and the valence on the cations in the solution increased.

Statistical analysis (Appendix C) of the mean particle sizes in Table V-4 also provide evidence of an effect on two of the three substrates due to the ionic strength. The starch and jack bean meal mean particle sizes varied significantly (greater than a 99% level of confidence) with changes in ionic strength. Changes in ionic medium had a lesser effect on starch but still recorded a significant statistical level of confidence (greater than 90%).

Specifically, the starch substrate mean particle size increased with an increase in ionic strength and cation valence. The jack bean meal substrate, however, had a rather peculiar reaction. For the NaCl ionic solutions, a larger mean particle size was exhibited at the higher ionic strength. The opposite occurred for the jack bean

meal suspended in the $CaCl_2$, i.e. the particle size decreased when placed in the higher ionic strength solution.

Activated Sludge

Introduction

Identical studies to those conducted on the substrates were made on the acclimated activated sludges. With similar data on both the substrate and the sludge, a favorable or unfavorable interaction between the two could be explained.

pH Effects

The results of the electrophoretic mobility studies in Figures V-42, V-43, and V-44, revealed that the effect of pH upon the three activated sludge cultures was small. This was expected since the isoelectric point of activated sludge has been reported in the literature [54] to lie between a pH of 1 and 3.

Ionic Strength, Ionic Medium, and Solids Concentrations

The electrophoretic mobility studies indicated an effect on the sludges due to ionic strength and medium. All the activated sludge mobilities dropped with an increase in ionic strength and cation valences.

Sludge mean particle sizes for the various conditions imposed are listed in Table V-5. Unlike the electrophoretic mobilities, the albumin and jack bean meal acclimated sludge particle sizes did not appear greatly affected by either ionic medium, ionic strength, or

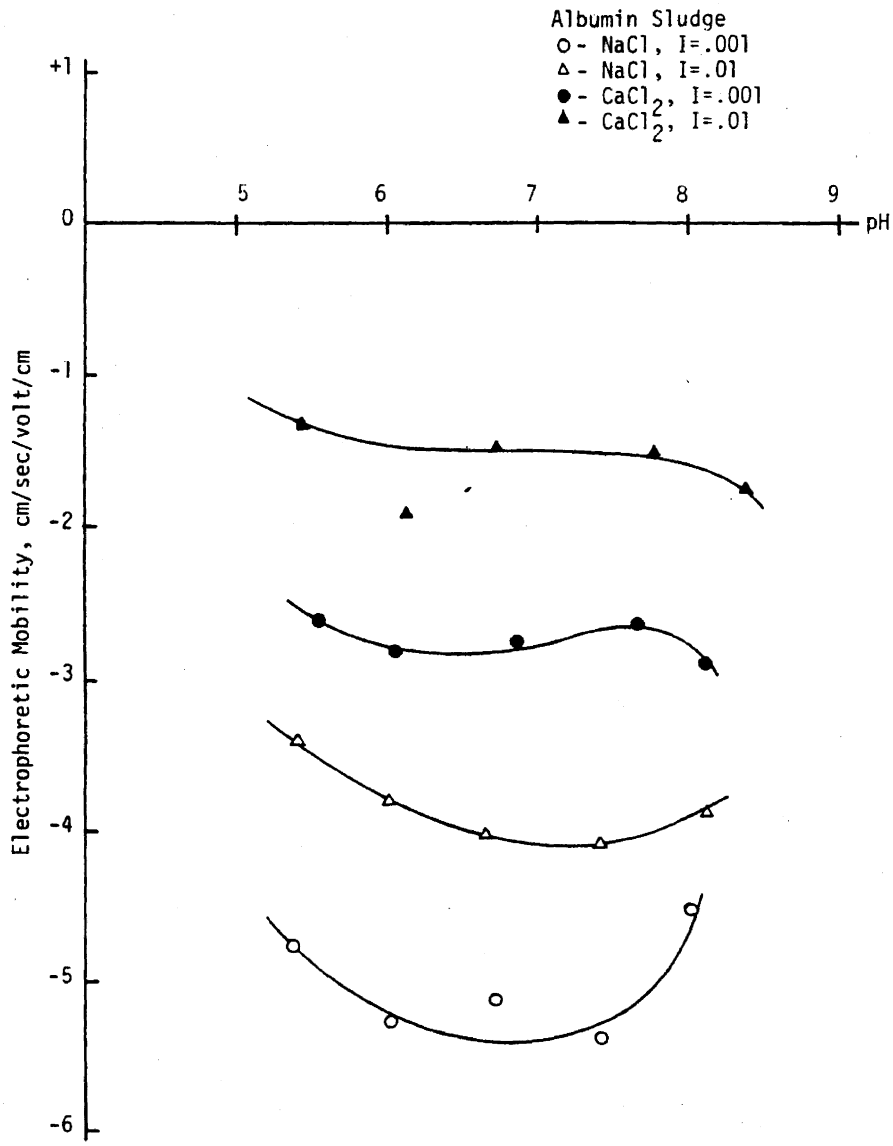


Figure V-42: Relationship Between Electrophoretic Mobilities of Albumin Sludge and pH in Two Ionic Mediums and Two Ionic Strengths

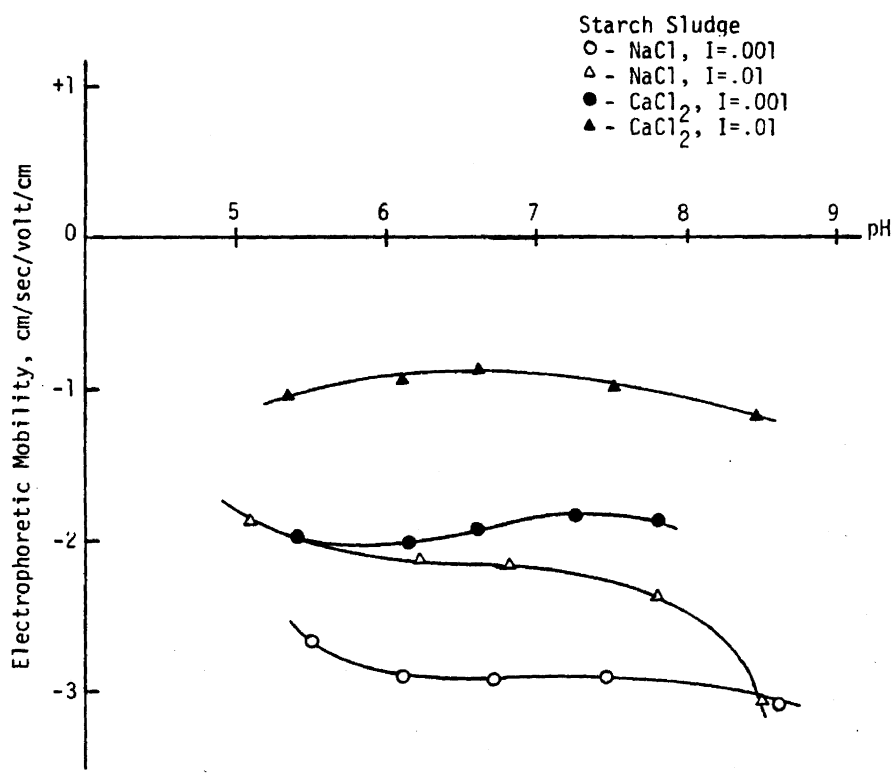


Figure V-43: Relationship Between Electrophoretic Mobilities of Starch Acclimated Activated Sludge and pH in Two Ionic Mediums and Ionic Strengths

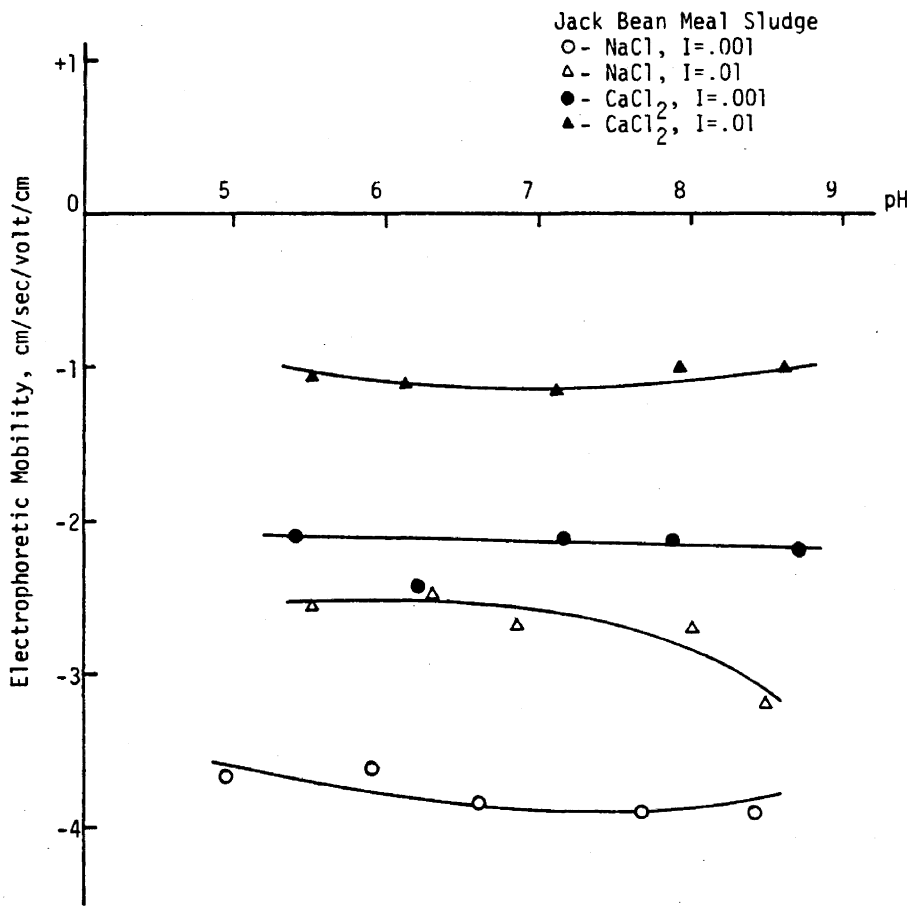


Figure V-44: Relationship Between Electorphoretic Mobilities of Jack Bean Meal Acclimated Activated Sludge and pH in Two Ionic Mediums and Two Ionic Strengths

Table V-5

Sludge Mean Particle Sizes

Substrate Acclimated Sludge	Ionic Medium/Strength	MLSS (mg/l)	Mean Particle Size (microns) pH 6	pH 7	pH 8
Albumin	NaCl/.01	1055	30.8	30.1	30.7
		2825	32.3	31.7	31.5
	$CaCl_2$/.01	925	18.9	17.8	18.4
		2770	26.1	24.7	24.6
Starch	NaCl/.001	990	18.9	17.8	18.4
		2725	31.5	28.7	27.7
	NaCl/.01	2650	24.2	18.8	20.6
		2750	--	22.6	22.2
	$CaCl_2$/.001	885	22.6	26.4	21.6
		2800	43.9	36.9	36.4
	$CaCl_2$.01	960	66.1	65.2	64.8
		2665	53.0	59.7	51.0
Jack Bean Meal	NaCl/.001	957	23.3	22.4	21.8
		2860	20.2	20.9	20.5
	NaCl/.01	1020	19.6	19.6	18.9
	$CaCl_2$/.001	933	23.9	22.5	22.3
		2740	24.0	21.8	19.4
		2645	24.0	25.0	25.4
	$CaCl_2$/.01	910	21.7	23.3	22.5
		2785	22.4	26.5	22.5

solids concentrations. However, the starch sludge did vary with all three of these parameters. The calcium ion appeared to be intimately involved in the floc structure of the starch sludge. An increase in calcium concentration increased the average diameter of the sludge by a factor of two. Solids levels also exerted an influence on the starch sludge. The data suggested that in the low ionic strength systems the particle size increased with an increase in solids concentration. This probably could be attributed to an increase in the number of collisions among sludge particles.

Albumin Adsorption Studies

Introduction

The purpose of this series of adsorption studies was to evaluate the adsorption capacity of the sludge and to simultaneously monitor changes in the turbidity of the settled sample and the physical characteristics of the sludge. By monitoring these last two phenomena, information concerning ways to improve effluent quality and adsorption of colloids in contact stabilization could potentially be obtained. Note that throughout the adsorption studies, mercury poisoned bacteria were used to prevent any metabolic uptake of the substrate.

Adsorption Isotherms

Isotherm plots for albumin are shown in Figure V-45 and V-46. Some very interesting responses were evident in these results. Initially, the lowest pH (~6) provided the greatest adsorption

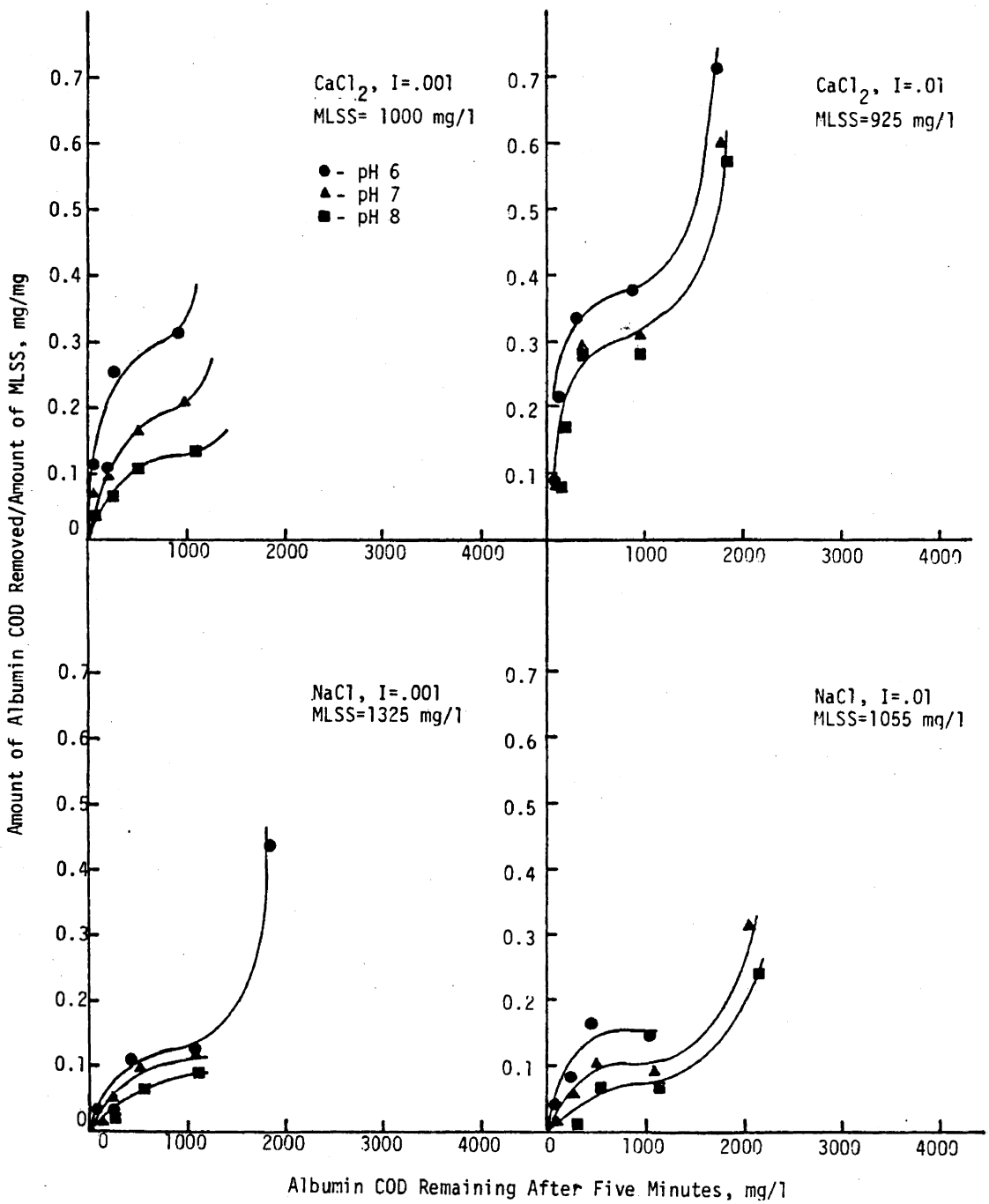


Figure V-45: Isotherms of the Combination of Albumin and Albumin Acclimated Activated Sludge Under Various Conditions and a Contact Time of Five Minutes

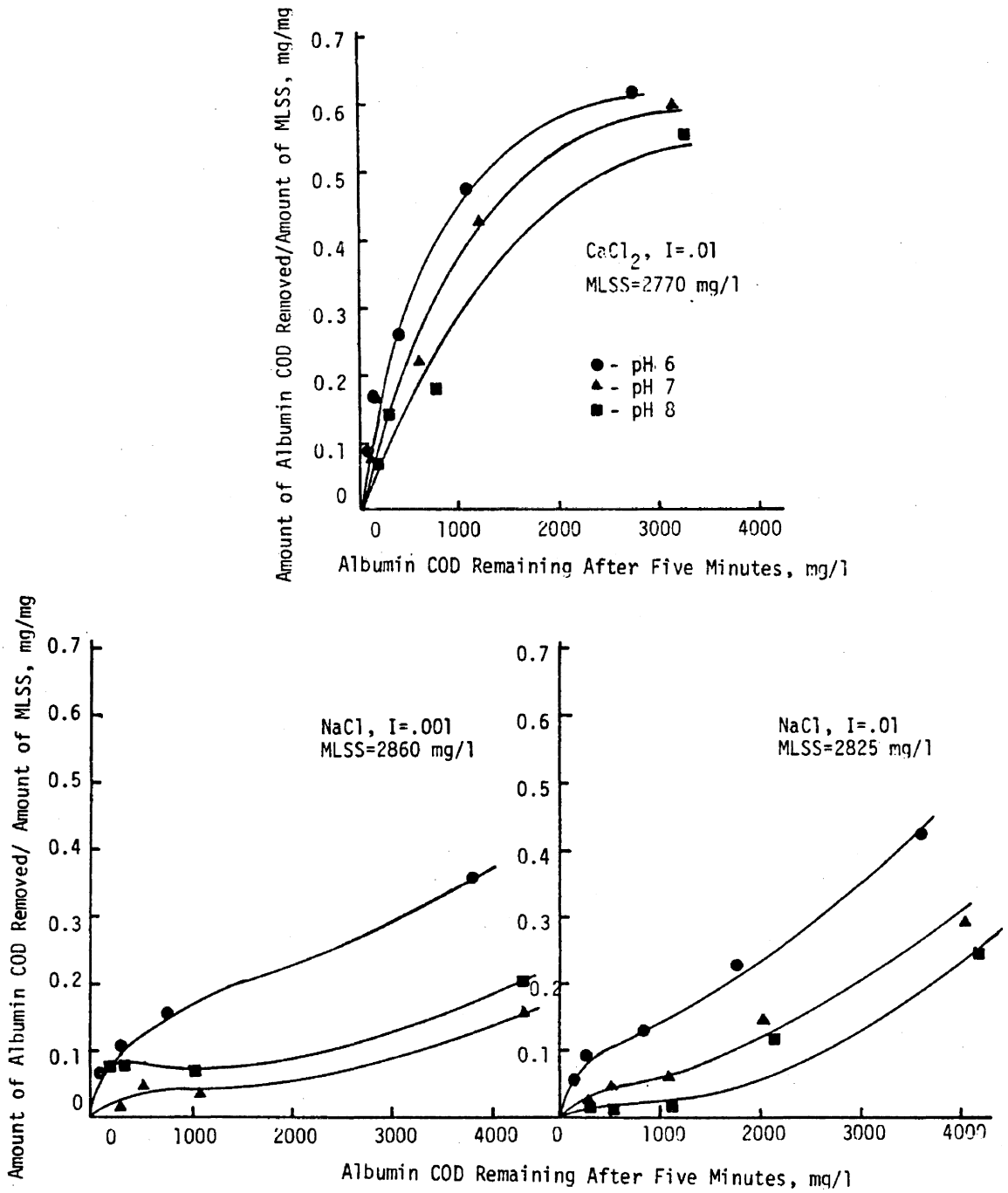


Figure V-46: Isotherms for the Combination of Albumin and Albumin Acclimated Activated Sludge Under Various Conditions and a Contact Time of Five Minutes

capacity, without exception. The data also revealed that increases in ionic strength enhanced the removal of the albumin. The removal capacities of the sludge appeared to be better in the $CaCl_2$ solutions than in the NaCl solutions. Finally, the unusual upward curves of the isotherms in Figure V-44 verified the isotherm drawn in Figure V-12 for the kinetic studies. However, this sharp increase was apparently tempered by increased MLSS levels as shown in Figure V-46. One set of isotherms performed in a .001 ionic strength $CaCl_2$ medium at a high MLSS concentration did not appear in Figure V-46. The COD data obtained was not plausible and was therefore discarded.

Particle Size Variations in the Adsorption Studies

Prior to discussion of the average particle size data, mention should be made of the patterns and variability of this parameter. The data for average particle size in all three sludges were, at times, easily explainable ; at other times, they were quite the contrary. However, this variance was linked to the method of measurement for the sludge-substrate mixtures. Specifically, the particle counter required stirring of a diluted sample of the mixtures, this stirring effect was believed to be the source of the problem. Apparently, for weakly attached colloid-sludge aggregates, shearing or separation of the couple occurred resulting in a smaller mean particle diameter. Even though this effect diminished the accuracy of the evaluation of the degree of interaction, it did

permit an evaluation of the strength of the substrate-sludge union.

Proceeding with the albumin study results, average particle size values for the sludge-substrate mixtures are shown in Figures V-47 and V-48. No data were taken for the two low strength ionic mediums. The idea of sludge particle counts was formulated as an after-thought and initiated after the four studies at the two lower ionic strength mediums had been performed. All the mean particle size values were plotted against the ratio of the substrate concentration to the MLSS level in hopes of normalizing the data.

Figure V-47 describes particle size variations for the substrate-sludge mixtures in a sodium chloride solution with an ionic strength of .01. There appeared to be a weak interaction between the sludge and substrates denoted by the steadily decreasing pattern in particle sizes. If there was a strong interaction, some type of increase would have been exhibited.

Increases in the average particle sizes are clearly depicted in Figure V-47. A calcium chloride of .01 ionic strength was used as the supporting medium. The results, however, did not exhibit a consistent pattern. For the studies with a MLSS concentration of 925 mg/l, the average particle sizes at pH 6 and 7 followed the same general S-curve but the pH 8 curve just increased in a curvilinear fashion. This response was somewhat puzzling. Study results at the higher MLSS level were quite acceptable though.

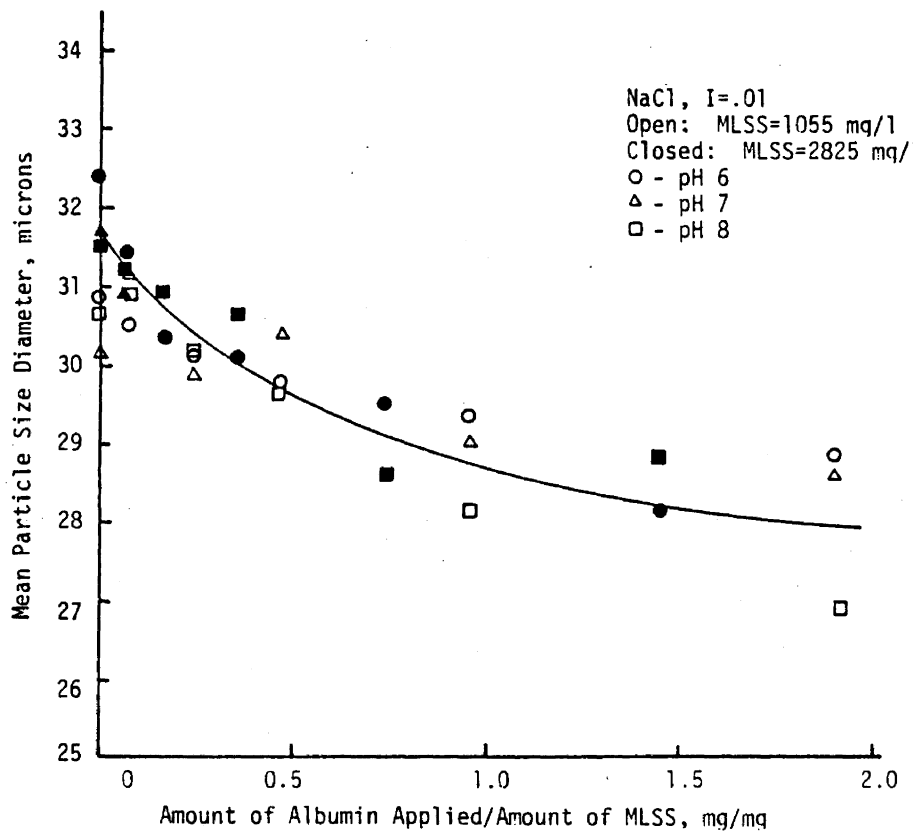


Figure V-47: Relationship Between Mean Particle Size of Albumin Acclimated Activated Sludge and the Amount of Albumin Applied per Unit of Sludge in a NaCl Solution (I=.01)

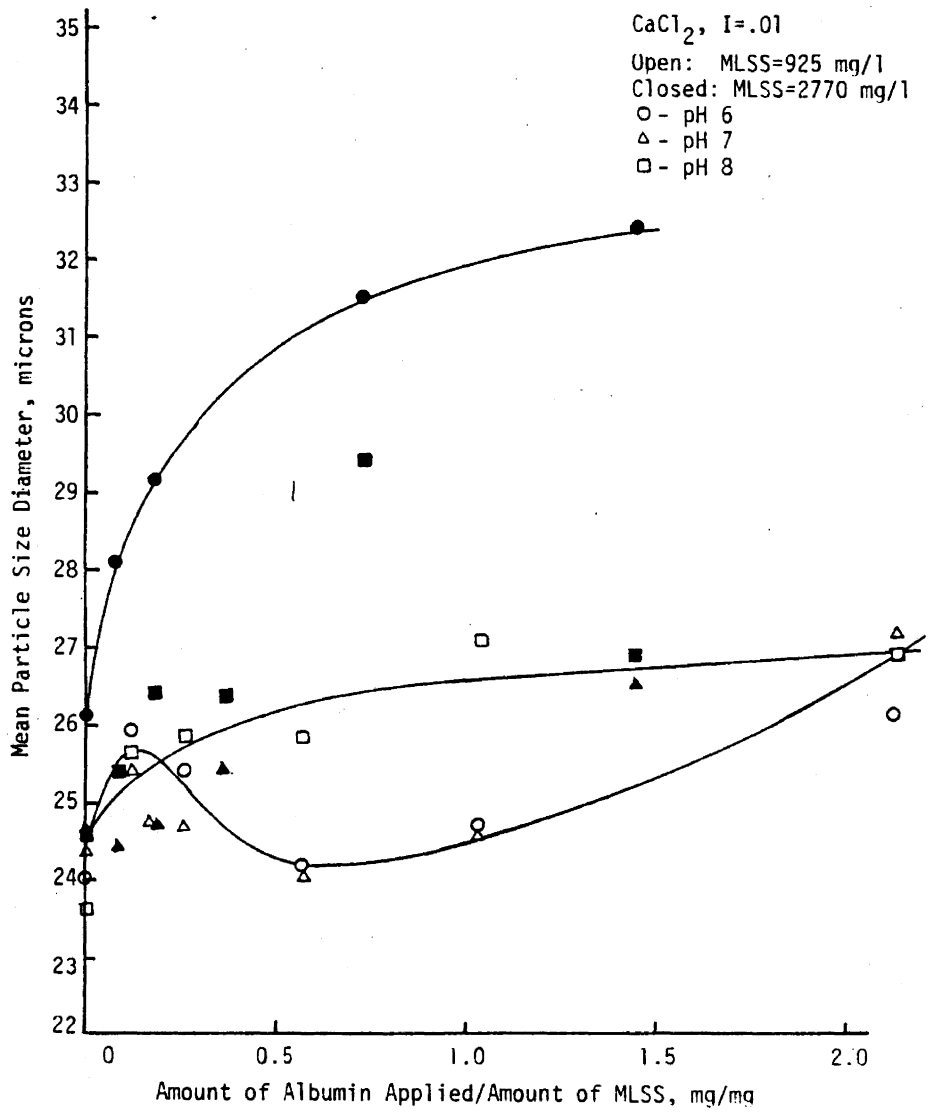


Figure 48: Relationship Between Mean Particle Size of Albumin Acclimated Activated Sludge and the Amount of Albumin Applied per Unit of Sludge in a $CaCl_2$ Solution (I=.01)

Particle sizes at pH 6 increased at a rapid rate with an increase in the substrate-sludge ratio. The data for pH 7 and 8 (represented by the middle line in Figure V-48) responded with a more moderate increase in particle size. Finally, no consistent pattern between the two MLSS levels was exhibited in the $CaCl_2$ solution except at pH 8.

Even with these variances present, a comparison of the NaCl and $CaCl_2$ studies described a distinct difference in the degree of interaction between the sludge and substrate colloids. For the albumin, the presence of $CaCl_2$ appeared to strengthen the physical union of the substrate and sludge as evidenced by the increases in mean particle sizes.

Turbidity and Supernatant Particle Counts

The turbidity in the supernatant was monitored to determine if colloid addition to activated sludge does improve the supernatant quality with respect to solids concentration. Graphs of turbidity versus albumin concentration are shown in Figures V-49 and V-50 for all the various combinations of ionic mediums and sludge concentrations. The solid lines represent the actual turbidity measured whereas the dotted segments denote what the turbidity would be if no albumin colloids were removed. Values for the dotted lines were obtained by adding the turbidities of a completely-mixed pure albumin mixture to the turbidity of a settled sludge supernatant with no substrate added. This is a

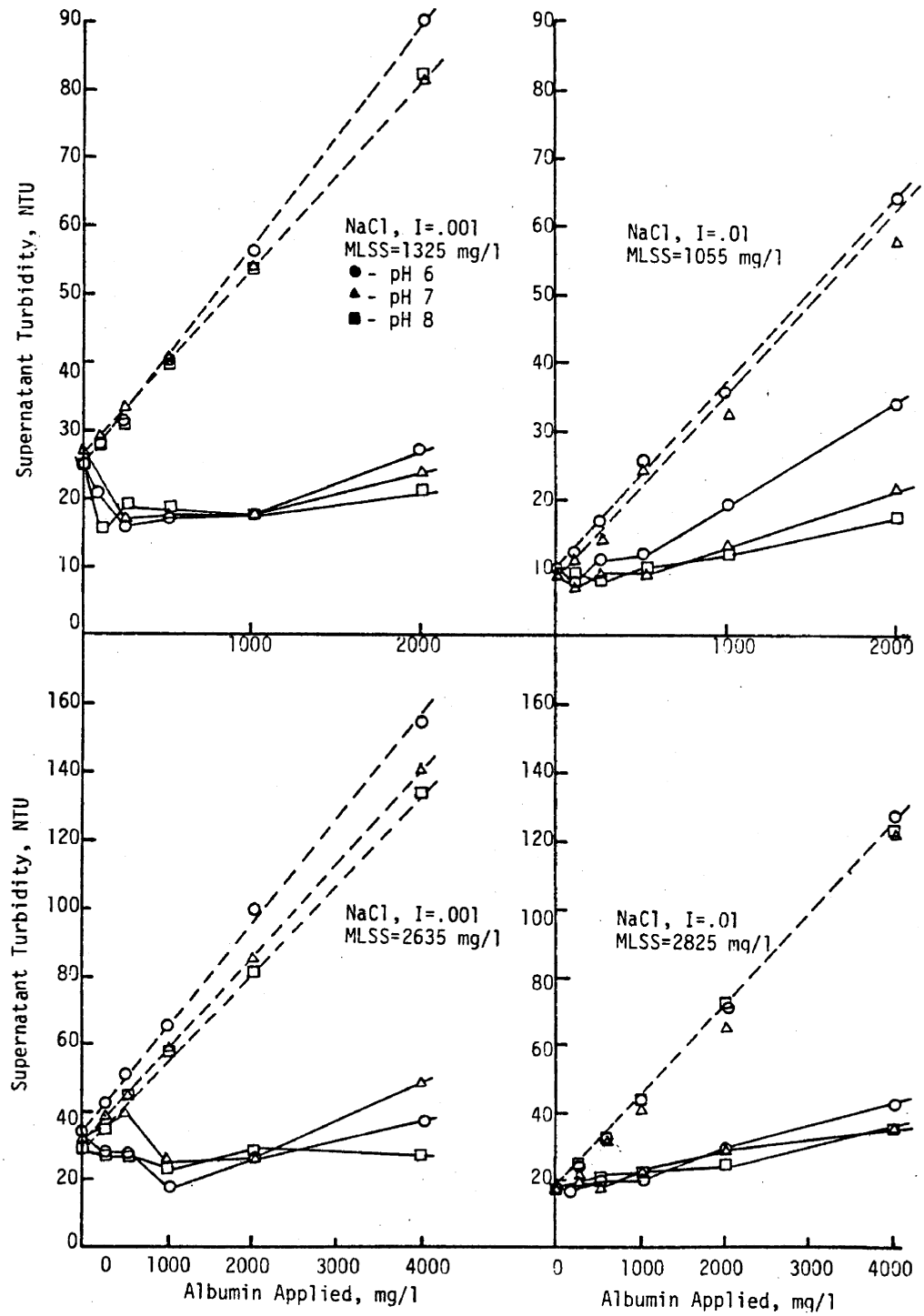


Figure V-49: Relationship Between Supernatant Turbidity and and Albumin Applied Under Various Conditions

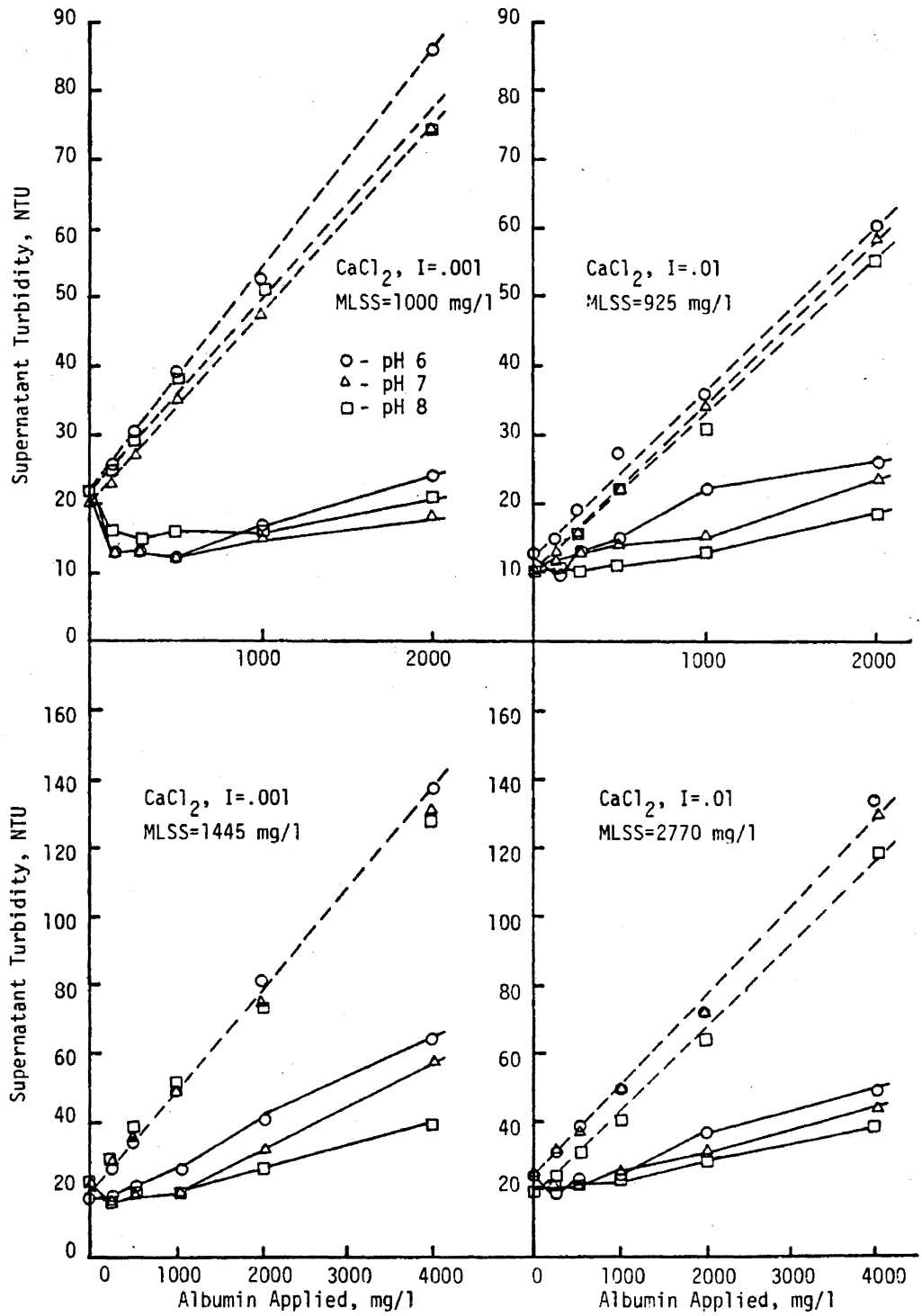


Figure V-50: Relationship Between Supernatant Turbidity and the Albumin Applied Under Various Conditions

liberal interpretation, yet it does point out the removal capacity of the sludge.

There are two points of interest in these plots. The first is that there were slight to very significant drops in turbidity at the low substrate additions. Secondly, the largest drops occurred at low ionic strengths and low MLSS concentrations. Addressing the initial point, turbidity shifts can be caused by changes in particle distribution without changing the particle count. Plots of the turbidity contrasted against particle counts of the supernatant are shown in Figures V-51 thru V-54. The particle counts correspond with the turbidity decreases only in the low ionic strength systems; the opposite was true in the high ionic strength systems. Even though the decrease in turbidity and particle count occurred only at the lower ionic strengths, it is significant that a reduction in supernatant suspended solids does occur with addition of substrate.

Starch Adsorption Studies

Adsorption Isotherms

Figure V-55 presents the isotherms obtained in studies with potato starch. The major mechanism for the physical removal of starch has been reported as adsorption [22,23], and these studies tend to support this hypothesis. Data on average particle size of the sludge are provided in Figures V-56 to V-59 and will be discussed in conjunction with the isotherm results.

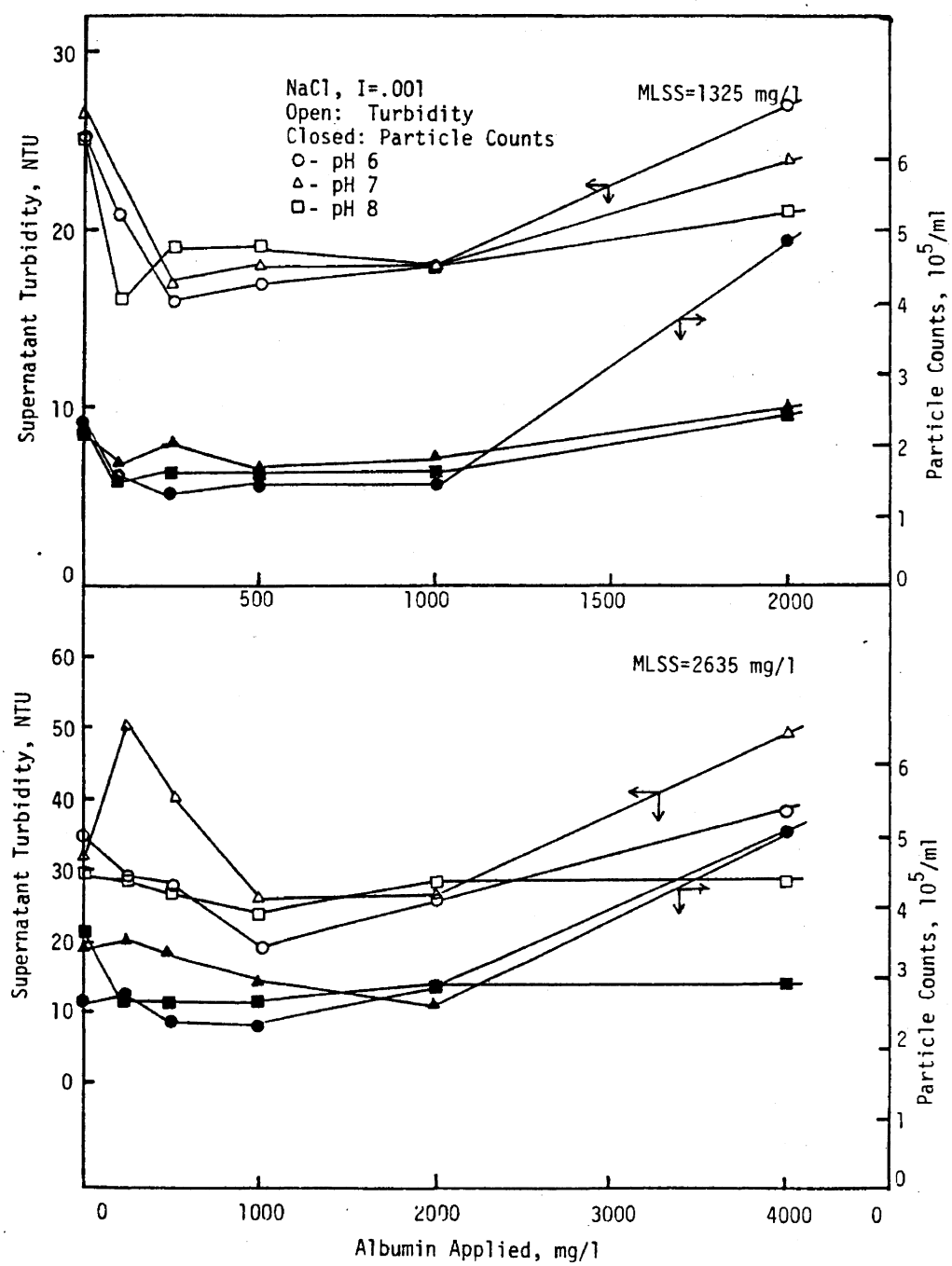


Figure V-51: Relationship Between Supernatant Turbidity and Particle Counts in the Supernatant and the Albumin Applied Under Various Conditions

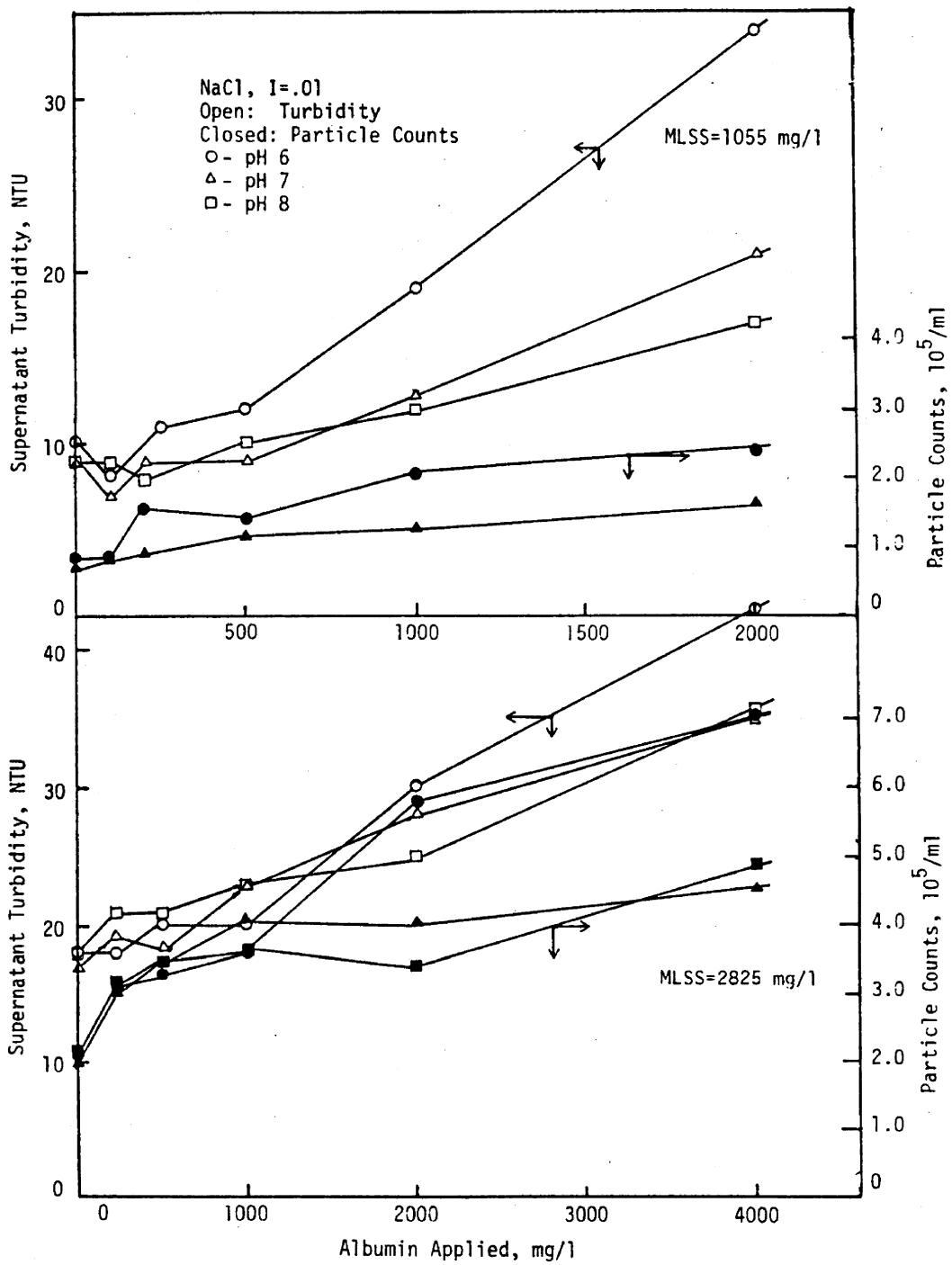


Figure V-52; Relationship Between Supernatant Turbidity and Particle Counts in the Supernatant and the Albumin Applied Under Various Conditions

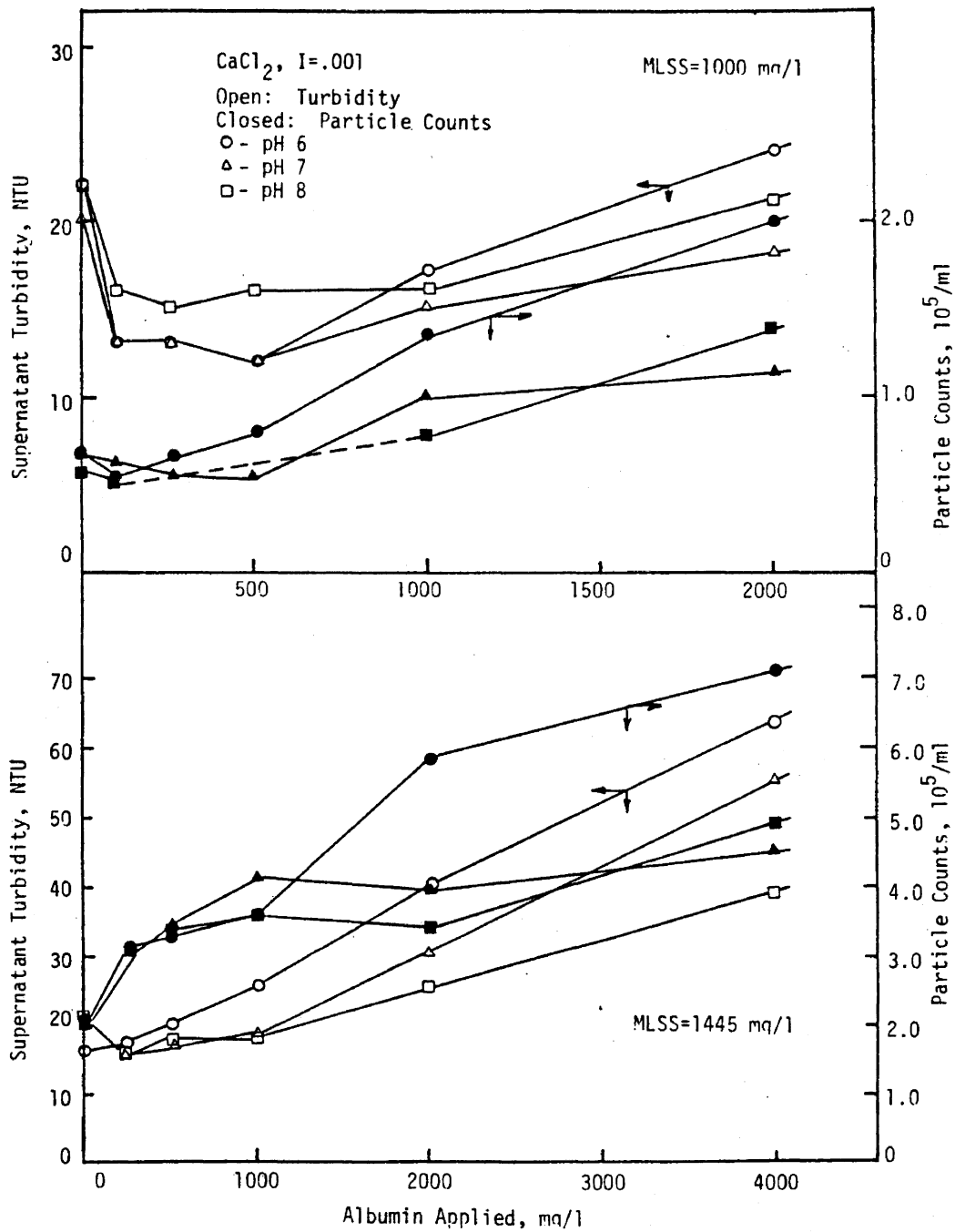


Figure V-53: Relationship Between Supernatant Turbidity and Particle Counts in Supernatant and the Albumin Applied Under Various Conditions

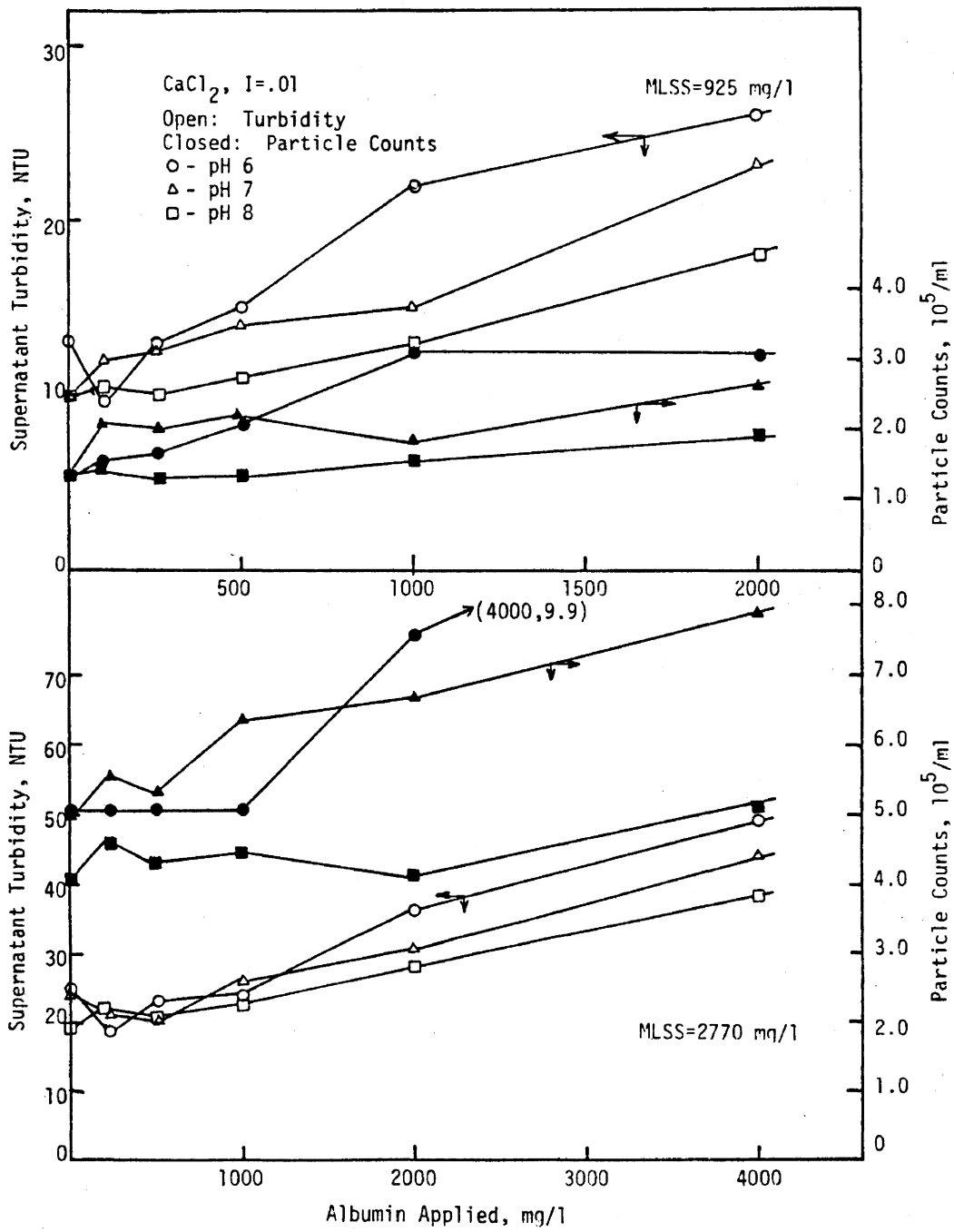


Figure V-54: Relationship Between Supernatant Turbidity and Particle Counts in Supernatant and the Albumin Applied Under Various Conditions

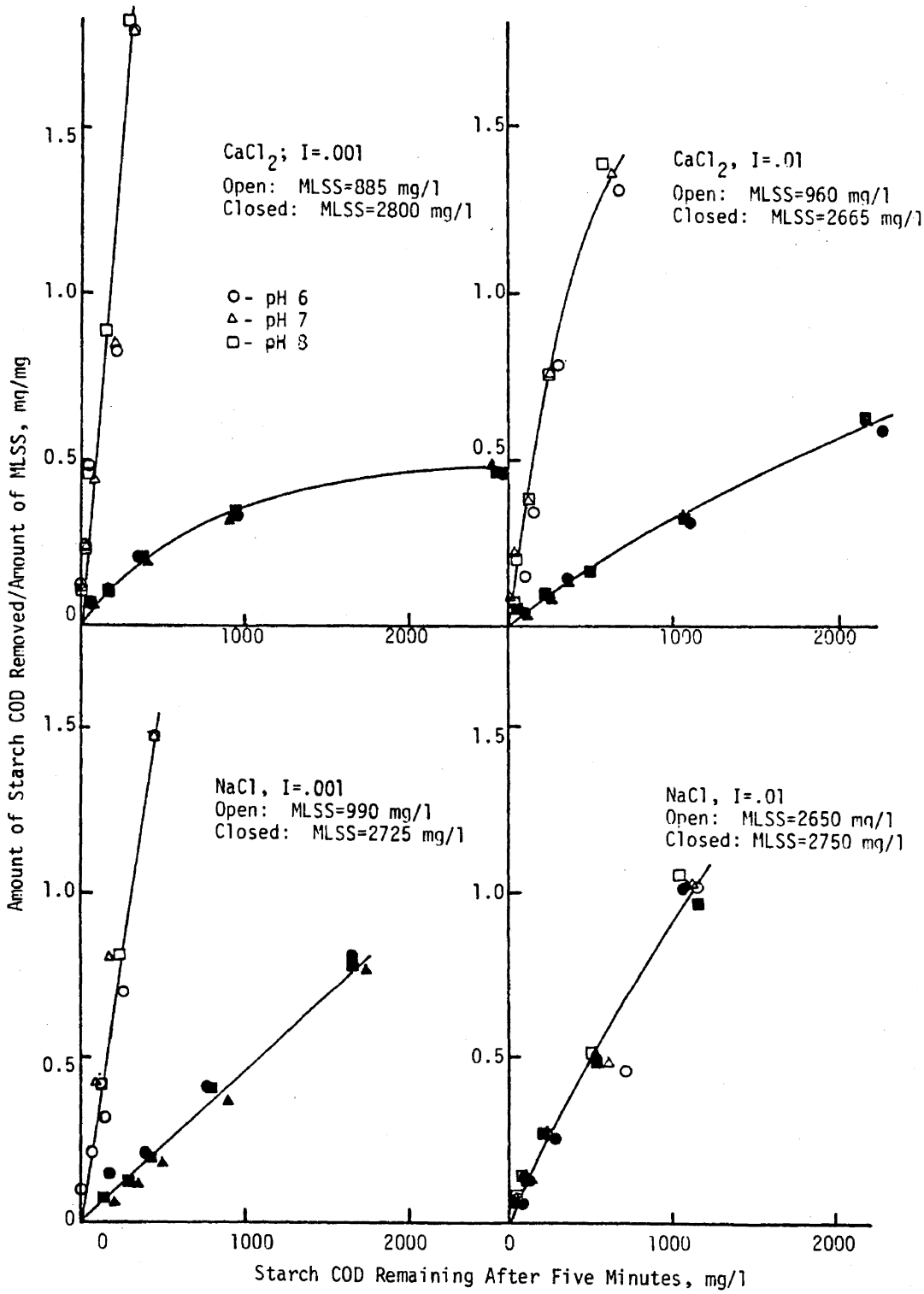


Figure V-55: Isotherms for the Combination of Starch and Starch Acclimated Activated Sludge Under Various Conditions and a Contact Time Five Minutes

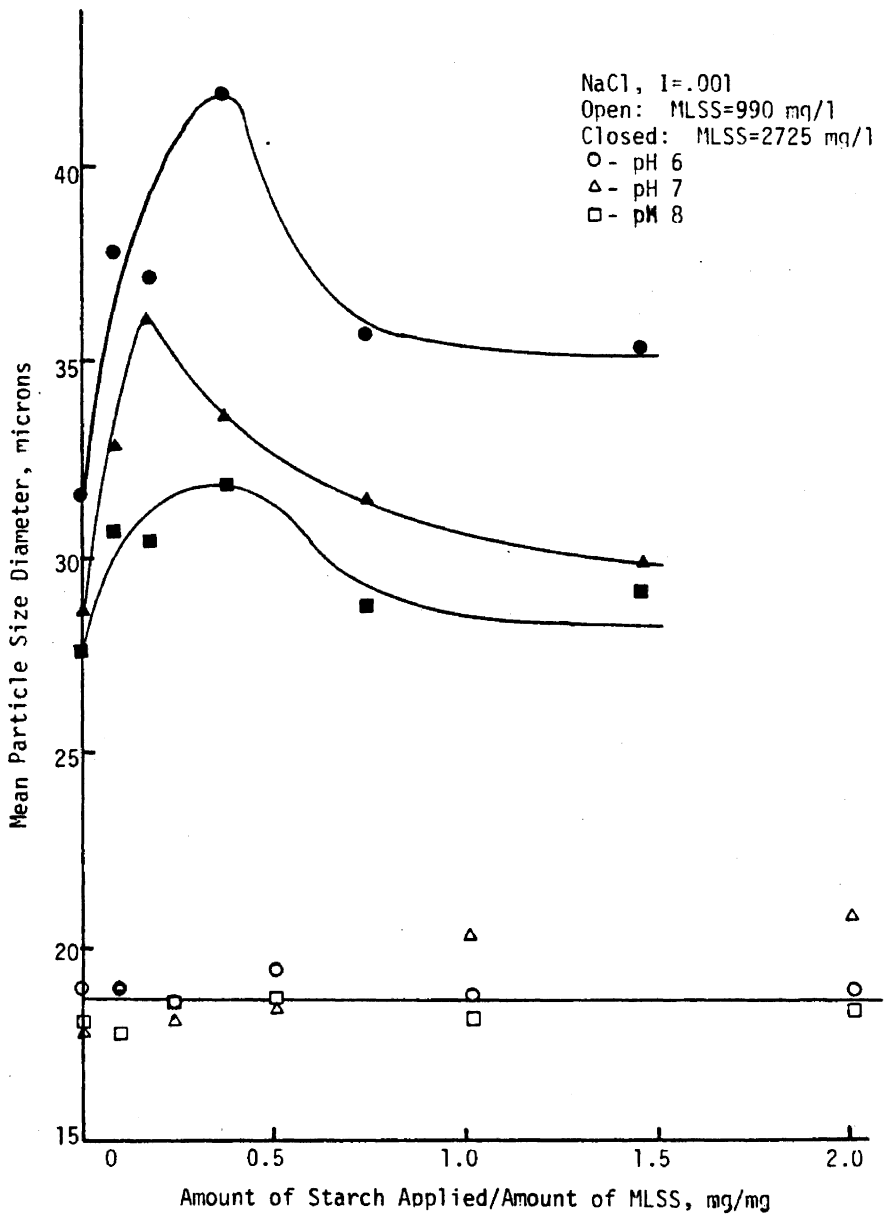


Figure V-56: Relationship Between Mean Particle Size of Starch Acclimated Activated Sludge and the Amount of Starch Applied per Unit of Sludge in a NaCl Solution (I=.001)

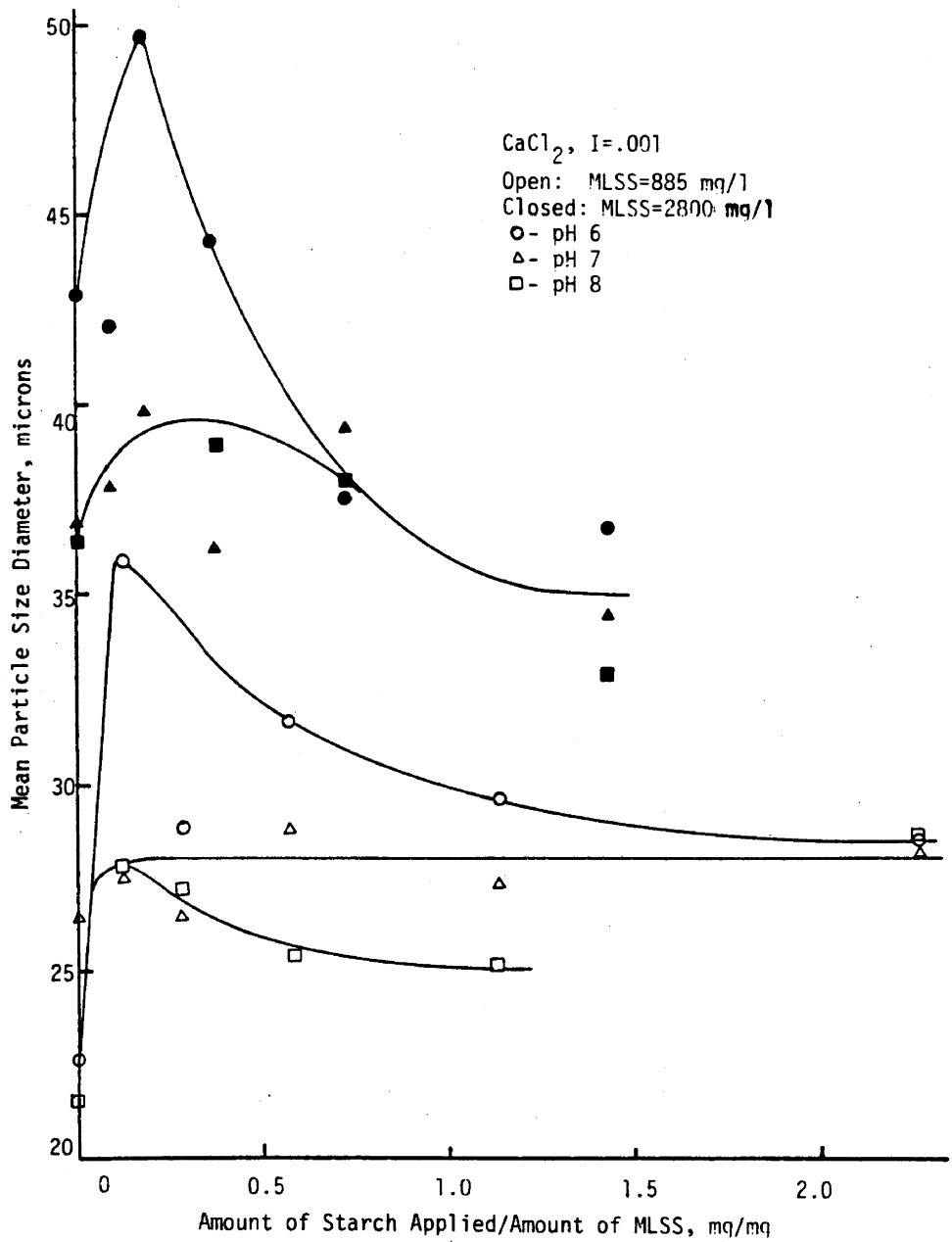


Figure V-57: Relationship Between Mean Particle Size of Starch Acclimated Activated Sludge and the Amount of Starch Applied per Unit of Sludge in a $CaCl_2$ Solution (I=.001)

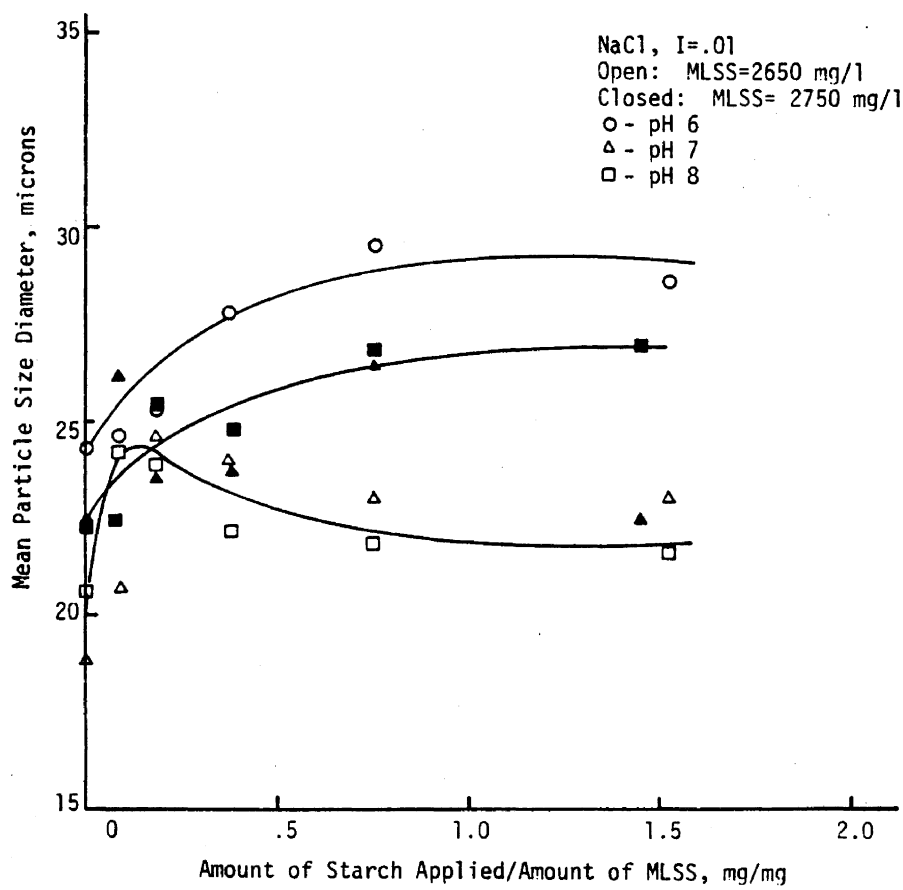


Figure V-58: Relationship Between Mean Particle Size of Starch Acclimated Activated Sludge and the Amount of Starch Applied per Unit of Sludge in a NaCl Solution (I=.01)

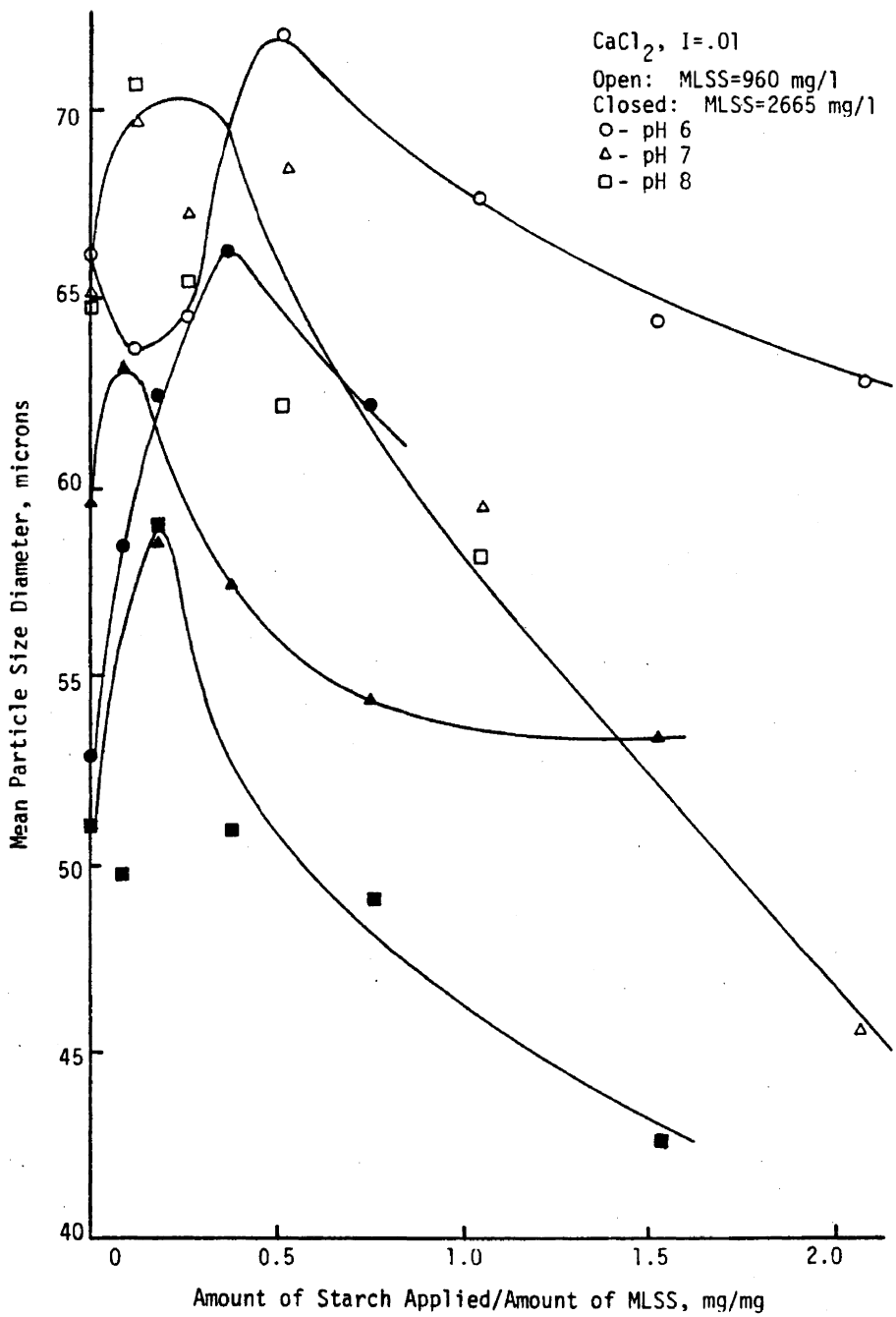


Figure V-59: Relationship Between Mean Particle Size of Starch Acclimated Activated Sludge and the Amount of Starch Applied per Unit of Sludge in a $CaCl_2$ Solution (I=.01)

Initially, the amount of adsorption occurring in the starch sludge-starch substrate mixture was quite high. It was not uncommon for the sludge to adsorb (or enmesh) one and one-half times its own weight in substrate. However, contrary to the albumin studies, no apparent pH effect was denoted for starch adsorption. The particle size data, though, suggested that, in most cases, the pH did affect the interaction. For almost all environmental conditions tested, a pH of 6 produced the largest particle sizes which tended to decrease as the pH values rose. Apparently, the interaction between the substrate colloids and sludge strengthened at lower pH values.

Another interesting comparison between the isotherm and particle size data concerns the adsorption capacity of the sludge at various MLSS concentrations. The higher sludge solids concentrations had a lower capacity for the adsorption of starch than the low solids level. For the series of studies conducted in both .001 ionic strength $CaCl_2$ and NaCl solutions, the low sludge solids concentration mixtures exhibited lower particle size diameters compared to the high solids concentration mixtures. Therefore, a greater area on the sludge flocs was available for adsorption. This hypothesis did not hold, though, for the .01 ionic strength $CaCl_2$ mixture as seen in Figure V-59.

The effect posed by the different ionic strengths appeared to be significant only for NaCl. Due to a mistake by the investigator,

the same MLSS concentration was used for a .01 NaCl ionic strength solution; however, this error did provide evidence of the reproducibility of the adsorption isotherms. The increase in the sodium chloride ionic strength improved the adsorption of starch to the sludge floc, whereas an increase in the calcium chloride ionic strength seemed to slightly decrease the substrate-sludge interaction. This latter effect though could be the result of experimental error.

The word "adsorption" has been used throughout this discussion for the mechanism responsible for starch removal. This is not without basis. Not only do the particle size results show responses compatible with adsorption, but Figure VI-60 provides visual evidence for the presence of the adsorption phenomena. This set of photographs shows a series of starch-acclimated sludge samples with starch added. The specific test conditions photographed were:

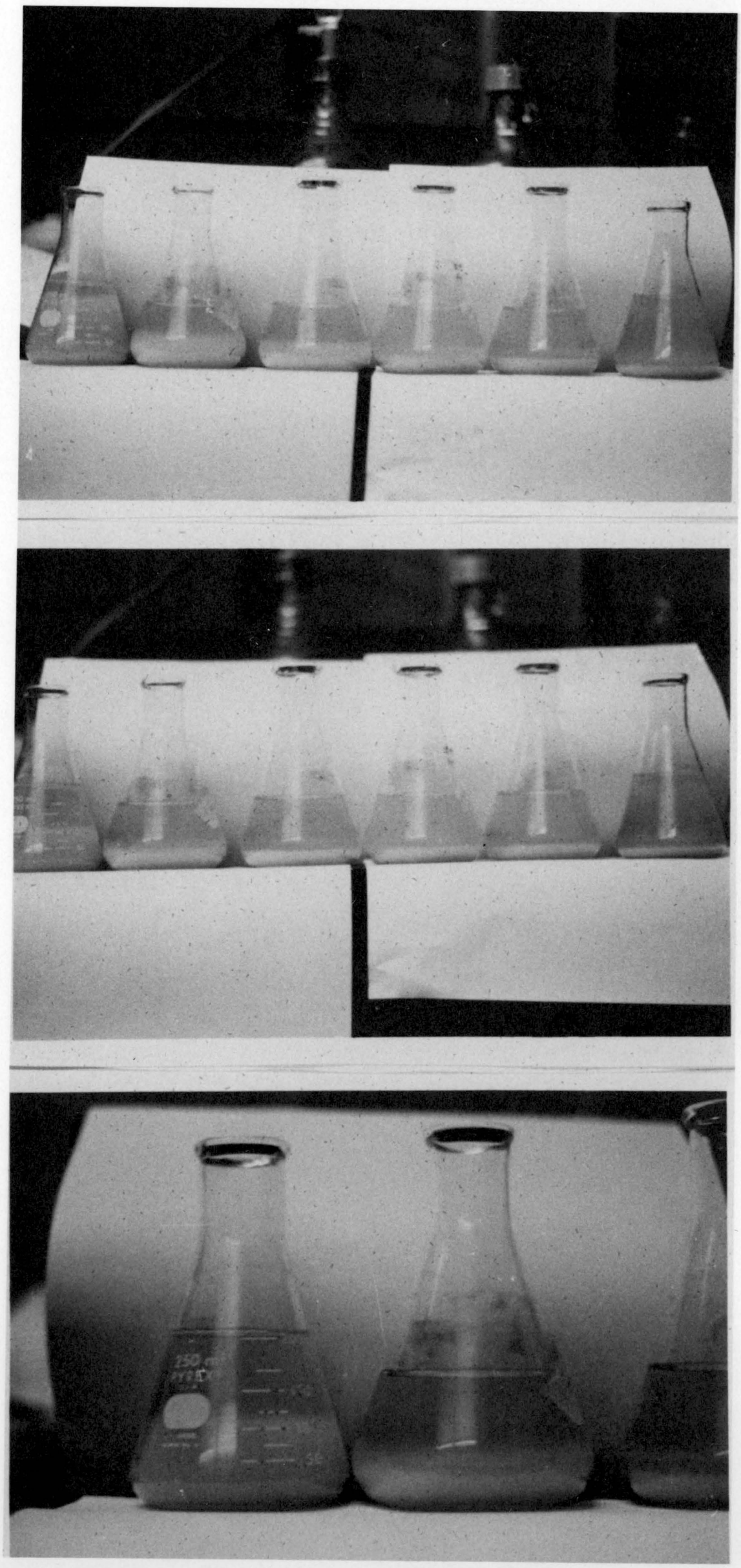
a) MLSS = 885 mg/l

b) .001 ionic strength $CaCl_2$ solution

c) pH 6

From left to right, the amount of starch added to each beaker was 2000, 1000, 500, 250, 100, and 0 mg/l. The top two photos were taken at 5 and 20 minutes, respectively, after mixing was stopped. This time lapse describes that not only did the large starch dosages decrease the sludge settling rate, but they also increased the sludge volume. The lower photo focuses on this increased volume

Figure V-60: Photographs of Starch-Acclimated Sludge Mixed With Various Amounts of Starch. Test Conditions Included: MLSS=885 mg/l; pH 6; $CaCl_2$ Solution, I=.001. Top Two Photos Represent a Time Lapse of 5 and 20 Minutes After Mixing was Stopped. The Lower Photo Shows the Increase in Sludge Volume Upon Starch Addition



of sludge for the two left-most samples. Sludge volume increases was due to the decrease in particle size of the substrate-sludge combination.

Finally, it should be again noted that some inconsistencies in the particle size data did exist. For instance, the variances in the .01 ionic NaCl solution for the MLSS concentrations of 2650 mg/l and 2750 mg/l were evident in Figure V-58. The only explanation that can be given was identical to that given for the albumin studies, i.e. the stirring required for the particle counting sheared the particles thereby producing these apparent differences.

Turbidity and Particle Counts

In Figures V-61 and V-62, the turbidities for the starch substrate-sludge mixture supernatants are presented. Turbidities for the sludge supernatant without substrate addition appeared to drop slightly as the ionic strength increased. As in the albumin studies, the turbidity in the supernatant usually decreased upon the addition of the smaller amounts of starch. The most apparent reductions occurred for low ionic strength systems. Particle count data in Figures V-63 to V-66 supported this observation.

Jack Bean Meal Adsorption Studies

Adsorption Isotherms

The jack bean meal studies posed some rather difficult problems due to a mistake made by this investigator in the experimental matrix.

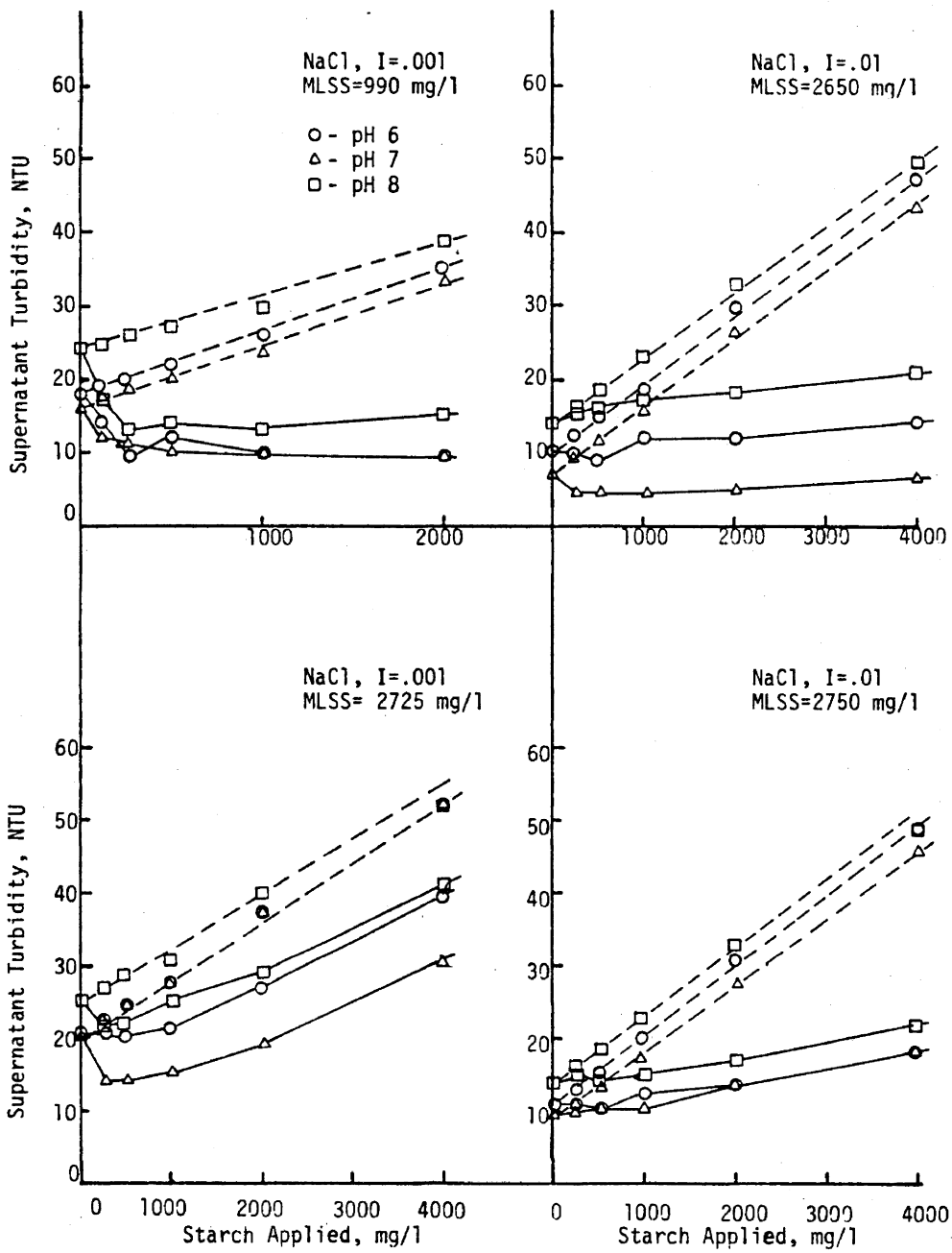


Figure V-61: Relationship Between Supernatant Turbidity and the Starch Applied Under Various Conditions

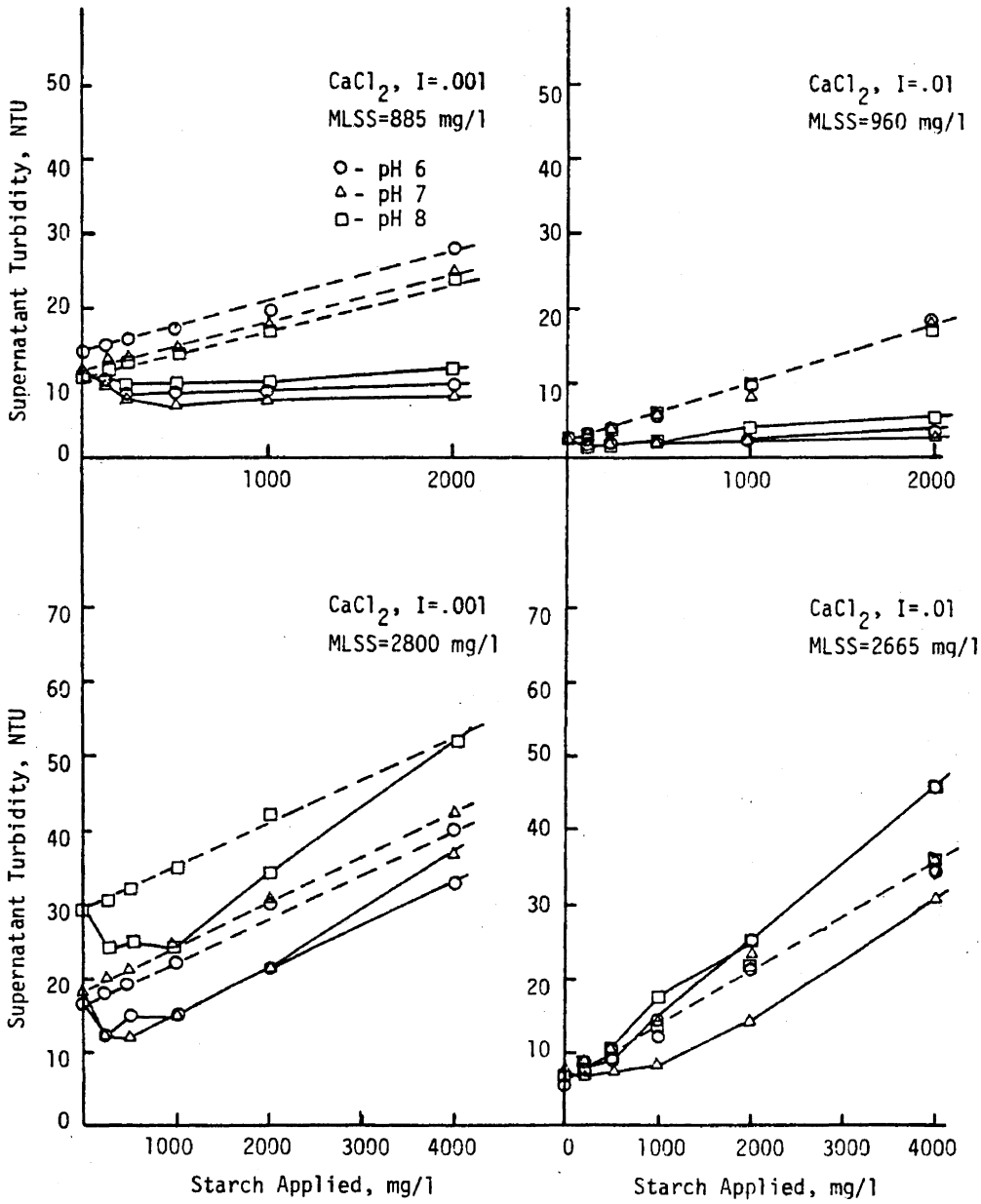


Figure V-62: Relationship Between Supernatant Turbidity and the Starch Applied Under Various Conditions

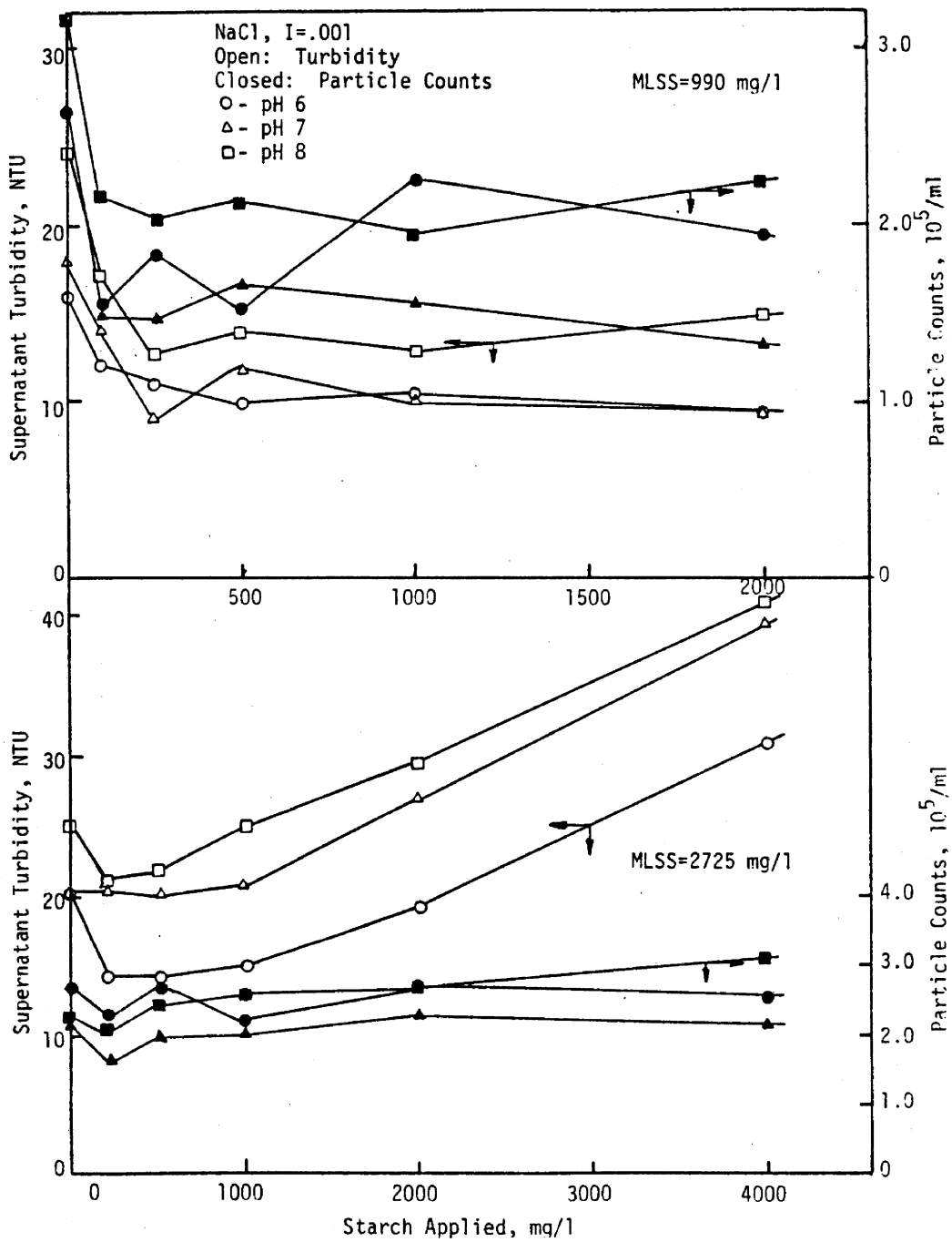


Figure V-63: Relationship Between Supernatant Turbidity and Particle Counts in the Supernatant and the Starch Applied Under Various Conditions

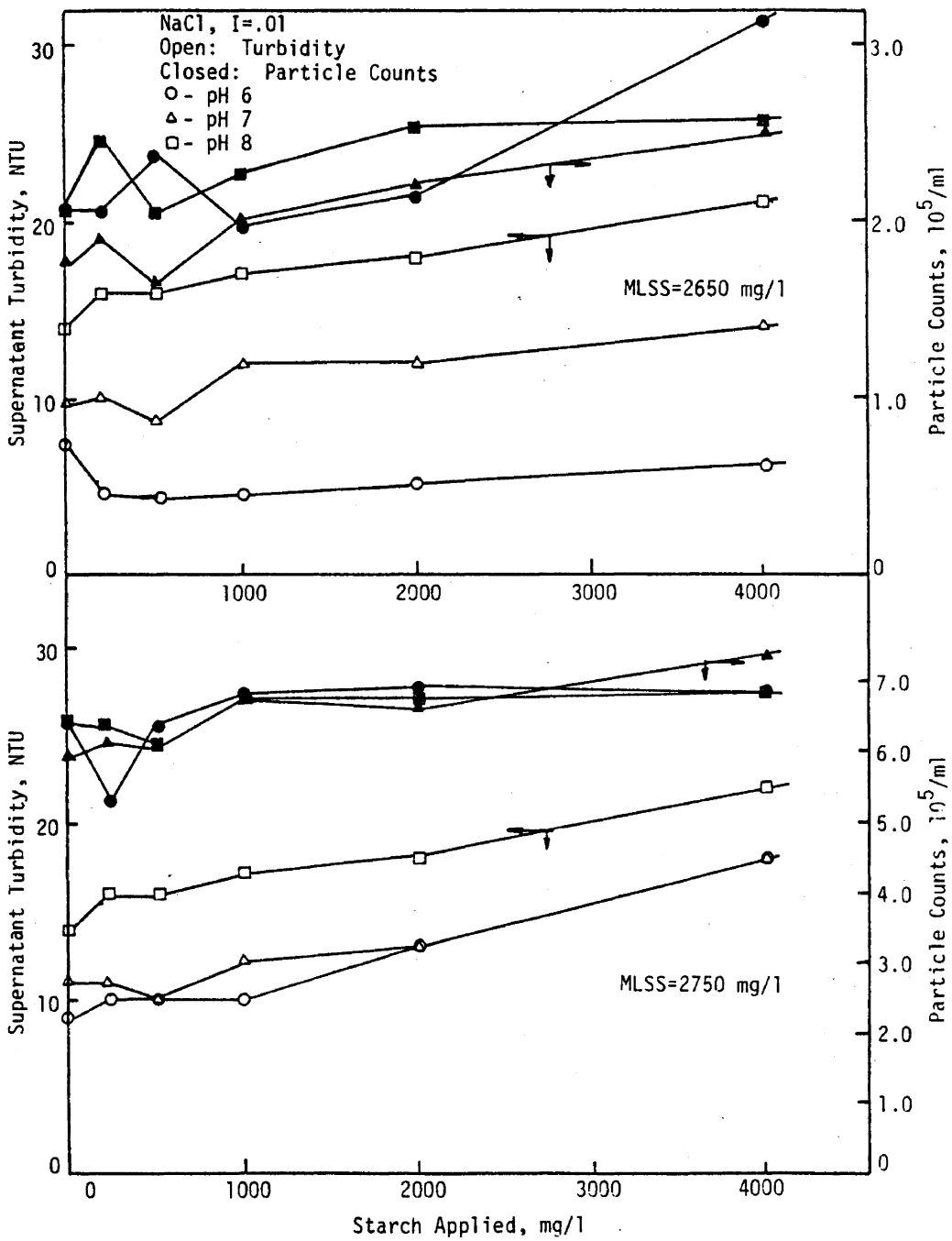


Figure V-64: Relationship Between Supernatant Turbidity and Particle Counts in the Supernatant and the Starch Applied Under Various Conditions

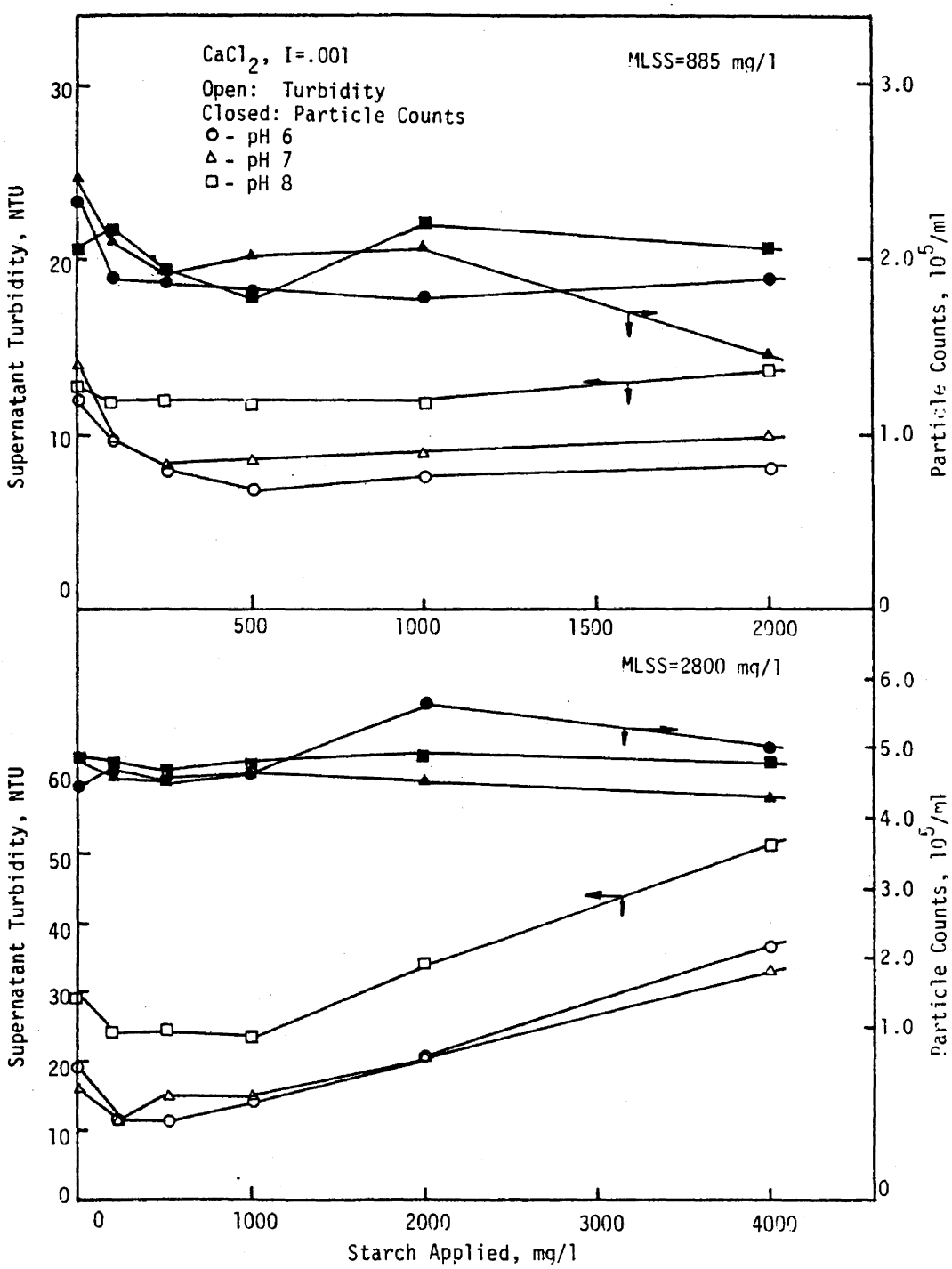


Figure V-65; Relationship Between Supernatant Turbidity and Particle Counts in the Supernatant and the Starch Applied Under Various Conditions

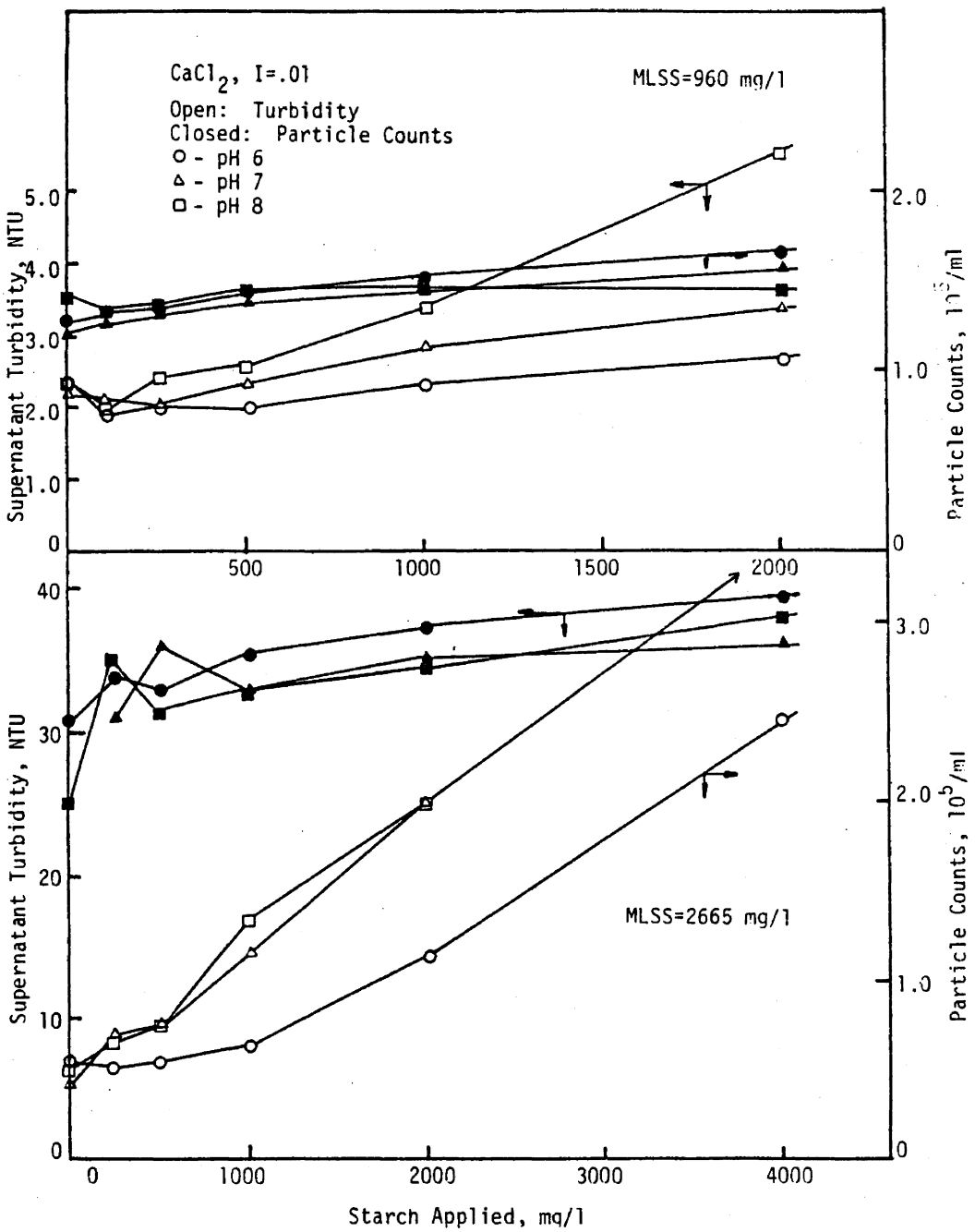


Figure V-66: Relationship Between Supernatant Turbidity and Particle Counts in the Supernatant and the Starch Applied Under Various Conditions

Upon viewing the data in Figures VI-67, no study was made in a .01 ionic strength sodium chloride solution at a high MLSS concentration. Instead, an extra run was made at 0.1 ionic strength $CaCl_2$ solution with a comparable MLSS value. This discrepancy was detected, however, after the acclimated jack bean meal culture was disposed of due to excess filamentous growth. This was unfortunate since this one additional study could have aided greatly in determining the major mechanism of colloid removal: enmeshment or physical adsorption.

Consulting Figure VI-67, pH affects adsorption at the lower ionic strengths more than at the higher levels. The lower the pH value, the more easily the jack bean meal was removed. In regard to ionic strength, the addition of a higher concentration of sodium chloride enhanced the adsorption. This may relate to the increase in the jack bean meal particle size with sodium chloride addition noted earlier in Table V-4. An increase in the presence of calcium chloride, though, decreased the adsorption capacity, according to the isotherms. This again may have been related to particle size since the average particle size of the jack bean meal decreased with increasing $CaCl_2$.

In an attempt to clarify the adsorption isotherm data, Figure V-68 was drawn. The amount of jack bean meal COD removed was plotted against the amount remaining in solution. The studies performed in $CaCl_2$ revealed that the amount of substrate removed did not vary with the concentration of the sludge (MLSS). This suggested that an

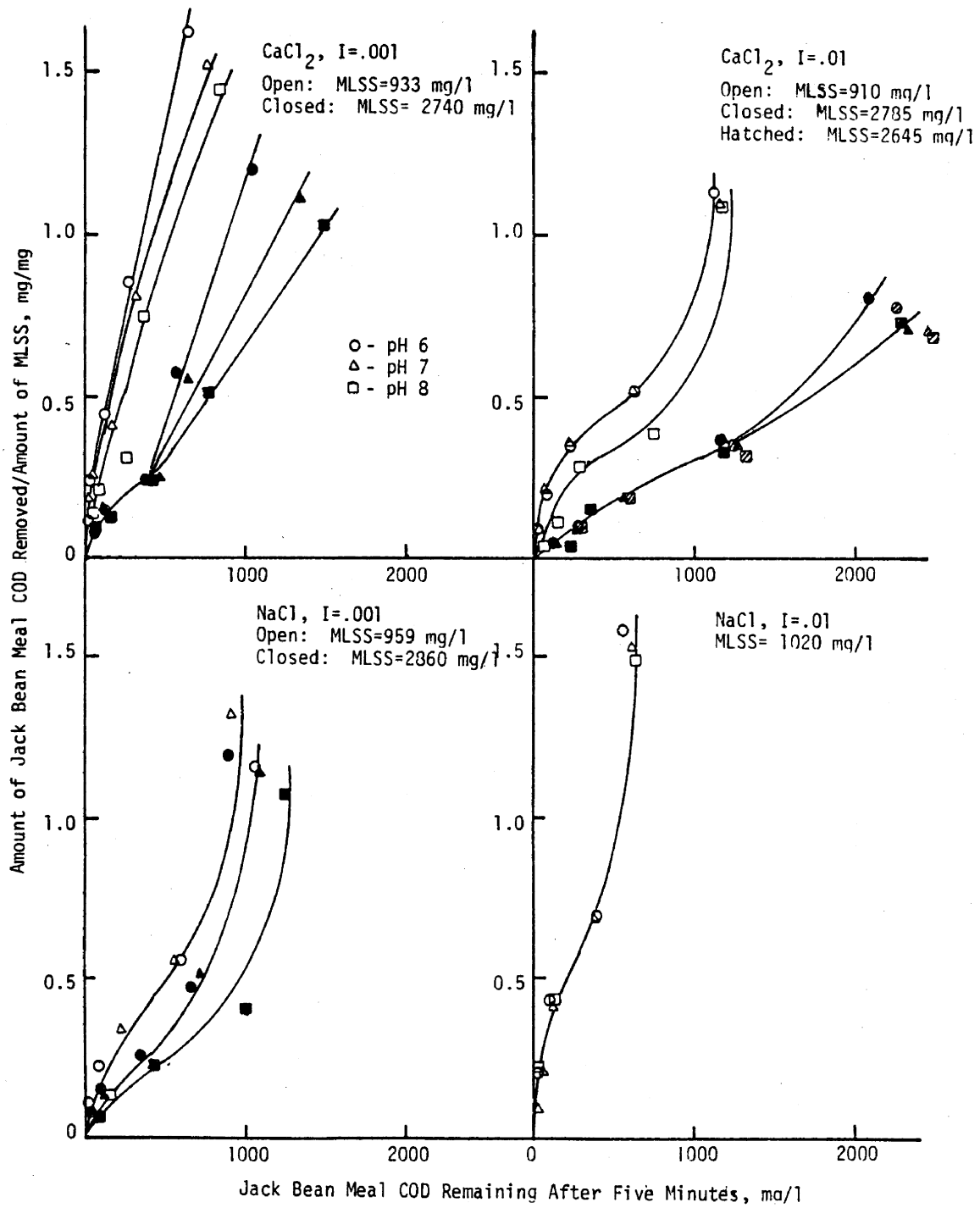


Figure V-67: Isotherms for the Combination of Jack Bean Meal and Jack Bean Meal Acclimated Activated Sludge Under Various Conditions and a Contact Time of Five Minutes

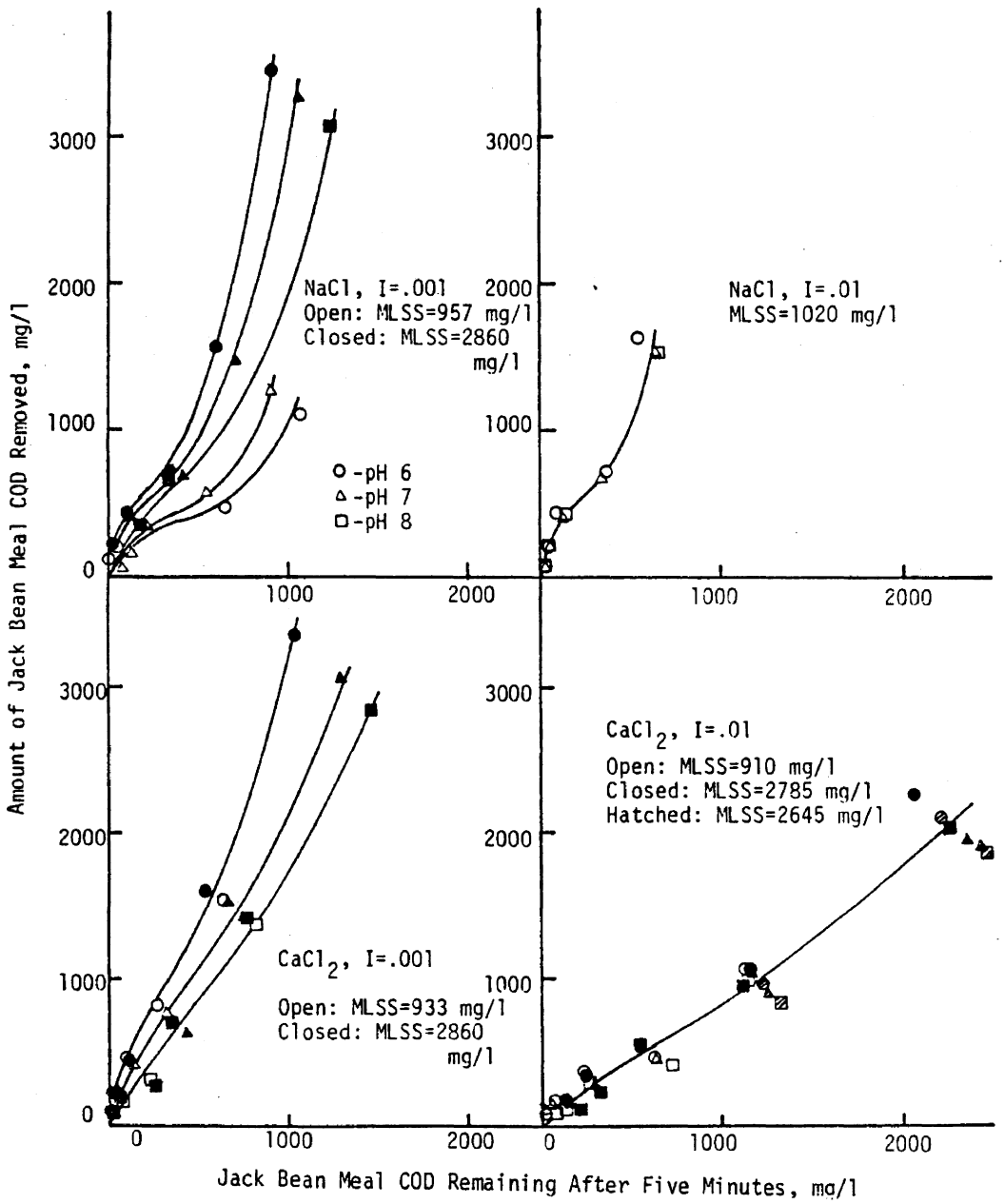


Figure V-68: Relationship Between the Jack Bean Meal Removed and the Amount Remaining in Solution After Five Minutes

enmeshment mechanism was dominant. However, no real conclusions could be put forth due to the "missing run" in .01 ionic strength NaCl. Adsorption isotherms in Figure V-68 suggested the possible presence of an adsorption mechanism due to the independence of the curves to the MLSS concentration.

Average particle size data of the substrate-sludge mixture are shown in Figures V-69 to V-72. Problems similar to those encountered previously arose in the interpretation of the results. General patterns existed, e.g., an initial increase in particle size with substrate addition followed by a decrease. However, some data could not be explained with any degree of certainty. Any conclusions resulting from this data will be made only with some type of corroboration evidence.

Turbidity and Particle Counts

The series of turbidity plots in Figures VI-73 and Figure VI-74 showed that significant amounts of jack bean meal were removed from solution upon addition to the sludge. High ionic strengths aided the turbidity removal. This is better exhibited in Figures VI-75 to VI-78 wherein turbidities were shown to drop from 15 to 40 NTU's to a range of 5 to 15 NTU's for the .01 ionic strength solutions. Particle counts supported this observation. As with the other substrates, the turbidities and particle counts

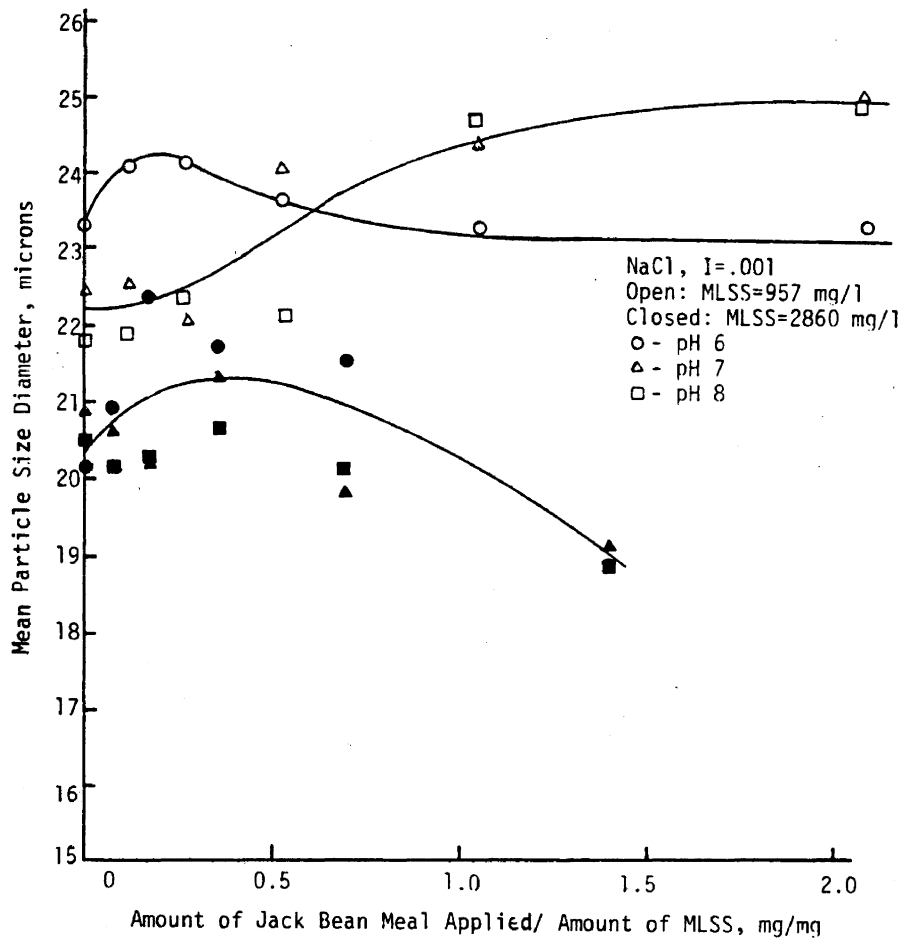


Figure V-69: Relationship Between Mean Particle Size of Jack Bean Meal Acclimated Activated Sludge and the Amount of Jack Bean Meal Applied per Unit of Sludge in a NaCl Solution (I=.001)

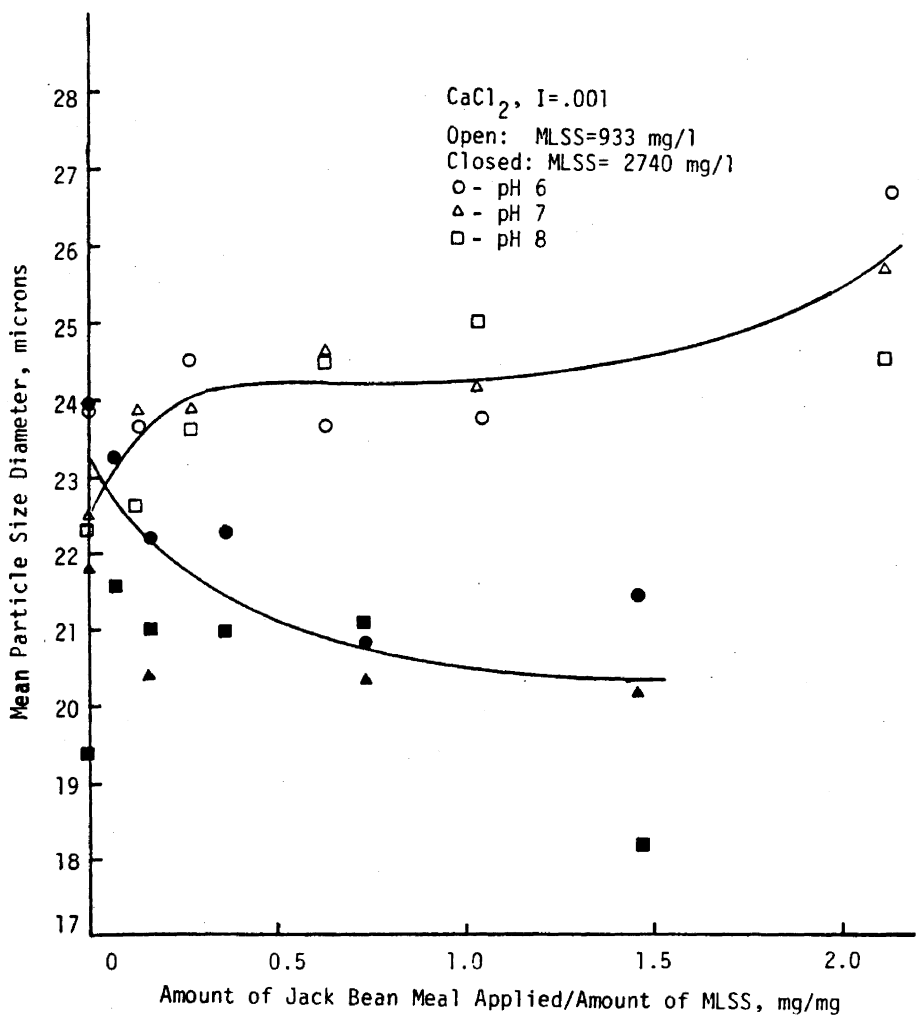


Figure 70: Relationship Between the Mean Particle Size of Jack Bean Meal Acclimated Activated Sludge and the Amount of Jack Bean Meal Applied per Unit of Sludge in a $CaCl_2$ Solution (I=.001)

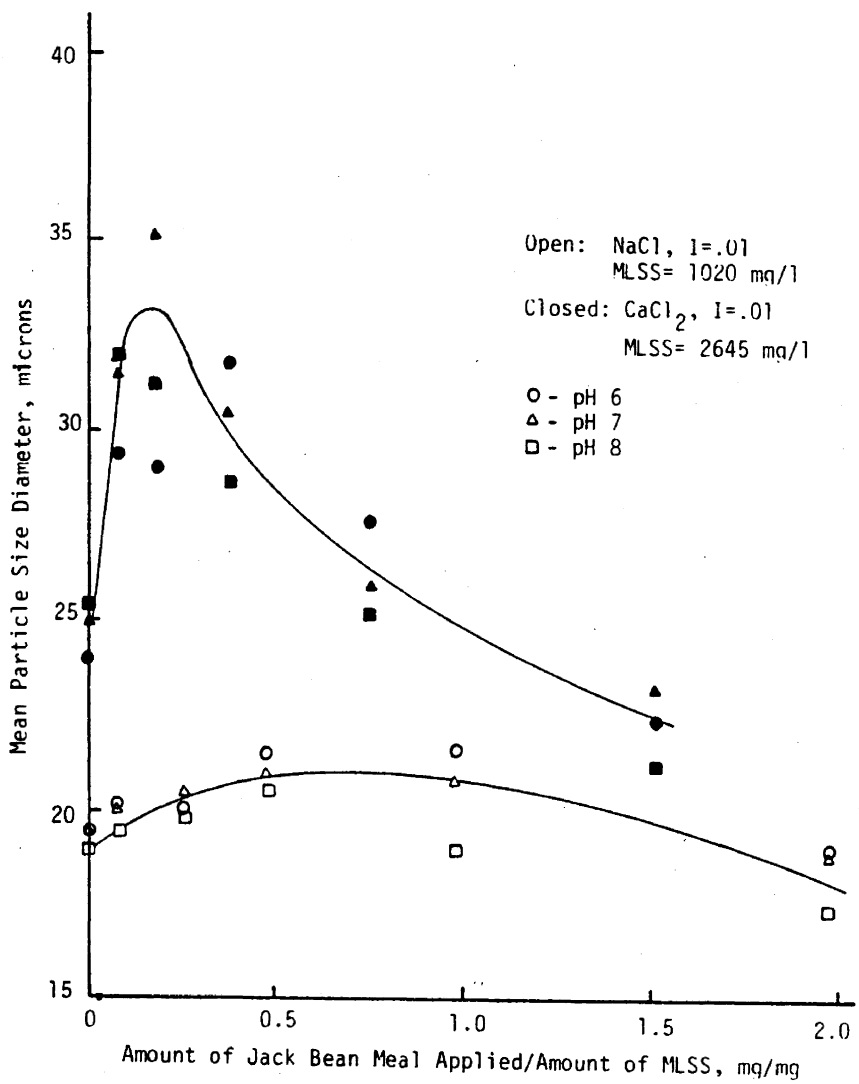


Figure 71: Relationship Between Mean Particle Size of Jack Bean Meal Acclimated Activated Sludge and the Amount of Jack Bean Meal Applied per Unit of Sludge Under Various Conditions

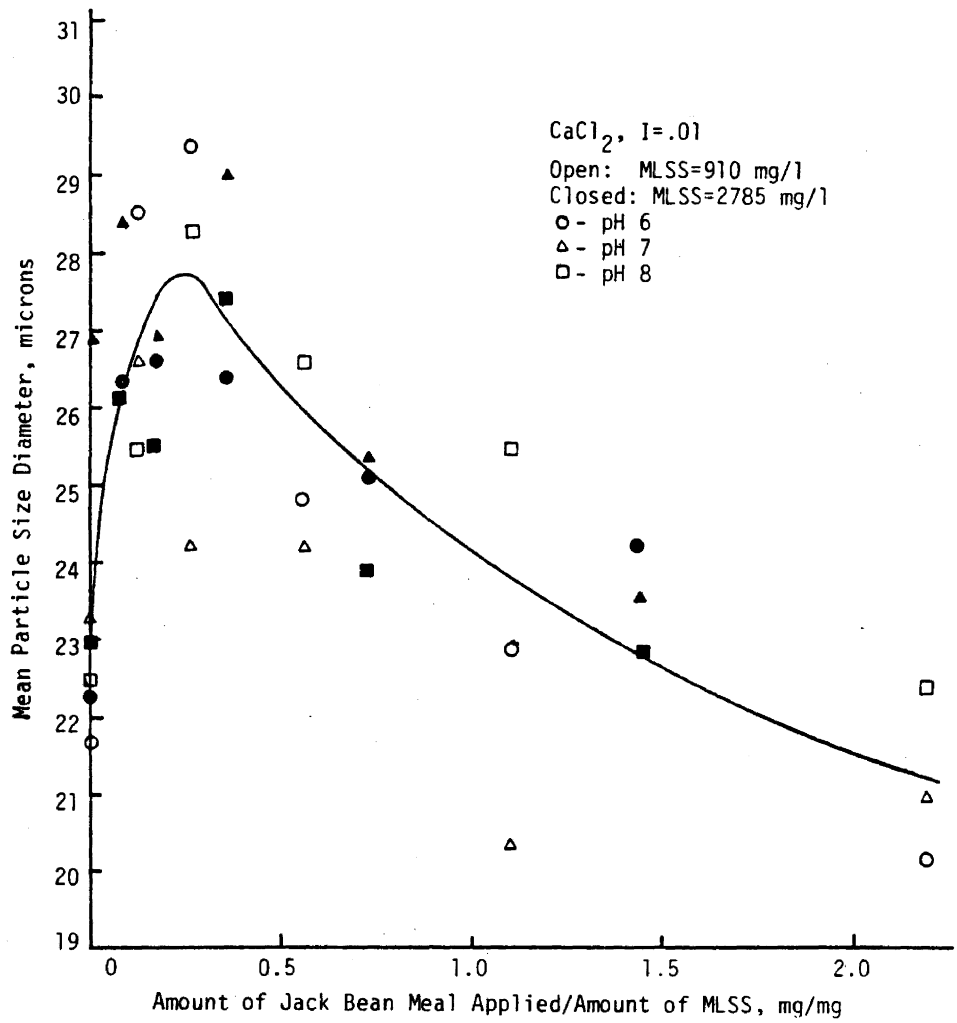


Figure V-72: Relationship Between Mean Particle Size of Jack Bean Meal Acclimated Activated Sludge and the Amount of Jack Bean Meal Applied per Unit of Sludge in a $CaCl_2$ Solution (I=.01)

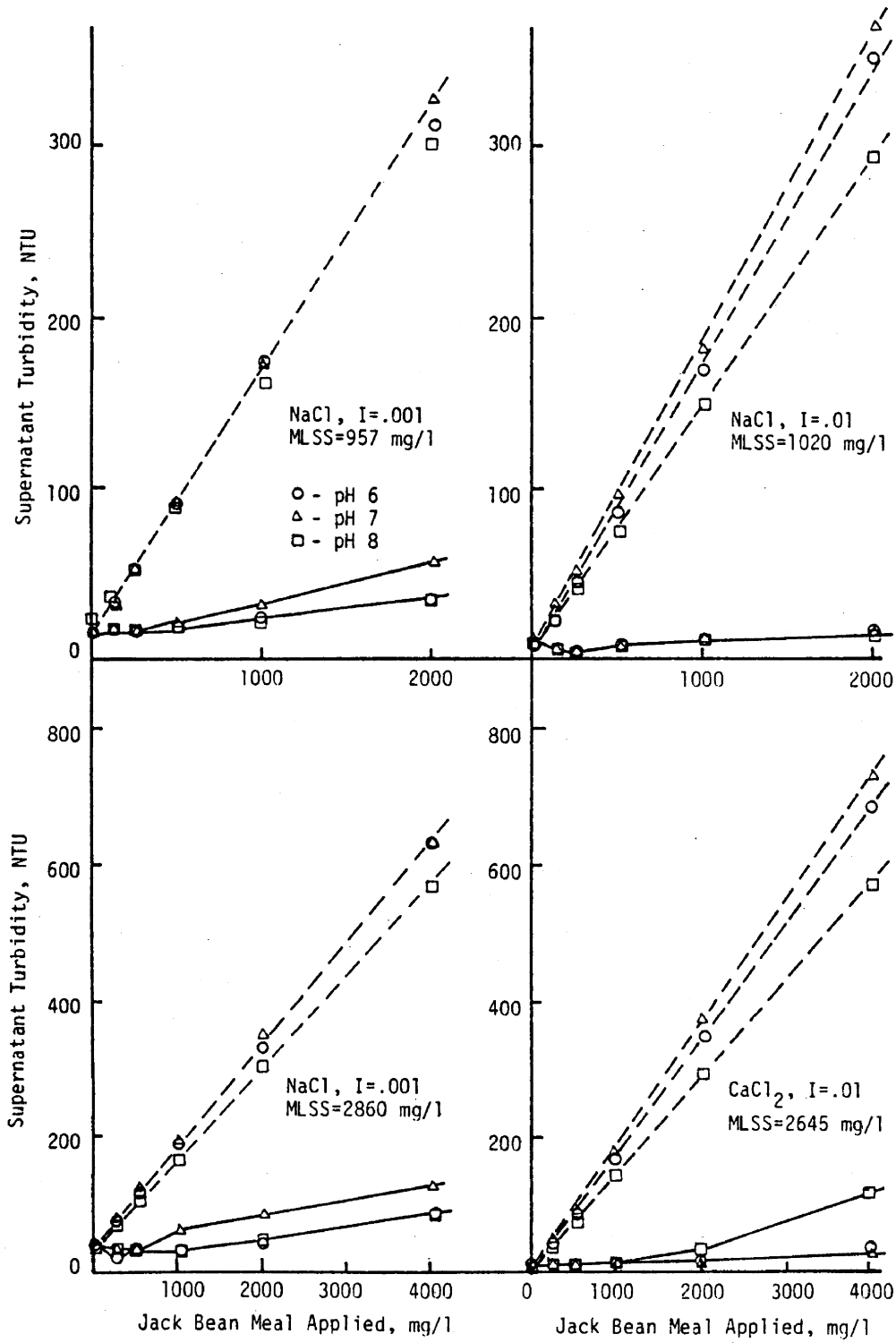


Figure V-73: Relationship Between Supernatant Turbidity and Jack Bean Meal Applied Under Various Conditions

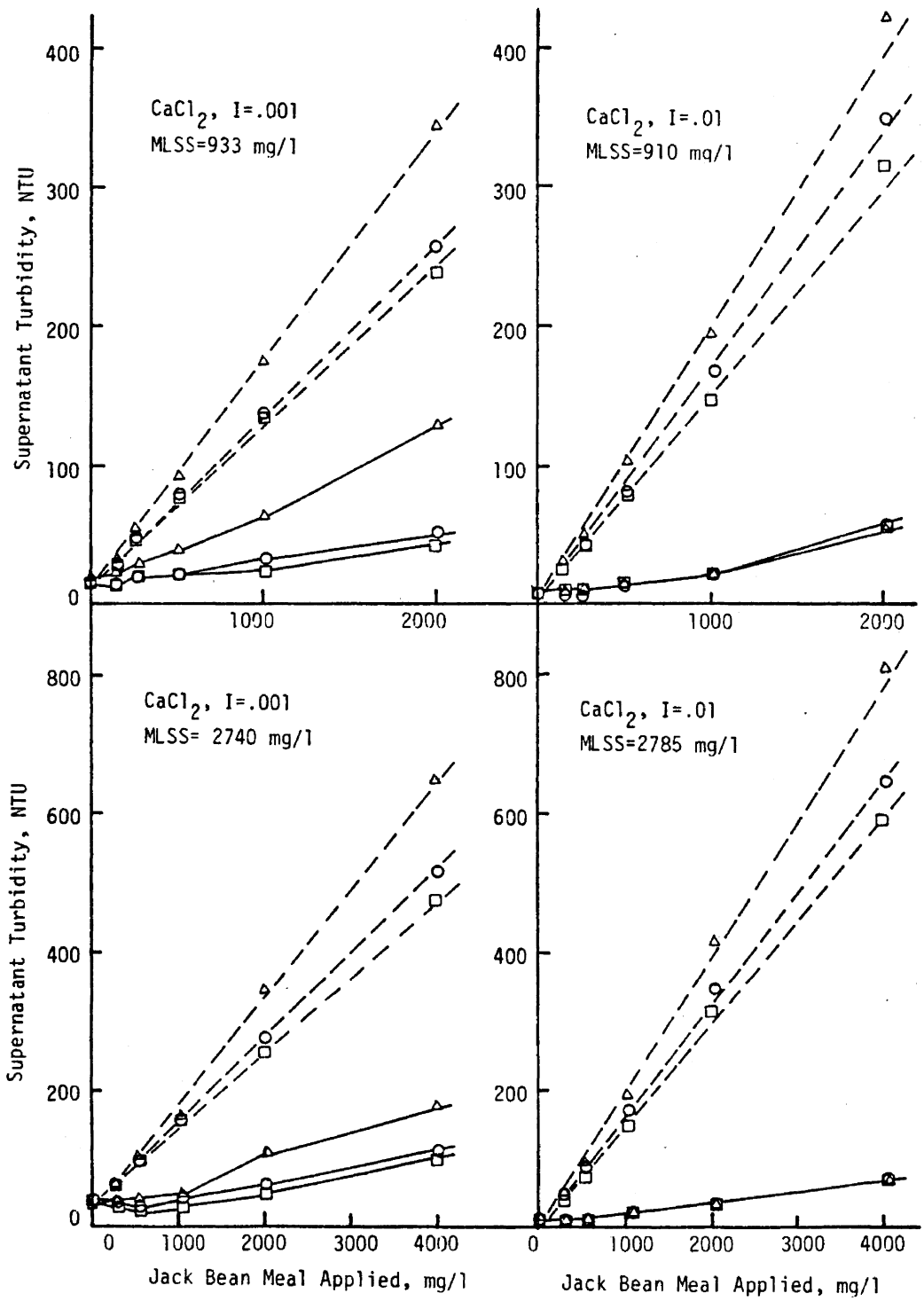


Figure V-74; Relationship Between Supernatant Turbidity and Jack Bean Meal Applied Under Various Conditions

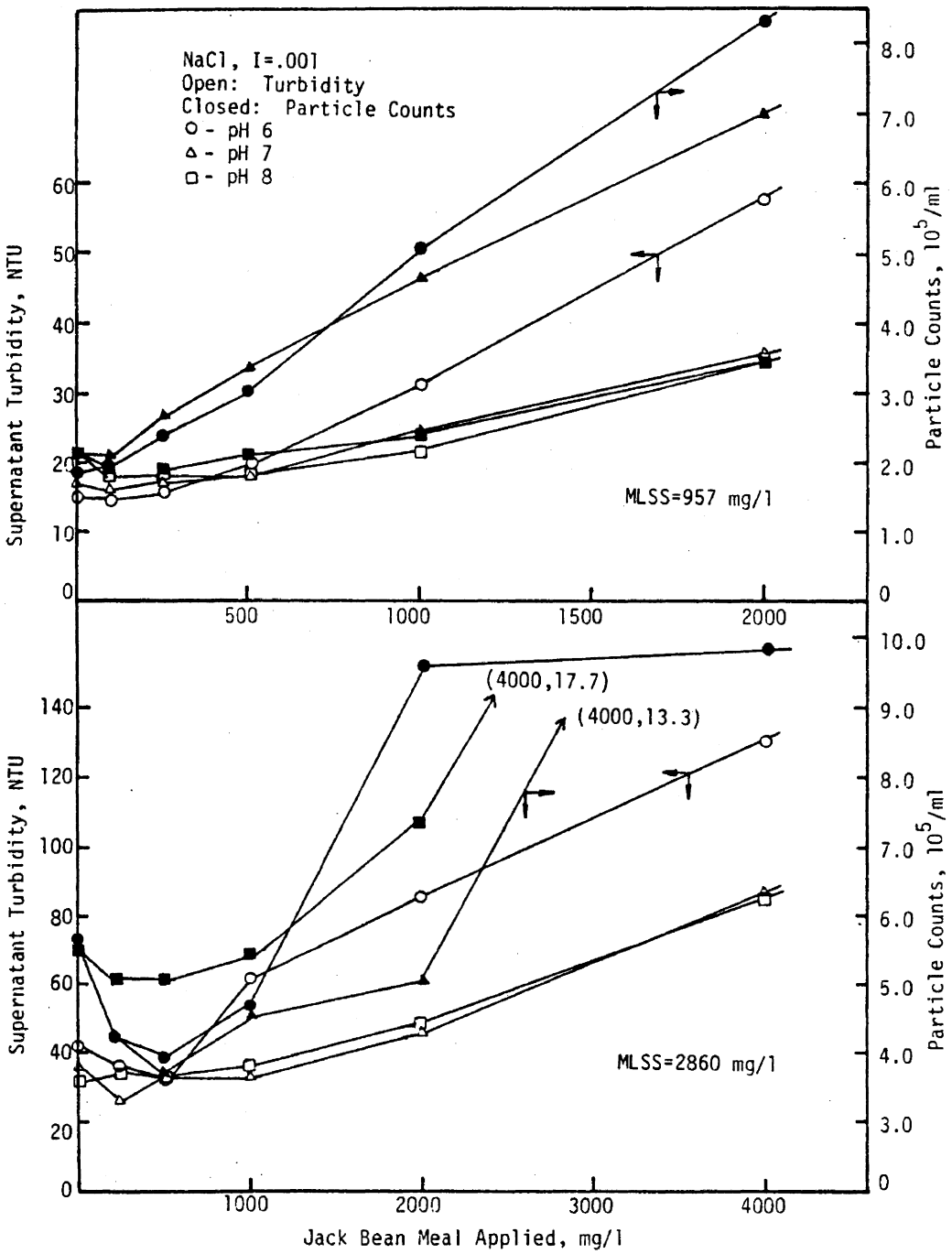


Figure V-75: Relationship Between Supernatant Turbidity and Particle Counts in Supernatant and the Jack Bean Meal Applied Under Various Conditions

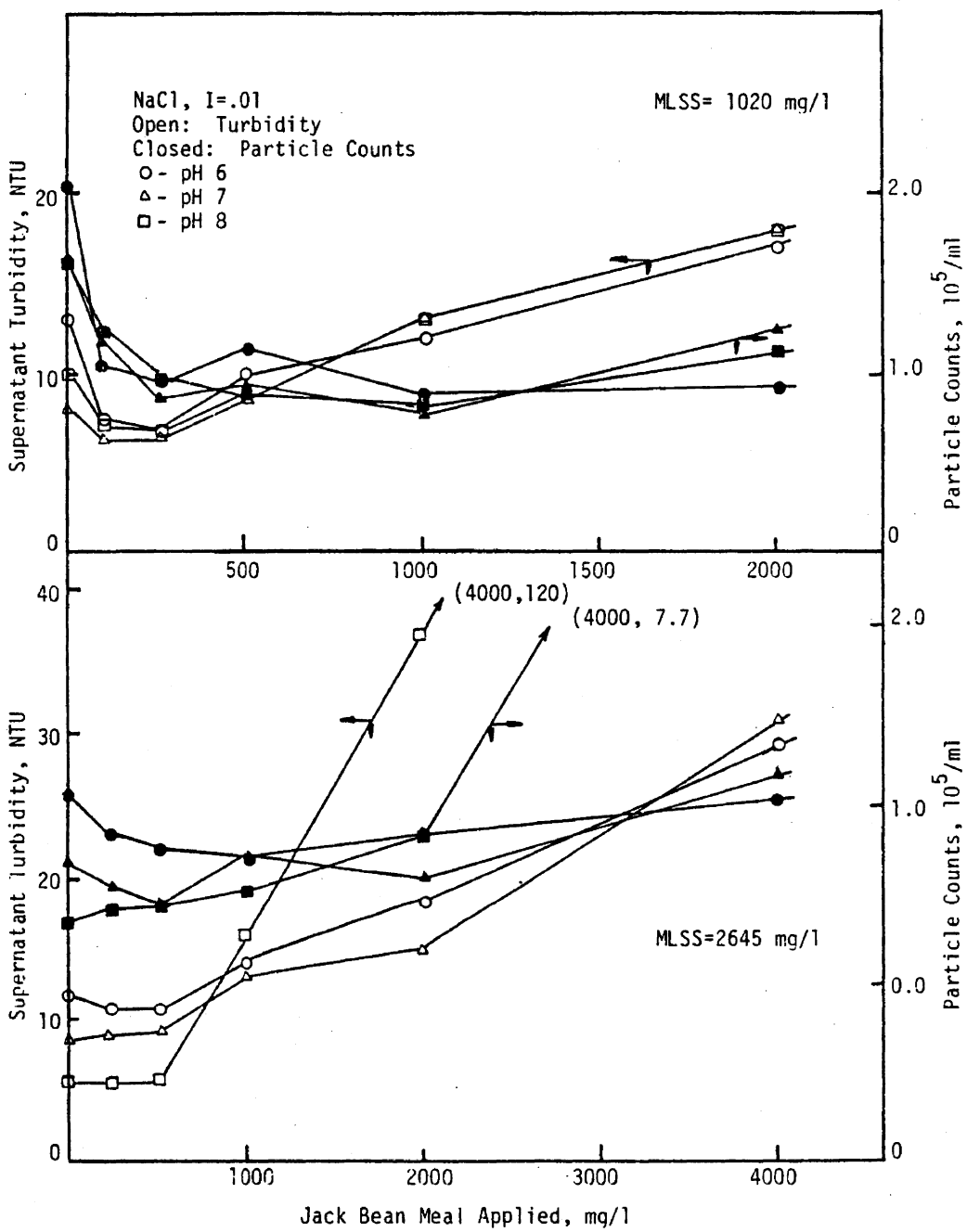


Figure V-76: Relationship Between Supernatant Turbidity and Particle Counts in Supernatant and the Jack Bean Meal Applied Under Various Conditions

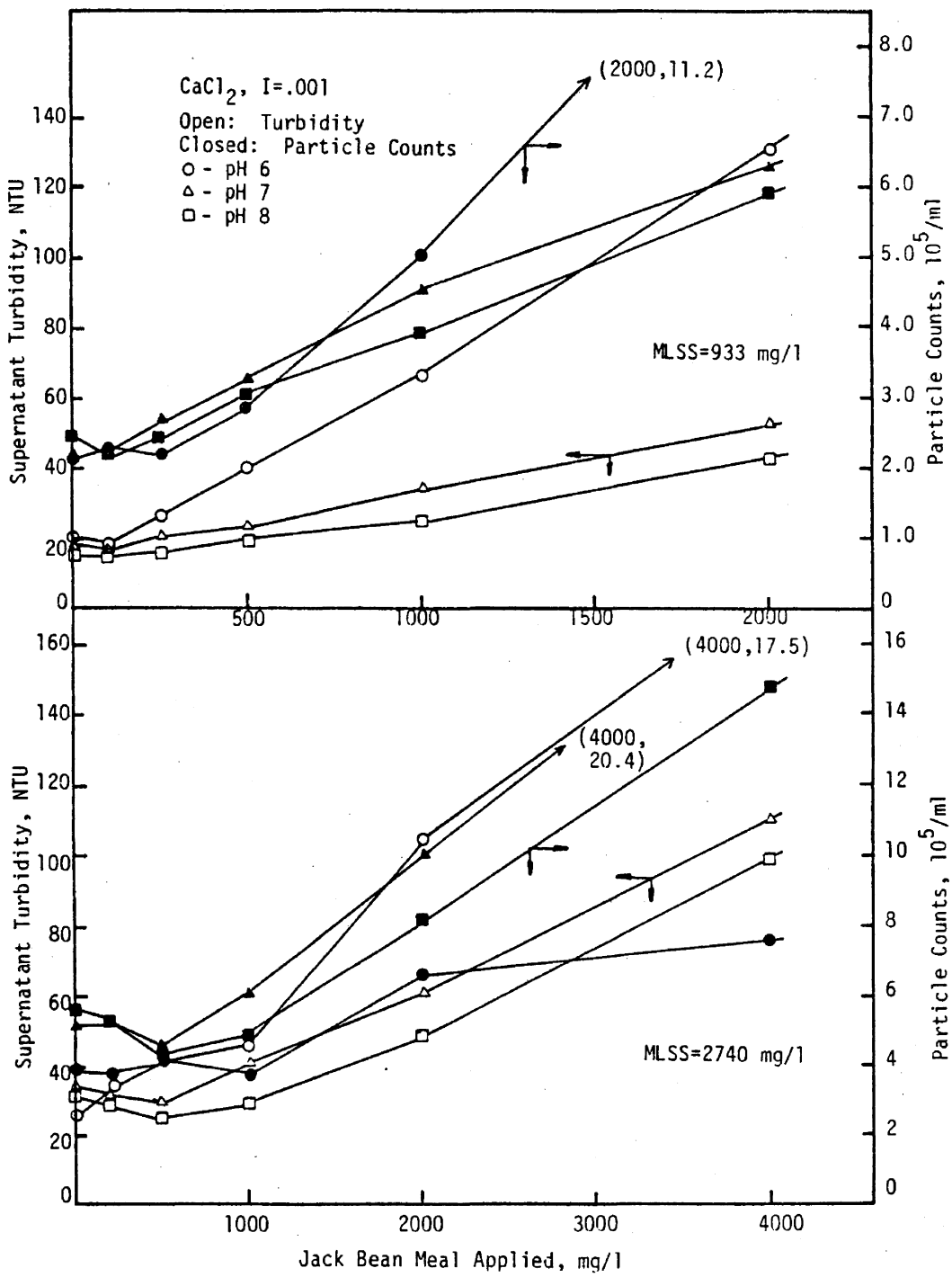


Figure V-77: Relationship Between Supernatant Turbidity and Particle Counts in the Supernatant and the Jack Bean Meal Applied Under Various Conditions

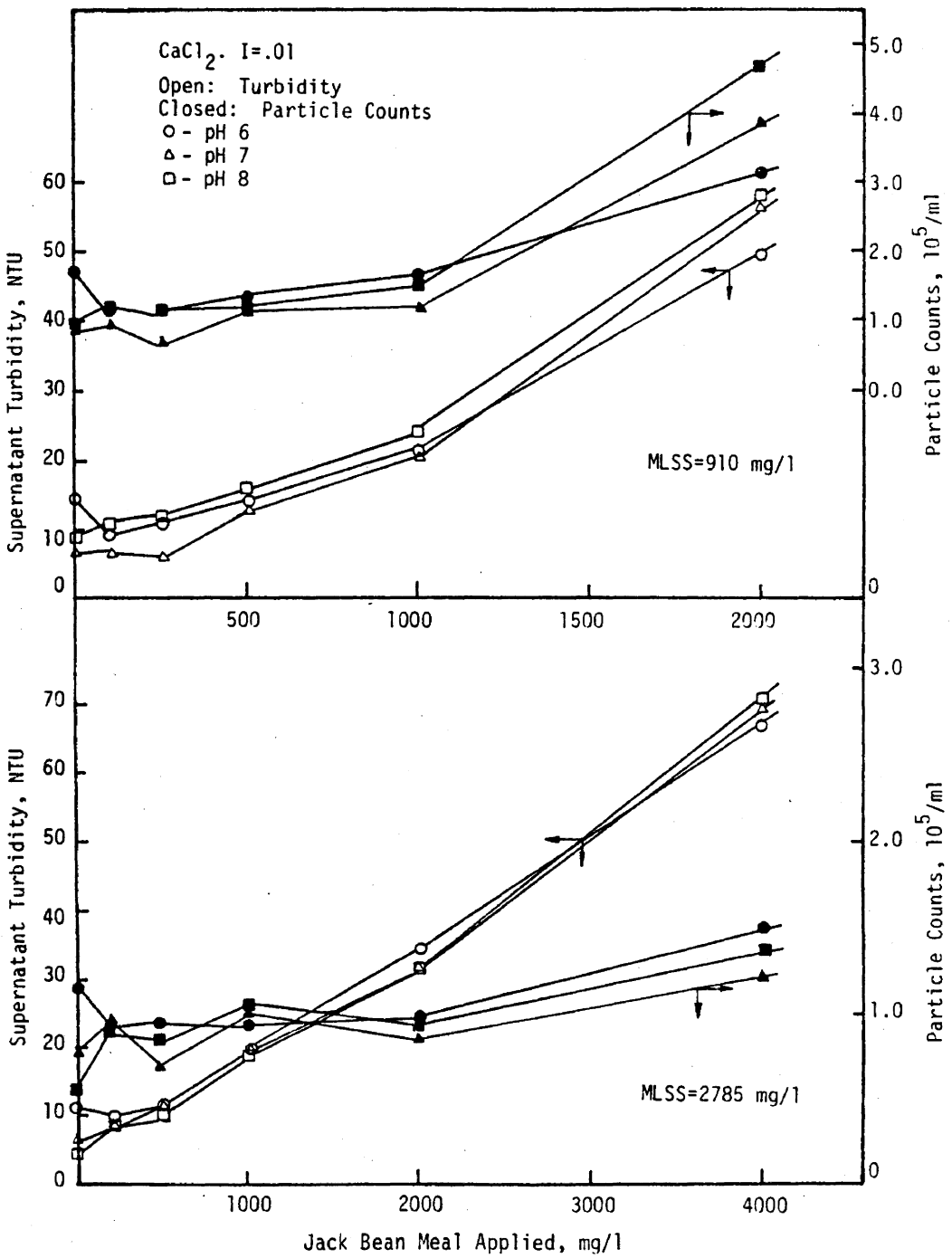


Figure V-78: Relationship Between Supernatant Turbidity and Particle Counts in the Supernatant and the Jack Bean Meal Applied Under Various Conditions

for certain conditions dropped for low additions of substrate from the control sample (sludge with no substrate added). This response was probably due to better settling characteristics from the additional weight of the jack bean meal colloids in the activated sludge flocs. No consistent pattern appeared with regard to which specific conditions would foster the turbidity drops.

CHAPTER VI

DISCUSSION OF RESULTS

Introduction

The results obtained during this investigation were analyzed from the standpoint of importance to the theory and operation of the contact stabilization process. Attempts were made to supplement explanation of previously reported phenomena and to provide some rules toward optimum design and operation of this variation of the activated sludge process.

Theory of Contact Stabilization

Process Schematic

The concept of the contact stabilization process has been presented previously. Historically, its use has been recommended for wastewater whose organic matter is predominately colloidal in nature. The dual tank arrangement of contact stabilization has been reported to remove these organics by first utilizing the soluble organics in the contact tank while adsorbing or enmeshing the colloids in the sludge flocs. The sludge is then settled, drawn from the bottom of the setting tanks and pumped to the stabilization basin wherein the colloids are removed. The results from this study supported the use of two tanks for the treatment of certain colloidal wastes. For each substrate tested, there existed a sequential utilization function i.e. the activated sludge cultures utilized the substrates in two or more distinct steps. This action was revealed by the oxygen

uptake and/or COD data in the kinetic studies. However, this statement should not be taken to mean that all colloidal wastewaters exhibiting a multi-step utilization should be treated by contact stabilization. The remainder of this chapter will deal with this particular point (when or when not to use contact stabilization) by analyzing the mechanisms involved.

Metabolic Factors

Colloid Substrate Utilization

All bacteria require food in a soluble form. If the substrate molecule is too large to pass through the cell membrane, the bacteria will produce extracellular enzymes to hydrolyze the molecule into smaller units. The manner of this hydrolysis appeared to play a role in the results obtained in this study.

Extracellular enzymes excreted by bacteria may attack large, polymer molecules either from the terminal ends (exoenzyme) or between the terminal ends (endoenzyme). The exoenzymes simply cleave one monomer at a time from the parent polymer molecule. These monomer units quite often are easily transported through the cell membrane without further alteration. However, the endoenzymes operate on the interior bonds in the polymer molecule breaking it into smaller and smaller polymer units until the bacteria are able to transport the fragments into the cell.

The scenario for the endoenzymes explains the uptake and release phenomena for the starch substrate quite well. It has been reported

that the amylase enzyme responsible for the hydrolysis of the starch molecule acts in a random manner along the chain. Apparently, the activated sludge bacteria adsorbed or enmeshed the starch colloids and then initiated breakdown of the molecule by use of the amylase enzymes. The random cleavage of the bonds along the chain resulted in a release of smaller polymer units into the solution, which then were measured by an increase in the COD during the study.

If exoenzymes are functioning on a colloidal particle, the resulting monomer units would likely be transported immediately into the cell. Therefore, no uptake and release phenomena would be exhibited unless the rate of hydrolysis surpassed the rate of transport.

McKinney [25] initially postulated that contact stabilization should be used for colloid wastes and that all colloidal wastes will exhibit the "uptake and release" phenomenon suggested for use as the contact tank detention time was the time required to remove any soluble substrate prior to the release of hydrolyzed substrate into the solution. From a theoretical viewpoint, this design procedure provided a very nice textbook problem; however from a practical viewpoint, it proved to be an operational nightmare. For this design approach to be successful, the plant would have to receive a wastewater consistent in both organic strength and hydraulic flow. Also the bacteria would have to cease functioning during settling in the secondary clarifier so that no release of substrate would

occur. The chance that all of these conditions would occur in a field operation is virtually nonexistant. Therefore, what type of response does signify advantageous use of contact stabilization?

One objective of the study was to investigate whether the typical kinetic response of contact stabilization is a function of the substrate chemical make-up. Since most wastewaters are composed of primarily protein and/or carbohydrate matter, the choices of albumin (protein), starch (carbohydrate), and jack bean meal (carbohydrate and protein) provided representative colloidal substrates. In the kinetic studies, the jack bean meal exhibited the best removal in the initial stages, probably because of its large insoluble fraction. Jack bean meal also showed a slight adsorption and release phenomenon following soluble substrate removal. Starch substrate produced the largest adsorption and release response However, in all cases, the hydrolysis of starch began immediately. Finally, the albumin acclimated culture exhibited a two step utilization of the albumin substrate as described by the oxygen uptake data in Figures V-1, V-2, and V-3. No adsorption and release of the albumin was observed and initial substrate removals were probably not high enough to justify use of contact stabilization. Finally, no real conclusion could be made with regard to the relationship between substrate composition and the classical contact stabilization uptake and release phenomenon.

However, the activated sludge cultures that were acclimated to the carbohydrate containing substrates did possess better adsorption and settling characteristics. This may have resulted from the attraction of the carbohydrate colloids to the carbohydrate exocellular bacterial matrix.

Effect of Glucose Upon Colloid Utilization

Glucose has been reported to be a strong catabolic repressor [9,10], and the observations of these studies reinforced this observation. The glucose supplementation affected not only the manner and rates of removal but also the adsorptive capacities of the sludges.

For both albumin and jack bean meal substrates, glucose supplementation produced a sequential uptake in the substrates--glucose first, followed by the colloidal organics. The glucose appeared to repress the production of one or more enzymes required for utilization of the organic material. The starch-glucose mixtures did not show any consistent signs of sequential uptake.

The effects of enzyme repression carried over to the removal rate and adsorption capacity results. Prior to the experimental work, it was assumed that the addition of the easily assimilatable glucose would increase the rate of total substrate removal. This supposition proved correct except in the case of albumin. For twenty percent glucose addition, the substrate removal rates significantly decreased from those measured for pure albumin. This result may be related to

the observed reduction in adsorption capacity of the sludge after glucose addition. Apparently, the glucose presence altered the nature of the bacterial floc surface. One hypothesis is that the glucose repressed production of cell-bound extracellular enzymes, which would result in a reduction in the number of receptor sites on the surfaces for the albumin colloids. Another hypothesis is that the glucose presence simply altered the bacterial population to one with less capacity for albumin adsorption. Since all cultures were acclimated before data collection, the latter mechanism is plausible. Albumin was not the only substrate affected by the glucose; the starch adsorption capacity also was decreased. Jack bean meal adsorption results, however, were inconclusive.

From generally accepted contact stabilization process theory, the substrate removal was hypothesized to be sequential, soluble then colloidal, resulting in potentially advantageous use of the two tank arrangement. Also, the adsorption or enmeshment of the colloidal matter by the sludge was assumed to be a key factor in the success of the contact stabilization process. The results from the studies with glucose qualified these assumptions. First, the mere presence of both soluble and colloidal organics in wastewater does not ensure a sequential uptake phenomenon. Wastewaters containing both soluble and colloidal substrates will have a unique response depending upon the nature of the two substrates, i.e. does the presence of soluble substrate repress the colloidal substrate utilization? If a

wastewater does not exhibit a sequential uptake, yet does show an instantaneous and significant "release" of substrate, a plug flow contact stabilization system should not be used since release of substrate would occur in the contact tank.

Secondly, the presence of certain soluble organic components may reduce the adsorption or enmeshment capacity of the sludge. Emphasis should be placed on the word "certain", since all soluble molecules do not display catabolic repression functions on bacteria. This study employed glucose which is a strong catabolic repressor. More study should be done on the effect of various soluble substrates upon the adsorption capacity of an activated sludge.

Yield Coefficients

The yield coefficients obtained in these studies ranged from 0.765 to 1.136 grams of MLSS per gram of COD utilized. These values were quite high when compared to typical yield coefficients reported in the literature for activated sludge (0.32-0.69 grams of cells/gram of COD). Possible reasons for this phenomenon may be the measurement of an inordinate amount of inorganic and nonbiodegradable organic matter in the solids test or the presence of a significant amount of unmetabolized colloidal substrate remaining in the floc matrix.

With respect to the first hypothesis, the jack bean meal substrate contained a large amount of insoluble organic material which more than likely contributed to the large yield coefficients

observed for the jack bean meal activated sludge. The starch and albumin cultures, however, did not possess large quantities of insoluble material, yet large yield coefficients were still measured for the two acclimated cultures. One explanation that seems reasonable for this phenomenon relies upon the storage of the substrate on the cell surface, or in the floc matrix. The question of whether this storage would be voluntary or involuntary was not answered by the data. However, if this extracellular storage or entrapment does occur, it may have serious implications for the adsorptive capacity of the activated sludge in a contact stabilization system. For example, when a sludge containing extracellular substrate is pumped into the contact tank, less adsorption of the colloidal material could occur because of the presence of the entrapped substrate colloids. This situation is analagous to the work of Walters, et. al. [30] wherein they concluded that the presence of intracellular stored metabolites in activated sludge decreased the substrate removal rate of the bacteria. Nonetheless, a properly chosen stabilization detention time can avert the problem of a reduced initial substrate removal potential whether the substrate is soluble or colloidal.

Physical Factors

Significance of Adsorption or Enmeshment of Colloids

The physical separation of colloidal substrate from wastewaters is essential to the success of the contact stabilization process. Adsorption and/or enmeshment are commonly cited as the mechanisms

responsible for the removal of the colloidal material in the contact tank. Results from these studies corroborated the existence of these mechanisms and aided evaluation of the effect of certain physical conditions on these mechanisms. Further, the facility of colloidal adsorption and enmeshment was determined to be a function of the separate substrate and sludge characteristics. The following discussion to follow will deal with the dependence of colloid removal on substrate characteristics and sludge characteristics separately.

Colloid Removal as Related to Substrate Characteristics

The adsorption or enmeshment of colloidal substrates in activated sludge flocs was found to be, in part, a direct function of certain substrate characteristics. For example, the type of substrate appears to be very important with regard to physical removal. The substrates chosen for these studies consisted of primarily protein (albumin), carbohydrate (starch), or was a protein-carbohydrate mixture (jack bean meal). The two substrates containing carbohydrate material exhibited a tendency to be easily removed from solution. Chian and DeWalle [64] reported that carbohydrate substrates were the first materials removed from a diluted leachate substrate when fed to an activated sludge system. They also stated that the carbohydrates were adsorbed to the cell wall resulting in flocculation of these cells. This affinity of activated sludge bacteria for carbohydrates may be related to the carbohydrate composition of their exocellular polymers. Since these polymers are

involved in cell flocculation, it is quite conceivable that free floating carbohydrate polymers would be attracted to the bacterial cell.

pH, ionic strength, and cation valence were some of the variables tested in these studies. The pH effect upon the substrate removal appeared to be small. Only the albumin was significantly affected by pH variations. Data obtained described a precipitation of the albumin from solution as the pH was progressively lowered toward its isoelectric point (pH = 4.6). Due to this precipitation, the albumin acclimated sludge exhibited a greater adsorption capacity with a drop in pH. The jack bean meal also displayed the same type of behavior at low ionic strength conditions. No precipitation of particles was observed for jack bean meal, but its electrophoretic mobility significantly decreased as the pH was lowered. In relation to the contact stabilization process, pH would probably play an effective role if the isoelectric point of the substrate lay in the operable range for activated sludge.

The ionic strength and ionic character influenced the removal of the colloidal substrates. Increases in ionic strength appeared to decrease the colloids inherent, repulsive negative charges described by the electrophoretic mobility data. From the isotherm data this reduction in charge generally encouraged better physical removals of these colloids. The ionic character of the solution also induced better colloid removal. However, there existed no consistent pattern with respect to the type of cation present (Na^{+} or Ca^{+2}) and the

degree of removal. For instance, the albumin colloids were better removed from the calcium solutions whereas the jack bean meal had enhanced removal in a sodium ion environment. The enhancement of albumin colloid removal in a calcium environment can be related to the particle count data in Figure V-38. The number of particles counted increased dramatically in the calcium solutions, apparently because of precipitation and aggregation of the albumin polymer from solutions. This particle number increase apparently relates to enhanced albumin removal. The jack bean meal removal appeared directly related to the mean size of the substrate colloids. Table V-4 described a larger mean particle size in .01 ionic strength NaCl solution than in the .001 ionic strength NaCl solution. Adsorption isotherms in Figure V-67 exhibited a concomitant increase in adsorption capacity of the jack bean meal sludge in the NaCl solutions. The same pattern existed from the $CaCl_2$ solutions with the exception that the mean particle size decreased with an increase in ionic strength. This was accompanied by a decrease in the jack bean meal adsorption capacity of the sludge. Finally, the degree of starch substrate removal appeared to be more a function of the sludge characteristics than the substrate characteristics. Further discussion follows in the next section.

Removal as Related to Sludge Characteristics

Two very interesting effects were noted in the adsorption studies related to sludge characteristics. The first concerns the

degree of substrate colloid removal and the solids concentration of the sludges, and the second involves how tightly the sludge holds the adsorbed or enmeshed substrate colloids. Prior to the studies, it was believed that a higher sludge solids concentration would augment substrate colloid removal. However, the adsorption isotherms described significant decreases in the amount of substrate removed per unit of sludge as the solids concentrations increased. Two causative factors were postulated to be:

1. For enmeshable substrates, the low MLSS concentrations (approximately 1000 mg/l) easily removed a major portion of the colloids. Any further increase in the MLSS concentration only slightly supplemented the colloidal substrate removal. This led to the disparity in the adsorption isotherms such that the adsorption capacity appeared to be reduced with an increase in MLSS. Jack bean meal and albumin substrates exhibited this mechanism.
2. If the adsorption mechanism was dominant, the substrate colloids were removed as a function of the surface area of the floc. For the low sludge concentrations, smaller sludge flocs were generated thereby increasing the surface area available for adsorption of the colloid. This variation in particle size was clearly seen for the starch sludge in Table V-5.

From the standpoint of total removal of substrate colloids, some very interesting patterns were noted as shown in Figures VI-1, VI-2, and VI-3. These data were obtained from the isotherm studies performed with the biologically inactive sludges. The plots describe the total amount of substrate COD removed for varying amounts of substrate applied to different levels of biomass solids. Only that data involving two MLSS concentrations at a pH of 6 were plotted for the purpose of visual clarity.

For the highly adsorptive starch substrate shown in Figure VI-2, an increase in the MLSS concentrations was detrimental to the total removal of the starch. This was initially thought to be due to a reduction in the surface area available for adsorption at the higher MLSS concentrations. However, simple calculations made for determination of total surface area showed that the total surface area was greater at the higher MLSS concentration. Alternate explanations formulated for this phenomenon included variations in the charge density on the bacterial surfaces and variations in the manner of adsorption (multilayer or single layer) as the MLSS concentration varied. However, the concept of a greater surface area was still believed to play a major role in the starch removals. This was based on the fact that the sludge particle sizes were calculated using particle counts obtained with a 5 to 300 micron sensor. Any particles with diameters less than five microns were not counted which may have contributed a major portion of the surface area

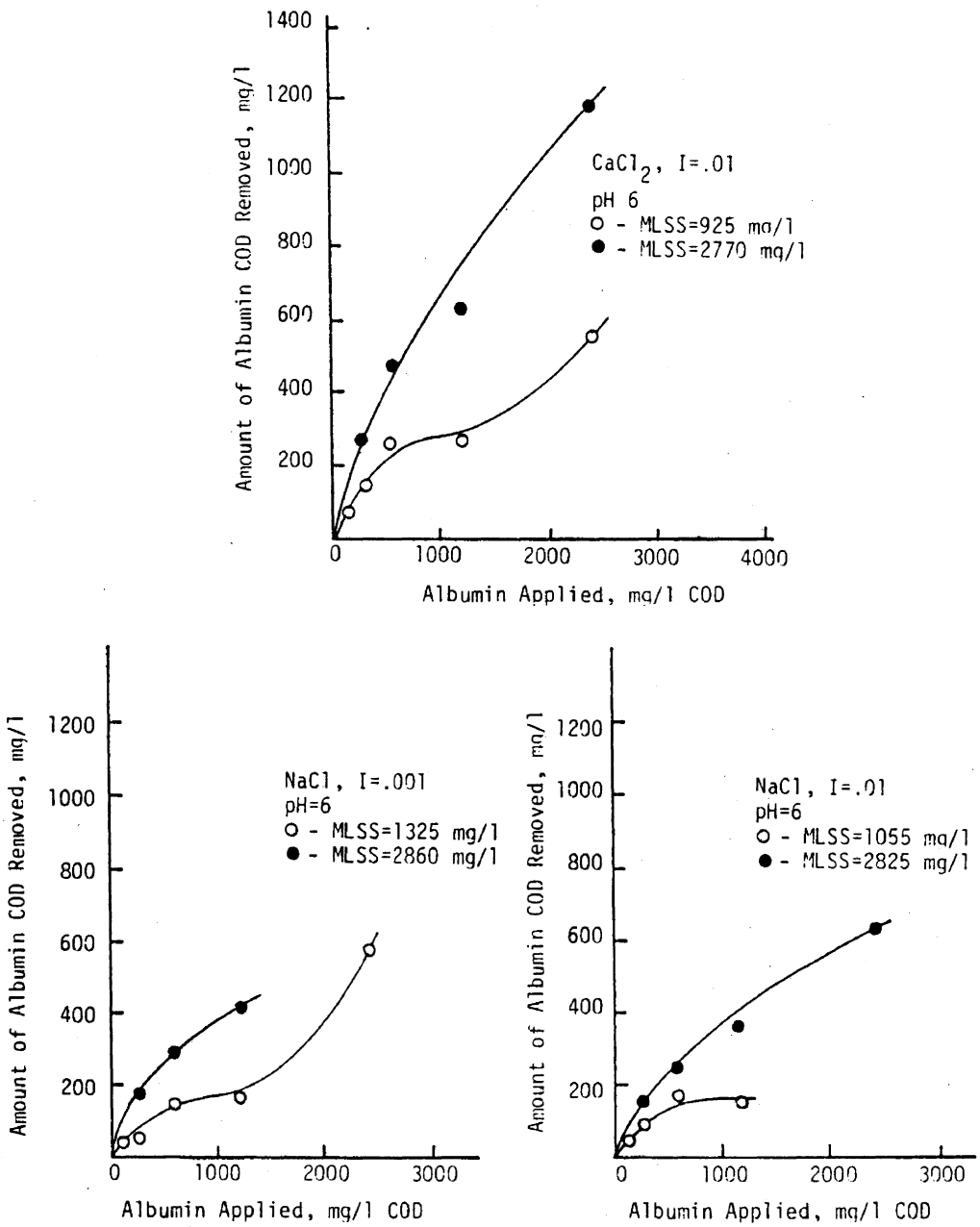


Figure VI-1: Relationship Between the Amount of Albumin Removed When Mixed With Activated Sludge (Poisoned) and the Amount of Albumin Applied

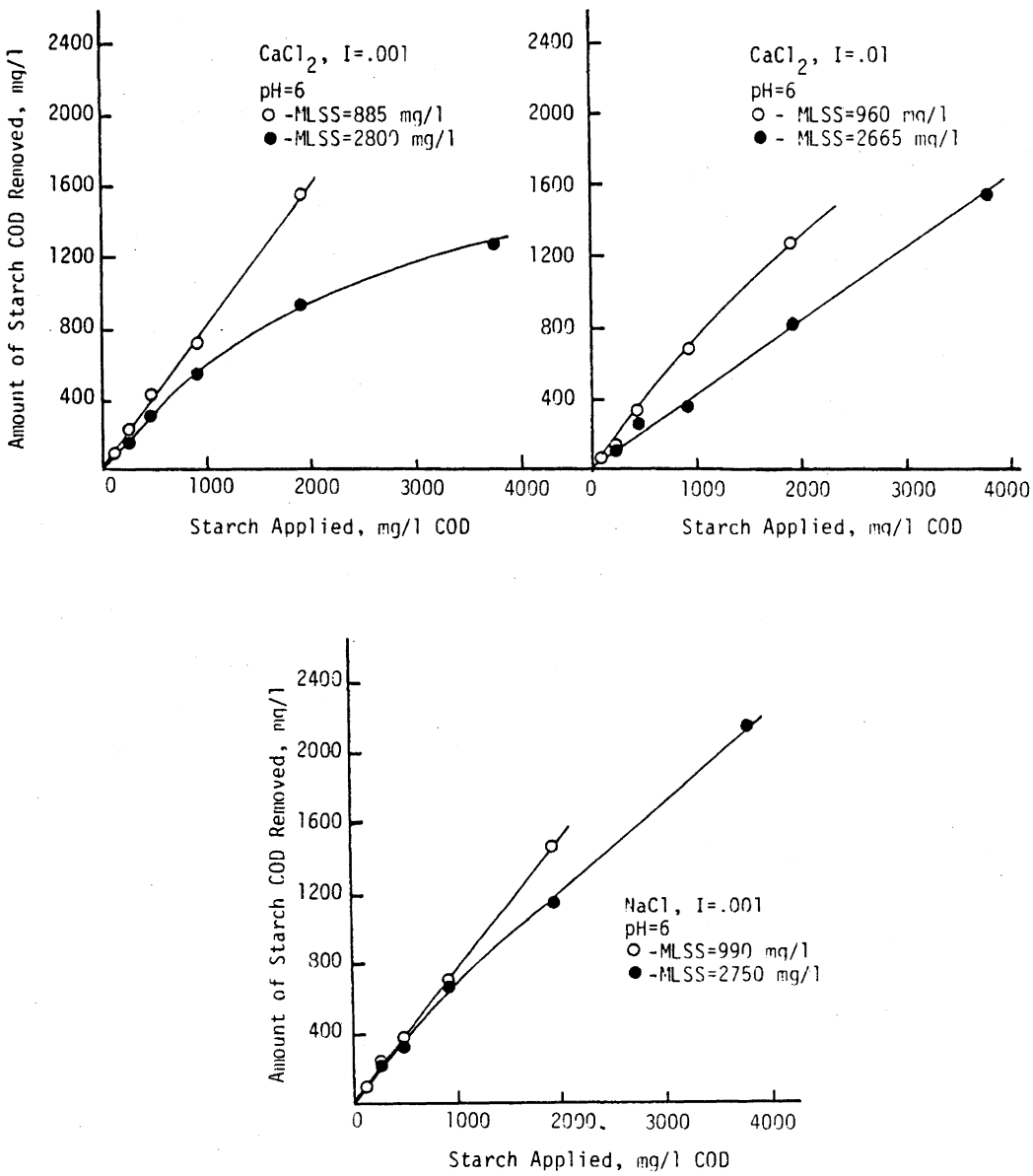
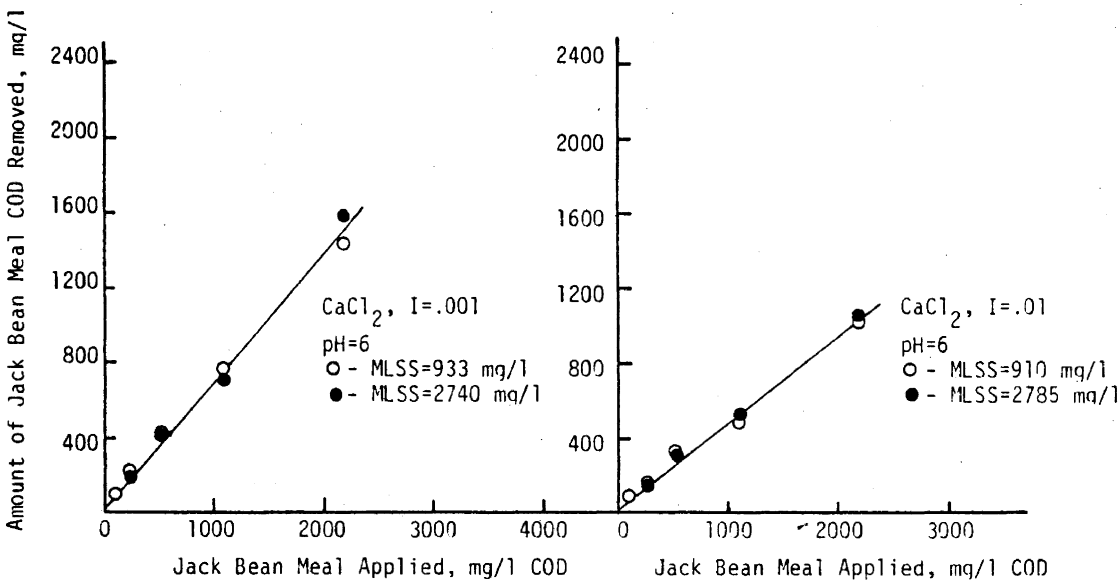


Figure VI-2: Relationship Between the Amount of Starch Removed When Mixed With Activated Sludge (Poisoned) and the Amount of Starch Applied



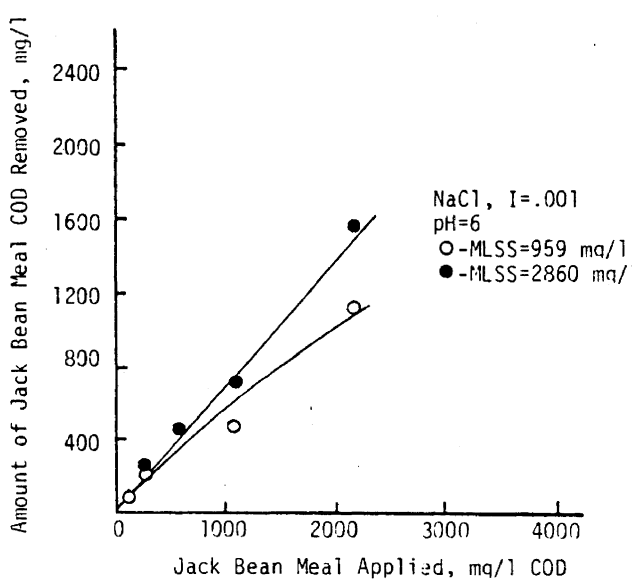


Figure VI-3: Relationship Between the Amount of Jack Bean Meal Removed When Mixed With Activated Sludge (Poisoned) and the Amount of Jack Bean Meal Applied

available for adsorption. Therefore, the surface area theory may still be a valid one.

Analysis of Figures VI-1 and VI-3 (albumin and jack bean meal, respectively), in some cases, describes better removals of substrate colloids at higher MLSS concentrations. For albumin, by approximately tripling the MLSS concentrations, removals of albumin substrate were doubled. The jack bean meal substrate was only slightly augmented, and, then, only in the sodium chloride solution.

For these two substrates and any other colloidal substrates that are removed via enmeshment, higher MLSS levels may augment the total colloidal removal. However, from a comparison of the jack bean meal and albumin data, there appeared to be a solids concentration above which little or no further removal of colloids was possible. For jack bean meal, this solids level appeared to have been reached at about 1000 mg/l or lower in a calcium environment. The solids level for maximum albumin removal, however, could not be pin-pointed.

From a design and operational standpoint, high MLSS levels in the contact tank of a contact stabilization process may or may not increase the system efficiency. If the wastewater contains substrate colloids of a highly adsorption nature, physical removals of the substrate may actually be enhanced by lowering the MLSS concentration in the aeration basin. On the other hand, enmeshable colloidal substrates will be removed in greater quantities with increases in MLSS concentrations. There is, however, a maximum

amount of substrate that can be removed no matter how high the biomass solids concentrations are increased.

The degree or strength of the substrate colloid-sludge floc was observed in the physical studies. There appeared to be either a strong or a weak bond between the colloid and the sludges whether enmeshment or adsorption was the mechanism involved. In all cases, both an increase in ionic strength and the presence of calcium ion strengthened this union. This agrees very well with the Novak model of bioflocculation [18,19] wherein the presence of divalent cations and the ionic strength of a solution play important roles in the aggregate of bacteria in activated sludge. The solution pH also appeared to affect this union--generally, the lower the pH, the stronger the interaction. This was probably a result of the decrease in the repulsive charge on the substrate colloids.

Application to the Contact Stabilization Process

One result not mentioned in this chapter but alluded to in the previous one, was the reduction in the supernatant particles after substrate addition. The substrate colloids were thought to add weight to or act as coagulants for the biological flocs resulting in better settling of the suspended biomass. This answers, at least partially, the question of why contact stabilization processes produce an effluent lower in suspended solids than a conventional typical complete mix activated sludge system.

It should be strongly emphasized that simply because a wastewater is predominately colloidal does not automatically suggest advantageous use of contact stabilization. Kharajian and Sherrard clearly demonstrated that the desired reactions did not occur when a colloidal yogurt waste was used [24]. Thorough laboratory testing of the wastewater and its suitability for use in contact stabilization should be made from both a metabolic and physical standpoint. Since the results of these studies did point to various factors affecting the substrate colloid-sludge union, an investigator could improve the efficiency of a contact stabilization system by simply adjusting the pH or adding a calcium salt to the wastewater.

The substrate to sludge ratio may be an effective parameter for operational control of the contact stabilization system. Commonly, for ratios less than one, the substrate sludge mean particle sizes were greater than in the control. This could potentially lead to better settling, thickening and dewatering of the sludge if wasting occurred from the clarifier. More testing is required to support this hypothesis though. Also, the supernatant suspended solids concentrations were decreased up to a ratio of one under certain conditions. At ratios greater than one, the supernatant usually became more turbid than the control.

CHAPTER VII

CONCLUSIONS AND RECOMMENDATIONS

The purpose of this study was to evaluate certain metabolic and physical functions of activated sludge bacteria upon selected colloidal substrates and to determine how these functions would impact a contact stabilization system. Specific conclusions were:

1) The carbohydrate substrates used in these studies (starch and jack bean meal) exhibited an "adsorption and release" phenomenon whereas no such response was noted for the albumin, a predominately proteinaceous compound. From the results obtained in this study, wastewaters containing major quantities of carbohydrates are quite likely to exhibit an adsorption and release phenomenon.
2) Two stage oxygen utilization exists during colloidal organic utilization. This was observed for the albumin and starch substrates. However, it remains unclear as to what type of substrate or conditions would stimulate this sequential utilization of oxygen.
3) The addition of glucose as a portion of the substrate decreased the sorption capacity of a metabolically active activated sludge. This was attributed to the repression of extracellular enzyme production by the glucose. In turn, enzyme repression reduced the active sites

on the bacterial cell surface for adsorption of the substrate.

4) The sorption capacity of an activated sludge culture was found to be a function of pH, ionic medium and ionic strength. pH effects were substrate specific whereas ionic strength and ionic medium affected both the sludge and the substrate.

5) Carbohydrate wastes exhibited better sorption characteristics on both metabolically active and inactive (mercury poisoned) bacterial flocs. Wastewaters containing large quantities of carbohydrates should be seriously considered for treatment by contact stabilization.

6) Increases in MLSS levels did not always augment the unit and total sorption capacity of the sludge. In fact, decreases in both of these capacities were observed. In all cases, the unit sorption capacity was the same or greater for the lower MLSS concentrations. However, the total sorption capacity varied with respect to the mechanism for removal of the substrate. For highly adsorptive substrates (starch), decreases in total removals were noted. However for enmeshmenable substrates (albumin and jack bean meal), total removals were augmented by increases in MLSS. The differences were attributed to the type of substrate being removed and the available surface area for adsorption.

7) Decreases in supernatant turbidity with substrate addition were commonly noted with decreases in ionic strength. This turbidity removal was attributed to the action of substrate colloids upon the sludge. Specifically, coagulation of dispersed bacteria by the organic colloids and the addition of weight to the sludge floc by the adsorbed or enmeshed colloid were believed to be the mechanisms involved.

8) The presence of calcium ions appeared to strengthen the substrate-sludge union. This provides supportive evidence for the Novak model of bioflocculation which proposes that enmeshment of colloids in bacterial flocs composed of biopolymers and cations is the major mechanism of bioflocculation. It is suggested that the cations act simply as binding agents between the polymers.

RECOMMENDATIONS

Further study should be performed on evaluating factors that affect the contact stabilization system. Specific areas for study are:

1) The effect of specific growth rate (sludge age) on the adsorption and/or enmeshment of substrate colloids. (This would provide valuable information to the operators of contact stabilization systems.)

2) The effect of various soluble substrates upon removals of the colloidal substrates by activated sludge. (One suggestion is to supplement filtered primary effluent in a defined colloidal substrate.)

3) A sensitivity analysis on the efficiency of a continuous flow contact stabilization system should be performed with respect to:

   a) Recycle ratio,

   b) Plug flow vs. complete mix regimes,

   c) Hydraulic flow fluctuations,

   d) pH and ionic character of the solutions,

   e) Contact tank detention time.

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APPENDIX A

APPENDIX A

Appendix	Description
A-1	Nutrient Solutions for Activated Sludge
A-2	Buffer Solutions

APPENDIX A-1

NUTRIENT SOLUTIONS FOR ACTIVATED SLUDGE

Solution	Constituent	Concentration (g/l)
A*	NH_4Cl	38.23
B	KH_2PO_4	107.00
	K_2HPO_4	215.00
C**	$MgSO_4 \cdot 7H_2O$	20.28
	$CaCl_2$	5.54
	$MnSO_4 \cdot H_2O$	6.08
	$FeCl_3 \cdot 6H_2O$	9.68

*10 mg of N/ml

**2 mg of cation/ml

APPENDIX A-2

BUFFER SOLUTIONS

Buffer Solution	pH	Buffer Solution Constituents	Concentration* (g/l)
Malonic Acid	6.1	$C_3H_4O_4$	10.41
		NaOH	6.00
Phosphate	7.2	KH_2PO_4	6.80
		K_2HPO_4	8.71
Tris	8.1	$C_4H_{11}NO_3$	12.11
		HCl	1.83
Boric Acid	9.2	H_3BO_4	4.38
		NaOH	2.00

*Concentrations shown are equivalent to 0.1M solutions of buffer.

APPENDIX B

APPENDIX B

Appendix	Description
B-1	Particle Counting Procedure
B-2	Particle Count Tables
B-3	Particle Count Analysis

APPENDIX B-1

PARTICLE COUNTING PROCEDURE

Dilution of Sample

The Hiac Particle Counter required dilution of highly concentrated particle solutions. For the supernatant samples, measured dilutions from .004 to .04 were used for passage thru the 1 to 60 micron sensor. The sludge samples also needed dilution for the 5 to 300 micron sensor. However, no measured dilutions were performed due to interest in only the distribution of the particles and not the particle count. The make-up of the dilution water was identical to the ionic character and strength of the sample solutions. The pH was also adjusted to equal that of the original sample.

Particle Counting

The diluted sample was placed in 125 ml erlenmeyer flasks and placed into the mechine for counting. The samples were continuously mixed during counting. Ten milliliter samples were measured and three repetitions of these counts were performed. The analyzer was adjusted to supply twelve separate values. Each value represented the number of particles in the sample with diameters in predetermined range. These ranges were set by the investigator and are shown in the particle count data later in the appendix. Finally, a particle count on the dilution water was performed to account for backround particles. These values were subtracted from the sample counts, and the particle counts listed later in this appendix reflect this.

APPENDIX B-2

Appendix	Description
B-2-a	Supernatant Particle Counts (1-60 micron)
B-2-b	Substrate-Sludge Particle Counts (5-300 micron)
B-2-c	Substrate Particle Counts (1-60 micron)
B-2-d	Substrate Particle Counts (5-300 micron)

APPENDIX B-2-a

Supernatant Particle Counts (1-60 micron)

SUBSTRATE: ALBUMIN
SLUDGE: ALBUMIN
SLUDGE CONCENTRATION: 1325.

IONIC MEDIUM: NACL
IONIC STRENGTH: 0.001

SUBSTRATE CONC.	PH	DILUTION	PARTICLE COUNTS BETWEEN SIZES 1-2	2-3	3-4	4-5	5-6	6-8	8-10	10-15	15-20	20-30	30-40	40-60
2000.	6	0.004	8121.	3794.	2658.	1467.	932.	1162.	618.	625.	221.	110.	17.	12.
1000.	6	0.020	12135.	6029.	4821.	3072.	2150.	3572.	2290.	2985.	988.	275.	30.	28.
500.	6	0.012	8450.	3282.	3388.	2013.	1235.	1819.	1054.	1143.	338.	97.	16.	12.
250.	6	0.020	8981.	4180.	3318.	2103.	1404.	2125.	1367.	1456.	407.	135.	21.	13.
100.	6	0.004	1879.	856.	700.	511.	346.	632.	410.	554.	200.	68.	8.	3.
0.	6	0.004	2713.	1365.	1199.	820.	599.	912.	599.	754.	255.	100.	11.	4.
2000.	7	0.004	4093.	1865.	1270.	696.	432.	640.	372.	472.	170.	61.	5.	1.
1000.	7	0.012	6942.	3858.	3178.	2038.	1413.	2241.	1260.	1282.	281.	54.	7.	4.
500.	7	0.012	7652.	3726.	2877.	1716.	1171.	1763.	952.	1013.	189.	39.	5.	3.
250.	7	0.012	7575.	3807.	3151.	2007.	1352.	2195.	1473.	1844.	615.	213.	16.	12.
100.	7	0.012	5639.	3087.	2811.	1938.	1420.	2417.	1540.	1656.	435.	100.	5.	1.
0.	7	0.006	4947.	2609.	2260.	1540.	1131.	1836.	1149.	1263.	294.	83.	4.	1.
2000.	8	0.008	6926.	3628.	2715.	1614.	1035.	1561.	887.	981.	293.	120.	13.	8.
1000.	8	0.012	7590.	3724.	2710.	1553.	1070.	1468.	756.	712.	172.	73.	24.	20.
500.	8	0.012	6706.	3489.	2691.	1687.	1142.	1842.	1105.	981.	194.	36.	3.	3.
250.	8	0.012	6218.	3408.	2605.	1900.	1312.	1997.	1104.	1038.	168.	24.	3.	0.
100.	8	0.012	5191.	3061.	2604.	1776.	1253.	1949.	1081.	969.	163.	51.	8.	8.
0.	8	0.008	4041.	2611.	2449.	1697.	1293.	2149.	1349.	1374.	364.	79.	3.	0.

SUBSTRATE: ALBUMIN
SLUDGE: ALBUMIN
SLUDGE CONCENTRATION: 2635.

IONIC MEDIUM: NACL
IONIC STRENGTH: 0.001

SUBSTRATE CONC.	PH	DILUTION	PARTICLE COUNTS BETWEEN SIZES 1-2	2-3	3-4	4-5	5-6	6-8	8-10	10-15	15-20	20-30	30-40	40-60
4000.	6	0.002	3545.	1649.	1132.	674.	484.	756.	557.	788.	337.	148.	10.	4.
2000.	6	0.004	3601.	1709.	1347.	946.	676.	1159.	750.	817.	230.	47.	10.	9.
1000.	6	0.012	8129.	3955.	4623.	2350.	1770.	2958.	1812.	1785.	334.	57.	10.	1.
500.	6	0.008	5144.	2364.	2107.	1624.	1311.	2301.	1526.	1769.	445.	86.	4.	4.
250.	6	0.008	5491.	2754.	2571.	2016.	1583.	2868.	1932.	2202.	576.	139.	7.	8.
0.	6	0.008	4430.	2419.	2366.	1928.	1615.	3056.	2224.	2642.	639.	120.	6.	1.
4000.	7	0.002	3238.	1439.	1113.	785.	559.	914.	656.	856.	308.	154.	26.	16.
2000.	7	0.008	6967.	3258.	2751.	1922.	1457.	2300.	1249.	955.	122.	16.	2.	0.
1000.	7	0.008	6095.	5349.	2947.	2089.	1574.	2451.	1365.	1131.	180.	40.	7.	7.
500.	7	0.004	2771.	1748.	1636.	1238.	976.	1801.	1315.	1513.	413.	76.	5.	5.
250.	7	0.004	2551.	1682.	1636.	1353.	1126.	2099.	1512.	1814.	446.	98.	3.	2.
0.	7	0.004	3032.	1796.	1734.	1351.	1060.	1937.	1273.	1383.	287.	59.	2.	0.
4000.	8	0.008	8247.	4395.	3280.	1906.	1305.	1974.	1026.	883.	144.	31.	9.	1.
2000.	8	0.004	4107.	1855.	1392.	956.	734.	1115.	596.	548.	131.	57.	15.	7.
1000.	8	0.004	3031.	1797.	1452.	1002.	752.	1210.	660.	544.	88.	21.	6.	4.
500.	8	0.008	4767.	2974.	2687.	2098.	1670.	3016.	1677.	1753.	260.	38.	4.	4.
250.	8	0.008	4413.	2909.	2790.	2132.	1758.	3063.	1993.	1771.	265.	43.	5.	4.
0.	8	0.004	2127.	1623.	1632.	1289.	1107.	2206.	1646.	2164.	695.	206.	8.	2.

SUBSTRATE: ALBUMIN
SLUDGE: ALBUMIN
SLUDGE CONCENTRATION: 1000.

IONIC MEDIUM: CACL2
IONIC STRENGTH: 0.001

SUBSTRATE CONC.	PH	DILUTION	PARTICLE COUNTS BETWEEN SIZES 1-2	2-3	3-4	4-5	5-6	6-8	8-10	10-15	15-20	20-30	30-40	40-60
2000.	6	0.008	7182.	3320.	1877.	903.	499.	697.	430.	597.	296.	163.	21.	4.
1000.	6	0.008	4539.	1966.	1153.	554.	349.	578.	402.	726.	431.	223.	24.	4.
500.	6	0.008	2607.	1009.	561.	328.	232.	411.	337.	583.	247.	77.	5.	0.
250.	6	0.008	1913.	796.	433.	274.	198.	430.	371.	580.	253.	92.	9.	2.
100.	6	0.008	1217.	473.	268.	224.	200.	440.	391.	669.	303.	130.	12.	1.
0.	6	0.008	1153.	407.	253.	257.	273.	599.	569.	1082.	579.	323.	50.	9.
2000.	7	0.008	4415.	1842.	676.	382.	246.	356.	284.	483.	240.	143.	17.	1.
1000.	7	0.008	3147.	1353.	773.	408.	302.	505.	408.	652.	302.	148.	10.	2.
500.	7	0.008	1341.	596.	366.	219.	192.	452.	372.	597.	215.	73.	2.	0.
250.	7	0.020	3070.	1257.	915.	613.	550.	1102.	957.	1563.	649.	254.	25.	2.
100.	7	0.020	3235.	1368.	1073.	829.	682.	1491.	1232.	1789.	618.	200.	4.	0.
0.	7	0.020	2275.	774.	773.	776.	730.	1700.	1561.	2639.	1220.	667.	76.	4.
2000.	8	0.020	11132.	4704.	3052.	1669.	1121.	1787.	1220.	1862.	801.	361.	25.	6.
1000.	8	0.020	5373.	2423.	1561.	946.	710.	1329.	1048.	1606.	634.	233.	16.	7.
500.	8	-----	------	------	------	------	------	------	------	------	-----	-----	-----	-----
250.	8	-----	------	------	------	------	------	------	------	------	-----	-----	-----	-----
100.	8	0.020	895.	641.	1035.	915.	861.	1635.	1444.	2076.	198.	301.	27.	8.
0.	8	0.020	192.	481.	771.	940.	916.	2105.	1825.	2815.	1179.	618.	74.	8.

SUBSTRATE: ALBUMIN
SLUDGE: ALBUMIN
SLUDGE CONCENTRATION: 1445.

IONIC MEDIUM: CACL2
IONIC STRENGTH: 0.001

SUBSTRATE CONC.	PH	DILUTION	PARTICLE COUNTS BETWEEN SIZES 1-2	2-3	3-4	4-5	5-6	6-8	8-10	10-15	15-20	20-30	30-40	40-60
4000.	6	0.008	30231.	13805.	8496.	3951.	2176.	2736.	1265.	1289.	397.	213.	42.	22.
2000.	6	0.008	21970.	11314.	6634.	2817.	1430.	1621.	728.	686.	242.	141.	26.	22.
1000.	6	0.008	13924.	7159.	4101.	1760.	947.	1105.	490.	482.	144.	73.	12.	9.
500.	6	0.008	9247.	4508.	2483.	1135.	652.	945.	484.	605.	250.	132.	16.	7.
250.	6	0.012	16013.	8281.	4694.	2090.	1069.	1193.	536.	654.	276.	123.	18.	10.
0.	6	0.016	9779.	5079.	3001.	1810.	1024.	1270.	651.	751.	262.	163.	23.	5.
4000.	7	0.012	16611.	7654.	4136.	1610.	754.	902.	491.	520.	193.	102.	23.	10.
2000.	7	0.012	19446.	9196.	5247.	2070.	1067.	1297.	653.	666.	200.	85.	15.	6.
1000.	7	0.020	18126.	8477.	2679.	2303.	1392.	1784.	897.	945.	312.	111.	15.	9.
500.	7	0.020	12891.	6285.	3772.	1945.	1085.	1436.	735.	894.	368.	192.	25.	11.
250.	7	0.020	12704.	6968.	4796.	2487.	1412.	1730.	757.	672.	155.	59.	9.	3.
0.	7	0.020	10084.	6270.	4733.	2645.	1662.	2252.	1246.	1709.	758.	321.	21.	5.
4000.	8	0.012	12536.	6661.	3804.	1569.	804.	1038.	531.	717.	336.	175.	27.	13.
2000.	8	0.012	10205.	5322.	3123.	1349.	772.	1041.	529.	630.	214.	119.	34.	20.
1000.	8	0.020	11671.	6355.	4608.	2443.	1437.	1785.	823.	766.	235.	81.	12.	5.
500.	8	0.020	8003.	4506.	3201.	1674.	961.	1139.	564.	552.	156.	63.	10.	2.
250.	8	0.020	10703.	6351.	4643.	2445.	1382.	1704.	809.	910.	293.	114.	15.	10.
0.	8	0.020	11628.	7338.	5820.	3341.	2045.	2743.	1538.	2017.	900.	364.	19.	3.

SUBSTRATE: ALBUMIN
SLUDGE: ALBUMIN
SLUDGE CONCENTRATION: 1055.

IONIC MEDIUM: NACL
IONIC STRENGTH: 0.010

SUBSTRATE CONC.	PH	DILUTION	PARTICLE COUNTS BETWEEN SIZES 1-2	2-3	3-4	4-5	5-6	6-8	8-10	10-15	15-20	20-30	30-40	40-60
2000.	6	0.020	20899.	9717.	5787.	2990.	1852.	2674.	1802.	2247.	630.	595.	123.	21.
1000.	6	0.020	16227.	7183.	4642.	2931.	2097.	3357.	1942.	2328.	865.	429.	32.	9.
500.	6	0.020	10867.	4637.	3578.	2429.	1699.	2422.	1245.	1407.	554.	281.	24.	6.
250.	6	0.020	13389.	5878.	3708.	2167.	1466.	2086.	1094.	1330.	551.	266.	32.	9.
100.	6	0.020	7350.	2771.	1983.	1217.	815.	1111.	628.	736.	280.	108.	5.	4.
0.	6	0.020	6719.	2638.	1918.	1211.	801.	1167.	714.	972.	455.	264.	39.	5.
2000.	7	0.020	15052.	6158.	3344.	1755.	1140.	1612.	1058.	1330.	510.	223.	22.	5.
1000.	7	0.020	11140.	4454.	2760.	1701.	1199.	1765.	904.	1025.	353.	163.	17.	5.
500.	7	0.020	8050.	3814.	3344.	2221.	1488.	1989.	1048.	1107.	357.	141.	5.	3.
250.	7	0.020	7135.	3055.	2445.	1537.	980.	1339.	763.	936.	347.	164.	10.	4.
100.	7	0.020	5782.	2966.	2384.	1488.	968.	1260.	675.	660.	199.	62.	9.	2.
0.	7	0.020	4994.	2539.	1924.	679.	804.	1093.	684.	905.	422.	213.	27.	8.
2000.	8	-----	------	------	------	------	------	------	------	------	-----	-----	-----	-----
1000.	8	-----	------	------	------	------	------	------	------	------	-----	-----	-----	-----
500.	8	-----	------	------	------	------	------	------	------	------	-----	-----	-----	-----
250.	8	-----	------	------	------	------	------	------	------	------	-----	-----	-----	-----
100.	8	-----	------	------	------	------	------	------	------	------	-----	-----	-----	-----
0.	8	-----	------	------	------	------	------	------	------	------	-----	-----	-----	-----

SUBSTRATE: ALBUMIN
SLUDGE: ALBUMIN
SLUDGE CONCENTRATION: 2825.

IONIC MEDIUM: NACL
IONIC STRENGTH: 0.010

SUBSTRATE CONC.	IB	DILUTION	PARTICLE COUNTS BETWEEN SIZES 1-2	2-3	3-4	4-5	5-6	6-8	8-10	10-15	15-20	20-30	30-40	40-60
4000.	6	0.008	22638.	11360.	7523.	4200.	2592.	3641.	1830.	1770.	511.	253.	39.	9.
2000.	6	0.008	20566.	8926.	6261.	3519.	2193.	2706.	1113.	952.	266.	111.	11.	5.
1000.	6	0.020	31190.	15308.	10708.	5678.	3206.	3628.	1503.	1408.	446.	201.	17.	7.
500.	6	0.020	30362.	13955.	9065.	4651.	2518.	2975.	1330.	1362.	430.	191.	20.	12.
250.	6	0.020	30322.	13433.	8192.	4044.	2248.	2673.	1206.	1288.	417.	193.	16.	4.
0.	6	0.020	21193.	8118.	4974.	2469.	1409.	1831.	925.	1058.	400.	204.	31.	4.
4000.	7	0.020	37775.	18480.	12863.	7806.	5276.	2190.	2757.	2128.	524.	191.	14.	5.
2000.	7	0.020	29972.	15467.	11802.	6922.	4220.	5214.	2392.	2578.	913.	430.	66.	29.
1000.	7	0.020	33157.	17090.	13000.	6966.	3862.	4367.	1763.	1751.	538.	206.	19.	9.
500.	7	0.020	31252.	13704.	9587.	5182.	2958.	3323.	1445.	1482.	576.	261.	29.	9.
250.	7	0.020	24673.	13109.	9269.	4876.	2702.	3209.	1386.	1414.	438.	159.	14.	3.
0.	7	0.020	17669.	8350.	5447.	2810.	1579.	1965.	98.	1111.	442.	227.	41.	8.
4000.	8	0.020	35739.	16623.	14031.	9474.	6345.	8175.	3393.	2741.	731.	255.	19.	3.
2000.	8	0.020	23257.	14243.	11877.	6779.	3813.	4267.	1728.	1566.	454.	168.	14.	4.
1000.	8	0.020	26427.	15941.	11756.	6310.	3443.	3960.	1706.	1658.	529.	203.	14.	6.
500.	8	0.020	25912.	14935.	11228.	5906.	3251.	3957.	1690.	1697.	532.	197.	15.	6.
250.	8	0.020	23919.	13774.	10136.	5320.	3091.	3660.	1697.	1605.	531.	200.	13.	3.
0.	8	0.020	16290.	9060.	6446.	3436.	1941.	2375.	1209.	1350.	485.	220.	18.	3.

SUBSTRATE: ALBUMIN
SLUDGE: ALBUMIN
SLUDGE CONCENTRATION: 925.

IONIC MEDIUM: CACL2
IONIC STRENGTH: 0.010

SUBSTRATE CONC.	PH	DILUTION	PARTICLE COUNTS BETWEEN SIZES 1-2	2-3	3-4	4-5	5-6	6-8	8-10	10-15	15-20	20-30	30-40	40-60
2000.	6	0.020	23628.	11777.	7289.	3721.	2499.	3873.	2552.	3798.	1807.	1081.	142.	14.
1000.	6	0.012	13545.	6573.	4212.	2415.	1644.	2640.	1963.	2792.	1253.	704.	118.	78.
500.	6	0.020	15463.	7081.	4066.	2491.	1820.	3240.	2222.	2953.	1005.	332.	14.	11.
250.	6	0.020	13223.	6485.	3874.	2250.	1622.	2482.	1418.	1579.	479.	135.	11.	6.
100.	6	0.020	14310.	6197.	3507.	1797.	1129.	1593.	916.	1043.	389.	177.	47.	27.
0.	6	0.020	11629.	4815.	2912.	1586.	1102.	1706.	1088.	1364.	577.	248.	21.	3.
2000.	7	0.020	21636.	10415.	5997.	3044.	2006.	3385.	2235.	3105.	1265.	616.	76.	31.
1000.	7	0.020	15363.	6697.	3770.	2037.	1445.	2472.	1704.	2302.	876.	385.	21.	5.
500.	7	0.020	16922.	7673.	4517.	2872.	2183.	3757.	2441.	2881.	764.	168.	26.	20.
250.	7	0.020	16645.	7394.	4317.	2689.	1940.	3079.	1762.	2116.	657.	203.	10.	7.
100.	7	0.020	17520.	7998.	4793.	2903.	2061.	2915.	1677.	1698.	543.	202.	43.	31.
0.	7	0.020	14303.	5392.	3024.	1772.	1148.	1589.	955.	1118.	387.	145.	12.	1.
2000.	8	0.020	17692.	7557.	4062.	2121.	1404.	2354.	1544.	2031.	771.	298.	32.	36.
1000.	8	0.020	13903.	5613.	2928.	1591.	1099.	1651.	1263.	1758.	690.	287.	15.	5.
500.	8	0.020	11899.	4877.	2635.	1626.	1197.	1917.	1207.	1455.	450.	146.	17.	13.
250.	8	0.020	13026.	4371.	2665.	1572.	1039.	1563.	944.	1024.	322.	95.	9.	3.
100.	8	0.020	12892.	5313.	2967.	1639.	1131.	1733.	1052.	1306.	502.	194.	24.	11.
0.	8	0.020	12568.	5276.	3008.	1683.	1137.	1673.	943.	1105.	350.	120.	9.	4.

SUBSTRATE: ALBUMIN
SLUDGE: ALBUMIN
SLUDGE CONCENTRATION: 2770.

IONIC MEDIUM: CACL2
IONIC STRENGTH: 0.010

SUBSTRATE CONC.	PH	DILUTION	PARTICLE COUNTS BETWEEN SIZES 1-2	2-3	3-4	4-5	5-6	6-8	8-10	10-15	15-20	20-30	30-40	40-60
4000.	6	0.006	29729.	15361.	10154.	5659.	3734.	5653.	3293.	3820.	1252.	498.	47.	13.
2000.	6	0.008	23621.	11776.	7663.	4538.	2989.	4256.	2341.	2473.	695.	191.	1.	3.
1000.	6	0.008	16699.	7158.	4695.	2889.	1948.	2516.	1150.	916.	155.	28.	0.	0.
500.	6	0.008	18564.	8193.	5443.	2683.	1620.	1862.	852.	749.	180.	40.	0.	0.
250.	6	0.008	20380.	8404.	4390.	2165.	1250.	1553.	811.	872.	266.	75.	0.	0.
0.	6	0.008	18374.	8123.	5180.	2842.	1649.	1999.	988.	1012.	291.	102.	0.	0.
4000.	7	0.008	25291.	11926.	7346.	4190.	2809.	4637.	2768.	3164.	900.	268.	4.	0.
2000.	7	0.012	31873.	15924.	10294.	6103.	4049.	5696.	3074.	2763.	527.	85.	0.	0.
1000.	7	0.012	31643.	15331.	9879.	5891.	3836.	5197.	2454.	2010.	360.	53.	0.	0.
500.	7	0.012	31028.	14193.	8231.	4073.	2236.	2432.	1007.	732.	101.	14.	0.	0.
250.	7	0.012	31492.	14494.	8349.	4336.	2515.	3044.	1312.	1033.	157.	21.	0.	0.
0.	7	0.012	26153.	12222.	6991.	3720.	2195.	2804.	1426.	1412.	321.	74.	0.	0.
4000.	8	0.016	36828.	15671.	9772.	5618.	3790.	5757.	3107.	2822.	558.	99.	6.	0.
2000.	8	0.016	30766.	12423.	7878.	4520.	3012.	4083.	2007.	1729.	295.	58.	0.	0.
1000.	8	0.016	31661.	14582.	8652.	4805.	3073.	4181.	2186.	1977.	463.	90.	0.	0.
500.	8	0.016	31573.	14543.	8599.	4582.	2740.	3562.	1702.	1489.	309.	58.	0.	0.
250.	8	0.016	33665.	15488.	9341.	5042.	3167.	4013.	2012.	1904.	394.	95.	0.	0.
0.	8	0.016	30496.	13697.	7683.	3836.	2257.	2889.	1446.	1489.	473.	170.	5.	0.

SUBSTRATE: STARCH
SLUDGE: STARCH
SLUDGE CONCENTRATION: 990.

IONIC MEDIUM: NACL
IONIC STRENGTH: 0.001

SUBSTRATE CONC.	PH	DILUTION	PARTICLE COUNTS BETWEEN SIZES 1-2	2-3	3-4	4-5	5-6	6-8	8-10	10-15	15-20	20-30	30-40	40-60
2000.	6	0.008	3984.	2615.	2390.	1572.	1094.	1584.	869.	949.	273.	82.	8.	2.
1000.	6	0.008	4929.	3014.	2612.	1758.	1178.	1780.	1003.	1171.	366.	136.	0.	0.
500.	6	0.016	4997.	3483.	3492.	2493.	1830.	2929.	1799.	2256.	789.	273.	8.	0.
250.	6	0.016	6353.	4460.	4138.	2949.	2092.	3354.	2030.	2576.	943.	386.	16.	0.
100.	6	0.016	4644.	3438.	3441.	2516.	1809.	3016.	1984.	2553.	983.	373.	13.	0.
0.	6	0.012	6452.	4416.	4425.	3204.	2401.	3963.	2468.	3004.	1010.	341.	13.	0.
2000.	7	0.020	5402.	3870.	3999.	3025.	2178.	3469.	2178.	2291.	634.	143.	0.	0.
1000.	7	0.020	6168.	4489.	4554.	3267.	2403.	3958.	2401.	2838.	805.	160.	0.	0.
500.	7	0.016	4450.	3522.	3682.	2802.	2159.	3663.	2379.	2970.	940.	290.	0.	9.
250.	7	0.016	5184.	3553.	3382.	2342.	1677.	2675.	1660.	2050.	750.	234.	4.	0.
100.	7	0.016	3583.	2686.	2778.	2245.	1807.	3346.	2360.	3208.	1187.	341.	4.	0.
0.	7	0.016	7397.	5552.	5604.	4249.	3395.	5899.	3786.	4697.	1449.	424.	4.	0.
2000.	8	0.016	40406.	31219.	32550.	24606.	18896.	31506.	18665.	20300.	5479.	1194.	13.	0.
1000.	8	0.016	5720.	4400.	4605.	3429.	2627.	4396.	2507.	2789.	697.	131.	0.	0.
500.	8	0.016	5559.	4607.	4969.	3637.	2980.	5069.	2993.	3117.	684.	93.	0.	0.
250.	8	0.020	6728.	5420.	5922.	4567.	3550.	6063.	3670.	4009.	1008.	172.	3.	2.
100.	8	0.020	6142.	5111.	5742.	4610.	3766.	6550.	4399.	5464.	1656.	378.	1.	0.
0.	8	0.020	9304.	7363.	8200.	6649.	5379.	9282.	6362.	7900.	2330.	574.	5.	0.

SUBSTRATE: STARCH
SLUDGE: STARCH
SLUDGE CONCENTRATION: 2725.

IONIC MEDIUM: NACL
IONIC STRENGTH: 0.001

SUBSTRATE CONC.	PH	DILUTION	PARTICLE COUNTS BETWEEN SIZES 1-2	2-3	3-4	4-5	5-6	6-8	8-10	10-15	15-20	20-30	30-40	40-60
4000.	6	0.020	19239.	11649.	10417.	5116.	1838.	1008.	237.	270.	115.	69.	14.	5.
2000.	6	0.020	20639.	12473.	10237.	5071.	2058.	1339.	364.	378.	157.	106.	24.	10.
1000.	6	0.020	17530.	10847.	8763.	3907.	1329.	686.	173.	198.	96.	62.	10.	4.
500.	6	0.020	20343.	12004.	10437.	5468.	2254.	1420.	396.	387.	187.	111.	52.	10.
250.	6	0.020	16541.	10662.	8630.	4202.	1545.	850.	195.	227.	110.	76.	23.	9.
0.	6	0.020	18603.	11139.	9675.	5034.	2169.	2019.	1048.	1668.	865.	651.	112.	24.
4000.	7	0.020	19981.	10996.	8500.	3343.	980.	632.	203.	231.	101.	62.	12.	8.
2000.	7	0.020	19203.	11684.	9184.	3864.	1394.	900.	279.	297.	134.	115.	33.	18.
1000.	7	0.020	17698.	10755.	7417.	2463.	724.	497.	209.	227.	116.	65.	12.	5.
500.	7	0.020	18967.	9949.	6687.	2272.	752.	595.	235.	260.	118.	87.	24.	9.
250.	7	0.020	16585.	8276.	4903.	1512.	512.	435.	267.	392.	219.	197.	63.	36.
0.	7	0.020	17295.	10326.	7354.	2809.	1099.	1109.	667.	1054.	527.	335.	47.	8.
4000.	8	0.012	18363.	9642.	4949.	1593.	682.	771.	353.	355.	109.	41.	9.	6.
2000.	8	0.020	24199.	13540.	7065.	2924.	1325.	1428.	687.	936.	440.	260.	54.	19.
1000.	8	0.020	23573.	12823.	6995.	2598.	1106.	1310.	659.	895.	439.	252.	42.	7.
500.	8	0.020	23671.	12116.	6397.	2330.	1043.	1082.	508.	676.	284.	166.	25.	6.
250.	8	0.020	19561.	10576.	5600.	2102.	1003.	1112.	542.	710.	300.	180.	38.	9.
0.	8	0.020	17422.	9618.	6011.	2723.	1558.	2179.	1427.	2265.	1211.	711.	115.	14.

SUBSTRATE: STARCH
SLUDGE: STARCH
SLUDGE CONCENTRATION: 2650.

IONIC MEDIUM: NACL
IONIC STRENGTH: 0.010

SUBSTRATE CONC.	PH	DILUTION	PARTICLE COUNTS BETWEEN SIZES 1-2	2-3	3-4	4-5	5-6	6-8	8-10	10-15	15-20	20-30	30-40	40-60
4000.	6	0.020	33473.	14454.	7691.	2949.	1290.	1187.	415.	361.	132.	76.	19.	7.
2000.	6	0.020	24500.	10201.	5179.	1819.	702.	615.	198.	141.	55.	25.	6.	3.
1000.	6	0.020	21706.	9363.	5022.	1802.	770.	666.	239.	196.	58.	42.	7.	2.
500.	6	0.020	25016.	11133.	6237.	2461.	1105.	1027.	384.	322.	102.	62.	11.	3.
250.	6	0.020	22876.	9915.	5274.	1984.	809.	733.	260.	212.	61.	31.	8.	0.
0.	6	0.020	21704.	9399.	5130.	2110.	989.	1120.	497.	533.	211.	129.	28.	4.
4000.	7	0.020	25500.	12128.	6665.	2609.	1126.	1123.	408.	349.	97.	62.	14.	3.
2000.	7	0.020	22376.	10518.	6022.	2417.	1077.	1005.	339.	265.	75.	42.	12.	5.
1000.	7	0.020	18596.	8856.	5706.	2672.	1365.	1472.	605.	562.	181.	88.	13.	4.
500.	7	0.020	17032.	7708.	4603.	1970.	920.	863.	322.	287.	94.	65.	20.	4.
250.	7	0.020	17902.	8526.	5329.	2496.	1304.	1423.	633.	613.	217.	105.	22.	3.
0.	7	0.020	16761.	7687.	4861.	2258.	1151.	1321.	621.	660.	231.	119.	17.	4.
4000.	8	0.020	24424.	12020.	7255.	3101.	1493.	1467.	550.	472.	98.	34.	7.	1.
2000.	8	0.020	23324.	12371.	7063.	3226.	1598.	1722.	680.	599.	159.	65.	11.	4.
1000.	8	0.020	20351.	10480.	6319.	2820.	1480.	1942.	882.	846.	237.	89.	9.	1.
500.	8	0.020	18628.	9715.	6042.	2671.	1426.	1711.	792.	683.	175.	66.	6.	0.
250.	8	0.020	21368.	11119.	7032.	3242.	1719.	2148.	1056.	1049.	321.	133.	19.	3.
0.	8	0.020	17646.	9178.	6006.	2919.	1623.	1818.	861.	923.	326.	190.	24.	4.

SUBSTRATE: STARCH
SLUDGE: STARCH
SLUDGE CONCENTRATION: 2750.

IONIC MEDIUM: NACL
IONIC STRENGTH: 0.010

SUBSTRATE CONC.	PH	DILUTION	PARTICLE COUNTS BETWEEN SIZES 1-2	2-3	3-4	4-5	5-6	6-8	8-10	10-15	15-20	20-30	30-40	40-60
4000.	6	0.020	59376.	36718.	24681.	9266.	3216.	2073.	437.	297.	98.	73.	19.	13.
2000.	6	0.020	60008.	37336.	25147.	9434.	3342.	2190.	514.	266.	77.	24.	5.	5.
1000.	6	0.020	57520.	35929.	25175.	9689.	3714.	2730.	727.	535.	170.	112.	26.	16.
500.	6	0.020	55634.	34344.	23442.	8893.	3205.	2171.	515.	304.	80.	40.	6.	2.
250.	6	0.020	55343.	33240.	2366.	8639.	3236.	2349.	633.	505.	197.	133.	23.	4.
0.	6	0.020	56993.	34472.	23232.	8750.	3157.	2199.	560.	371.	139.	82.	20.	4.
4000.	7	0.020	63728.	39131.	26826.	10382.	3725.	2505.	600.	365.	96.	45.	12.	7.
2000.	7	0.020	60918.	35247.	23496.	8580.	2881.	1803.	393.	188.	38.	12.	2.	2.
1000.	7	0.020	59556.	36137.	23966.	9126.	3306.	2407.	641.	407.	98.	24.	4.	1.
500.	7	0.020	54693.	32260.	21052.	7718.	2754.	2003.	549.	283.	76.	24.	3.	2.
250.	7	0.020	55614.	32738.	21407.	8035.	3059.	2384.	700.	545.	144.	57.	8.	3.
0.	7	0.020	51321.	31653.	20727.	7995.	3037.	2544.	867.	701.	224.	77.	6.	3.
4000.	8	0.020	59019.	37116.	24400.	8916.	3226.	2369.	700.	501.	142.	152.	10.	4.
2000.	8	0.020	57112.	36228.	24611.	9249.	3435.	2538.	692.	558.	193.	113.	38.	17.
1000.	8	0.020	56485.	35723.	24130.	9564.	3671.	3039.	1039.	876.	327.	198.	48.	13.
500.	8	0.020	52668.	32801.	22020.	8518.	3240.	2603.	826.	715.	266.	153.	39.	9.
250.	8	0.020	54303.	33961.	22836.	8969.	3485.	2671.	967.	826.	297.	178.	40.	11.
0.	8	0.020	54429.	34010.	23513.	9186.	3619.	2800.	851.	722.	275.	194.	71.	21.

SUBSTRATE: STARCH
SLUDGE: STARCH
SLUDGE CONCENTRATION: 885.

IONIC MEDIUM: CACL2
IONIC STRENGTH: 0.001

SUBSTRATE CONC.	PH	DILUTION	PARTICLE COUNTS BETWEEN SIZES 1-2	2-3	3-4	4-5	5-6	6-8	8-10	10-15	15-20	20-30	30-40	40-60
2000.	6	0.020	14189.	9367.	7536.	3358.	1394.	1060.	442.	461.	209.	130.	29.	10.
1000.	6	0.020	13737.	8834.	6858.	2923.	1141.	963.	400.	500.	233.	144.	25.	5.
500.	6	0.020	14071.	8165.	7073.	3333.	1413.	1051.	375.	406.	187.	112.	14.	2.
250.	6	0.020	13834.	9002.	7226.	3232.	1294.	1075.	499.	644.	308.	208.	30.	4.
100.	6	0.020	13704.	9252.	7114.	3227.	1408.	1404.	144.	1016.	463.	230.	16.	0.
0.	6	0.020	15738.	9485.	7711.	4000.	2085.	2606.	1528.	1985.	770.	335.	18.	1.
2000.	7	0.020	11037.	6733.	5586.	2526.	1056.	831.	403.	501.	209.	152.	23.	1.
1000.	7	0.020	14990.	9788.	8489.	3713.	1472.	1217.	536.	662.	320.	219.	30.	3.
500.	7	0.020	15313.	9747.	7922.	3406.	1396.	1164.	522.	689.	296.	171.	17.	2.
250.	7	0.020	14412.	9425.	7565.	3214.	1284.	1055.	484.	560.	239.	138.	24.	2.
100.	7	0.020	14668.	9772.	8095.	3644.	1610.	1694.	937.	1295.	554.	241.	7.	3.
0.	7	0.020	15113.	10156.	8700.	4372.	2412.	3169.	1903.	2501.	914.	315.	7.	1.
2000.	8	0.020	15710.	10114.	7275.	3795.	1626.	1423.	680.	876.	279.	266.	50.	10.
1000.	8	0.020	16637.	10762.	8670.	3867.	1665.	1534.	674.	852.	392.	263.	55.	11.
500.	8	0.020	13663.	8187.	6524.	3049.	1341.	1235.	566.	783.	444.	297.	42.	4.
250.	8	0.020	14559.	9649.	7461.	3106.	1257.	1276.	636.	879.	399.	229.	26.	3.
100.	8	0.020	15765.	10428.	8070.	3571.	1641.	1693.	909.	1132.	462.	228.	15.	4.
0.	8	0.020	14534.	9664.	7383.	3308.	1523.	1772.	993.	1380.	598.	311.	26.	3.

SUBSTRATE: STARCH
SLUDGE: STARCH
SLUDGE CONCENTRATION: 2800.

IONIC MEDIUM: CACL2
IONIC STRENGTH: 0.001

SUBSTRATE CONC.	PH	DILUTION	PARTICLE COUNTS BETWEEN SIZES 1-2	2-3	3-4	4-5	5-6	6-8	8-10	10-15	15-20	20-30	30-40	40-60
4000.	6	0.020	24273.	18135.	17900.	9626.	4429.	3323.	899.	726.	294.	268.	100.	46.
2000.	6	0.020	22401.	16861.	16604.	9274.	4102.	2969.	697.	373.	99.	116.	30.	7.
1000.	6	0.020	22745.	16840.	16540.	9173.	4202.	3170.	758.	439.	163.	145.	46.	17.
500.	6	0.020	22263.	16029.	15583.	8767.	3953.	2942.	671.	342.	116.	125.	36.	17.
250.	6	0.020	23061.	16213.	16377.	9146.	4223.	3439.	925.	566.	185.	192.	55.	26.
0.	6	0.020	22609.	14896.	13652.	7504.	3652.	3302.	1161.	1141.	592.	555.	176.	48.
4000.	7	0.020	26900.	16495.	12932.	6100.	2539.	1822.	474.	325.	143.	107.	23.	7.
2000.	7	0.020	25409.	16281.	14244.	6564.	3415.	2642.	693.	462.	158.	156.	38.	11.
1000.	7	0.020	23086.	17132.	16047.	8419.	3828.	3163.	884.	591.	207.	183.	53.	11.
500.	7	0.020	22355.	16361.	15576.	8239.	3760.	2974.	729.	372.	165.	213.	88.	32.
250.	7	0.020	23467.	17126.	15944.	8192.	3618.	2966.	767.	433.	159.	154.	48.	12.
0.	7	0.020	22730.	16748.	15555.	8295.	4092.	4095.	1704.	1923.	912.	686.	125.	17.
4000.	8	0.020	24485.	17830.	16204.	8036.	3696.	3254.	922.	648.	195.	101.	14.	1.
2000.	8	0.020	24409.	18209.	16744.	8543.	4033.	3615.	1026.	722.	260.	229.	54.	12.
1000.	8	0.020	22716.	16761.	16063.	8502.	4225.	3935.	1220.	903.	335.	284.	64.	11.
500.	8	0.020	22159.	16822.	15582.	8200.	4150.	3600.	1180.	834.	323.	265.	72.	13.
250.	8	0.020	21638.	16412.	15747.	8935.	4393.	4136.	1264.	869.	360.	383.	131.	43.
0.	8	0.020	20946.	15844.	15349.	8721.	4690.	5028.	2110.	2217.	1049.	1051.	328.	82.

SUBSTRATE: STARCH
SLUDGE: STARCH
SLUDGE CONCENTRATION: 960.

IONIC MEDIUM: CACL2
IONIC STRENGTH: 0.010

SUBSTRATE CONC.	PH	DILUTION	PARTICLE COUNTS BETWEEN SIZES 1-2	2-3	3-4	4-5	5-6	6-8	8-10	10-15	15-20	20-30	30-40	40-60
2000.	6	0.025	16932.	10788.	7706.	3147.	1164.	860.	251.	191.	70.	50.	10.	6.
1000.	6	0.050	29713.	20369.	15127.	6544.	2537.	1764.	398.	262.	63.	35.	7.	11.
500.	6	0.050	27659.	18474.	13778.	5794.	2225.	1530.	372.	236.	63.	33.	6.	3.
250.	6	0.050	26402.	17613.	12891.	5302.	2033.	1358.	302.	207.	62.	29.	8.	7.
100.	6	0.050	25692.	17392.	12687.	5313.	2003.	1381.	323.	222.	60.	36.	27.	4.
0.	6	0.050	24879.	17119.	12892.	5520.	2089.	1354.	321.	236.	82.	75.	28.	14.
2000.	7	0.025	16241.	10640.	7227.	2872.	1091.	778.	200.	151.	41.	20.	6.	3.
1000.	7	0.050	27324.	18668.	14137.	6295.	2610.	1978.	569.	465.	157.	86.	24.	18.
500.	7	0.050	26757.	18386.	13450.	5793.	2266.	1576.	393.	290.	90.	51.	15.	6.
250.	7	0.050	25543.	17270.	12704.	5425.	2225.	1588.	444.	320.	88.	59.	14.	6.
100.	7	0.050	24377.	16900.	12512.	5207.	2024.	1378.	311.	211.	65.	32.	7.	4.
0.	7	0.050	23840.	16312.	12065.	5018.	1863.	1253.	258.	248.	58.	42.	9.	3.
2000.	8	0.050	29132.	19155.	13412.	5346.	2044.	1472.	433.	331.	190.	57.	6.	2.
1000.	8	0.050	28130.	19299.	14315.	5970.	2291.	1506.	348.	250.	66.	35.	7.	2.
500.	8	0.050	26144.	19332.	14406.	6294.	2482.	1666.	408.	314.	96.	49.	9.	5.
250.	8	0.050	26369.	18288.	13836.	5957.	2474.	1736.	434.	284.	76.	38.	10.	5.
100.	8	0.050	26386.	17915.	13083.	5591.	2130.	1427.	334.	262.	70.	35.	12.	5.
0.	8	0.050	27050.	17879.	13664.	6231.	2554.	1938.	440.	338.	94.	67.	16.	9.

SUBSTRATE: STARCH
SLUDGE: STARCH
SLUDGE CONCENTRATION: 2665.

IONIC MEDIUM: CACL2
IONIC STRENGTH: 0.010

SUBSTRATE CONC.	PH	DILUTION	PARTICLE COUNTS BETWEEN SIZES 1-2	2-3	3-4	4-5	5-6	6-8	8-10	10-15	15-20	20-30	30-40	40-60
4000.	6	0.020	36379.	16271.	6865.	1770.	561.	364.	114.	101.	50.	44.	12.	9.
2000.	6	0.020	35373.	14877.	6232.	1664.	533.	336.	107.	85.	31.	21.	4.	4.
1000.	6	0.020	34131.	14004.	5830.	1482.	438.	225.	55.	47.	17.	9.	0.	1.
500.	6	0.020	32897.	12738.	5407.	1323.	365.	192.	31.	25.	9.	11.	1.	1.
250.	6	0.020	33417.	13351.	5427.	1334.	401.	221.	67.	43.	24.	18.	5.	3.
0.	6	0.020	30144.	12036.	5115.	1346.	389.	238.	64.	69.	31.	34.	12.	7.
4000.	7	0.020	34963.	13930.	6582.	1768.	525.	346.	68.	48.	18.	10.	4.	1.
2000.	7	0.020	33102.	13936.	6582.	1768.	525.	346.	68.	48.	18.	10.	4.	1.
1000.	7	0.020	30747.	13164.	6269.	1764.	514.	314.	69.	57.	19.	22.	5.	4.
500.	7	0.020	34372.	14851.	6082.	1503.	408.	200.	42.	30.	8.	13.	1.	2.
250.	7	0.020	30357.	12229.	4795.	1197.	377.	246.	70.	59.	24.	11.	2.	1.
0.	7	0.020	10507.	3428.	969.	184.	63.	9.	9.	22.	13.	8.	4.	1.
4000.	8	0.020	36461.	15520.	6317.	1620.	469.	279.	72.	67.	29.	17.	6.	2.
2000.	8	0.020	33417.	13639.	5552.	1371.	386.	274.	61.	58.	22.	14.	7.	4.
1000.	8	0.020	31538.	13038.	5274.	1357.	411.	274.	86.	87.	38.	23.	7.	4.
500.	8	0.020	30911.	12450.	4790.	1208.	360.	223.	59.	54.	21.	10.	4.	3.
250.	8	0.020	37438.	10602.	4082.	1022.	2294.	197.	63.	71.	30.	42.	9.	11.
0.	8	0.020	25382.	9522.	3623.	925.	302.	195.	73.	70.	32.	28.	13.	6.

SUBSTRATE: JACK BEAN MEAL
SLUDGE: JACK BEAN MEAL
SLUDGE CONCENTRATION: 957.

IONIC MEDIUM: NACL
IONIC STRENGTH: 0.001

SUBSTRATE CONC.	PH	DILUTION	PARTICLE COUNTS BETWEEN SIZES 1-2	2-3	3-4	4-5	5-6	6-8	8-10	10-15	15-20	20-30	30-40	40-60
2000.	6	0.012	35557.	20086.	15827.	6877.	6887.	7575.	2489.	3178.	790.	262.	16.	3.
1000.	6	0.020	33608.	19274.	14603.	8550.	5533.	7929.	4378.	4851.	1645.	644.	56.	15.
500.	6	0.020	21231.	11124.	7920.	4640.	3053.	4720.	2818.	3458.	1199.	412.	17.	3.
250.	6	0.020	15260.	7737.	5596.	3597.	2559.	4330.	2916.	3988.	1489.	580.	43.	3.
100.	6	0.020	12117.	6009.	4041.	2678.	2025.	3817.	2768.	4054.	1581.	685.	43.	4.
0.	6	0.020	11106.	5239.	3606.	2525.	1953.	3628.	2751.	4082.	1618.	767.	82.	6.
2000.	7	0.020	47820.	26366.	21130.	12637.	8225.	11270.	5598.	5111.	1331.	447.	25.	7.
1000.	7	0.020	30954.	16527.	12796.	7672.	5199.	7776.	4438.	5168.	1650.	556.	181.	7.
500.	7	0.020	20019.	9578.	7614.	5074.	3793.	6629.	4722.	6841.	2689.	1010.	59.	3.
250.	7	0.020	15447.	7794.	6128.	4060.	3065.	5458.	4026.	5623.	2107.	761.	47.	6.
100.	7	0.040	23330.	11617.	8449.	5821.	4693.	9024.	7296.	11045.	4361.	1536.	80.	5.
0.	7	0.040	22080.	10565.	8165.	6009.	4750.	9608.	7161.	9932.	3648.	1317.	67.	4.
2000.	8	0.020	16388.	10981.	10367.	7198.	5234.	8020.	4369.	4376.	1079.	311.	22.	3.
1000.	8	0.020	11662.	7434.	6636.	4703.	3413.	5734.	3465.	4165.	1361.	495.	33.	3.
500.	8	0.020	8466.	5337.	5152.	3776.	2984.	5350.	3568.	4561.	1498.	487.	37.	2.
250.	8	0.040	14870.	8808.	8358.	6338.	5163.	10000.	7194.	10187.	3706.	1408.	114.	5.
100.	8	0.040	14319.	8211.	7943.	6248.	5236.	10536.	7904.	11188.	4200.	1657.	137.	8.
0.	8	0.040	14240.	8199.	7895.	6300.	5248.	10887.	8263.	12499.	5044.	2331.	245.	21.

SUBSTRATE: JACK BEAN MEAL
SLUDGE: JACK BEAN MEAL
SLUDGE CONCENTRATION: 2860.

IONIC MEDIUM: NACL
IONIC STRENGTH: 0.001

SUBSTRATE CONC.	PH	DILUTION	PARTICLE COUNTS BETWEEN SIZES 1-2	2-3	3-4	4-5	5-6	6-8	8-10	10-15	15-20	20-30	30-40	40-60
4000.	6	0.004	13902.	7961.	5731.	3233.	2012.	2887.	1510.	1518.	413.	136.	12.	7.
2000.	6	0.004	13108.	7320.	5586.	3349.	2217.	3103.	1560.	1505.	383.	95.	9.	3.
1000.	6	0.008	11151.	6278.	5025.	3408.	2464.	3967.	2293.	2390.	644.	199.	15.	5.
500.	6	0.012	12774.	7242.	6234.	4438.	3371.	5579.	3255.	3505.	966.	290.	9.	3.
250.	6	0.020	16295.	11565.	10925.	8367.	6622.	11342.	6858.	7754.	2271.	645.	20.	4.
0.	6	0.016	18261.	12042.	12022.	9429.	7365.	12612.	7761.	8248.	2269.	589.	16.	2.
4000.	7	0.004	18213.	10567.	8329.	5054.	3237.	4301.	1901.	1434.	216.	36.	3.	3.
2000.	7	0.008	13009.	7076.	5602.	3687.	2573.	3867.	2034.	1991.	475.	115.	4.	3.
1000.	7	0.012	14637.	8341.	7536.	5448.	4108.	6488.	3581.	3295.	650.	101.	3.	0.
500.	7	0.016	14884.	8725.	8080.	6053.	4528.	7485.	4248.	4170.	951.	181.	6.	1.
250.	7	0.020	16793.	12326.	12277.	9554.	7258.	12099.	6602.	5745.	918.	111.	5.	0.
0.	7	0.016	17730.	12084.	12461.	9879.	7626.	12564.	7047.	6748.	1292.	228.	11.	8.
4000.	8	0.004	23000.	14052.	11836.	7374.	4735.	5989.	2292.	1380.	143.	22.	7.	2.
2000.	8	0.008	17695.	10324.	8993.	5954.	4184.	6043.	2927.	2181.	319.	38.	6.	1.
1000.	8	0.012	17202.	10557.	10011.	6932.	5082.	7764.	4034.	3251.	531.	75.	2.	1.
500.	8	0.016	19343.	12255.	11894.	8983.	6661.	10413.	5570.	4583.	708.	94.	5.	2.
250.	8	0.020	20599.	13968.	14452.	11405.	8922.	14638.	8130.	7096.	1205.	143.	3.	0.
0.	8	0.016	19590.	13346.	13526.	10301.	7799.	11911.	6111.	4588.	662.	77.	3.	1.

SUBSTRATE: JACK BEAN MEAL
SLUDGE: JACK BEAN MEAL
SLUDGE CONCENTRATION: 933.

IONIC MEDIUM: CACL2
IONIC STRENGTH: 0.001

SUBSTRATE CONC.	PH	DILUTION	PARTICLE COUNTS BETWEEN SIZES 1-2	2-3	3-4	4-5	5-6	6-8	8-10	10-15	15-20	20-30	30-40	40-60
2000.	6	0.008	28402.	17891.	14562.	8587.	5335.	6678.	3497.	3156.	962.	399.	72.	24.
1000.	6	0.012	20293.	11331.	8856.	5142.	3222.	4454.	2420.	2734.	1036.	552.	66.	9.
500.	6	0.020	17243.	9862.	7923.	4699.	3161.	4657.	3037.	4038.	1620.	783.	72.	12.
250.	6	0.020	10650.	6431.	5076.	3180.	2296.	4041.	2911.	4594.	2106.	1088.	135.	14.
100.	6	0.020	10539.	6226.	4960.	3274.	2555.	4860.	3664.	5799.	2461.	1129.	112.	9.
0.	6	0.020	8633.	4839.	3985.	2819.	2364.	4887.	3895.	6398.	2953.	1553.	199.	20.
2000.	7	0.020	37814.	24045.	20540.	12503.	8230.	11160.	5531.	5204.	1300.	480.	37.	9.
1000.	7	0.020	27832.	16997.	13624.	8351.	5444.	7619.	4369.	5022.	1757.	686.	49.	4.
500.	7	0.020	18246.	10870.	8763.	5539.	3828.	6115.	3980.	5331.	2059.	832.	64.	5.
250.	7	0.020	13423.	8269.	6740.	4404.	3240.	5636.	4046.	5752.	2304.	1006.	89.	11.
100.	7	0.020	10242.	6282.	5127.	3348.	2666.	4950.	3775.	5505.	2274.	900.	59.	4.
0.	7	0.020	9404.	5702.	4589.	3199.	2556.	4880.	3783.	5947.	2576.	1176.	111.	11.
2000.	8	0.020	36270.	21572.	17667.	11020.	7331.	10499.	5487.	5515.	1533.	544.	35.	2.
1000.	8	0.020	23492.	13529.	10974.	6992.	4796.	7147.	4366.	5138.	1782.	738.	64.	14.
500.	8	0.020	16552.	9646.	7882.	5133.	3693.	6155.	4151.	5563.	2133.	850.	57.	5.
250.	8	0.020	12640.	7270.	5943.	3991.	2932.	5145.	3649.	5201.	1982.	830.	66.	4.
100.	8	0.020	10337.	5958.	4985.	3451.	2720.	5067.	3813.	5631.	2213.	898.	61.	6.
0.	8	0.020	10053.	6060.	5228.	3618.	3108.	6047.	4684.	6625.	2576.	1044.	101.	12.

SUBSTRATE: JACK BEAN MEAL
SLUDGE: JACK BEAN MEAL
SLUDGE CONCENTRATION: 2740.

IONIC MEDIUM: CACL2
IONIC STRENGTH: 0.001

SUBSTRATE CONC.	PH	DILUTION	PARTICLE COUNTS BETWEEN SIZES 1-2	2-3	3-4	4-5	5-6	6-8	8-10	10-15	15-20	20-30	30-40	40-60
4000.	6	0.008	21038.	12389.	9319.	5225.	3152.	4059.	1976.	1728.	370.	124.	23.	8.
2000.	6	0.016	23077.	16247.	15579.	11206.	8362.	13411.	7689.	7184.	1610.	454.	54.	22.
1000.	6	0.020	16632.	10956.	10694.	7996.	6163.	10267.	6051.	5888.	1403.	379.	35.	15.
500.	6	0.020	15060.	10537.	10850.	8707.	6939.	11966.	7317.	7813.	2092.	594.	33.	8.
250.	6	0.020	13592.	9618.	10166.	8291.	6608.	11315.	7051.	7150.	1668.	354.	14.	7.
0.	6	0.020	15234.	11072.	11693.	9134.	1196.	12020.	7081.	6844.	1483.	286.	12.	4.
4000.	7	0.004	27228.	17697.	1588.	10354.	6799.	9774.	4562.	3346.	464.	81.	10.	12.
2000.	7	0.012	26767.	19754.	16795.	13243.	9704.	14998.	7896.	6400.	1048.	159.	11.	6.
1000.	7	0.016	21096.	14471.	14560.	11183.	8435.	13162.	7036.	5503.	826.	103.	8.	2.
500.	7	0.020	19392.	13369.	13752.	10424.	8062.	12866.	6948.	5730.	880.	124.	15.	5.
250.	7	0.020	17746.	13177.	14494.	11954.	9613.	16367.	9602.	8484.	1552.	223.	7.	2.
0.	7	0.020	16492.	12657.	14071.	11839.	9583.	16785.	10168.	9661.	1831.	316.	7.	4.
4000.	8	0.004	19369.	11603.	9741.	5956.	3902.	4918.	2143.	1452.	186.	53.	6.	2.
2000.	8	0.010	23937.	15215.	13722.	8962.	6148.	8140.	3398.	2072.	195.	25.	4.	2.
1000.	8	0.016	21681.	14010.	13190.	8971.	6135.	8295.	3342.	1610.	132.	19.	2.	0.
500.	8	0.020	19302.	13767.	14196.	10620.	7848.	11168.	5075.	3363.	377.	44.	4.	2.
250.	8	0.020	22262.	16058.	16504.	13089.	9765.	14514.	6935.	4826.	570.	86.	6.	4.
0.	8	0.020	20499.	14861.	16012.	12721.	9984.	16261.	9007.	7653.	1271.	160.	9.	7.

SUBSTRATE: JACK BEAN MEAL
SLUDGE: JACK BEAN MEAL
SLUDGE CONCENTRATION: 1020.

IONIC MEDIUM: NACL
IONIC STRENGTH: 0.010

SUBSTRATE CONC.	PH	DILUTION	PARTICLE COUNTS BETWEEN SIZES 1-2	2-3	3-4	4-5	5-6	6-8	8-10	10-15	15-20	20-30	30-40	40-60
2000.	6	0.020	6501.	3295.	2631.	1562.	1032.	1515.	790.	909.	310.	156.	33.	25.
1000.	6	0.020	4791.	2756.	2337.	2714.	1090.	1163.	1067.	1314.	504.	206.	21.	6.
500.	6	0.020	6269.	3507.	2901.	1935.	1346.	2188.	1422.	1766.	633.	241.	29.	13.
250.	6	0.020	4631.	2940.	2607.	1643.	1303.	2158.	1306.	1536.	450.	128.	5.	6.
100.	6	0.020	1977.	4183.	3768.	2504.	1745.	2828.	1701.	1862.	531.	146.	18.	11.
0.	6	0.020	10332.	6950.	6266.	4313.	3029.	4630.	2603.	2503.	544.	110.	7.	4.
2000.	7	0.020	6110.	3852.	3588.	2478.	1805.	2875.	1674.	1819.	553.	215.	26.	11.
1000.	7	0.020	4381.	2617.	2219.	1480.	990.	1557.	928.	1037.	323.	133.	12.	2.
500.	7	0.020	4632.	2958.	2706.	1850.	1320.	2146.	1266.	1333.	331.	80.	7.	5.
250.	7	0.020	4540.	2886.	2595.	1721.	1177.	1889.	1040.	1082.	279.	51.	2.	1.
100.	7	0.020	6054.	4035.	3679.	2551.	1787.	2702.	1467.	1332.	241.	49.	6.	7.
0.	7	0.020	7955.	5562.	5150.	3565.	2500.	3772.	2070.	1990.	452.	79.	3.	1.
2000.	8	0.020	5391.	3576.	3326.	2289.	1610.	2577.	1433.	1636.	547.	205.	22.	6.
1000.	8	0.020	4056.	2637.	2386.	1649.	1160.	1835.	1057.	1202.	448.	187.	11.	2.
500.	8	0.020	4209.	2787.	2569.	1748.	1230.	1970.	1236.	1435.	440.	139.	9.	2.
250.	8	0.020	4699.	3149.	2956.	2025.	1450.	2231.	1261.	1270.	297.	65.	3.	2.
100.	8	0.020	5600.	4070.	3672.	2561.	1792.	2802.	1533.	1504.	325.	69.	2.	2.
0.	8	0.020	8049.	5456.	5037.	3474.	2439.	3647.	1941.	1801.	394.	76.	0.	1.

SUBSTRATE: JACK BEAN MEAL
SLUDGE: JACK BEAN MEAL
SLUDGE CONCENTRATION: 910.

IONIC MEDIUM: CACL2
IONIC STRENGTH: 0.010

SUBSTRATE CONC.	PH	DILUTION	PARTICLE COUNTS BETWEEN SIZES 1-2	2-3	3-4	4-5	5-6	6-8	8-10	10-15	15-20	20-30	30-40	40-60
2000.	6	0.012	10543.	6754.	5480.	3464.	2399.	3545.	1634.	1872.	674.	380.	62.	12.
1000.	6	0.020	12276.	6623.	4114.	2140.	1452.	2142.	1279.	1601.	650.	337.	47.	13.
500.	6	0.020	11511.	5293.	2747.	1338.	910.	1414.	976.	1339.	528.	243.	28.	10.
250.	6	0.020	10535.	4651.	2347.	1161.	817.	1370.	996.	1406.	565.	251.	25.	6.
100.	6	0.020	10184.	4660.	2509.	1152.	800.	1381.	997.	1409.	571.	249.	23.	3.
0.	6	0.020	11846.	5797.	3584.	2148.	1609.	2909.	2031.	2851.	1156.	572.	79.	10.
2000.	7	0.012	10131.	6975.	7149.	5551.	4240.	6304.	3003.	2423.	596.	200.	12.	2.
1000.	7	0.020	7576.	3758.	2553.	1848.	1436.	2210.	1332.	1381.	541.	276.	31.	6.
500.	7	0.020	7915.	3761.	2266.	1497.	1150.	1929.	1220.	1468.	552.	237.	19.	3.
250.	7	0.020	6585.	2663.	1011.	476.	390.	554.	383.	597.	313.	205.	34.	7.
100.	7	0.020	8170.	3146.	1370.	668.	565.	1148.	935.	1404.	588.	252.	20.	3.
0.	7	0.020	7779.	3071.	1296.	544.	426.	763.	595.	1010.	539.	326.	57.	15.
2000.	8	0.016	17175.	11076.	10708.	8328.	6415.	9958.	5192.	4928.	1356.	515.	36.	7.
1000.	8	0.020	11214.	5026.	3206.	2076.	1536.	2503.	1565.	2007.	756.	359.	39.	5.
500.	8	0.020	9585.	3935.	2097.	1312.	1062.	1941.	1424.	2147.	801.	353.	38.	3.
250.	8	0.020	9662.	4052.	2055.	1215.	937.	1768.	1263.	1843.	744.	327.	25.	4.
100.	8	0.020	9673.	3750.	2106.	1205.	994.	1905.	1498.	2204.	1002.	503.	52.	3.
0.	8	0.020	8018.	3236.	1699.	991.	740.	1369.	943.	1426.	613.	306.	43.	2.

SUBSTRATE: JACK BEAN MEAL
SLUDGE: JACK BEAN MEAL
SLUDGE CONCENTRATION: 2645.

IONIC MEDIUM: CACL2
IONIC STRENGTH: 0.010

SUBSTRATE CONC.	PH	DILUTION	PARTICLE COUNTS BETWEEN SIZES 1-2	2-3	3-4	4-5	5-6	6-8	8-10	10-15	15-20	20-30	30-40	40-60
4000.	6	0.020	7544.	4369.	3674.	1949.	1044.	1244.	566.	610.	244.	131.	21.	6.
2000.	6	0.025	8035.	4815.	3634.	1799.	880.	940.	442.	510.	194.	102.	21.	6.
1000.	6	0.025	6823.	4083.	3168.	1372.	618.	576.	271.	291.	115.	62.	6.	3.
500.	6	0.025	7713.	4578.	3370.	1460.	688.	629.	283.	332.	134.	81.	15.	5.
250.	6	0.025	8518.	4932.	3563.	1550.	699.	697.	340.	390.	157.	89.	17.	5.
0.	6	0.025	10573.	6443.	4262.	1808.	820.	799.	376.	420.	170.	133.	31.	12.
4000.	7	0.025	7782.	5559.	5064.	3139.	1940.	2670.	1367.	1486.	554.	221.	28.	3.
2000.	7	0.025	5114.	3234.	2628.	1262.	633.	687.	365.	467.	223.	159.	38.	12.
1000.	7	0.025	6516.	3938.	3004.	1411.	677.	756.	414.	525.	230.	141.	31.	6.
500.	7	0.025	4507.	2647.	2030.	791.	387.	369.	181.	260.	107.	75.	22.	8.
250.	7	0.025	5469.	3172.	2394.	1049.	487.	546.	324.	413.	176.	117.	21.	4.
0.	7	0.025	7230.	3717.	2646.	1156.	532.	651.	367.	470.	199.	121.	30.	7.
4000.	8	0.010	36122.	16487.	9609.	4471.	2517.	3261.	1767.	2023.	640.	242.	19.	3.
2000.	8	0.025	8252.	4225.	3298.	1870.	1055.	1538.	935.	1200.	473.	250.	25.	6.
1000.	8	0.025	4679.	2599.	1954.	950.	507.	755.	492.	652.	262.	140.	17.	4.
500.	8	0.025	4226.	2339.	1644.	678.	347.	457.	305.	416.	162.	100.	18.	9.
250.	8	0.025	4225.	2241.	1591.	599.	264.	345.	225.	328.	159.	87.	16.	3.
0.	8	0.025	4085.	2107.	1411.	533.	222.	235.	148.	262.	118.	95.	27.	12.

SUBSTRATE: JACK BEAN MEAL
SLUDGE: JACK BEAN MEAL
SLUDGE CONCENTRATION: 2785.

IONIC MEDIUM: CACL2
IONIC STRENGTH: 0.010

SUBSTRATE CONC.	PH	DILUTION	PARTICLE COUNTS BETWEEN SIZES 1-2	2-3	3-4	4-5	5-6	6-8	8-10	10-15	15-20	20-30	30-40	40-60
4000.	6	0.008	4949.	2492.	1701.	684.	504.	636.	293.	317.	133.	73.	15.	7.
2000.	6	0.020	7960.	4061.	2681.	1322.	745.	897.	498.	593.	241.	131.	20.	5.
1000.	6	0.020	7364.	3647.	2652.	1296.	783.	1012.	522.	650.	245.	130.	9.	1.
500.	6	0.020	7463.	3877.	2680.	1302.	753.	1010.	540.	702.	284.	129.	12.	1.
250.	6	0.020	6977.	3476.	2471.	1307.	742.	1110.	652.	899.	356.	191.	16.	4.
0.	6	0.020	7310.	4111.	3134.	1781.	1201.	1829.	1103.	1548.	684.	398.	57.	9.
4000.	7	0.020	9294.	4685.	3422.	1907.	1229.	1669.	809.	804.	236.	123.	20.	5.
2000.	7	0.020	7026.	3738.	2432.	1141.	645.	799.	391.	451.	215.	132.	22.	7.
1000.	7	0.020	7441.	4118.	2832.	1448.	848.	1189.	674.	799.	290.	138.	17.	2.
500.	7	0.020	5483.	3060.	2065.	997.	532.	674.	344.	432.	187.	95.	11.	4.
250.	7	0.020	6793.	3927.	2858.	1483.	860.	1258.	647.	833.	286.	114.	11.	1.
0.	7	0.020	5325.	3212.	2309.	1212.	702.	1018.	549.	724.	318.	192.	22.	4.
4000.	8	0.020	9166.	4968.	3734.	2283.	1490.	2246.	1163.	1240.	428.	201.	25.	4.
2000.	8	0.020	6655.	3573.	2616.	1425.	893.	1248.	654.	817.	334.	158.	15.	1.
1000.	8	0.020	7248.	4107.	3027.	1765.	1099.	1550.	877.	1054.	394.	151.	14.	3.
500.	8	0.020	5386.	3194.	2386.	1334.	869.	1211.	765.	862.	320.	137.	6.	2.
250.	8	0.020	5025.	2995.	2388.	1415.	897.	1395.	876.	1163.	477.	230.	19.	0.
0.	8	0.020	3458.	2095.	1517.	849.	550.	800.	478.	714.	344.	227.	28.	8.

APPENDIX B-2-b

Substrate-Sludge Particle Counts (5-300 micron)

SUBSTRATE: ALBUMIN
SLUDGE: ALBUMIN
SLUDGE CONCENTRATION: 1055.

IONIC MEDIUM: NACL
IONIC STRENGTH: 0.010

SUBSTRATE CONC.	PH	PARTICLE COUNTS BETWEEN SIZES 5-10	10-15	15-20	20-25	25-30	30-40	40-50	50-60	60-80	80-100	100-200	200-300
2000.	6	2529.	1424.	1113.	925.	707.	1045.	627.	330.	491.	226.	263.	30.
1000.	6	1950.	1146.	971.	762.	584.	837.	478.	290.	369.	182.	195.	15.
500.	6	1349.	885.	789.	636.	479.	667.	423.	232.	309.	155.	168.	15.
250.	6	1291.	948.	846.	685.	518.	736.	428.	241.	337.	170.	168.	14.
100.	6	1234.	959.	849.	667.	497.	778.	412.	259.	330.	174.	172.	16.
0.	6	1240.	987.	882.	734.	554.	798.	461.	267.	339.	182.	180.	22.
2000.	7	2544.	1518.	1292.	1028.	780.	1058.	629.	374.	483.	250.	266.	23.
1000.	7	1896.	1248.	1078.	886.	639.	900.	521.	308.	415.	194.	214.	19.
500.	7	1462.	1117.	959.	789.	605.	800.	503.	280.	373.	190.	213.	19.
250.	7	1415.	1065.	932.	756.	551.	772.	477.	253.	344.	164.	188.	19.
100.	7	1291.	1046.	923.	727.	551.	765.	462.	294.	354.	197.	213.	15.
0.	7	1265.	1004.	849.	692.	509.	713.	442.	237.	329.	152.	188.	12.
2000.	8	1518.	933.	776.	577.	440.	569.	357.	193.	244.	124.	124.	10.
1000.	8	1444.	978.	847.	677.	490.	686.	409.	201.	242.	146.	162.	13.
500.	8	1416.	1113.	962.	769.	555.	776.	493.	266.	319.	179.	190.	15.
250.	8	1371.	1063.	942.	736.	541.	764.	518.	265.	364.	172.	190.	15.
100.	8	1331.	1062.	953.	764.	574.	771.	484.	271.	375.	190.	213.	19.
0.	8	1375.	1101.	953.	771.	566.	823.	497.	275.	360.	205.	203.	19.

SUBSTRATE: ALBUMIN
SLUDGE: ALBUMIN
SLUDGE CONCENTRATION: 2825.

IONIC MEDIUM: NACL
IONIC STRENGTH: 0.010

SUBSTRATE CONC.	PH	PARTICLE COUNTS BETWEEN SIZES 5-10	10-15	15-20	20-25	25-30	30-40	40-50	50-60	60-80	80-100	100-200	200-300
4000.	6	2901.	1599.	1238.	1023.	755.	1089.	691.	374.	539.	262.	261.	24.
2000.	6	2561.	1671.	1353.	1059.	849.	1160.	730.	455.	585.	281.	284.	25.
1000.	6	1964.	1350.	1150.	988.	745.	1026.	621.	359.	475.	253.	260.	15.
500.	6	1808.	1276.	1136.	937.	716.	1006.	610.	354.	465.	228.	243.	19.
250.	6	1714.	1299.	1123.	964.	716.	1032.	659.	364.	481.	256.	267.	28.
0.	6	2048.	1547.	1356.	1153.	924.	1285.	779.	451.	606.	325.	372.	36.
4000.	7	2589.	1589.	1335.	1072.	805.	1121.	688.	410.	529.	281.	259.	21.
2000.	7	2424.	1649.	1463.	1166.	881.	1225.	745.	429.	576.	279.	280.	27.
1000.	7	2046.	1496.	1320.	1044.	806.	1079.	694.	394.	510.	257.	262.	19.
500.	7	2253.	1698.	1515.	1254.	968.	1329.	841.	476.	634.	333.	326.	30.
250.	7	2074.	1597.	1446.	1194.	922.	1216.	782.	448.	614.	309.	295.	27.
0.	7	2030.	1599.	1439.	1205.	884.	1247.	779.	443.	623.	319.	343.	33.
4000.	8	2576.	1762.	1416.	1169.	854.	1169.	729.	427.	555.	269.	276.	26.
2000.	8	2242.	1631.	1365.	1109.	804.	1159.	721.	413.	266.	267.	285.	19.
1000.	8	2112.	1613.	1431.	1144.	837.	1190.	749.	437.	586.	296.	311.	21.
500.	8	2072.	1539.	1385.	1118.	808.	1150.	734.	421.	581.	293.	304.	29.
250.	8	2065.	1595.	1438.	1189.	858.	1244.	795.	447.	603.	314.	320.	24.
0.	8	1973.	1520.	1310.	1096.	789.	1101.	671.	403.	568.	291.	330.	33.

SUBSTRATE: ALBUMIN
SLUDGE: ALBUMIN
SLUDGE CONCENTRATION: 925.

IONIC MEDIUM: CACL2
IONIC STRENGTH: 0.010

SUBSTRATE CONC.	PH	PARTICLE COUNTS BETWEEN SIZES 5-10	10-15	15-20	20-25	25-30	30-40	40-50	50-60	60-80	80-100	100-200	200-300
2000.	6	3148.	2559.	2107.	1627.	1259.	1692.	957.	450.	512.	221.	214.	34.
1000.	6	3233.	2799.	2370.	1746.	1249.	1755.	844.	398.	438.	177.	184.	25.
500.	6	2799.	2270.	1861.	1328.	912.	1209.	605.	295.	343.	150.	154.	22.
250.	6	2532.	2068.	1646.	1169.	858.	1111.	588.	268.	347.	155.	186.	32.
100.	6	1910.	1603.	1298.	961.	724.	949.	490.	216.	282.	131.	144.	30.
0.	6	2738.	2120.	1589.	1064.	805.	1026.	494.	241.	277.	139.	160.	33.
2000.	7	3214.	2944.	2651.	2074.	1610.	2182.	1160.	577.	667.	251.	274.	43.
1000.	7	3001.	2577.	2180.	1543.	1134.	1456.	768.	344.	394.	174.	180.	22.
500.	7	3298.	2684.	2113.	1450.	1017.	1309.	671.	317.	379.	169.	192.	31.
250.	7	2906.	2371.	1866.	1307.	947.	1210.	642.	304.	361.	169.	178.	34.
100.	7	2320.	1941.	1561.	1167.	774.	1045.	549.	267.	325.	134.	177.	29.
0.	7	1999.	1630.	1181.	802.	576.	804.	416.	194.	214.	102.	123.	24.
2000.	8	2524.	2196.	2087.	1613.	1254.	1730.	908.	439.	498.	209.	196.	25.
1000.	8	2181.	1956.	1849.	1407.	1054.	1400.	743.	368.	404.	181.	211.	27.
500.	8	2145.	1756.	1566.	1113.	817.	1096.	562.	261.	310.	1133.	144.	19.
250.	8	2052.	1632.	1450.	1008.	799.	1050.	562.	258.	315.	127.	150.	21.
100.	8	1965.	1528.	1270.	879.	642.	922.	470.	239.	277.	125.	140.	22.
0.	8	2721.	2049.	1547.	1047.	737.	922.	466.	232.	272.	128.	144.	30.

SUBSTRATE: ALBUMIN
SLUDGE: ALBUMIN
SLUDGE CONCENTRATION: 2770.

IONIC MEDIUM: CACL2
IONIC STRENGTH: 0.010

SUBSTRATE CONC.	PH	PARTICLE COUNTS BETWEEN SIZES 5-10	10-15	15-20	20-25	25-30	30-40	40-50	50-60	60-80	80-100	100-200	200-300
4000.	6	3039.	2394.	2145.	1670.	1266.	1870.	1076.	575.	732.	361.	499.	176.
2000.	6	3786.	3059.	2555.	1964.	1524.	2285.	1325.	724.	956.	443.	621.	135.
1000.	6	3762.	2935.	2406.	1828.	1397.	1927.	1046.	528.	633.	276.	346.	49.
500.	6	2746.	2060.	1701.	1217.	908.	1343.	708.	372.	467.	222.	351.	89.
250.	6	3587.	2768.	2360.	1733.	1281.	1819.	962.	506.	605.	282.	392.	88.
0.	6	4214.	3105.	2414.	1696.	1294.	1752.	925.	436.	537.	263.	345.	78.
4000.	7	3661.	2728.	2245.	1628.	1250.	1696.	935.	474.	527.	257.	289.	61.
2000.	7	3980.	2916.	2249.	1598.	1128.	1526.	767.	400.	450.	194.	236.	47.
1000.	7	3763.	2813.	2172.	1505.	1146.	1545.	809.	392.	471.	220.	271.	48.
500.	7	3568.	2603.	2000.	982.	984.	1327.	704.	344.	402.	187.	221.	46.
250.	7	3257.	2427.	1873.	928.	930.	1231.	578.	309.	369.	155.	204.	38.
0.	7	3034.	2266.	1782.	919.	921.	1187.	599.	280.	369.	162.	194.	34.
4000.	8	3366.	2656.	2275.	1686.	1272.	1785.	958.	462.	583.	244.	285.	53.
2000.	8	3082.	2670.	2283.	1822.	1371.	1967.	1115.	564.	723.	310.	416.	71.
1000.	8	2975.	2374.	2009.	1474.	1054.	1475.	824.	383.	467.	209.	235.	40.
500.	8	3279.	2607.	2120.	1517.	1162.	1594.	828.	417.	507.	228.	264.	52.
250.	8	2908.	2153.	1725.	1205.	854.	1144.	638.	285.	343.	162.	216.	46.
0.	8	2934.	2098.	1591.	1106.	827.	1062.	552.	266.	344.	150.	181.	37.

SUBSTRATE: STARCH
SLUDGE: STARCH
SLUDGE CONCENTRATION: 990.

IONIC MEDIUM: NACL
IONIC STRENGTH: 0.001

SUBSTRATE CONC.	PH	PARTICLE COUNTS BETWEEN SIZES 5-10	10-15	15-20	20-25	25-30	30-40	40-50	50-60	60-80	80-100	100-200	200-300
2000.	6	2985.	2489.	2032.	1495.	1022.	1108.	381.	114.	70.	21.	15.	1.
1000.	6	3793.	3414.	2789.	1997.	1353.	1362.	476.	149.	100.	25.	14.	1.
500.	6	3702.	3337.	2954.	2096.	1430.	1573.	592.	179.	101.	29.	14.	1.
250.	6	3749.	3464.	2931.	2055.	1270.	1259.	407.	131.	92.	25.	13.	0.
100.	6	3546.	3368.	2914.	2019.	1299.	1390.	467.	147.	92.	25.	15.	0.
0.	6	4373.	4092.	3341.	2387.	1557.	1649.	576.	164.	106.	38.	17.	1.
2000.	7	2773.	2816.	2685.	2117.	1576.	1740.	612.	169.	119.	36.	12.	1.
1000.	7	2447.	2284.	2062.	1532.	1044.	1256.	501.	169.	103.	22.	9.	0.
500.	7	3559.	3230.	2667.	1860.	1151.	1210.	383.	122.	83.	23.	9.	0.
250.	7	3105.	2755.	2186.	1503.	931.	914.	310.	89.	66.	17.	13.	0.
100.	7	3370.	3157.	2702.	2035.	1333.	1436.	254.	150.	103.	26.	17.	0.
0.	7	4267.	3718.	2874.	1941.	1174.	1168.	403.	103.	94.	21.	16.	1.
2000.	8	2737.	2698.	2380.	1771.	1144.	779.	351.	84.	55.	18.	11.	0.
1000.	8	2476.	2340.	1961.	1342.	830.	836.	248.	67.	48.	13.	10.	0.
500.	8	3580.	3367.	2676.	2068.	1304.	1299.	432.	122.	85.	26.	16.	0.
250.	8	4359.	4077.	3472.	2414.	1544.	1547.	500.	161.	100.	33.	19.	1.
100.	8	3664.	3397.	2816.	1878.	1136.	1044.	323.	86.	63.	18.	11.	1.
0.	8	4684.	4296.	3618.	2505.	1530.	1474.	457.	152.	93.	21.	19.	0.

SUBSTRATE: STAICH
SLUDGE: STARCH
SLUDGE CONCENTRATION: 2725.

IONIC MEDIUM: NACL
IONIC STRENGTH: 0.001

SUBSTRATE CONC.	PH	PARTICLE COUNTS BETWEEN SIZES 5-10	10-15	15-20	20-25	25-30	30-40	40-50	50-60	60-80	80-100	100-200	200-300
4000.	6	1450.	1507.	1574.	1518.	1496.	2895.	2198.	2265.	463.	485.	221.	1.
2000.	6	631.	668.	677.	804.	717.	1414.	1097.	1244.	291.	314.	135.	2.
1000.	6	775.	839.	785.	710.	690.	1410.	1291.	1734.	483.	605.	304.	3.
500.	6	733.	811.	870.	830.	819.	1604.	1339.	1463.	316.	340.	146.	1.
250.	6	1048.	1111.	1164.	1153.	1091.	2105.	1818.	2096.	471.	536.	229.	3.
0.	6	1364.	1483.	1578.	1547.	1368.	2473.	1657.	1362.	254.	241.	120.	3.
4000.	7	1606.	1793.	1708.	1440.	1312.	2339.	1562.	1300.	227.	204.	100.	1.
2000.	7	1596.	1754.	1575.	1634.	1469.	2654.	1842.	1591.	272.	268.	123.	3.
1000.	7	1348.	1648.	1632.	1470.	1309.	2469.	1906.	1965.	373.	388.	163.	0.
500.	7	1263.	1421.	1444.	1261.	1220.	2361.	1937.	2170.	461.	471.	216.	4.
250.	7	1261.	1558.	1667.	1564.	1422.	2633.	1897.	1794.	317.	326.	134.	3.
0.	7	2192.	2430.	2551.	2260.	2004.	3317.	1982.	1494.	249.	227.	116.	1.
4000.	8	2155.	2266.	2259.	1992.	1770.	2949.	1862.	1513.	247.	247.	133.	3.
2000.	8	1508.	1552.	1548.	1341.	1196.	1903.	1304.	1020.	156.	148.	78.	1.
1000.	8	1748.	1877.	1862.	1690.	1544.	2819.	2043.	1847.	342.	317.	159.	3.
500.	8	1710.	1795.	1703.	1458.	1216.	2117.	1531.	1441.	277.	268.	118.	0.
250.	8	1619.	1900.	1899.	1628.	1357.	2468.	1714.	1594.	299.	306.	132.	1.
0.	8	2195.	2448.	2474.	2219.	1900.	2997.	1772.	1312.	204.	194.	84.	1.

SUBSTRATE: STARCH
SLUDGE: STARCH
SLUDGE CONCENTRATION: 885.

IONIC MEDIUM: CACL2
IONIC STRENGTH: 0.001

SUBSTRATE CONC.	PH	PARTICLE COUNTS BETWEEN SIZES 5-10	10-15	15-20	20-25	25-30	30-40	40-50	50-60	60-80	80-100	100-200	200-300
2000.	6	1614.	1837.	2192.	2221.	2123.	3324.	1895.	747.	479.	86.	36.	1.
1000.	6	1358.	1429.	1519.	1531.	1301.	2233.	1376.	631.	550.	125.	63.	1.
500.	6	1781.	1803.	1989.	1988.	1832.	3307.	2149.	1094.	1069.	264.	88.	2.
250.	6	1369.	1387.	1529.	1505.	1310.	2158.	1259.	573.	480.	116.	47.	1.
100.	6	1493.	1571.	1773.	1818.	1750.	3080.	2096.	4034.	940.	245.	104.	1.
0.	6	1637.	1508.	1430.	1271.	982.	1260.	532.	174.	120.	22.	15.	0.
2000.	7	1273.	1310.	1578.	1560.	1426.	2461.	1418.	616.	461.	83.	30.	1.
1000.	7	2114.	2092.	2191.	2124.	1824.	2980.	1613.	686.	494.	90.	44.	1.
500.	7	1856.	2009.	2200.	2057.	1884.	3074.	1786.	759.	621.	132.	58.	2.
250.	7	2331.	2364.	2472.	2249.	1954.	3045.	1649.	695.	432.	99.	40.	1.
100.	7	1726.	1672.	1784.	1713.	1501.	2400.	1371.	546.	435.	100.	40.	1.
0.	7	2337.	2343.	2529.	2352.	2036.	3198.	1662.	646.	435.	76.	36.	1.
2000.	8	1486.	1675.	1947.	1249.	1717.	2866.	1625.	651.	455.	72.	38.	1.
1000.	8	2510.	2395.	2394.	2119.	1815.	2712.	1334.	489.	345.	68.	38.	1.
500.	8	2092.	2100.	2079.	1883.	1620.	2404.	1257.	482.	305.	61.	26.	0.
250.	8	2420.	2386.	2514.	2401.	2137.	3375.	1894.	756.	555.	111.	46.	0.
100.	8	2071.	2052.	2238.	2147.	1974.	3208.	1844.	766.	545.	95.	32.	0.
0.	8	2030.	1748.	1541.	1282.	1006.	1259.	535.	167.	113.	19.	15.	0.

SUBSTRATE: STARCH
SLUDGE: STARCH
SLUDGE CONCENTRATION: 2000.

IONIC MEDIUM: CACL2
IONIC STRENGTH: 0.001

SUBSTRATE CONC.	PH	PARTICLE COUNTS BETWEEN SIZES 5-10	10-15	15-20	20-25	25-30	30-40	40-50	50-60	60-80	80-100	100-200	200-300
4000.	6	1118.	811.	774.	720.	696.	1503.	1243.	739.	890.	298.	155.	2.
2000.	6	965.	739.	761.	733.	730.	1504.	1263.	763.	867.	299.	153.	4.
1000.	6	1475.	1059.	1102.	1170.	1212.	2731.	2548.	1715.	2472.	1006.	533.	8.
500.	6	1090.	685.	658.	649.	664.	1497.	1342.	925.	1350.	582.	325.	325.
250.	6	1103.	813.	799.	779.	829.	1724.	1501.	1021.	1387.	601.	335.	2.
0.	6	1649.	1505.	1574.	1535.	1501.	2876.	2143.	1143.	7226.	386.	157.	3.
4000.	7	2003.	1618.	1556.	1532.	1460.	3051.	2436.	1308.	1392.	394.	156.	2.
2000.	7	1550.	1218.	1230.	1234.	1284.	2889.	2566.	1576.	1898.	614.	256.	4.
1000.	7	1790.	1372.	1344.	1385.	1394.	2848.	2260.	1298.	1505.	453.	187.	2.
500.	7	1706.	1242.	1273.	1387.	1415.	3159.	2737.	1723.	2184.	752.	272.	3.
250.	7	1122.	754.	740.	751.	783.	1623.	1360.	823.	966.	359.	157.	3.
0.	7	1456.	1207.	1358.	1381.	1374.	2928.	2360.	1373.	1516.	441.	163.	1.
4000.	8	2171.	1726.	1729.	1641.	1581.	2993.	2257.	1198.	1221.	342.	143.	1.
2000.	8	1376.	1107.	1113.	1098.	1152.	2435.	2027.	1242.	1443.	494.	188.	3.
1000.	8	1612.	1286.	1301.	1324.	1377.	2877.	2561.	1620.	1924.	635.	258.	2.
500.	8	1478.	1136.	1156.	1228.	1276.	2878.	2568.	1669.	2108.	730.	308.	5.
250.	8	1678.	1182.	1209.	1263.	1294.	2777.	2389.	1525.	1890.	630.	252.	3.
0.	8	1394.	1198.	1284.	1333.	1321.	2720.	2252.	1260.	1376.	397.	145.	2.

SUBSTRATE: STARCH
SLUDGE: STARCH
SLUDGE CONCENTRATION: 2650.

IONIC MEDIUM: NACL
IONIC STRENGTH: 0.010

SUBSTRATE CONC.	PH	PARTICLE COUNTS BETWEEN SIZES 5-10	10-15	15-20	20-25	25-30	30-40	40-50	50-60	60-80	80-100	100-200	200-300
4000.	6	2345.	2197.	2139.	2077.	1676.	3268.	2018.	906.	761.	156.	55.	1.
2000.	6	1716.	1537.	1541.	1358.	1256.	2251.	1478.	731.	666.	140.	44.	0.
1000.	6	1999.	1864.	1845.	1694.	1472.	2421.	1500.	662.	569.	124.	41.	2.
500.	6	2861.	2632.	2733.	2439.	2000.	3076.	1609.	602.	463.	107.	37.	0.
250.	6	3373.	3012.	2780.	2336.	1989.	3035.	1525.	586.	474.	100.	43.	0.
0.	6	2896.	2519.	2357.	1994.	1564.	2420.	1241.	458.	355.	73.	35.	1.
4000.	7	4368.	3707.	3226.	2603.	2009.	2909.	1435.	576.	453.	97.	32.	0.
2000.	7	3428.	2944.	2479.	1990.	1502.	2150.	1129.	470.	377.	74.	27.	0.
1000.	7	3749.	3132.	2608.	2059.	1634.	2475.	1384.	625.	530.	109.	38.	1.
500.	7	3167.	2743.	2410.	1978.	1601.	2361.	1339.	564.	455.	96.	39.	1.
250.	7	3930.	3213.	2977.	2188.	1587.	1920.	763.	265.	189.	44.	24.	1.
0.	7	3789.	2964.	2260.	1553.	1067.	1258.	482.	181.	115.	28.	14.	0.
4000.	8	4036.	3421.	2775.	2168.	1648.	2168.	1046.	386.	289.	61.	30.	0.
2000.	8	3704.	3068.	2496.	1872.	1477.	1959.	964.	360.	314.	65.	34.	1.
1000.	8	4140.	3462.	2767.	2121.	1622.	2266.	1090.	453.	379.	78.	31.	0.
500.	8	3149.	2589.	2119.	1640.	1304.	1980.	1126.	522.	447.	88.	30.	1.
250.	8	2865.	2255.	1886.	1481.	1135.	1791.	1055.	486.	426.	95.	28.	0.
0.	8	3505.	3090.	2567.	1922.	1380.	1739.	715.	246.	180.	46.	21.	1.

SUBSTRATE: STARCH
SLUDGE: STARCH
SLUDGE CONCENTRATION: 2750.

IONIC MEDIUM: NACL
IONIC STRENGTH: 0.010

SUBSTRATE CONC.	PH	PARTICLE COUNTS BETWEEN SIZES 5-10	10-15	15-20	20-25	25-30	30-40	40-50	50-60	60-80	80-100	100-200	200-300
4000.	6	1867.	1474.	1307.	1069.	924.	1615.	1276.	866.	1212.	323.	226.	0.
2000.	6	2053.	1671.	1530.	1326.	1306.	2566.	2109.	1405.	1927.	791.	392.	2.
1000.	6	1969.	1781.	1774.	1636.	1537.	2949.	2181.	1258.	1458.	480.	190.	1.
500.	6	1621.	1479.	1481.	1420.	1356.	2707.	2199.	1389.	1911.	747.	409.	2.
250.	6	1517.	1427.	1499.	1383.	1376.	2842.	2365.	1470.	2021.	760.	380.	2.
0.	6	1745.	1369.	1192.	1058.	1002.	2131.	1874.	1267.	1928.	914.	520.	2.
4000.	7	2944.	2508.	2152.	1724.	1361.	1843.	870.	310.	273.	64.	23.	1.
2000.	7	3240.	2968.	2664.	2323.	1981.	3150.	1780.	760.	666.	150.	63.	1.
1000.	7	3156.	2611.	2251.	1825.	1500.	2261.	1111.	463.	382.	93.	35.	0.
500.	7	2596.	2159.	1935.	1600.	1246.	1788.	919.	370.	330.	67.	27.	0.
250.	7	3114.	2895.	2629.	2318.	1968.	3193.	1718.	784.	641.	130.	59.	0.
0.	7	2809.	2422.	2075.	1632.	1242.	1773.	865.	346.	263.	59.	21.	1.
4000.	8	3119.	2702.	2516.	2267.	1841.	3028.	1779.	843.	775.	172.	68.	1.
2000.	8	3311.	2874.	2645.	2284.	2009.	3168.	1904.	876.	755.	180.	70.	1.
1000.	8	2434.	2047.	1757.	1487.	1260.	1869.	1047.	463.	383.	91.	33.	2.
500.	8	3677.	3121.	2796.	2282.	1976.	3000.	1698.	736.	680.	148.	56.	1.
250.	8	4169.	3484.	3052.	2439.	1900.	2617.	1194.	486.	356.	81.	37.	0.
0.	8	3681.	3033.	2541.	1990.	1539.	2185.	1015.	401.	283.	70.	27.	1.

SUBSTRATE: STARCH
SLUDGE: STARCH
SLUDGE CONCENTRATION: 960.

IONIC MEDIUM: CACL2
IONIC STRENGTH: 0.010

SUBSTRATE CONC.	PH	PARTICLE COUNTS BETWEEN SIZES 5-10	10-15	15-20	20-25	25-30	30-40	40-50	50-60	60-80	80-100	100-200	200-300
2000.	6	646.	209.	129.	101.	86.	264.	343.	357.	838.	692.	569.	6.
1000.	6	429.	119.	104.	101.	124.	323.	448.	451.	974.	817.	640.	3.
500.	6	457.	143.	110.	107.	115.	312.	434.	409.	964.	825.	909.	4.
250.	6	382.	120.	110.	109.	116.	350.	396.	430.	925.	653.	498.	1.
100.	6	523.	201.	181.	191.	217.	617.	769.	762.	1629.	1105.	725.	5.
0.	6	515.	173.	164.	179.	227.	630.	822.	819.	1718.	1174.	883.	5.
2000.	7	899.	383.	213.	139.	110.	285.	334.	309.	651.	408.	233.	1.
1000.	7	552.	250.	192.	192.	192.	513.	637.	566.	1162.	770.	545.	3.
500.	7	520.	172.	147.	145.	167.	449.	602.	549.	1271.	978.	911.	3.
250.	7	591.	197.	161.	186.	201.	525.	703.	600.	1367.	1066.	978.	5.
100.	7	489.	168.	157.	151.	168.	469.	610.	570.	1238.	1032.	969.	6.
0.	7	495.	204.	199.	201.	262.	799.	1061.	943.	2080.	1395.	905.	5.
2000.	8	2343.	1432.	1022.	569.	382.	648.	645.	589.	1113.	742.	478.	1.
1000.	8	1908.	827.	524.	338.	310.	764.	851.	739.	1462.	1122.	1123.	8.
500.	8	1044.	400.	256.	215.	233.	569.	753.	725.	1611.	1213.	1044.	4.
250.	8	771.	249.	187.	186.	195.	483.	576.	512.	1223.	1054.	968.	3.
100.	8	555.	206.	152.	149.	181.	433.	584.	548.	1269.	1120.	1106.	4.
0.	8	485.	179.	166.	196.	254.	698.	887.	828.	1755.	1516.	732.	3.

SUBSTRATE: STARCH
SLUDGE: STARCH
SLUDGE CONCENTRATION: 2665.

IONIC MEDIUM: CACL2
IONIC STRENGTH: 0.010

SUBSTRATE CONC.	PH	PARTICLE COUNTS BETWEEN SIZES 5-10	10-15	15-20	20-25	25-30	30-40	40-50	50-60	60-80	80-100	100-200	200-300
4000.	6	145.	85.	81.	87.	117.	376.	575.	544.	1102.	641.	337.	1.
2000.	6	205.	70.	177.	197.	240.	616.	774.	711.	1431.	884.	425.	3.
1000.	6	84.	68.	72.	64.	91.	277.	434.	407.	899.	548.	287.	1.
500.	6	164.	133.	135.	147.	179.	501.	707.	667.	1355.	779.	380.	0.
250.	6	541.	364.	302.	318.	332.	977.	1220.	1117.	2133.	1230.	622.	3.
0.	6	538.	369.	357.	366.	418.	1102.	1267.	1036.	1864.	880.	384.	1.
4000.	7	911.	439.	313.	296.	324.	927.	1136.	1019.	1938.	963.	457.	1.
2000.	7	511.	414.	394.	437.	474.	1269.	1371.	1112.	1995.	1075.	520.	3.
1000.	7	341.	286.	315.	334.	361.	922.	1061.	905.	1716.	1009.	510.	0.
500.	7	291.	251.	278.	316.	350.	1011.	1163.	1022.	1955.	1093.	505.	2.
250.	7	217.	161.	201.	210.	259.	767.	933.	876.	1749.	1072.	594.	1.
0.	7	424.	326.	339.	364.	431.	1206.	1463.	1326.	2602.	1445.	721.	4.
4000.	8	1644.	1049.	866.	841.	835.	2024.	2027.	1467.	2194.	847.	295.	1.
2000.	8	776.	542.	567.	630.	677.	1737.	1811.	1366.	2216.	965.	385.	1.
1000.	8	747.	609.	669.	712.	786.	1848.	1975.	1530.	2574.	1276.	528.	2.
500.	8	246.	263.	321.	372.	470.	1317.	1596.	1317.	2570.	1332.	619.	0.
250.	8	715.	678.	772.	861.	943.	2146.	2190.	1706.	2742.	1269.	511.	1.
0.	8	410.	435.	501.	562.	634.	1606.	1650.	1289.	2046.	965.	364.	0.

SUBSTRATE: JACK BEAN MEAL
SLUDGE: JACK BEAN MEAL
SLUDGE CONCENTRATION: 957.

IONIC MEDIUM: NACL
IONIC STRENGTH: 0.001

SUBSTRATE CONC.	PH	PARTICLE COUNTS BETWEEN SIZES 5-10	10-15	15-20	20-25	25-30	30-40	40-50	50-60	60-80	80-100	100-200	200-300
2000.	6	4659.	3057.	2192.	1737.	1385.	1925.	999.	445.	467.	198.	158.	4.
1000.	6	3885.	3016.	2259.	1707.	1257.	1856.	933.	473.	457.	212.	185.	7.
500.	6	3121.	2521.	1787.	1284.	893.	1276.	673.	308.	339.	172.	152.	7.
250.	6	2530.	2162.	1480.	1136.	744.	986.	549.	235.	313.	152.	168.	7.
100.	6	2215.	1874.	1340.	953.	653.	874.	440.	212.	258.	143.	144.	8.
0.	6	3205.	2623.	1916.	1273.	859.	1161.	538.	232.	294.	177.	227.	10.
2000.	7	4406.	2967.	2176.	1629.	1221.	1776.	1000.	476.	603.	306.	244.	9.
1000.	7	3828.	2854.	2122.	1537.	1101.	1567.	825.	436.	486.	237.	209.	8.
500.	7	3121.	2545.	1809.	1256.	914.	1179.	670.	324.	364.	183.	184.	7.
250.	7	2960.	2436.	1652.	1084.	750.	934.	481.	227.	249.	139.	130.	6.
100.	7	3033.	2618.	1802.	1168.	782.	1041.	471.	244.	270.	155.	164.	6.
0.	7	3155.	2709.	1771.	1156.	789.	1032.	469.	206.	268.	151.	182.	7.
2000.	8	4392.	3198.	2355.	1668.	1235.	1731.	947.	485.	645.	340.	247.	8.
1000.	8	3762.	2846.	2064.	1496.	1020.	1417.	762.	406.	518.	280.	229.	6.
500.	8	3614.	2795.	1961.	1229.	791.	1071.	555.	262.	327.	175.	171.	4.
250.	8	3374.	2789.	1892.	1192.	771.	1038.	544.	262.	323.	162.	170.	7.
100.	8	3109.	2524.	1710.	1057.	710.	898.	445.	193.	248.	139.	163.	5.
0.	8	3380.	2884.	1972.	1225.	799.	1041.	461.	205.	271.	159.	169.	6.

SUBSTRATE: JACK BEAN MEAL
SLUDGE: JACK BEAN MEAL
SLUDGE CONCENTRATION: 2860.

IONIC MEDIUM: NACL
IONIC STRENGTH: 0.001

SUBSTRATE CONC.	PH	PARTICLE COUNTS BETWEEN SIZES 5-10	10-15	15-20	20-25	25-30	30-40	40-50	50-60	60-80	80-100	100-200	200-300
4000.	6	7162.	4299.	2642.	1736.	1180.	1411.	583.	241.	280.	124.	179.	16.
2000.	6	4841.	3277.	2163.	1414.	952.	1207.	563.	286.	352.	151.	231.	17.
1000.	6	5278.	3674.	2471.	1561.	1037.	1199.	601.	299.	389.	190.	261.	18.
500.	6	5359.	3894.	2669.	1705.	1038.	1232.	606.	315.	427.	234.	352.	22.
250.	6	5110.	3456.	2263.	1410.	868.	938.	427.	214.	300.	165.	276.	20.
0.	6	5730.	3796.	2430.	1518.	890.	900.	385.	185.	310.	171.	286.	24.
4000.	7	6562.	4182.	2552.	1615.	1108.	1272.	555.	246.	299.	136.	184.	13.
2000.	7	6164.	4012.	2499.	1500.	1009.	1209.	542.	264.	325.	136.	218.	18.
1000.	7	6183.	4431.	2899.	1792.	1136.	1300.	601.	305.	400.	199.	350.	25.
500.	7	5667.	3847.	2442.	1485.	876.	975.	428.	237.	295.	164.	278.	16.
250.	7	5279.	3550.	2280.	1383.	817.	906.	408.	216.	304.	162.	276.	26.
0.	7	5364.	3811.	2611.	1733.	1066.	1134.	463.	245.	345.	196.	243.	28.
4000.	8	5437.	3261.	2025.	1230.	799.	891.	388.	174.	243.	120.	155.	6.
2000.	8	5670.	3795.	2485.	1450.	932.	1051.	495.	251.	338.	149.	231.	15.
1000.	8	5804.	4021.	2639.	1581.	935.	1044.	499.	263.	347.	191.	299.	20.
500.	8	5671.	3875.	2523.	1471.	837.	920.	420.	235.	303.	173.	284.	17.
250.	8	5290.	3557.	2277.	1298.	749.	812.	369.	191.	264.	148.	281.	17.
0.	8	5892.	4021.	2560.	1544.	927.	958.	387.	216.	306.	182.	334.	25.

SUBSTRATE: JACK BEAN MEAL
SLUDGE: JACK BEAN MEAL
SLUDGE CONCENTRATION: 935.

IONIC MEDIUM: CACL2
IONIC STRENGTH: 0.001

SUBSTRATE CONC.	PH	PARTICLE COUNTS BETWEEN SIZES 5-10	10-15	15-20	20-25	25-30	30-40	40-50	50-60	60-80	80-100	100-200	200-300
2000.	6	3658.	2192.	1540.	1183.	992.	1556.	861.	462.	532.	245.	281.	14.
1000.	6	3944.	2927.	2298.	1651.	1263.	1822.	944.	406.	449.	166.	186.	6.
500.	6	3332.	2748.	2049.	1448.	1016.	1442.	756.	320.	354.	155.	194.	9.
250.	6	2909.	2494.	1833.	1245.	916.	1285.	639.	298.	349.	162.	222.	10.
100.	6	2641.	2451.	1751.	1191.	788.	1124.	520.	218.	292.	143.	186.	10.
0.	6	2548.	2329.	1724.	1137.	840.	1202.	496.	197.	277.	134.	200.	7.
2000.	7	5027.	3341.	2562.	1915.	1465.	2163.	1223.	623.	744.	321.	325.	20.
1000.	7	4162.	3225.	2390.	1708.	1247.	1728.	943.	447.	510.	229.	232.	8.
500.	7	3300.	2800.	2111.	1466.	1037.	1328.	729.	350.	431.	217.	235.	10.
250.	7	3278.	2983.	2225.	1398.	1005.	1280.	632.	313.	366.	185.	242.	13.
100.	7	3021.	2816.	2006.	1324.	902.	1210.	564.	264.	333.	175.	231.	11.
0.	7	2735.	2521.	1768.	1073.	769.	1049.	415.	167.	221.	123.	177.	10.
2000.	8	4979.	3481.	2592.	1837.	1332.	1836.	971.	490.	619.	307.	317.	11.
1000.	8	4433.	3482.	2683.	1982.	1411.	1819.	1025.	508.	647.	285.	310.	13.
500.	8	3661.	3074.	2274.	1597.	1061.	1376.	740.	363.	489.	215.	278.	13.
250.	8	2823.	2509.	1789.	1164.	798.	1023.	503.	249.	328.	151.	202.	8.
100.	8	2646.	2396.	1665.	1053.	702.	916.	440.	200.	239.	118.	168.	4.
0.	8	3054.	2659.	2048.	1286.	879.	1192.	495.	198.	223.	132.	182.	9.

SUBSTRATE: JACK BEAN MEAL
SLUDGE: JACK BEAN MEAL
SLUDGE CONCENTRATION: 2740.

IONIC MEDIUM: CACL2
IONIC STRENGTH: 0.001

SUBSTRATE CONC.	PH	PARTICLE COUNTS BETWEEN SIZES 5-10	10-15	15-20	20-25	25-30	30-40	40-50	50-60	60-80	80-100	100-200	200-300
4000.	6	4986.	3904.	2994.	2055.	1384.	1650.	717.	292.	338.	148.	199.	16.
2000.	6	4336.	2864.	1808.	1153.	837.	997.	429.	188.	229.	123.	210.	10.
1000.	6	4808.	3113.	1985.	1367.	888.	1090.	457.	216.	320.	205.	321.	21.
500.	6	5533.	3839.	2386.	1535.	944.	1032.	475.	267.	396.	249.	397.	23.
250.	6	4146.	2716.	1712.	1159.	710.	822.	382.	223.	310.	192.	356.	25.
0.	6	4615.	3080.	2171.	1500.	926.	1056.	544.	311.	433.	262.	387.	25.
4000.	7	5720.	3944.	2480.	1654.	1119.	1400.	566.	234.	259.	139.	222.	14.
2000.	7	6053.	4140.	2691.	1823.	1148.	1309.	507.	232.	269.	167.	266.	17.
1000.	7	3599.	2352.	1595.	1098.	729.	896.	443.	225.	293.	174.	266.	18.
500.	7	6218.	4333.	2764.	1715.	970.	1060.	482.	236.	334.	190.	313.	30.
250.	7	6030.	4645.	3277.	2140.	1224.	1233.	532.	280.	379.	223.	368.	31.
0.	7	5666.	3545.	2199.	1425.	799.	856.	430.	244.	366.	236.	363.	25.
4000.	8	6548.	4052.	2569.	1668.	1003.	1135.	521.	230.	236.	104.	115.	7.
2000.	8	5417.	3725.	2612.	1747.	1111.	1357.	628.	286.	356.	172.	240.	12.
1000.	8	6013.	4331.	2786.	1765.	1054.	1224.	583.	262.	325.	177.	346.	26.
500.	8	6380.	4878.	3292.	1949.	1183.	1260.	592.	291.	397.	249.	351.	20.
250.	8	5871.	4538.	3022.	1929.	1131.	1132.	551.	273.	389.	238.	388.	21.
0.	8	5739.	3577.	2041.	1171.	651.	675.	378.	195.	286.	163.	238.	17.

SUBSTRATE: JACK BEAN MEAL
SLUDGE: JACK BEAN MEAL
SLUDGE CONCENTRATION: 1020.

IONIC MEDIUM: NACL
IONIC STRENGTH: 0.010

SUBSTRATE CONC.	PH	PARTICLE COUNTS BETWEEN SIZES 5-10	10-15	15-20	20-25	25-30	30-40	40-50	50-60	60-80	80-100	100-200	200-300
2000.	6	4849.	4782.	4068.	2512.	1393.	1419.	545.	206.	222.	82.	64.	3.
1000.	6	3260.	3205.	2824.	1962.	1153.	1137.	507.	222.	308.	135.	135.	1.
500.	6	3374.	3044.	2504.	1676.	977.	1060.	457.	212.	305.	145.	140.	1.
250.	6	3737.	3041.	2400.	1447.	809.	822.	369.	185.	278.	111.	122.	4.
100.	6	4456.	3609.	2713.	1680.	962.	967.	434.	219.	315.	138.	150.	3.
0.	6	5283.	4158.	3111.	1922.	1173.	1120.	474.	228.	326.	144.	164.	2.
2000.	7	4940.	4944.	4007.	2364.	1214.	1109.	468.	221.	276.	109.	87.	2.
1000.	7	4525.	4641.	4079.	2665.	1509.	1418.	559.	296.	392.	178.	165.	3.
500.	7	5185.	5033.	4203.	2796.	1606.	1643.	668.	335.	434.	205.	194.	2.
250.	7	4813.	4389.	3444.	2216.	1322.	1283.	565.	280.	334.	161.	159.	4.
100.	7	4891.	3930.	3003.	1880.	1079.	1071.	467.	246.	316.	131.	160.	3.
0.	7	5212.	4185.	3122.	1931.	1156.	1234.	460.	231.	280.	137.	163.	1.
2000.	8	5241.	4639.	3323.	1696.	850.	779.	373.	162.	193.	71.	44.	1.
1000.	8	5108.	4890.	3858.	2224.	1158.	1135.	477.	228.	270.	130.	119.	1.
500.	8	5427.	5225.	4371.	2732.	1483.	1533.	638.	298.	392.	194.	187.	4.
250.	8	5459.	4707.	3765.	2354.	1358.	1326.	567.	285.	323.	158.	158.	5.
100.	8	5331.	4244.	3219.	2055.	1138.	1207.	506.	237.	286.	128.	163.	4.
0.	8	5014.	3644.	2731.	1571.	933.	972.	385.	190.	241.	115.	135.	4.

SUBSTRATE: JACK BEAN MEAL
SLUDGE: JACK BEAN MEAL
SLUDGE CONCENTRATION: 2645.

IONIC MEDIUM: CACL2
IONIC STRENGTH: 0.010

SUBSTRATE CONC.	PH	PARTICLE COUNTS BETWEEN SIZES 5-10	10-15	15-20	20-25	25-30	30-40	40-50	50-60	60-80	80-100	100-200	200-300
4000.	6	4168.	2847.	2307.	1786.	1438.	2084.	1054.	382.	276.	99.	81.	2.
2000.	6	2600.	2019.	1684.	1418.	1171.	1982.	1246.	592.	601.	196.	135.	4.
1000.	6	1821.	1499.	1437.	1259.	1124.	1983.	1313.	662.	780.	289.	203.	5.
500.	6	2326.	1960.	1793.	1579.	1375.	2135.	1299.	613.	651.	252.	190.	3.
250.	6	1761.	1667.	1674.	1478.	1344.	2112.	1255.	558.	558.	187.	150.	6.
0.	6	3802.	3175.	2805.	1635.	1825.	2516.	1195.	416.	395.	156.	163.	3.
4000.	7	3870.	2519.	2087.	1631.	1328.	2015.	1058.	404.	322.	103.	102.	4.
2000.	7	2241.	1554.	1407.	1194.	1031.	1599.	885.	366.	303.	111.	94.	3.
1000.	7	1689.	1703.	1672.	1514.	1312.	2170.	1361.	652.	706.	265.	169.	3.
500.	7	1300.	1202.	1259.	1245.	1113.	1919.	1425.	721.	909.	387.	237.	6.
250.	7	1422.	1422.	1459.	1414.	1277.	2078.	1304.	620.	637.	223.	185.	6.
0.	7	3610.	3220.	2898.	2428.	1917.	2760.	1403.	518.	432.	179.	185.	5.
4000.	8	3114.	2332.	1805.	1387.	1019.	1359.	633.	212.	178.	59.	62.	1.
2000.	8	2946.	2525.	2204.	1802.	1461.	2257.	1135.	485.	417.	131.	112.	2.
1000.	8	2021.	1926.	1868.	1633.	1390.	2173.	1287.	555.	565.	216.	148.	6.
500.	8	1683.	1754.	1739.	1569.	1433.	2318.	1434.	681.	742.	296.	190.	4.
250.	8	1532.	1497.	1535.	1414.	1245.	2099.	1230.	628.	703.	300.	208.	5.
0.	8	3168.	3014.	2921.	2513.	2060.	2871.	1323.	455.	424.	181.	177.	8.

SUBSTRATE: JACK BEAN MEAL
SLUDGE: JACK BEAN MEAL
SLUDGE CONCENTRATION: 910.

IONIC MEDIUM: CACL2
IONIC STRENGTH: 0.010

SUBSTRATE CONC.	PH	PARTICLE COUNTS BETWEEN SIZES 5-10	10-15	15-20	20-25	25-30	30-40	40-50	50-60	60-80	80-100	100-200	200-300
2000.	6	5983.	4303.	3433.	2291.	1478.	1643.	726.	327.	366.	169.	136.	8.
1000.	6	3835.	2843.	2206.	1509.	1038.	1222.	608.	310.	395.	191.	190.	9.
500.	6	2913.	2253.	1712.	1164.	737.	977.	502.	292.	353.	204.	239.	12.
250.	6	2020.	1501.	1153.	790.	519.	679.	405.	239.	388.	255.	321.	14.
100.	6	2180.	1689.	1236.	803.	486.	662.	404.	241.	390.	258.	319.	15.
0.	6	4007.	3265.	2313.	1416.	828.	826.	363.	192.	261.	187.	282.	12.
2000.	7	6206.	4015.	2974.	2035.	1348.	1758.	825.	399.	429.	174.	184.	7.
1000.	7	4746.	2818.	1926.	1257.	850.	982.	513.	245.	292.	144.	148.	8.
500.	7	2541.	1770.	1267.	868.	575.	749.	437.	222.	299.	161.	162.	12.
250.	7	2864.	2138.	1625.	1096.	684.	904.	487.	245.	356.	171.	201.	12.
100.	7	1866.	1502.	999.	640.	395.	538.	320.	198.	281.	178.	215.	10.
0.	7	3887.	3475.	2721.	1759.	1106.	1296.	568.	260.	355.	222.	317.	19.
2000.	8	3971.	2732.	2191.	1566.	1135.	1394.	884.	332.	374.	154.	115.	2.
1000.	8	3795.	3011.	2498.	1832.	1277.	1693.	907.	459.	574.	275.	293.	15.
500.	8	2666.	2024.	1463.	1022.	722.	911.	528.	267.	432.	237.	280.	10.
250.	8	2559.	1933.	1324.	854.	536.	691.	1263.	230.	353.	242.	321.	15.
100.	8	2394.	1806.	1227.	751.	459.	527.	300.	168.	299.	192.	263.	13.
0.	8	4498.	3925.	3012.	1959.	1249.	1326.	548.	246.	348.	215.	332.	20.

SUBSTRATE: JACK BEAN MEAL
SLUDGE: JACK BEAN MEAL
SLUDGE CONCENTRATION: 2765.

IONIC MEDIUM: CACL2
IONIC STRENGTH: 0.010

SUBSTRATE CONC.	PH	PARTICLE COUNTS BETWEEN SIZES 5-10	10-15	15-20	20-25	25-30	30-40	40-50	50-60	60-80	80-100	100-200	200-300
4000.	6	4516.	3460.	3067.	2548.	1983.	2525.	1189.	528.	594.	238.	192.	5.
2000.	6	4036.	3440.	3222.	2646.	1875.	2428.	1126.	552.	638.	263.	246.	4.
1000.	6	2892.	2720.	2576.	2132.	1588.	1995.	959.	496.	599.	278.	227.	4.
500.	6	2386.	2295.	2244.	1829.	1315.	1638.	823.	398.	534.	244.	216.	1.
250.	6	3129.	2954.	2781.	2193.	1490.	1842.	936.	481.	661.	302.	279.	4.
0.	6	3178.	2771.	2476.	1804.	1213.	1301.	553.	254.	332.	143.	138.	3.
4000.	7	3970.	3353.	3046.	2504.	1905.	2377.	1032.	427.	490.	179.	142.	3.
2000.	7	2670.	2402.	2245.	1864.	1358.	1735.	838.	375.	501.	167.	156.	3.
1000.	7	1868.	1854.	1910.	1655.	1204.	1694.	883.	456.	579.	272.	231.	4.
500.	7	2576.	2526.	2478.	2067.	1420.	1717.	858.	437.	628.	276.	245.	4.
250.	7	2677.	2718.	2713.	2331.	1691.	2151.	1107.	561.	785.	355.	337.	7.
0.	7	2746.	2726.	2765.	2373.	1682.	2125.	1014.	484.	575.	267.	274.	10.
4000.	8	3892.	3361.	3056.	2455.	1658.	1926.	773.	330.	361.	147.	111.	3.
2000.	8	2372.	2168.	2011.	1580.	1176.	1363.	625.	275.	340.	122.	104.	2.
1000.	8	2277.	2253.	2245.	1696.	1338.	1795.	894.	451.	571.	241.	228.	3.
500.	8	2671.	2897.	2762.	2170.	1528.	1867.	843.	412.	582.	237.	213.	2.
250.	8	3000.	2980.	2875.	2345.	1664.	2006.	923.	466.	643.	288.	255.	4.
0.	8	3467.	3296.	3005.	2298.	1550.	1651.	680.	302.	404.	173.	170.	3.

APPENDIX B-2-c

Substrate Particle Counts (1-60 micron)

SUBSTRATE: ALBUMIN

IONIC MEDIUM: NACL
IONIC STRENGTH: 0.001

SUBSTRATE CONC.	PH	DILUTION	PARTICLE COUNTS BETWEEN SIZES 1-2	2-3	3-4	4-5	5-6	6-8	8-10	10-15	15-20	20-30	30-40	40-60
2000.	6	0.010	16306.	6948.	4995.	2754.	1705.	2242.	1035.	926.	292.	153.	30.	11.
500.	6	0.025	8984.	4042.	2912.	1648.	1013.	1319.	611.	588.	197.	106.	16.	8.
100.	6	0.025	2044.	969.	664.	371.	236.	298.	133.	169.	60.	39.	4.	2.
4000.	7	0.005	11383.	5799.	4351.	2502.	1580.	2061.	1011.	944.	263.	146.	27.	7.
1000.	7	0.012	6462.	3350.	2444.	1436.	878.	1130.	566.	534.	144.	76.	20.	5.
250.	7	0.025	3136.	1604.	1177.	666.	419.	528.	238.	248.	80.	47.	8.	3.
2000.	8	0.010	10695.	5532.	4178.	2410.	1512.	2009.	927.	902.	275.	163.	39.	14.
500.	8	0.025	7082.	3692.	2709.	1521.	922.	1272.	586.	599.	208.	124.	25.	8.
100.	8	0.025	1536.	774.	571.	348.	178.	243.	121.	123.	43.	22.	6.	2.

SUBSTRATE: ALBUMIN

IONIC MEDIUM: NACL
IONIC STRENGTH: 0.010

SUBSTRATE CONC.	PH	DILUTION	PARTICLE COUNTS BETWEEN SIZES 1-2	2-3	3-4	4-5	5-6	6-8	8-10	10-15	15-20	20-30	30-40	40-60
2000.	6	0.010	25141.	10004.	6523.	3542.	2067.	2589.	1192.	1118.	346.	161.	41.	20.
500.	6	0.010	3355.	1605.	1172.	651.	373.	481.	237.	209.	71.	36.	9.	5.
100.	6	0.025	4702.	2124.	1369.	690.	357.	402.	180.	176.	51.	25.	8.	4.
4000.	7	0.005	13489.	6732.	4866.	2704.	1674.	2194.	1096.	1234.	496.	259.	61.	27.
1000.	7	0.025	13274.	6654.	4880.	2888.	1805.	2445.	1239.	1282.	381.	166.	30.	14.
250.	7	0.025	3158.	1499.	1063.	576.	333.	434.	208.	227.	65.	35.	3.	0.
2000.	8	0.001	10988.	5568.	4174.	2453.	1546.	2112.	1088.	1219.	479.	299.	71.	35.
500.	8	0.025	5864.	2853.	2054.	1154.	688.	926.	449.	465.	126.	53.	13.	5.
100.	8	0.025	2504.	1145.	783.	413.	250.	333.	169.	258.	118.	76.	0.	0.

SUBSTRATE: ALBUMIN

IONIC MEDIUM: CACL2
IONIC STRENGTH: 0.001

SUBSTRATE CONC.	PH	DILUTION	PARTICLE COUNTS BETWEEN SIZES 1-2	2-3	3-4	4-5	5-6	6-8	8-10	10-15	15-20	20-30	30-40	40-60
4000.	6	0.005	18207.	7558.	5322.	3085.	1875.	2556.	1270.	1175.	355.	194.	56.	26.
1000.	6	0.010	6087.	2992.	2262.	1375.	867.	1147.	587.	555.	184.	114.	28.	15.
250.	6	0.025	4606.	2288.	1696.	997.	625.	815.	384.	421.	154.	89.	23.	8.
2000.	7	0.005	5056.	2374.	1736.	1050.	677.	900.	447.	435.	140.	74.	17.	6.
1000.	7	0.010	5172.	2557.	1863.	1127.	703.	983.	465.	449.	151.	71.	13.	6.
100.	7	0.025	1832.	908.	660.	380.	237.	279.	126.	153.	58.	40.	13.	2.
4000.	8	0.005	10011.	4860.	3649.	2158.	1348.	1810.	864.	906.	299.	161.	39.	14.
250.	8	0.025	6121.	3336.	2320.	1373.	894.	1158.	599.	586.	192.	109.	24.	10.
100.	8	0.025	4484.	2186.	1592.	964.	600.	756.	344.	386.	149.	86.	16.	9.

SUBSTRATE: ALBUMIN

IONIC MEDIUM: CACL2
IONIC STRENGTH: 0.010

SUBSTRATE CONC.	PH	DILUTION	PARTICLE COUNTS BETWEEN SIZES 1-2	2-3	3-4	4-5	5-6	6-8	8-10	10-15	15-20	20-30	30-40	40-60
2000.	6	0.005	25798.	9844.	5327.	2405.	1344.	1662.	736.	726.	215.	136.	31.	14.
500.	6	0.025	19735.	6678.	3929.	2026.	1196.	1549.	702.	688.	211.	116.	25.	9.
100.	6	0.025	3299.	1427.	941.	542.	305.	385.	174.	172.	55.	37.	7.	10.
4000.	7	0.002	15132.	4981.	3000.	1523.	942.	1188.	573.	510.	158.	81.	16.	8.
1000.	7	0.012	13570.	4998.	3333.	1875.	1174.	1476.	693.	656.	197.	105.	24.	13.
250.	7	0.025	5252.	2382.	1694.	886.	536.	676.	320.	317.	115.	65.	17.	10.
4000.	8	0.002	14909.	5127.	3209.	1688.	1004.	1328.	629.	593.	159.	95.	24.	11.
1000.	8	0.020	10576.	4169.	2933.	1557.	994.	1344.	616.	533.	147.	80.	18.	8.
250.	8	0.025	3975.	1827.	1273.	326.	431.	530.	245.	212.	70.	33.	9.	3.

SUBSTRATE: STARCH

IONIC MEDIUM: NACL
IONIC STRENGTH: 0.001

SUBSTRATE CONC.	PH	DILUTION	PARTICLE COUNTS BETWEEN SIZES 1-2	2-3	3-4	4-5	5-6	6-8	8-10	10-15	15-20	20-30	30-40	40-60
2000.	6	0.001	9721.	1792.	1130.	560.	326.	480.	237.	325.	161.	157.	33.	9.
500.	6	0.005	14660.	5306.	4129.	5150.	8359.	9919.	331.	192.	68.	48.	9.	2.
100.	6	0.025	12303.	4486.	2997.	1981.	1881.	2229.	333.	385.	163.	99.	21.	13.
4000.	7	0.001	9062.	917.	531.	308.	183.	248.	128.	160.	96.	73.	15.	0.
1000.	7	0.025	9284.	867.	504.	296.	189.	349.	159.	219.	117.	84.	23.	1.
250.	7	0.010	9923.	1158.	514.	363.	202.	306.	159.	227.	122.	100.	24.	6.
2000.	8	0.001	7779.	293.	0.	0.	0.	24.	23.	73.	69.	54.	16.	2.
500.	8	0.002	8807.	730.	278.	96.	15.	43.	24.	75.	48.	34.	6.	1.
100.	8	0.020	9586.	1591.	954.	473.	288.	339.	160.	218.	101.	45.	14.	4.

SUBSTRATE: STARCH

IONIC MEDIUM: NACL
IONIC STRENGTH: 0.010

SUBSTRATE CONC.	PH	DILUTION	PARTICLE COUNTS BETWEEN SIZES 1-2	2-3	3-4	4-5	5-6	6-8	8-10	10-15	15-20	20-30	30-40	40-60
4000.	6	0.001	3046.	969.	652.	408.	290.	461.	251.	403.	272.	396.	165.	53.
1000.	6	0.002	2522.	794.	584.	332.	210.	307.	181.	284.	199.	251.	128.	40.
250.	6	-----	------	------	------	------	------	------	------	------	-----	-----	-----	-----
2000.	7	0.002	4029.	1172.	845.	523.	477.	482.	273.	414.	296.	430.	213.	81.
500.	7	0.005	2209.	651.	459.	302.	290.	295.	156.	244.	183.	216.	89.	24.
100.	7	0.005	841.	378.	285.	163.	65.	114.	32.	70.	55.	67.	12.	6.
4000.	8	0.002	9481.	3208.	2216.	1322.	845.	1276.	775.	1185.	835.	1047.	485.	136.
1000.	8	0.005	3240.	1477.	990.	579.	362.	506.	304.	409.	242.	226.	56.	12.
250.	8	0.005	1933.	643.	454.	278.	306.	311.	203.	346.	225.	207.	73.	18.

SUBSTRATE: STARCH

IONIC MEDIUM: CACL2
IONIC STRENGTH: 0.001

SUBSTRATE CONC.	PH	DILUTION	PARTICLE COUNTS BETWEEN SIZES 1-2	2-3	3-4	4-5	5-6	6-8	8-10	10-15	15-20	20-30	30-40	40-60
4000.	6	0.002	11012.	1319.	766.	469.	305.	461.	264.	394.	246.	204.	66.	18.
1000.	6	0.005	13225.	1812.	1168.	647.	432.	658.	381.	540.	319.	257.	81.	29.
250.	6	0.020	19166.	3104.	1873.	1003.	629.	816.	395.	480.	214.	149.	34.	8.
2000.	7	0.001	6028.	909.	628.	336.	232.	298.	166.	255.	135.	137.	35.	11.
500.	7	0.002	4512.	640.	428.	222.	135.	196.	104.	159.	93.	77.	22.	8.
100.	7	0.010	4159.	662.	463.	231.	142.	185.	96.	117.	56.	39.	13.	4.
4000.	8	0.001	9560.	1582.	1029.	601.	389.	543.	320.	425.	221.	199.	60.	12.
1000.	8	0.002	8773.	1471.	992.	548.	350.	528.	313.	416.	225.	179.	49.	12.
250.	8	0.005	6842.	1317.	799.	445.	273.	370.	174.	181.	89.	60.	15.	5.

SUBSTRATE: JACK BEAN MEAL

IONIC MEDIUM: NACL
IONIC STRENGTH: 0.001

SUBSTRATE CONC.	PH	DILUTION	PARTICLE COUNTS BETWEEN SIZES 1-2	2-3	3-4	4-5	5-6	6-8	8-10	10-15	15-20	20-30	30-40	40-60
2000.	6	0.002	4183.	3141.	3865.	3418.	3074.	6202.	4792.	6887.	2809.	1775.	650.	446.
500.	6	0.005	3111.	2043.	2225.	1801.	1594.	3159.	2423.	3395.	1313.	867.	335.	234.
100.	6	0.010	4990.	2373.	1952.	1216.	884.	1468.	1010.	1357.	567.	380.	166.	110.
2000.	7	0.002	3375.	2531.	2553.	2969.	2671.	5681.	4537.	6673.	2764.	1685.	662.	457.
500.	7	0.005	2123.	1357.	1596.	1369.	1212.	2449.	1984.	3168.	1369.	938.	383.	251.
100.	7	0.010	2703.	1552.	1363.	1014.	774.	1339.	911.	1226.	517.	393.	155.	103.
2000.	8	0.002	7472.	4855.	5325.	4415.	3653.	7116.	5068.	6869.	2566.	1497.	625.	400.
500.	8	0.005	3716.	2537.	2859.	2332.	1933.	3602.	2413.	2757.	1061.	586.	342.	253.
100.	8	0.010	3262.	1559.	1405.	893.	750.	1322.	902.	1195.	439.	311.	133.	103.

SUBSTRATE: JACK BEAN MEAL

IONIC MEDIUM: NACL
IONIC STRENGTH: 0.010

SUBSTRATE CONC.	PH	DILUTION	PARTICLE COUNTS BETWEEN SIZES 1-2	2-3	3-4	4-5	5-6	6-8	8-10	10-15	15-20	20-30	30-40	40-60
2000.	6	0.002	6807.	3999.	3795.	2899.	2305.	4351.	3380.	5622.	3114.	2487.	775.	364.
500.	6	0.005	3703.	2137.	1928.	1407.	1065.	2046.	1484.	2494.	1358.	1207.	428.	220.
100.	6	0.005	1027.	559.	540.	339.	268.	476.	347.	582.	310.	276.	88.	52.
4000.	7	0.002	10573.	6669.	1695.	5026.	4052.	7728.	5889.	9711.	5257.	3929.	1249.	577.
1000.	7	0.005	6844.	3967.	3845.	2785.	2175.	4088.	3040.	4966.	2647.	2120.	705.	443.
250.	7	0.005	2096.	1178.	1121.	840.	666.	1203.	878.	1361.	708.	564.	212.	116.
2000.	8	0.005	13005.	8001.	7768.	5851.	4649.	8733.	6305.	6892.	4811.	3695.	1309.	656.
500.	8	0.005	3178.	1959.	1920.	1431.	1123.	1999.	1435.	2080.	974.	776.	299.	179.
100.	8	0.025	4363.	2569.	2467.	1861.	1364.	2548.	1739.	2468.	1145.	954.	391.	218.

SUBSTRATE: JACK BEAN MEAL

IONIC MEDIUM: CACL2
IONIC STRENGTH: 0.001

SUBSTRATE CONC.	PH	DILUTION	PARTICLE COUNTS BETWEEN SIZES 1-2	2-3	3-4	4-5	5-6	6-8	8-10	10-15	15-20	20-30	30-40	40-60
2000.	6	0.002	6790.	4734.	4808.	3702.	2913.	5145.	3513.	4837.	2101.	1374.	390.	187.
500.	6	0.010	11866.	7837.	7648.	5566.	4065.	6357.	3686.	4354.	1767.	1498.	640.	381.
100.	6	0.025	3979.	2392.	2244.	1505.	1113.	1824.	1119.	1527.	707.	521.	167.	70.
4000.	7	0.002	14946.	10022.	9512.	6690.	4883.	7901.	4756.	5876.	2737.	2520.	1048.	537.
1000.	7	0.005	8190.	5032.	4471.	3046.	2207.	3575.	2212.	2909.	1395.	1320.	585.	321.
250.	7	0.025	9483.	6236.	5852.	4129.	2904.	4682.	2764.	3544.	1677.	1622.	679.	401.
2000.	8	0.005	16201.	10674.	9753.	6685.	4757.	7424.	4375.	5498.	2704.	2699.	1290.	690.
500.	8	0.010	7630.	5144.	4833.	3350.	2400.	3449.	2247.	2421.	1285.	1274.	582.	321.
100.	8	0.025	5184.	3340.	3025.	1985.	1370.	2133.	1209.	1511.	667.	603.	274.	160.

SUBSTRATE: JACK BEAN MEAL

IONIC MEDIUM: CACL2
IONIC STRENGTH: 0.010

SUBSTRATE CONC.	PH	DILUTION	PARTICLE COUNTS BETWEEN SIZES 1-2	2-3	3-4	4-5	5-6	6-8	8-10	10-15	15-20	20-30	30-40	40-60
4000.	6	0.001	3730.	3010.	3903.	4023.	3835.	8522.	6554.	9033.	3345.	1754.	415.	182.
1000.	6	0.005	4721.	3735.	4477.	4299.	4172.	8650.	6606.	9125.	3528.	1915.	470.	235.
250.	6	0.010	3888.	2983.	2118.	3110.	2671.	5246.	3572.	4345.	1512.	870.	204.	110.
2000.	7	0.001	2863.	2547.	3290.	3027.	2588.	4841.	3124.	3657.	1324.	748.	224.	104.
500.	7	0.005	3655.	2987.	3628.	3179.	2643.	4750.	2944.	3365.	1201.	754.	213.	100.
100.	7	0.025	7238.	5394.	5626.	4195.	3067.	4907.	2808.	3036.	1095.	711.	200.	99.
4000.	8	0.001	6459.	5460.	6739.	5987.	5084.	8999.	5627.	6542.	2407.	1506.	381.	177.
1000.	8	0.005	5957.	4952.	6810.	5289.	4559.	8334.	5338.	6426.	2382.	1516.	404.	197.
250.	8	0.010	3465.	2927.	3545.	3010.	2499.	4401.	2727.	3070.	1156.	726.	190.	92.

APPENDIX B-2-d

Substrate Particle Counts (5-300 micron)

SUBSTRATE: ALBUMIN

IONIC MEDIUM: NACL
IONIC STRENGTH: 0.001

SUBSTRATE CONC.	PH	PARTICLE COUNTS BETWEEN SIZES 5-10	10-15	15-20	20-25	25-30	30-40	40-50	50-60	60-80	80-100	100-200	200-300
2000.	6	2658.	887.	344.	143.	74.	55.	26.	4.	3.	1.	1.	0.
250.	6	596.	200.	80.	31.	10.	15.	7.	1.	4.	1.	1.	0.
100.	6	404.	159.	64.	24.	12.	12.	3.	1.	1.	1.	0.	0.
4000.	7	4671.	1695.	664.	267.	134.	113.	33.	14.	13.	5.	3.	0.
1000.	7	2120.	716.	279.	94.	48.	39.	12.	5.	5.	1.	0.	0.
250.	7	640.	200.	88.	33.	17.	13.	3.	1.	1.	1.	0.	0.
2000.	8	4598.	1680.	659.	265.	108.	109.	34.	11.	11.	3.	2.	0.
500.	8	1079.	322.	111.	52.	21.	18.	8.	4.	4.	1.	1.	0.
100.	8	324.	108.	56.	25.	12.	4.	7.	2.	3.	1.	1.	0.

SUBSTRATE: ALBUMIN

IONIC MEDIUM: NACL
IONIC STRENGTH: 0.010

SUBSTRATE CONC.	PH	PARTICLE COUNTS BETWEEN SIZES 5-10	10-15	15-20	20-25	25-30	30-40	40-50	50-60	60-80	80-100	100-200	200-300
2000.	6	1961.	589.	219.	80.	42.	38.	11.	4.	3.	1.	1.	0.
500.	6	423.	138.	68.	22.	16.	11.	7.	0.	1.	2.	0.	0.
100.	6	147.	55.	25.	12.	5.	9.	3.	1.	2.	1.	1.	0.
4000.	7	7385.	2671.	984.	367.	186.	176.	72.	15.	23.	6.	4.	0.
1000.	7	2250.	737.	242.	88.	45.	46.	12.	7.	7.	3.	3.	0.
250.	7	512.	207.	79.	34.	11.	14.	5.	2.	3.	1.	0.	1.
2000.	8	3547.	1132.	399.	139.	75.	72.	22.	8.	11.	3.	5.	0.
500.	8	915.	310.	123.	46.	27.	29.	8.	3.	6.	1.	1.	0.
100.	8	198.	78.	26.	16.	12.	15.	7.	3.	2.	1.	0.	0.

SUBSTRATE: ALBUMIN

IONIC MEDIUM: CACL2
IONIC STRENGTH: 0.001

SUBSTRATE CONC.	PH	PARTICLE COUNTS BETWEEN SIZES 5-10	10-15	15-20	20-25	25-30	30-40	40-50	50-60	60-80	80-100	100-200	200-300
4000.	6	9098.	3471.	1335.	536.	307.	314.	110.	45.	37.	10.	5.	0.
1000.	6	2567.	857.	326.	134.	85.	81.	35.	11.	14.	4.	4.	0.
250.	6	657.	183.	83.	37.	27.	23.	6.	2.	3.	0.	0.	0.
2000.	7	4253.	1473.	556.	255.	119.	124.	46.	15.	16.	4.	2.	0.
500.	7	1332.	444.	202.	82.	46.	36.	15.	6.	7.	1.	0.	0.
100.	7	430.	133.	55.	8.	9.	5.	1.	0.	1.	0.	1.	0.
4000.	8	7857.	3132.	1235.	530.	280.	266.	110.	34.	28.	7.	4.	0.
1000.	8	2193.	689.	252.	103.	61.	65.	22.	7.	7.	1.	1.	0.
250.	8	1070.	350.	122.	44.	25.	26.	10.	2.	6.	2.	2.	0.

SUBSTRATE: ALBUMIN

IONIC MEDIUM: CACL2
IONIC STRENGTH: 0.010

SUBSTRATE CONC.	PH	PARTICLE COUNTS BETWEEN SIZES 5-10	10-15	15-20	20-25	25-30	30-40	40-50	50-60	60-80	80-100	100-200	200-300
2000.	6	6669.	2104.	815.	332.	158.	138.	55.	22.	15.	4.	5.	0.
500.	6	1331.	391.	153.	60.	32.	29.	12.	9.	3.	1.	1.	0.
100.	6	339.	115.	48.	21.	11.	9.	14.	2.	3.	0.	1.	0.
4000.	7	5628.	1927.	727.	324.	147.	150.	48.	17.	18.	6.	3.	0.
1000.	7	2723.	896.	366.	153.	78.	71.	29.	9.	8.	2.	3.	0.
250.	7	696.	226.	93.	45.	22.	21.	9.	3.	3.	3.	2.	0.
4000.	8	5341.	1836.	734.	305.	152.	139.	61.	19.	21.	6.	5.	0.
1000.	8	2422.	791.	307.	127.	56.	56.	43.	8.	5.	2.	3.	0.
250.	8	638.	195.	76.	31.	18.	9.	6.	2.	4.	2.	0.	0.

SUBSTRATE: STARCH

IONIC MEDIUM: NACL
IONIC STRENGTH: 0.001

SUBSTRATE CONC.	PH	PARTICLE COUNTS BETWEEN SIZES 5-10	10-15	15-20	20-25	25-30	30-40	40-50	50-60	60-80	80-100	100-200	200-300
2000.	6	4213.	1533.	535.	140.	38.	16.	0.	1.	0.	0.	0.	0.
500.	6	1322.	425.	160.	51.	16.	13.	2.	2.	2.	0.	0.	0.
100.	6	291.	101.	28.	10.	2.	3.	0.	0.	0.	0.	0.	0.
4000.	7	4178.	1521.	516.	144.	37.	12.	4.	4.	3.	1.	0.	0.
1000.	7	2014.	598.	210.	54.	16.	7.	0.	0.	1.	0.	0.	0.
250.	7	437.	133.	49.	14.	0.	3.	0.	0.	0.	0.	0.	0.
2000.	8	4525.	1646.	670.	191.	53.	21.	1.	0.	0.	0.	0.	0.
500.	8	662.	201.	61.	16.	4.	3.	0.	0.	0.	0.	0.	0.
100.	8	302.	146.	59.	20.	5.	5.	0.	0.	0.	0.	0.	0.

SUBSTRATE: STARCH

IONIC MEDIUM: NACL
IONIC STRENGTH: 0.010

SUBSTRATE CONC.	PH	PARTICLE COUNTS BETWEEN SIZES 5-10	10-15	15-20	20-25	25-30	30-40	40-50	50-60	60-80	80-100	100-200	200-300
4000.	6	4087.	2976.	2129.	1144.	541.	335.	39.	2.	0.	0.	0.	0.
1000.	6	1331.	862.	519.	258.	125.	65.	6.	1.	0.	0.	0.	0.
250.	6	760.	404.	245.	110.	51.	14.	4.	2.	1.	0.	0.	0.
2000.	7	4055.	2892.	2093.	1109.	490.	280.	27.	2.	0.	0.	0.	0.
500.	7	1391.	824.	473.	205.	93.	43.	4.	1.	1.	0.	0.	0.
100.	7	263.	186.	97.	46.	19.	11.	1.	2.	3.	1.	0.	0.
4000.	8	4117.	2916.	1950.	978.	451.	266.	29.	3.	4.	1.	2.	0.
1000.	8	2580.	1616.	1005.	455.	183.	90.	9.	1.	0.	0.	0.	0.
250.	8	846.	462.	229.	108.	39.	19.	0.	0.	0.	0.	0.	0.

SUBSTRATE: STARCH

IONIC MEDIUM: CACL2
IONIC STRENGTH: 0.001

SUBSTRATE CONC.	PH	PARTICLE COUNTS BETWEEN SIZES 5-10	10-15	15-20	20-25	25-30	30-40	40-50	50-60	60-80	80-100	100-200	200-300
4000.	6	4888.	2722.	1454.	531.	180.	76.	5.	1.	0.	0.	0.	0.
1000.	6	2841.	1412.	666.	249.	85.	36.	3.	1.	0.	0.	0.	0.
250.	6	749.	327.	135.	55.	18.	9.	1.	1.	0.	0.	0.	0.
2000.	7	4314.	2109.	1006.	379.	124.	51.	5.	2.	2.	0.	0.	0.
500.	7	1520.	713.	287.	103.	34.	16.	2.	1.	0.	0.	0.	0.
100.	7	255.	114.	54.	19.	6.	4.	2.	0.	0.	0.	0.	0.
4000.	8	4627.	2947.	1745.	733.	255.	100.	9.	2.	0.	0.	0.	0.
1000.	8	2602.	1160.	540.	170.	58.	25.	2.	1.	0.	1.	0.	0.
250.	8	601.	232.	103.	32.	10.	6.	0.	0.	0.	0.	0.	0.

SUBSTRATE: STARCH

IONIC MEDIUM: CACL2
IONIC STRENGTH: 0.010

SUBSTRATE CONC.	PH	PARTICLE COUNTS BETWEEN SIZES 5-10	10-15	15-20	20-25	25-30	30-40	40-50	50-60	60-80	80-100	100-200	200-300
4000.	6	4036.	3329.	2801.	1709.	864.	574.	84.	8.	3.	1.	1.	0.
1000.	6	2685.	1953.	1473.	843.	367.	244.	33.	2.	1.	0.	0.	0.
250.	6	789.	313.	173.	71.	30.	17.	3.	0.	0.	0.	0.	0.
2000.	7	4264.	3345.	2649.	1495.	732.	471.	52.	4.	3.	0.	0.	0.
500.	7	1620.	874.	497.	210.	98.	51.	7.	0.	1.	0.	0.	0.
100.	7	378.	165.	85.	40.	18.	9.	3.	1.	1.	0.	0.	0.
4000.	8	4242.	3317.	2563.	1492.	764.	488.	70.	4.	1.	0.	0.	0.
1000.	8	2934.	1702.	1093.	511.	227.	144.	14.	2.	0.	1.	0.	0.
250.	8	790.	397.	189.	75.	37.	20.	5.	1.	1.	1.	0.	0.

SUBSTRATE: JACK BEAN MEAL

IONIC MEDIUM: NACL
IONIC STRENGTH: 0.001

SUBSTRATE CONC.	PH	PARTICLE COUNTS BETWEEN SIZES 5-10	10-15	15-20	20-25	25-30	30-40	40-50	50-60	60-80	80-100	100-200	200-300
2000.	6	7078.	4381.	1952.	927.	591.	621.	134.	29.	22.	7.	4.	0.
500.	6	6985.	3577.	1592.	865.	573.	582.	134.	24.	26.	6.	4.	0.
100.	6	4068.	1787.	687.	358.	252.	255.	57.	15.	13.	4.	3.	0.
4000.	7	6400.	3672.	1526.	725.	462.	460.	90.	24.	17.	3.	4.	0.
1000.	7	6827.	4079.	1812.	894.	594.	628.	133.	31.	23.	9.	13.	0.
250.	7	7712.	3689.	1714.	969.	692.	748.	162.	37.	29.	12.	10.	0.
2000.	8	7002.	3891.	1637.	768.	518.	530.	121.	21.	20.	4.	4.	0.
1000.	8	6983.	2934.	1294.	739.	535.	605.	126.	27.	24.	8.	9.	0.
250.	8	7488.	3229.	3248.	893.	614.	684.	151.	39.	25.	12.	10.	0.

SUBSTRATE: JACK BEAN MEAL

IONIC MEDIUM: NACL
IONIC STRENGTH: 0.010

SUBSTRATE CONC.	PH	PARTICLE COUNTS BETWEEN SIZES 5-10	10-15	15-20	20-25	25-30	30-40	40-50	50-60	60-80	80-100	100-200	200-300
2000.	6	7011.	5699.	4346.	2573.	1453.	1343.	290.	74.	53.	22.	16.	0.
500.	6	5567.	4244.	2807.	1614.	913.	830.	180.	38.	32.	14.	9.	0.
100.	6	3026.	2103.	1341.	647.	370.	298.	57.	22.	16.	5.	8.	0.
4000.	7	7554.	6298.	4591.	2627.	1545.	1464.	329.	77.	78.	21.	19.	0.
1000.	7	7212.	5948.	4296.	2499.	1416.	1304.	282.	64.	51.	18.	9.	0.
250.	7	6256.	4104.	2491.	1325.	787.	737.	155.	35.	29.	12.	8.	0.
2000.	8	8098.	5965.	3884.	2100.	1312.	1360.	293.	64.	53.	25.	19.	0.
500.	8	6494.	3851.	2210.	1192.	750.	702.	150.	36.	34.	13.	7.	1.
100.	8	3356.	1742.	869.	449.	278.	250.	55.	15.	10.	5.	3.	0.

SUBSTRATE: JACK BEAN MEAL

IONIC MEDIUM: CACL2
IONIC STRENGTH: 0.001

SUBSTRATE CONC.	PH	PARTICLE COUNTS BETWEEN SIZES 5-10	10-15	15-20	20-25	25-30	30-40	40-50	50-60	60-80	80-100	100-200	200-300
2000.	6	7802.	4991.	2904.	1715.	1169.	1325.	354.	77.	61.	22.	22.	0.
500.	6	8977.	5227.	3033.	1774.	1304.	1526.	445.	103.	82.	32.	25.	0.
100.	6	2167.	1158.	680.	387.	255.	319.	76.	21.	19.	5.	9.	0.
4000.	7	6395.	3395.	1960.	1292.	917.	999.	228.	56.	38.	13.	6.	0.
1000.	7	6257.	1859.	1877.	1249.	963.	1113.	305.	64.	42.	17.	16.	0.
250.	7	4073.	1076.	1094.	726.	580.	658.	191.	36.	26.	12.	7.	0.
2000.	8	5797.	2924.	1761.	1264.	943.	1111.	290.	61.	47.	20.	11.	0.
500.	8	6917.	3676.	2154.	1519.	1183.	1383.	419.	87.	66.	28.	20.	0.
100.	8	1994.	837.	493.	337.	245.	258.	68.	18.	12.	3.	4.	0.

SUBSTRATE: JACK BEAN MEAL

IONIC MEDIUM: CACL2
IONIC STRENGTH: 0.010

SUBSTRATE CONC.	PH	PARTICLE COUNTS BETWEEN SIZES 5-10	10-15	15-20	20-25	25-30	30-40	40-50	50-60	60-80	80-100	100-200	200-300
4000.	6	6665.	4043.	1839.	705.	304.	279.	88.	31.	21.	6.	5.	0.
1000.	6	8856.	4778.	1397.	780.	357.	319.	110.	34.	35.	12.	9.	0.
250.	6	10241.	5237.	2424.	969.	468.	416.	137.	45.	45.	20.	16.	0.
2000.	7	7815.	4058.	1755.	703.	327.	296.	97.	30.	27.	10.	7.	0.
500.	7	8086.	3620.	1558.	647.	312.	308.	101.	31.	34.	12.	10.	0.
100.	7	4870.	1742.	728.	315.	166.	143.	48.	19.	18.	6.	6.	0.
4000.	8	6395.	3261.	1595.	660.	325.	253.	96.	26.	23.	9.	8.	0.
1000.	8	8022.	3671.	1587.	654.	318.	286.	97.	34.	22.	12.	9.	0.
250.	8	8760.	3988.	1787.	768.	383.	318.	110.	42.	40.	17.	18.	0.

APPENDIX B-3

PARTICLE COUNT ANALYSIS

Only two variables resulting from the particle count data were utilized in analyzing the research project. They were total particle count per milliliter of sample and the mean particle size. The initial value was calculated by simply summing the number of particles counted in each size range for each sample. The dilution factor (if measured) was then applied after which the resulting value was divided by the volume measured (in milliliters).

The mean particle size was calculated from frequency distribution of the particle counts. The number of particles in each size range was divided by the total particle count. These quotients represented the fractional percentage of particles in the respective size ranges. The mean particle size was then determined by multiplying the fractional amounts by the average (arithmetic) particle size of each size interval.

APPENDIX C

APPENDIX C

Appendix	Description
C-1	Explanation of Statistical Analysis
C-2	Fixed Effects Model
C-3	Albumin Mean Particle Size ANOVA
C-4	Starch Mean Particle Size ANOVA
C-5	Jack Bean Meal Mean Particle Size ANOVA

APPENDIX C-1

EXPLANATION OF STATISTICAL ANALYSIS

The mean particle size values for each substrate were analyzed using a three-way analysis of variance (ANOVA). Ionic medium, ionic strength, and pH were the variables tested for effects upon the particle sizes. Each variable was assumed to be a fixed variable. Four tables follow in this appendix. The first represents the general model for a fixed effects analysis which was used to test for significant differences. The other three list the ANOVA for each substrate.

APPENDIX C-2

FIXED EFFECTS MODEL

Source of Variation	Model
Ionic Medium (IM)	$\sigma^2+nbc(\frac{\varepsilon\alpha^2}{a-1})$
pH	$\sigma^2+nac(\frac{\Sigma\beta^2}{b-1})$
Ionic Strength (I)	$\sigma^2+nab(\frac{\Sigma\gamma^2}{c-1})$
IMX pH Interaction	$\sigma^2+nc(\frac{\Sigma(\alpha\beta)^2}{ab-a-b-b+1})$
IMxI Interaction	$\sigma^2+nb(\frac{\Sigma(\alpha\gamma)^2}{ac-a-c+1})$
pHxI Interaction	$\sigma^2+na(\frac{\Sigma(\beta\gamma)^2}{bc-b-c+1})$
IMxIxpH Interaction	$\sigma^2+n(\frac{\Sigma(\alpha\beta\gamma)^2}{abc-ab-ac-bc+a+b+c-1})$
Error	σ^2

APPENDIX C-3

ALBUMIN MEAN PARTICLE SIZE ANOVA (THREE-WAY)

Source*	df**	SS***	MS****	F*****	Pr>F******
IM	1	0.111	0.111	0.23	0.638
pH	2	0.811	0.406	0.83	0.449
I	1	0.444	0.444	0.91	0.350
IMxpH	2	0.394	0.197	0.40	0.673
IMxI	1	0.588	0.588	1.20	0.284
IxpH	2	0.191	0.096	0.19	0.825
IMxIxpH	2	0.681	0.341	0.69	0.509
Error	24	11.760	0.490		
Total	35	114.979			

*see Appendix C-2 for explanation of source abbreviations
**df = degrees of freedom
***SS = sum of squares
****MS = mean square
*****F = F value; a statistical function used to describe statistical differences
******Pr>F = represents the level of statistical significance, e.g. [1-(Pr>F value)]x100 equals the significant effect by the corresponding variable

APPENDIX C-4

STARCH MEAN PARTICLE SIZE ANOVA (THREE-WAY)*

Source	df	SS	MS	F	Pr>F
IM	1	4.271	4.271	3.86	0.0612
pH	2	0.382	0.191	0.17	0.8425
I	1	57.254	57.254	51.70	0.0001
IMxpH	2	0.509	0.255	0.23	0.7965
IMxI	1	1.778	1.778	1.61	0.2173
IxpH	2	0.542	0.276	0.24	0.7848
IMxIxpH	2	0.176	0.088	0.08	0.9240
Error	24	26.580	1.108		
Total	35	91.49			

*See footnotes, Table C-3 for explanation of column heading symbols.

APPENDIX C-5

JACK BEAN MEAL MEAN PARTICLE SIZE ANOVA (THREE-WAY)*

Source	df	SS	MS	F	Pr>F
IM	1	0.694	0.694	1.90	0.1805
pH	2	0.249	0.125	0.34	0.7145
I	1	5.601	5.601	15.35	0.0007
IMxpH	2	0.962	0.481	1.32	0.2863
IMxI	1	61.884	61.884	169.55	0.0001
IxpH	2	0.896	0.448	1.23	0.3110
IMxIxpH	2	0.862	0.431	1.18	0.3241
Error	24	8.76	0.365		
Total	35	79.909			

*See footnotes, Table C-3 for explanation of column heading symbols.

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MECHANISMS OF CONTACT STABILIZATION SUBSTRATE REMOVAL

by

Victor Gulas

(ABSTRACT)

The purpose of this study was to investigate the interaction between colloidal substances and activated sludge and attempt to relate this information to the performance of activated sludge processes, particularly the contact stabilization process. Protein and carbohydrate organic colloids and a combination substrate were separately studied to determine if the type or classification of substrate colloids is a factor in the colloid-sludge interaction.

Albumin, potato starch, and jack bean meal were the substrates chosen. Two series of studies were performed. The first involved monitoring metabolic uptake of the colloidal substrates alone and then with glucose supplementation. The second series used mercury poisoned activated sludge to investigate the physical-chemical removal of different loadings of the colloidal substrates. During these experiments, conditions of pH, ionic strength, and cationic valence were varied to determine their role in the physical interactions between the colloids and the sludge.

Data obtained from the metabolic studies were monitored for unusual responses in substrate and oxygen utilization. Other factors determined included yield and oxygen utilization constants, substrate removal rates, and initial removal capacities of the sludge. Physical studies on the metabolically inactive sludge yielded data on the facility of organic colloid removal by

activated sludge under a variety of different environmental conditions. Removal capacities were recorded as well as any particle size variations of the activated sludge after colloid addition. Further information was obtained on the settleability of the colloid-sludge mixtures.

An adsorption and release phenomenon was observed for the two carbohydrate containing substrates while a two step oxygen utilization was observed for albumin and starch. Glucose addition was hypothesized to repress extracellular enzyme production thereby decreasing the sorption capacities of the activated sludge. Studies with the metabolically inactive sludges indicated that variations in pH, ionic strength, and cation valence play important roles in the physical removal of organic colloids by activated sludge. Sorption capacities of sludge varied with MLSS concentration. Unit sorption capacities for all three substrates decreased as MLSS levels increased. Total sorption capacities increased for albumin and jack bean meal with an increase in MLSS; the starch removal capacity however still decreased. The type of sorption occurring (adsorption or enmeshment) was believed to be important for these variances. Better quality supernatants were noted after substrate colloid addition. It was hypothesized that dispersed bacteria in the supernatant were coagulated by the organic colloids. Finally, carbohydrate colloids responded in a manner consistent with contact stabilization theory. Specifically the carbohydrates exhibited better sorption characteristics as well as an adsorption and release phenomenon.