EFFECTS OF SELECTED ORGANIC POLYELECTROLYTES ON BIOLOGICAL SYSTEMS

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PREFACE

Bacterial flocculation can occur by natural autoflocculation or induced chemical flocculation and is an important part of the biological treatment process. Studies have been conducted on bacterial flocculation by the addition of an organic polyelectrolyte where the investigators were interested mainly in the concentration of the polyelectrolyte necessary to cause optimum flocculation. Large dosages of polyelectrolyte were generally required for optimum flocculation in the previous studies; however, the metabolic responses of the bacterial cells in the presence of the polyelectrolytes were not determined. The purpose of this study is not only to determine the effectiveness of a selected cationic and anionic polyelectrolyte under various aspects of flocculation, but also the metabolic response of the bacterial cells to various concentration of the polyelectrolytes. Acclimation of bacterial cells to the cationic polyelectrolyte was also attempted in these studies.

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CHAPTER I

INTRODUCTION

The Water Problem

On the basis of total water demand, there is no water shortage in the United States. This country receives an average rainfall of roughly thirty inches a year. After the needs of natural vegetation have been satisfied, there are approximately 7500 gallons per day for every person in the United States. With industry, irrigation, and human consumption taking their needs, roughly one-fifth of this available supply is being used; however, water is unevenly distributed, and the greatest demand for water is in areas of heaviest pollution. Since pollution is helping to destroy the usefulness of the remaining water supplies, steps must be taken to protect this water or to find new sources of supply. Since the latter expedient appears to be too expensive, and it behoves the population to treat its waste in order to protect the environment, the most practical solution appears to be treatment and re-use of water.

For the past fifty years, a number of treatment

processes for the separation of impurities from waste waters have been employed. The impurities may be soluble or insoluble, organic or inorganic matter. The insoluble organic and inorganic matter may be dense enough to settle under normal gravitational conditions and be removed mechanically from the system. The remaining insoluble material can be removed by biological or chemical methods. The soluble organics can best be removed by biological processes.

With continuing emphasis on higher degrees of treatment, and with increasing demands for water, efficient operation of existing treatment processes must be practiced while working toward more advanced treatment methods. The biological treatment process as it is known today is an efficient process when followed by gravity settling for the removal of organic contaminants. Needless-to-say, if the biological process is not working properly, the settling process cannot be expected to remove soluble pollutants. On the other hand, when bacteria convert soluble organics to insoluble organic matter, conditions for bacterial flocculation must be present or chemically induced so as to remove organic solids in the settling unit.

Chemical flocculation with inorganic chemicals has been used to some degree of success in removing insoluble solids from waste; however, the large amount of these chemicals required for efficient removal has not made this

a practical solution. Since the introduction of organic polyelectrolytes, thought has been given to the use of these organic polymers as an aid to the biological process by complimenting bacterial flocculation when there is a reduction in efficiency of the settling process. Polyelectrolytes might also be used to remove organic matter in the primary settling unit, thereby reducing the organic load on, for example, activated sludge process.

Scope and Purpose of the Present Research

In order to remove organic matter from waste water, bacteria must metabolize soluble organic matter and subsequently be separated along with the insoluble organic fraction from the waste water. Efficient settling of bacteria can be obtained only after they have flocculated. There are a number of factors which affect bacterial flocculation, and although no attempt to resolve these factors was made in the present study, they are still of interest because of their affect on the biological treatment system. Organic polyelectrolytes have been proven effective in recent years for the removal of insoluble organic matter from waste waters; however, if they are toxic or inhibitory to bacterial metabolism, they can never be used to any great extent.

The major purpose of this study was to determine inhibitory effects of selected polyelectrolytes to

biological solids grown under both batch and steady state conditions. Other aspects of this research were involved with the effects of these polyelectrolytes on various analytical tests used to measure growth or substrate removal, flocculation and settling characteristics of biological solids and acclimation of the biological solids to the polyelectrolyte.

Both a cationic and an anionic polyelectrolyte (Purifloc C-31 and A-21 respectively) were used in this research work. These two polyelectrolytes were chosen because of their opposite charge and because they have been shown to be effective in widely varying field applications (1), (2), (3).

CHAPTER II

REVIEW OF LITERATURE

Nature of Bacteria

1. The bacterial cell

The bacterial cell is made up of distinctive parts. The main cover of the cell is the cell wall which is below the extracellular capsule or slime layer and peripheral to the cytoplasmic membrane. Bacterial cell walls vary in thickness from 100 to 250 Angstroms and account for 10 to 40% of the cell dry weight (4). The major constituents of the bacterial cell wall are amino sugars, amino acids, sugars and lipids which are joined together to form complex polymeric substances. Common monomeric substances used to form the complex polymers are D-alanine, D-glutamic acid, muramic acid, glucosamine, and diaminopimelic acid (4). The cell wall of gram negative bacteria is composed of large amounts of lipid and protein and small amounts of polysaccharide, while the gram positive bacteria has a cell wall which contains large amounts of polysaccharide and small amounts of lipid and protein.

The cytoplasmic membrane is just beneath the cell wall, and it comprises roughly 10% of the cell weight (4). It is made up of a lipoprotein complex. The major function of the membrane is to control the passage of material into and out of the cell wall.

The cytoplasm is found inside the cytoplasmic membrane. The key component within the cytoplasm is the nucleus which contains the cellular nucleic acid.

In addition to units which make up the active portion of the cell, there is a capsule coating or inactive slime layer around the cell wall on most bacteria. The slime layer is largely polysaccharide complex with some lipoprotein components.

2. Cell composition

The chemical composition of bacteria resembles that of other organisms in the nature of elements and compounds present. An elemental analysis of bacteria reveals oxygen, hydrogen, carbon, nitrogen and phosphorous as major constituents. These elements, together with lesser quantities of potassium, magnesium, calcium, sodium and chloride ions constitute the bulk of the elementary composition (4). Present in still smaller or trace quantities are iron, aluminum, manganese, copper, boron and a variety of other elements. The ash content varies from 1 to 14%. The major constituent of bacteria is water, ranging from 75 to 90% of the total weight. The organic matter is composed

of 40 to 80% protein, 1 to 36% carbohydrates and 1 to 39% lipid (4). A portion of the proteinaceous material is in the form of peptides. In certain species, free amino acids occur and the amount varies with nutrient conditions during growth more extensively than do bound amino acids.

Luria (4) has found that the acid soluble organic fraction of bacteria consists of all metabolic intermediates such as phosphorylated sugars, ATP and amino acids. The acid soluble fraction constitutes about 8% of the cell carbon and 20% of the phosphorus. The acid insoluble fraction was divided by Luria (4) into a number of basic components. The total lipid content of a cell varies from 10 to 15% of the cell carbon and approximately 10% of the cell dry weight. The lipid content comprises as much as 20% of the cell dry weight when the substrate consists of a fatty acid. Beta hydroxbutarate is the form in which 10 to 25% of the lipid is stored in the cell. Ribonucleic acid represents between 10 and 20% of the cell dry weight with larger values being attained during the log growth phase of the cell. The RNA has been measured between 5×10^{-15} grams for an old cell to 3×10^{-14} grams for a young cell (4). Proteins in the bacterial cell represents approximately 50% of the dry cell weight, 60% of the cell carbon and 70% of the cell sulfur. The carbohydrate content of a bacterial cell is mainly in the form of nucleic acids and oligosaccharides. In the gram positive

bacteria, approximately 45% of the carbohydrate is in the cell wall while in gram negative bacteria, only 15 to 20% of the cell carbohydrate is in the cell wall. Polyphosphates found in bacterial cells are highly polymerized phosphoric acid which is formed by enzyme catalysis from adenosine triphosphate (4).

3. Aspects of bacterial growth

Since this research pertains to addition of organic polyelectrolytes to a synthetic waste, it seems important to review kinetics and mechanisms of bacterial metabolism so any inhibitory or toxic effect due to the polyelectrolyte could be discussed in light of basic metabolic considerations.

The two basic types or operational units currently used in activated sludge research are the batch or plug flow unit and the continuous flow completely mixed unit. Operation of the batch or plug flow type unit involves inoculating a fresh medium with a mixed microbial population and allowing the cells to grow under aerobic conditions at the expense of available substrate. After the inoculation of cells into this medium, there is a period of time in which total number of cells remain constant while the total dry weight of the cells continues to increase (5). This period is called the lag phase and is the time required for bacteria to induce enzymes required for metabolism. The next phase is the logarithmetic or

exponential phase in which cells divide at a steady rate determined by the generation time of each particular species. During this phase, the total dry weight of cells continues to increase while substrate is being depleted. Once the available substrate is depleted, cell growth remains fairly stationary. During this period, cell reproduction is balanced with an equivalent death rate, but the total dry cell weight starts to decline (5). Following the stationary phase, bacteria die at a rate which exceeds the rate of reproduction. This is called the declining or death phase. The total dry cell weight continues to decline during this period. Bacteria do not abruptly change from one phase to the next but proceed gradually from one phase of growth to the next (6).

Growth may be limited by a limiting concentration of an essential substrate. Monod (7) has shown that the specific growth rate μ, was a function of the concentration of a limiting growth substance and represented this relation by the following expression:

$$\mu = \frac{\mu_m S}{k_s + S} \qquad (1)$$

where S is the concentration of the limiting growth factor and k_s is the saturation constant which is numerically equal to the substrate concentration at which μ is equal to half of the maximum logarithmic growth rate, μ_m. It is interesting to note that this equation is similar to the

Michaelis-Menten equation for enzyme reactions. Microbial growth can be considered as the result of a series of enzymatic reactions in which the overall reaction rate is controlled by the slowest reaction.

When bacterial cells are in the log growth phase in either a batch system or a steady state system, increase in cell population can be expressed by the equation:

$$\frac{dX}{dt} = \mu X_o$$

which upon integration gives the following form

$$X_t = X_o e^{\mu t} \qquad (2)$$

where X_t is cell population at time t and X_o is the initial population of cells.

In completely mixed continuous flow units, establishment of a steady state may be expected. Steady state refers to a condition in which the rate of growth of organisms in the reactor is equal to the rate at which they leave the reactor (8). The steady state condition indicates not only a constant concentration of cells but also a constant effluent substrate value. In other words, under steady state conditions, the logarithmic rate of growth μ, is equal to dilution rate, D. Substituting the Monod equation for μ, the rate of change in population density may be expressed as:

$$\frac{dX}{dt} = \mu X = X\mu_m \frac{S}{k_s + S} \qquad (3)$$

Cell concentration X, may be expressed as number of cells or dry weight of cells.

The cell yield Y, is assumed to remain constant during the log growth phase and is usually expressed as the dry weight of cells produced from a given amount of substrate (8):

$$Y = \frac{dX}{dS} \tag{4}$$

Substituting dX from equation 4 into equation 3 provides a formula for the rate of substrate utilization:

$$\frac{dS}{dt} = \mu_m \frac{X}{Y} \frac{S}{k_s + S} \tag{5}$$

Equation 3 and 5 can be solved for X and S under steady state conditions by equating the differential to zero.

A heterogeneous culture may contain a countless variety of organisms, but by the action of a number of factors, such as type and concentration of substrate, intermediate production, pH changes, etc. the predominance could shift to a more restricted type of population. Heterogeneous microbial systems are more complex than pure culture systems, and periodic changes in predominance may be expected. Such changes may disrupt the steady state; regardless of these factors, some activated sludge processes have been operated at conditions which approach steady state with respect to cell and substrate concentration.

4. Change of bacterial surface charge

It is known that the composition of the bacterial cell may vary widely; however, the cell composition can be controlled to some degree by controlling the physiological state of the bacteria (4). Under different physiological conditions, it may be reasonable to surmise that the surface charge characteristics of the cell will change (9). The change of surface charge may not be from negative to positive but may involve a change in intensity of negative charge; i.e. surface characteristics may change from one containing all negative sites to one containing mainly negative sites and a few positive sites, thus reducing the net negative charge. Herein may be one of the explanations to the phenomenon of bacterial flocculation.

Riddick (9) in measuring the zeta potential in a large number of active microbial suspensions, noted that the individual particle charges varied from -16 to about -30 millivolts. In this work with unsterilized systems, prolonged microbial activity of the system lowered zeta potential of the system to where agglomeration of discrete particles occurred. This was followed by a build up of particle size and finally sedimentation. In the sterilized systems, no change in zeta potential was ever noted. It was not clearly determined whether the bacteria lowered the surface charge of other colloids or whether the bacterial surface charge itself was lowered.

In any case, the bacterial population appeared to be responsible for reducing zeta potential in the system. In the same article, Riddick (9), while discussing the pathways of microbial decomposition, used an example of decarboxylation where CO_2 was driven off the carboxyl group leaving hydrogen which rendered the net negative charge of the particle less electronegative.

Lamanna and Mollette (10) have reported that surface charge of Escherichia coli culture varied with the age of the bacterial culture. After this organism was inoculated into fresh peptone broth, growth was accompanied by an increase (more negative) in zeta potential; the maximal value coinciding in time with the log growth phase of the culture. Potential values began to decrease (less negative) after the period of greatest multiplication was past and reached minimal value when the population of bacterial cells became stabilized in the so-called stationary phase. Increase in charge, according to this investigator, may be proportional to the increase in surface area during the growth period; however, decrease in net charge after the log growth period to some more positive value would indicate the bacterial surface charge does change with age.

Abramson (11) and Moyer (12) also stated that changes in the charge density of bacteria do occur with changes in the age of the culture.

Chemical composition of the slime layer was believed by McKinney (13) to be responsible for the electrical charge on bacterial surfaces. McKinney stated that it was possible polymeric substances were always excreted, but under prolific growth conditions, new surfaces were created faster than they could become covered with these substances, and the net negative charge increased. In the declining growth and endogeneous respiration phase, the net negative charge decreased as the amount of surface slime increased.

5. Possible deleterious effects of polyelectrolytes on bacterial cells

The addition of a high molecular weight organic polyelectrolyte to a biological system may have a deleterious effect on the bacterial cells. These water soluble organic polymers exert an electrical charge. The charge sites on the polymer may be attracted to the bacterial cell and adsorbed on the cell by bridging or charge neutralization (14). Yu (15) believed that polymer molecule was too large to penetrate into the cell but was attracted to the cell surface because of the opposite charges. He believed that a reduced rate of substrate utilization in the presence of a high concentration of polyelectrolyte was caused by either metabolic inhibition or reduction in available entry space for substrate because of increased flocculation. The attraction of the

polymer molecule to the bacterial surface may lead to possible deleterious effects such as metabolic inhibition and damage to the cell wall (lysing). Cell flocculation itself could decrease the substrate removal capability of the cells.

a. Metabolic inhibition

The addition of an organic polyelectrolyte could, in response to the change in its environment, alter the growth of bacteria. In a sense, the polyelectrolyte could be likened to a qualitative shock load on the bacterial system. According to Gaudy (16), selection of species, change in metabolic pathway, and induction of required enzymes are three major factors which can govern the response to qualitative shock loading. Time may be required for bacteria to respond to the sudden change in environment, i.e. an acclimation period may be required in order for the bacteria to utilize the polymer as a substrate by inducing the proper enzymes or to develop a resistance to any harmful chemical effect.

During the course of glucose metabolism, permeases may be necessary to transport glucose into the cell (15). When the polymer becomes adsorbed on the bacterial cell and bridging and/or neutralization occurs, the polymer molecule may cover a portion of the permease sites and prevent to some degree the formation of glucose-permease complexes or the entry of glucose into the cell (15).

This could prevent, to some extent, utilization of the glucose and formation of new cellular material.

b. Lysing of bacterial cells

Yu (15) in discussing the effects of addition of Purifloc C-31 to a biological system, observed a reduction in the total dry cell weight of the sludge along with an immediate increase in soluble COD in the medium. He accounted for this by possible lysing of some bacterial cells and leakage of cellular material into the system. Kincannon and Gaudy (17) observed this same decrease in cell weight along with the increase in COD in their studies on the effects of NaCl in biological systems. These findings may have been, to a greater or lesser degree, similar in both of these experiments; however, this does not mean that organic polyelectrolytes and NaCl inhibit bacterial cells by the same mechanism.

c. Flocculated biological solids

Flocculation of biological solids may result in a reduction in the total surface area of the bacterial cells. Yu (15) hypothesized that the reduction in surface area by flocculation may only allow the outer surface area to have contact with the substrate. If it was possible for the flocculated cell to be compacted to a state in which the substrate was excluded from the interior of the floc mass, then the rate of substrate utilization should be substantially reduced. If new cells are produced and

released from the floc the rate of removal should increase.

Theories of Bacterial Floc Formation

There appears to be considerable uncertainty in regard to mechanisms whereby finely divided suspended matter and bacterial cells coalesce. This lack of clear understanding of the process has resulted in confusion pertaining to adequate terminology. The terms flocculation, bioflocculation and bioprecipitation have all been used interchangeably to describe this mechanism (18). In a biological waste treatment process, the term bioflocculation might best describe the removal of finely divided suspended matter by biologically active material. Heukelekian (18) distinguished bioprecipitation from bioflocculation as the removal of soluble material rather than suspended matter. The term flocculation has traditionally been used when physical forces (mechanical agitation) play a primary role in separating liquid and solid phases.

1. Zoogleal organisms

In 1935, Butterfield (19) noticed that whenever an activated sludge had been developed, floc was formed which contained considerable amounts of zooglea. When the process was working most efficiently, zoogleal masses predominated in the sludge. These findings, along with observations of previous workers, pointed to the

importance of this type of organism.

Butterfield mentioned in his report that in 1892, Winogradsky observed that nitrite-forming bacteria existed in both the motile and zoogleal stages. The zoogleal form was believed to be representative of a resting stage for the bacteria. This may have been true for the nitrifying bacteria observed by Winogradsky, but Butterfield showed that the zoogleal stage, although immotile, was an active phase in terms of metabolic activity. Butterfield (19) observed that flocculation was the result of sticking together of particles by gelatinous material secreted by bacteria. He concluded that flocculation was a direct result of the presence of a zooglea-producing bacterium, Zooglea ramigera. In addition to the well supported bacterial concept of zooglea formation, he also stated that protozoa were responsible for floc formation through a gelatinous slime excreted to trap the bacteria before ingestion (19). Butterfield's observation that flocculation occurred in the presence of zooglea was undoubtedly true. The zoogleal mass may have reduced the negative charge of the bacteria or could have contained some positive charge sites itself. Both factors could aid in flocculation of bacterial cells. Since no chemical definition for zooglea or zoogleal mass has been given, it is impossible to comment on its charge characteristics. From

Butterfield's discussion, this material appeared to be similar to the polysaccharide found in bacterial slime layers. McKinney (20) has reported isolating a number of different floc-producing bacteria from activated sludge processes. Microscopic examination of the floc formed by different bacteria, grown on a soluble substrate, indicated little difference in floc characteristics. All flocs were composed of discrete bacterial cells clumped together. As the flocs aged, there was a disappearance of the definite bacteria structures, and the floc appeared as an amorphous mass with a few individual bacteria on the surface. McKinney (20) claimed that special zooglea-producing bacteria were not necessary to form a floc of the same type as activated sludge.

Crabtree, et al (21) in an investigation of carbohydrate metabolism by cultures of Zooglea ramigera, observed that they accumulated a large amount of sudanophilic granules, suggestive of polybetahydroxybutyric acid which is an endogenous metabolite unique to certain bacteria. The extracellular accumulation of this polymer was considered interesting in that the organisms always flocculated when the polymer became visible in the Sudan Black stain under microscopic examination. This material may be the zooglea which Butterfield (19) described.

2. Enzyme coagulation of carbohydrates and related compounds

The coagulation, or rendering insoluble of a variety of substances in growing cultures, has been ascribed by Buchanan (22) to the activity of enzymes. In some cases, the essential change may be dehydration caused by polymerization leading to formation of an insoluble compound. The enzymes, which were designated as producers of "coagulations" of carbohydrates, were regarded as syntheases (22).

3. Bacteria surfaces

Investigations into the nature of bacterial surfaces have indicated that a polysaccharide slime layer or capsule normally surrounds the bacterial cell. Dubos (23) stated that capsular production was not dependent on the bacterial strain but was a factor of the conditions under which the culture was growing. This was in disagreement with Butterfield's (19) statement that the slime layer could only be produced by Zooglea ramigera. After reviewing the work of other investigators, Dubos (23) concluded that capsular material was a high molecular weight polysaccharide containing acidic, acetyl and amino groups in varying amounts.

Tenney, et al (24) have reported that natural bacterial flocculation was a direct result of the presence of certain extracellular material. When they removed this material from the flocculated cells and the cells were resuspended, stable dispersions resulted. They also

found that surface charge reduction and/or reversal were not necessary to cause flocculation. The author is of the opinion it was possible that production of the extracellular material could have reduced the surface charge of the bacteria sufficiently before flocculation occurred. It has been observed in working with a pure culture of Aerobacter in the Oklahoma State University Bio-Engineering Laboratories, that flocculation of the organism occurred during an active growth phase while there was no evidence of slime or extracellular material present as indicated by the india ink staining method.

Aspects of colloidal chemistry are important to better understanding of bacterial flocculation. Actually, bacteria are larger than "true colloids" but they are subject to many of the forces which affect colloids. Colloidal particles can be divided into two main classes; hydrophobic and hydrophillic, depending on their surface characteristics (20). Hydrophobic colloids generally lack stability in the presence of small quantities of electrolytes and are primarily inorganic. Hydrophilic colloids have a strong attraction for water and are primarily organic. Adsorbed water prevents the hydrophilic colloids from flocculating readily (20). In general, these hydrophilic colloids carry a net negative charge within the normal pH range in which the activated sludge process operates. Since surface charge of bacteria is

believed to be acquired through acid-base interactions of functional inorganic groups, surface charge density can be expected to be strongly pH dependent (20). As two colloids of like charge approach each other, the electrokinetic force of repulsion rises rapidly. Operating contrary to the electrokinetic forces of repulsion are the gravitational Van der Waals forces which tended to attract the two colloids. Electrokinetic forces increase as the reciprocal of the square of the distance separating the colloids, whereas Van der Waals forces increase as the reciprocal of the seventh power of the distance between the colloids. Thus it can be seen that when two colloids approach close enough, Van der Waals attractive forces predominate and the two colloids can stick together. When electrokinetic potential is high, it might not be possible to bring the cells close enough to floc. For most hydrophobic colloids, the "critical" potential, above which flocculation would not occur, was found by McKinney (20) to be between 20 and 30 millivolts.

Abramson (11) found that bacteria had a definite cell potential and reduction of this potential resulted in agglutination of bacteria. The reduction of cell potential was brought about by addition of various salts to the solution. Lowering the surface charge below fifteen millivolts resulted in autoagglutination of pure bacterial suspensions (20). Thus it can be seen that electrical

charge can play an important role in flocculation of organisms.

4. Gases

Agglomeration of suspended solids and bacterial cells in sewage has been explained by Williams (25) as the action of bubbling various gases through the liquid. He observed that coagulation obtained by air, oxygen, nitrogen and hydrogen over a six hour period was not materially different. Over a period of twenty-four hours, the following order of efficiency was observed: oxygen, air, nitrogen, and hydrogen. He concluded from these observations that chemical or biological effects or both were present, though small in comparison with physical effects. Bubbling different gases through sewage at different temperatures gave little if any coagulation at 0° and 10°C. At 25° and 37°C, clarification of suspended matter by different gases was greatly increased. These results might indicate a biological factor. At 80°C, slightly better coagulation was obtained than at 25° or 37°C, possibly due to heat coagulation. Removal of suspended material by gas bubbles, he concluded, would appear to be due to the combined action of two different processes; namely, biological changes operative only at temperatures of 25° and 37°C, and coagulation by physical means alone at 80°C. Since normal sewage temperatures never reach 80°C, the last factor may be disregarded.

Rudolfs and Gehm (26) stirred sewage in the presence of air, oxygen, ozone, nitrogen, carbon dioxide and hydrogen peroxide and also subjected it to bubbles of the same gases without the aid of stirring. Increased removals of BOD, turbidity and suspended solids over plain settling were observed. During short periods of one-half hour, all gases showed approximately the same removals with no reduction in soluble BOD. It was suggested that BOD removals were caused by removal of finely divided material by physical agglomeration. Continued treatment led to BOD reductions in the soluble fraction when gases containing oxygen were used. Removal of suspended solids on continued treatment was the same with all gases. Oxidation was greater with diffusion of air when compared with stirring, but turbidity removal was somewhat poorer. It would appear that the action observed by these investigators could be the result of biological and possibly chemical action instead of mainly physical action as they suggested. The observations made at various temperatures by Williams (25) tends to substantiate the idea of bacterial and possibly chemical action. Chemical action could take place when using different gases by forming new compounds which result directly in flocculation or by charge neutralization. For instance, in a slightly acid medium, a negative carbonyl group could combine with hydrogen to form a more neutral carboxyl group:

$$-COO^- + H^+ \longrightarrow -COOH \qquad (7)$$

Also in a slightly acidic solution, the amino group might be effected as follows:

$$-NH_2 + H^+ \longrightarrow -NH_3^+ \qquad (8)$$

5. Energy concept

McKinney (20) has proposed a theory of bacterial flocculation based on the concept of energy. From his work on biological flocculation, he states that microscopic examinations of nonflocculating bacteria have shown that all were extremely motile, indicating, according to McKinney, that active metabolism of the substrate was still in progress. Floc-forming bacteria were characterized by a complete lack of motility indicating little to no metabolic activity; however, he failed to show substrate concentration data which would indicate the lack of energy (in the case of flocculation) or the presence of energy (in the case of dispersion). Also his arguments lead one to believe that any motile organism would not flocculate while there was any available energy source in the substrate and that any non-motile organism would always flocculate regardless of the energy in the system. McKinney stated that if flocculation was a normal phenomenon which resulted when the bacterial food supply was exhausted then all bacteria which he isolated would eventually flocculate. In his studies, daily microscopic

examinations were made on all isolated bacteria until they flocculated. All these cultures flocculated within seventeen days and all organisms showed considerable activity before floc formation and little to no activity after flocculation. From this work, McKinney concluded that flocculation did not result until bacteria went into the endogenous phase of metabolism since ample energy was available during the growth phase to maintain motility. Observations of bacteria during floc formation showed that bacteria became a part of a floc only when they lacked sufficient energy (for motility) to break away from the floc (20).

6. Other theories

In some of his early work, Butterfield (19) stressed the importance of an inert foreign substance such as cotton fibers acting as a binder or framework for the floc formation in pure culture work. He indicated that inert foreign materials such as silt and clay may form the nuclei around which the organisms build their gelatinous colonies. This may account for some of the differences in flocculation observed while working with completely soluble wastes and wastes which contain both soluble and insoluble fractions. Using the electron microscope, Duguid and Wilkinson (27) have observed fine appendages 5-10 mu thick known as "fimbriae" which occur in many strains of Enterobacteriaceae. There were usually 100 to

300 around the cell periphery. Their function was not determined, but they seemed to confer certain adhesive properties including agglutinability (27). This strongly adhesive property of cells in the fimbriated phase may play a roll in bacterial cell flocculation.

Pelczar and Reid (28) have stated that when a bacterial cell divided, the two daughter cells could separate at once or remain attached to one another by their cell membranes. When this incomplete separation persisted during many successive cell divisions, cell aggregates (flocs) were produced. Aggregates formed in this way were characteristic of certain bacterial species. Staphlococci grew in irregular groups or clusters like bunches of grapes, while streptococci took the appearance of a chain. Sarcina, on the other hand, grew in cubes. This action has only been observed for these particular organisms but may, in some way, be related to cell flocculation.

Nature of Colloidal Surfaces

Pauling (29) attributed stability of colloidal particles in aqueous suspensions to hydration and electrostatic charge. According to him, both of these phenomena depend primarily on chemical composition of the substrate. The particle presents to the suspending medium an electronic or electrostatic capacity, i.e., it can lose,

gain or share electrons by forming bonds such as covalent, ionic, hydrogen, dipolar, or induced dipolar (29). These types were classified in terms of bond energies which are expressed as kilocalories per mole (29). The ionic crystal bonds are the strongest (150 to 200), covalent are next (50 to 100), hydrogen follows next (1 to 10) and then polar bonds (less than 5). Similar to polar bonds, induced polarization from Van der Waals forces produced bonds of low energy.

In most coagulation processes, covalent bonds are neither made nor broken, but the system is subject to ionic equilibrium in solution. Interaction among colloidal particles is influenced by ionic bonding, electronegativity and dipolar bonding (30). The term electronegativity expresses an affinity for electron deficient hydrogen atoms because of unshared or resonating electrons. Forces of this type account for the adsorption of oppositely oriented water dipoles and preferential adsorption of ions to establish, by concentration gradients, an electrostatic potential gradient or zeta potential (30). After the stabilizing effects of hydration and electrostatic charges have been removed, flocculation may occur.

1. Instability factors

The two most important instability factors are Brownian movement and Van der Waals forces of attraction. Brownian movement is the movement imparted to suspend solid particles

because of their impact with invisible, rapidly moving particles in the medium. It is essentially a thermal effect that tends to drive particles closer to each other and even causes them to penetrate the force fields surrounding each particle (29). Although the nature of the Van der Waals force is not well understood, it may be described as molecular cohesive forces that increase in intensity as the particles approach each other.

2. Stability factors

The most important stabilizing factors are hydration and zeta potential (29). Hydration is the property possessed by some particles which enhances attraction of relatively large numbers of solvent molecules to their surface. When the solvent is water, such particles are called hydrophilic. Zeta potential is much more complicated and is effected by a number of different factors. According to Brinton (30), solvation and ion adsorption are opposing forces because of high ionic strength limits solvation and vice versa. Also, positive ions in aqueous systems are solvated to a higher degree than negative ions; therefore, negative ions predominate at the interface either by being excluded from the bulk of the solution or by increased compaction by adsorption on the particle surface. This argument has been put forth to explain the fact that most colloids found in nature are negatively

charged (30). Brinton stated that negative ions predominate at the interface; however, this would not seem to eliminate the possibility that although a colloid does have a (net) negative charge there may still be some positive sites on its surface. This last statement might also help explain why some colloids have a more negative charge than others when measured by electrophoresis.

If the particle surface posses a negative charge, and the bulk of the solution is electrically neutral, then a potential gradient must exist between the particle surface and a point in the bulk of solution some distance away. Figure 1 was used by Priesing (31) in reviewing the factors affecting zeta potential. This figure shows the potential decay as a function of distance from the particle surface. According to Priesing (31) a fixed layer of counterions surrounds the particle, which may originate from within the colloidal mass itself, or by preferential adsorption from solution. This layer, which is sometimes called the Stern layer or compact double layer, would in the absence of Brownian motion, constitutes the entire double layer, and therefore, agree more closely with the early theories of Helmholtz. Brownian motion, plus synthetically induced velocity gradients, prevented the Stern layer from establishing electroneutrality within itself. As a result, this fluid motion diffuses counterions out into the bulk of the solution (31).

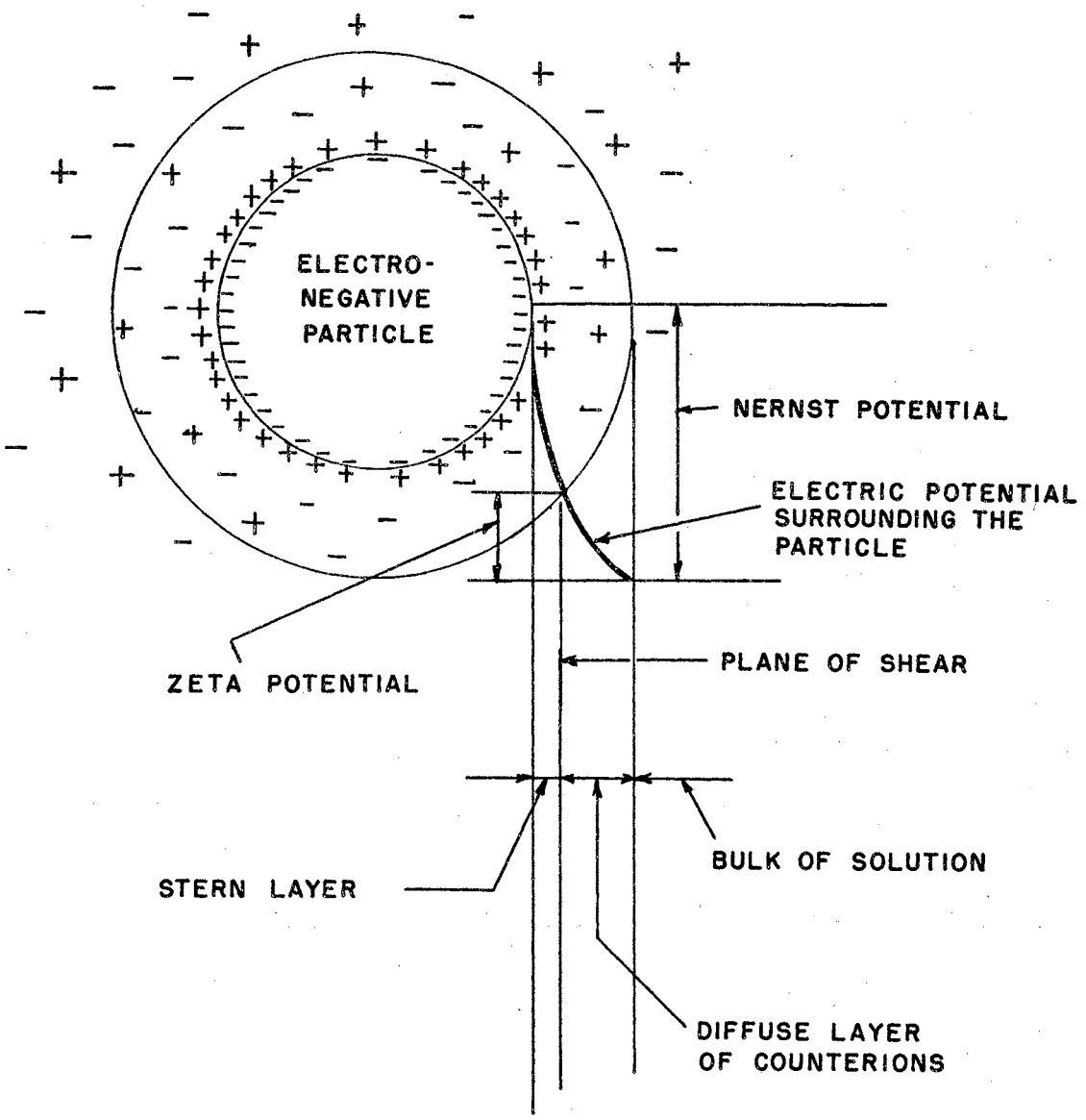


Fig. 1 - Factors Affecting Zeta Potential

Electroneutrality is, therefore, established at some finite distance away. Because counterions are so strongly attracted to the particle surface, their high concentration within the Stern layer shields the particle from other counterions which are then less strongly attracted. As a result, the potential drops sharply in the Stern layer but gradually thereafter. As the concentration of the counterions decreases within the double layer, the concentration of similar ions, which are repulsed at the particle surface, increase from the outer edge of the Stern layer to the bulk of the solution where electroneutrality with counterions exists. This region between the Stern layer and the solution proper is called the diffuse double layer or the Gouy-Chapman layer and the potential gradient over this zone is called the zeta potential (31). When the particle is placed in motion with a direct current potential gradient (electrophoresis), the Stern layer, is believed to migrate rigidly with the particle. Accordingly the dividing line between the Stern and Gouy-Chapman layers is called the plane of shear. There is not complete agreement about the exact location of this plane of shear or of the real boundry between the double layers.

Because potential depends on surface charge density and thickness of the double layer, it may be increased by increasing the charge density of the particle. Thickness has an inverse relationship to electrolyte charge density

and concentration, therefore increasing either charge density or concentration of similar ions or counterions decreases potential (9). By using electrophoretic velocities, measurable by a number of techniques, the zeta potential can be determined directly according to the relationship established by Helmholtz and Von Smoluchowski:

$$\text{Zeta potential} = \frac{4\pi\eta\mu}{E} \qquad (9)$$

when η is the viscosity of the medium, E the dielectric constant and μ the electrophoretic velocity.

Induced Chemical Flocculation

1. Multivalent metal ions

Typical metal coagulants such as aluminum and iron have been used for many years in domestic sewage plants as flocculating agents to remove solids from waste water. They have also been used as sludge conditioners in dewatering of sludge solids. In flocculation of microorganisms, efficiency of the metal coagulants has been found to be dependent largely on the pH of the medium and on the stoichiometric relationship between the concentration of microorganisms and dosage of metal ion (32). The optimum pH range for effective flocculation according to Tenney and Stumm (32) was approximately pH 5 to 6 for aluminum and pH 4.5 to 5.5 for iron. Within this pH range, all the aluminum and iron ions undergo rapid hydrolytic reactions

and form hydroxopolymers. At pH values lower than this optimum range, where free metal ions (Al^{+}_{3}, Fe^{+}_{3}) were prevalent, and in the neutral and alkaline pH range where hydrolysis led immediately to hydrous metal oxides, ready flocculation of microorganisms did not occur (32). It was especially interesting to note that in the acid range of pH (less than 4) the charge of the microbial surface was less negative than in neutral solutions and thus supposedly more amenable to flocculation. Large dosages of either iron or aluminum under these conditions would not coagulate microbial cells.

The fact that these workers found hydrolysis products alone and not free metal cations or hydrous metal oxides produced agglomeration of microorganisms suggested that the aggregation produced by aluminum or iron salts was caused by interaction of linear polymers resulting from hydrolysis of these salts with dispersed cells. Metal ions at the end of these short chain polymers could be bounded predominantly by chemical forces onto the ionic groups on the microbial surface.

2. Polymers of biological origin

Polymers of biological origin, especially polymers which carry electrical charges, are of great importance in organization of biological structure. Proteins, protamines, nucleic acids, pectic acids, alginic acids and numerous polyacids of bacterial origin are natural

polyelectrolytes (9). It thus appears possible that bioflocculation might be interpreted in terms of polyelectrolyte interactions. It has been reported by McKinney (13) that natural polymers such as complex polysaccharides and polyamino acids are excreted or exposed at the surface of the cell predominantly during the declining growth and the endogenous respiration phase.

3. Synthetic polyelectrolytes

An important development in colloidal chemistry within the last ten to twenty years has been the production of synthetic polymers with high molecular weight which are similar in general properties to biocolloids or organic compounds mentioned earlier. Synthetic polymers contain many recurring units of low molecular weight which are chemically combined to form a molecule of colloidal size. Each of the recurring units carries one or more electrical charges or ionizable groups. Because these compounds have the characteristics of polymers and electrolytes when in solution, they are called polymeric electrolytes, or more often polyelectrolytes.

Approximately fifteen years ago, the synthetic polyelectrolyte came into prominence as coagulants for colloidal suspensions and were instrumental in reviving interest in the older metal coagulants. The mechanism of action of metal coagulants had to be more fully

understood before work on the polyelectrolyte could progress. Ruehrwein and Ward (14) compared the effects of polycations and polyanions or clay suspensions. Their experiments showed that polycations were effective coagulants alone while polyanions were good coagulant aids after a metal coagulant had been added. In order to explain their results, they postulated that cationic polymer would flocculate by charge neutralization or polymer bridging between particles. Polyanions worked alone by a process of anion interchange with clay where the carboxyl ions replaced absorbed anions such as OH on the clay surface; however, if a metal coagulant was added first and increased the number of positive sites on the solid, the anionic polymer would be much more effective in bridging between particles (14).

In 1954, Michaels (33, 34) showed that in order for a polymer to contribute directly to flocculation of a suspension, it had to become adsorbed on the solid surface. Flocculation could then occur by two basically different phenomena: (1) the electrokinetic potential of the particle surface may be reduced sufficiently to permit aggregation by residual valence forces; (2) polymer molecules may adsorb on more than one particle and thus aggregate by bridging. For a polymer to cause flocculation by reducing the electrokinetic potential of a solid, it must first have an ionic charge and it must also have a

charge opposite to that of the net charge on the solid. Polymers carrying a neutral charge could never work in this manner. Cationic polymers, in addition to causing flocculation by reducing the electrokinetic potential, could also cause flocculation by interparticle briding. Anionic polymers cannot be responsible for flocculation by alteration of the electrokinetic potential, since they carry charges of the same sign as electronegative colloids. Also, the anionic groups on these polymers cannot easily be visualized as undergoing strong adsorption on negatively charged surfaces. Thus, it was felt that if bridging does indeed take place, it must occur by virtue of other active groups either in the polymer molecule or on the particle surface (33).

In explaining flocculation of clays by polyanions, Michaels (34) stressed the possibility that polyvalent cations such as Ca^{++} usually present in clays, may act as electrostatic bridges between the solid surface and the polyanion. Michaels also thought that adsorption of polyanions on clay occurred by hydrogen bonding between the solid and hydroxyl, amide or un-ionized carboxyl groups on the polymer. The sole function of ionic groups on the polymer in this case was to extend the polymer chain and keep it from coiling on itself (34).

Katchalsky (35) did much work on the molecular shapes of polyelectrolytes and their reactions. There are many

others who have contributed to developments in polymer chemistry. These works are too numerous to mention; however, the major theories of polyelectrolyte action have been presented.

a. Flocculation theory

Flocculation is the agglomeration of particulate matter into larger masses, thus promoting a more rapid rate of separation from the liquid than could be accomplished with unflocculated particles.

In flocculation, primary concern is usually given to insoluble colloidal particles having a diameter of from ten angstroms to one micron and supracolloidal particles having diameters ranging from one to one-hundred microns (31). Larger particles are readily removed by conventional sedimentation processes while materials smaller than ten angstroms are considered to be soluble and generally not amenable to flocculation with polymers. Although there is much work to be accomplished before establishing unifying theoretical principles to explain flocculation in the presence of polyelectrolytes, some generalized hypotheses may be synthesized for the existing literature.

When flocculation occurs in the presence of polymers, adsorption, charge neutralization and/or interparticle bridging are possible causitive mechanisms (35). When these materials are added to suspended solids systems, they are adsorbed primarily by the suspended inorganic and

organic matter present. This adsorption occurs due to formation of ionic bonds which are thought to be irreversible due to the large number of sites involved in the reaction. This adsorption is also accompanied by a measurable gain or loss of charged sites or by charge neutralization. When the electrostatic repulsive forces have been sufficiently suppressed by charge neutralization, the weaker Van der Waals forces, assisted by mechanical or natural agitation, can produce flocculation. In addition, although a particle has the polymer adsorbed to the charged sites on its surface, the polymer may bridge to adjacent particles and therefore greatly enlarge the suspended solids particles.

In a recent article, Birkner (36) reported on work pertaining to flocculation kinetics of dispersed latex particles. These latex particles were flocculated by cationic polyelectrolytes and a stoichiometric relationship existed between particle concentration and optimum polymer concentration. Polymer dosage smaller or larger than optimum produced incomplete coagulation. As the particle concentration and velocity gradients were varied, changes in polymer dosages resulted in flocs of different sizes. Birkner felt that the particle concentration and velocity gradient determined the number of collisions which were necessary for particle bridging while polymer dosage governed the number of particle-polymer bridges which

could form; however, the velocity gradient at the optimum polymer dosage also determined the maximum aggregate size before the floc starts to break up (36).

Cohen, et al (37) in their work with polyelectrolytes, have shown that a polymer dosage higher than necessary for optimum flocculation resulted in some dispersion of the solids. This was explained for the cationic polymers by a reversal of the net negative charge of the system to a net positive charge. This effect was similar to that caused by a less than optimum dosage of cationic polymer where the net charge of the system remained negative. Optimum flocculation occurred in a system having a net charge of 0 $\pm$ 3 millivolts (9).

b. Application of the polyelectrolytes to biological flocculation

In their work on the flocculation of microorganisms, Tenney and Stumm (32) thought of the bacteria as being negatively charged colloids. Their work indicated that the flocculation of microorganisms was dependent on the physiological condition of the organism, pH of the solution, degree of agitation and the flocculant dose. It appeared from their work that these were the only variables that were investigated. Tenney and Stumm concluded that Purifloc 602 (renamed by the Dow Chemical Company to Purifloc C-32), was most efficient at a dosage of 100 mg/l in flocculation of microorganisms. Purifloc 602 (C-32) is a

polyethyleneimine compound (38).

Purifloc 602, to the author's knowledge, has generally shown activity 1.4 to 1.5 times as great as that of Purifloc C-31 for this and similar types of applications. The work of Tenney and Stumm would, at least in part, therefore be in agreement with the work of Yu (15) who observed good flocculation of microorganisms in the range of 140 mg/l with Purifloc C-31. In both investigations, heterogeneous cultures grown on glucose were used. The microorganism concentration was in the range of 200 - 300 mg/l and the cells were in a young physiological condition.

Singer, Pipes and Herman (2), in laboratory work on bulking activated sludge, showed that polyelectrolytes generally enhance the settling characteristics of the bulked activated sludge. Their work indicated that optimum removal of biological solids in a bulking condition occurred at a dosage of 2 to 3 mg/l of Purifloc 601 (renamed by the Dow Chemical Company to Purifloc C-31).

Walker and Dougherty (39), without identifying the specific polymers, showed that cationic, anionic and nonionic polymers at various dosages could improve the settling rate of activated sludges. Their work on the BOD test with polymers indicated a slowdown of oxygen utilization when using the cationic and one anionic polymer while no slowdown in oxygen consumption was shown for the remaining anionic and nonionic polymers; however, they

only gave the volume and not the dosages of the polymer at which this occurred.

Jordon and Hatch (3) have reported recently on using these polyelectrolytes in various applications in an activated sludge plant. The increased efficiencies obtained in these applications indicated the versatility of the polymers.

CHAPTER III

MATERIALS AND METHODS

Development of a Heterogeneous Bacterial Population

An activated sludge was developed in a laboratory batch unit from an initial seed taken from the effluent of the primary clarifier at the municipal waste water treatment plant at Stillwater, Oklahoma. The synthetic waste used in all of the experiments was composed of the following constituants:

1000 mg/l glucose
15 mg/l 1.0 M potassium phosphate buffer
500 mg/l of $(NH_4)_2 SO_4$
200 mg/l of $Mg SO_4 \cdot 7H_2O$
20 mg/l of $Mn SO_4 \cdot 2 H_2O$
15 mg/l of $Ca Cl_2 \cdot 2 H_2O$
1 mg/l of $Fe Cl_3 \cdot 6 H_2O$
100 mls/l of tap water

Distilled water was added to bring the synthetic waste to the desired volume.

1. Batch unit

A batch activated sludge culture (9 liters) was grown

in a glass tank. Twenty-five mls of the sewage seed were added per liter of synthetic waste and aeration was started. Air was delivered to the medium through two porous glass diffusers, at a total rate of approximately 8000 cc/min.

In the standard batch unit, the daily feeding procedure was to waste one-third of the mixed liquor volume. The remaining mixed liquor was allowed to settle under quiescent conditions. After one hour of settling, one half of the remaining volume was removed from the supernatant. The remaining mixed liquor was then brought to its original volume and synthetic waste concentration and the air restarted. This procedure was followed daily.

The feeding procedure for the standard batch unit was changed for the experiment shown in figures 6 through 9 in order to obtain a high mixed liquor solids concentration so that after dilutions were made a wide range of solids concentrations would be available for experimentation. Daily, the air was turned off and the solids were allowed to settle for one hour. After this time, two-thirds of the volume in the unit were removed as supernatant. The remaining mixed liquor was returned to its original volume and synthetic waste concentration and the air was restarted; thus, very few biological solids were wasted. This unit was operated two to three weeks in this manner before the experiments were performed.

2. Continuous flow

The continuous flow process was conducted in a glass cylinderical reactor, which had an approximate volume of 2.4 liters, into which the standard synthetic waste was added at a predetermined rate. A constant volume was maintained in the reactor since the reactor volume was fixed and the air aggitation was the same throughout the experiments. As a given volume of synthetic waste was added, an equal volume of liquid containing the products and unreacted materials of the biochemical process, left the reactor. When the products and unreacted material in the effluent were numerically equal to those in the reactor, the unit was considered to be at a steady state.

Daily, twenty liters of synthetic waste were made up for one of the continuous flow units. This was sufficient volume for two days feeding with some excess. Two units were operated so this procedure was repeated daily. Each unit had two pumps and feed lines. While not being used for adding the synthetic waste, the feed line and pump were disinfected one day by recirculating a dilute chlorine solution, and the next day were washed out with tap water. When not in use, the container holding the synthetic waste was disinfected using cleaning solution.

In starting the continuous flow units, 50 mls of sewage seed was placed in the aeration tank and the air and synthetic waste feed were turned on. The air feed was

set at a rate of 2000 cc/min/liter. Synthetic waste was pumped into the aeration tank at a rate which yielded a theoretical detention time of 6.0 hours. Prior to running the experiments, complete mixing in the unit was checked by running a dilute-in and dilute-out curve with methylene blue dye. This curve is shown in Figure 2.

In the dilute-in curve, the chemostat or continuous flow reactor was filled with distilled water and a methylene blue dye solution was pumped into the chemostat. In running the dilute-out curve, the dye solution was put into the chemostat and distilled water was pumped in. Samples of the effluent were collected at various times, and the optical density was measured at 515 mμ wavelength. Wavelengths were scanned and 515 mμ appeared to give maximum adsorption on a flat portion of the resulting curve.

The theoretical values were calculated for the dilute-out curve by the formula

$$X = X_o e^{-Dt}$$

where $X_o = 1000$, $D = 0.1667$ hrs.$^{-1}$ and t was the time of run in hours. The dilute-in curve theoretical values were calculated by the formula

$$X = X_o (1 - e^{-Dt})$$

The actual values were figured by the formula

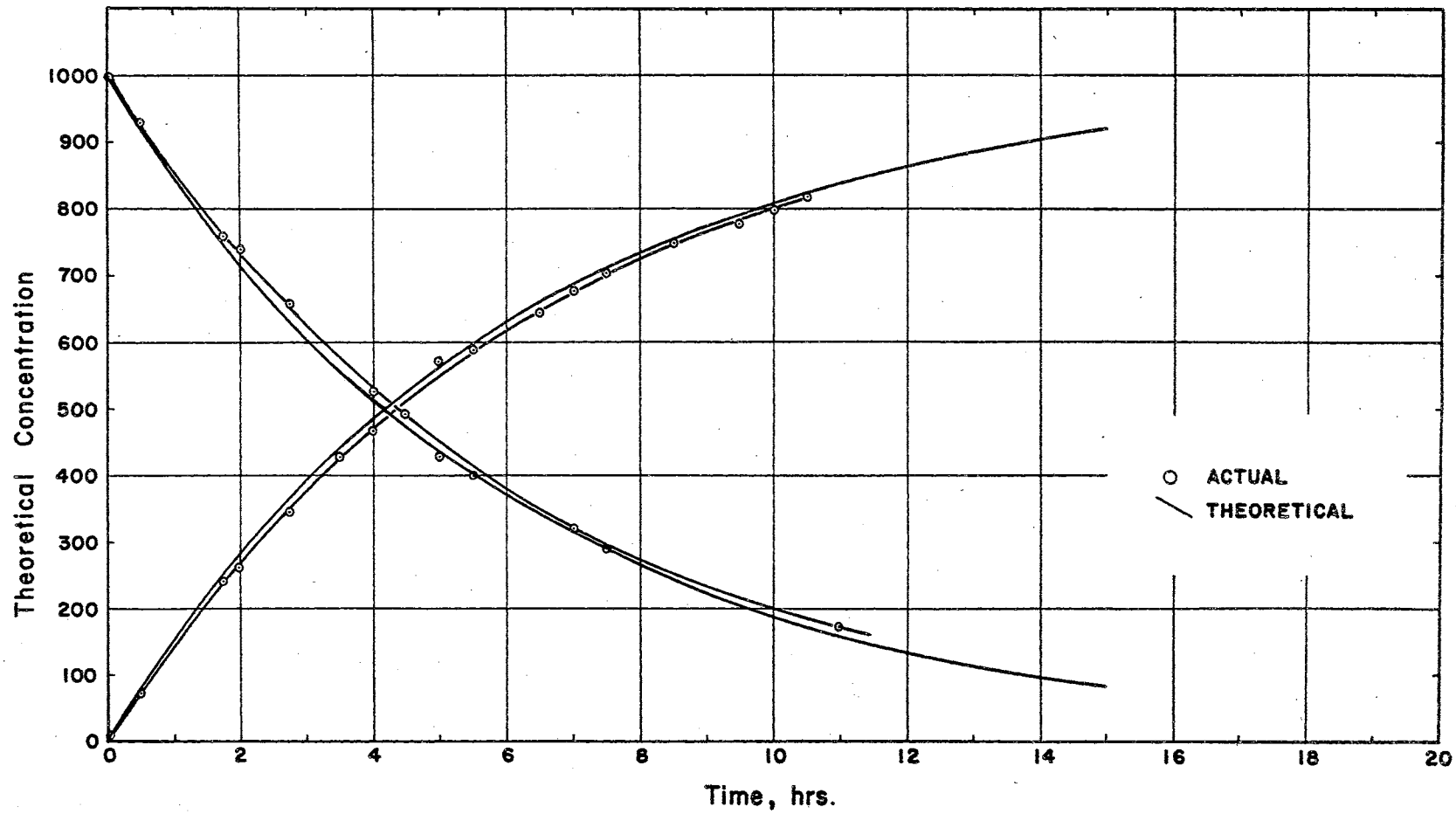


Fig. 2 - Dilute-in and Dilute-out Curve for Continuous Flow Units

$$X_t = 1000 \frac{O.D._t}{O.D._o}$$

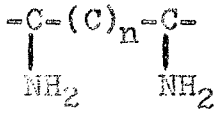
where $O.D._t$ was the optical density at any time t, and $O.D._o$ was the optical density of the dye solution at the start of the experiment.

Nature of the Organic Polyelectrolytes Used

In these studies, organic polyelectrolytes were used as "tools" to increase the flocculation efficiency of biological solids. No effort was made in this research to determine the most efficient polyelectrolyte, but a decision was made to limit the work to two polymers (one cationic and one anionic) that were widely used in the field (1), (2), (3). The polymers selected were Purifloes C-31 and A-21.

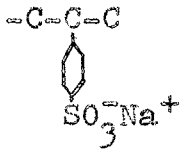
1. Purifloc C-31

Purifloc C-31 has been identified as polyalkaline polyamine (40). It is an organic, high molecular weight, cationic polyelectrolyte which is soluble in water (41). This organic polymer is available as a viscous solution having a specific gravity of approximately 1.25 at 25°C. The sample of Purifloc C-31 that was used in these experiments has a molecular weight in the range of 50 - 100,000 as determined by viscosity measurements. Very little information is available on its exact chemical structure; however, it may be visualized as follows:



2. Purifloc A-21

Purifloc A-21 is a synthetic, organic, very high molecular weight anionic polyelectrolyte (42). In its commercial form, it is a white, granular free-flowing powder. It is water soluble and can be made up to as much as a 5% solution by weight with special dispersing equipment. This polymer is essentially non-corrosive in the dilute form to most standard materials of construction with the exception of zinc and aluminum or their alloys. It is recommended that 1.0% stock solutions be made up for laboratory investigations and these diluted to 0.1% for working solutions (42). The sample of Purifloc A-21 that was used in these investigations has a molecular weight in the range of 10×10^6 as determined by viscosity measurements. An anionic polymer has been shown by Pugh and Heller (43) with a carbon backbone having sodium sulfonated aromatic rings attached as pictured below:



1. Biological solids determination

a. The membrane filter technique

All mass or biological solids concentration were determined using the membrane filter technique (0.45 μ pore size) as outlined in Standard Methods (44).

Before filtering, samples were centrifuged at 18,000 rpm for ten minutes in a refrigerated Sorvall Centrifuge (model RC 2-B). All gross and tare filter weights were made using a Mettler Gram-atic Balance.

b. Optical density measurements

Optical density was used as a means of measuring the turbidity of colloidal suspensions. Optical density also was used as a quick, approximate measure of the concentration of biological solids. The instrument used was a Coleman Junior Spectrophotometer model 6D (45). Light transmittance was measured and then converted to optical density using a conversion table. The relation between optical density and light transmittance can be expressed by the equation O.D. = $\log_{10}$ T, where O.D. represents optical density and T. represents the percent light transmittance. All measurements for biological solids were made at 540 mμ wavelength.

c. Protein determination

The biuret test was carried out during selected experiments to determine any change in protein content of the cells. The procedure followed was outlined by

Gaudy (46). As part of this test, the samples were centrifuged at 5,000 rpm for three minutes and the light transmittance of the supernatent was measured on the Coleman Junior Spectrophotometer (model 6D) at 540 mμ wavelength and compared to the standards.

2. Substrate determinations

a. Chemical oxygen demand (COD) test

The detailed procedure used in running the COD test is given in Standard Methods (44). This test is used for determining the efficiency of operation of treatment facilities because of the speed with which results can be obtained. It has been pointed out by a number of investigators that the BOD and COD tests are two distinctly different parameters, but most investigators agree that on a waste of relatively constant composition, the ratio between these two tests should be the same. The principle of the COD test involves oxidation of all the organic components to CO_2 and H_2O by the action of the strong oxidizing agent, potassium dichromate, under acid conditions. The major limitation of the COD test is its inability to differentiate between biologically inert and biologically oxidizable organic matter.

b. Glucose

The Glucostat test was run on the membrane filtrate. This test is specific for glucose according to the procedure outlined by the Worthington Biochemical

Corporation (47). Optical density was measured on the Coleman Junior Spectophotometer (model 6D) at 400 mμ and compared to standards.

c. Carbohydrate

The anthrone test was run on the membrane filtrate according to the procedure outlined by Gaudy (46). This test is specific for carbohydrates. Optical density of the test samples were run on the Coleman Junior Spectrophotometer (model 6D) at 540 mμ wavelength and compared to standards.

d. Biochemical oxygen demand (BOD test)

This test was run according to the procedure outlined in Standard Methods (44). It was used only to determine the oxygen demand exerted by the bacteria in using the organic polyelectrolytes as a substrate.

Experiment Protocol

1. Relation between optical density and solids concentration

In order to estimate solids concentration quickly and determine the degree of clarification in flocculation and settling experiments, a plot of cell concentration verses optical density was made as shown in Figure 3. During the course of the work in both batch and continuous flow units, wherever solids concentration and optical density were determined on the same sample,

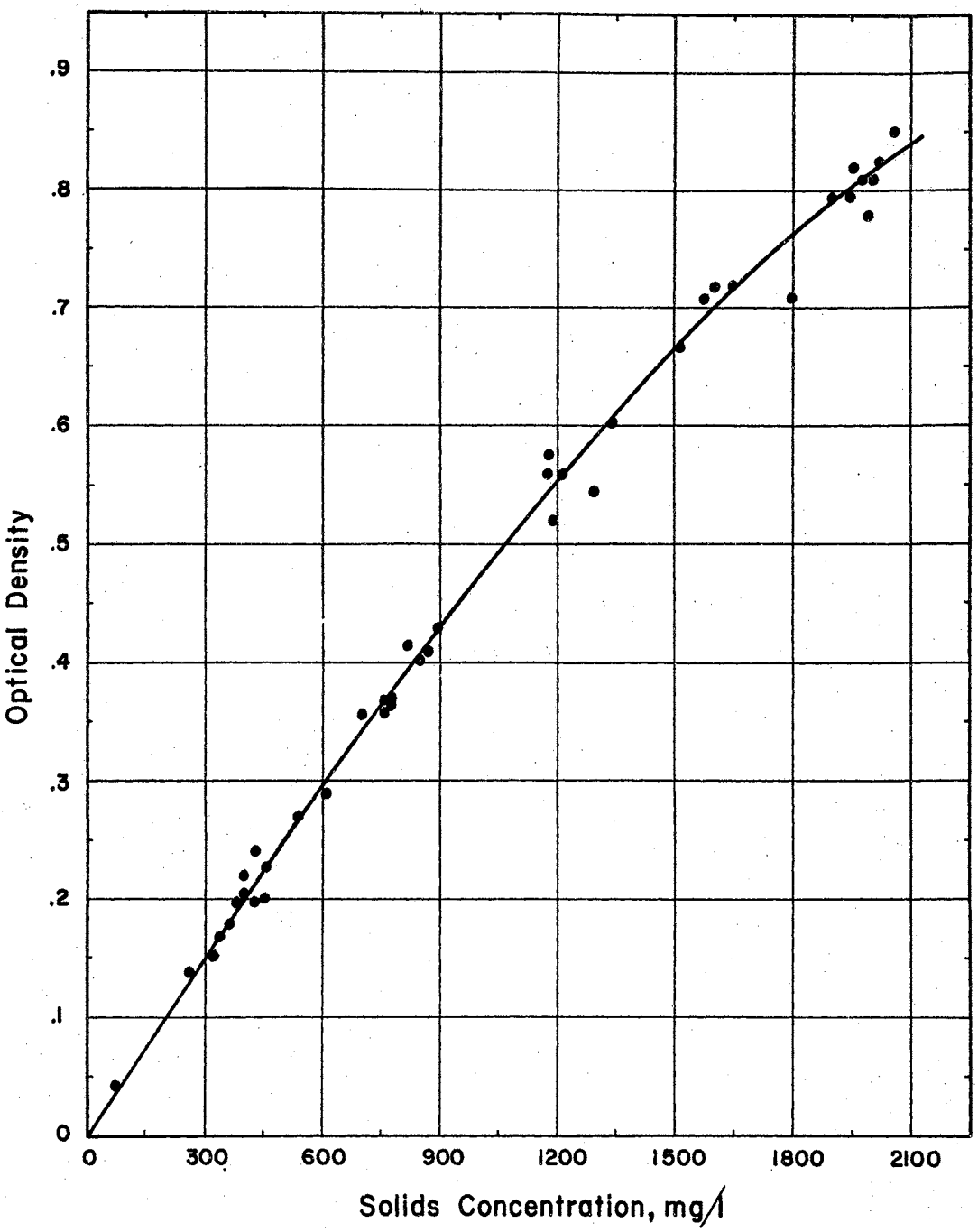


Fig. 3 - Optical Density Versus Solids Concentration

the data was used to define this curve.

2. Testing of polyelectrolytes

Various concentration of the two organic polymers used in this research, namely Puriflocs A-21 and C-31, were subjected to all the analytical procedures used in these experiments in order to determine if the polyelectrolytes would interfer with the analyses.

3. Flocculation of biological solids

In all of the batch and continuous flow units where flocculation by polyelectrolyte addition or natural agglomeration was measured, a standard procedure was adopted from a recommended procedure of the Dow Chemical Company (48). Two hundred fifty mls of mixed liquor was put into each of six 600 ml beakers and placed on a Phipps and Bird 6-paddle stirrer. If a polymer was to be added, it was added at this point. Next, the stirrer was started and operated for one minute at 80 rpm and then reduced to 30 rpm for four minutes. Settling was then allowed to take place by reducing the stirrer speed to 5-10 rpm. It was felt that solids that would settle under these dynamic conditions were more indicative of a truly flocculated solid. After five minutes of settling, a 10 ml sample was drawn from just below the surface of the liquid using a 10 ml pipette with a large opening. Only one sample was taken from each beaker. The optical density was then determined for each sample.

a. Dosage requirements

When comparing the effects of various dosages of both polymers, the desired dosage of the polymer was placed in a vial and brought to a volume of 5 mls with distilled water. The contents of each vial was emptied into the appropriate beaker at the time outlined in the procedure.

b. Effect of growth stage on flocculation

In making flocculation and settling rate determinations under batch growth conditions, it was desirable to use cells harvested at various stages of growth. To determine if the flocculation efficiency was caused in part by a change in natural flocculation ability due to the stage of growth or solely to polyelectrolyte addition, flocculation tests were run on the mixed liquor without any polymer addition. These tests were run at four different locations along the growth curve in a batch operated unit. These locations were selected to encompass the stage of bacterial growth to be used in the other flocculation experiments.

c. Effect of solids concentration

In this particular group of experiments, the efficiency of the polymers was determined as a function of the solids concentration. The solids concentration was varied by taking 250, 200, 150, 100 and 50 mls of mixed liquor for beakers one through five respectively, and

then adjusting the total volume in each beaker to 250 mls by the addition of buffered distilled water containing the same phosphate concentration as in the synthetic waste medium. The solids concentration in each beaker was estimated by measuring the optical density. The standard flocculation tests were performed using the same amount of polymer in each beaker. Since the solids concentration varied, an expression for polymer dosage in terms of solid concentration; namely, mg polymer/gm of solids, was used. In varying the solids concentration, it was impossible to compare the optical density in each beaker directly; therefore, a control was run for each beaker and the percent relative flocculation efficiency was determined. It was calculated by subtracting the optical density of the treated beaker from the optical density of the control and dividing by the optical density of the control (15).

d. Settling characteristics

The optimum time for settling under the dynamic settling conditions employed was determined by running the standard flocculation test and taking a sample from each beaker after various times of settling. This experiment was performed on cells from both the continuous flow unit under steady state conditions and the batch unit.

4. Inhibitory effects of polyelectrolytes due to shock loading

a. Batch unit shocked with Purifloc C-31

The bacterial cells used in these experiments were grown in a batch unit but under different conditions than the cells harvested from the standard batch unit. For these experiment, 200 mls. of supernatant were taken from the standard batch unit after one hour of settling and used as seed material. Two days before the experiment was to be run, this seed material was fed double the regular concentration of synthetic waste and brought to a total volume of 8 liters. On the day before the experiment was to be run, double the standard glucose, buffer and mineral salts was again added to this unit without removing any of the cells or substrate. On the day of the experiment, the unit was fed the standard concentration of glucose, buffer and mineral salts, again without removing any of the cells or substrate. Two liter portions of this mixed liquor were then added immediately to each of four cylinders containing the desired amount of polymer. Aeration was begun at a rate of 2000 cc/min/l and the sampling program was initiated.

b. Batch unit shocked with Puriflic A-21

The procedure used in this group of experiments was similar to the procedure outlined above except that on the day of experiment the mixed liquor was diluted with distilled water to an approximate solids concentration of 400 mg/l. The standard concentration of glucose, buffer and mineral salts were added, and the mixed liquor was

divided into 2 liter portions. The cylinders contained the desired amount of polymer and after the air was turned on at the rate of 2000 cc/min/l, the sampling program was started.

c. Continuous flow units

The continuous flow units were operated as previously described until they reached a steady state. After a few days operation at steady state, the unit was shocked with a known amount of polymer to give a desired concentration in the reactor. Before the polymer was added, the same volume of mixed liquor was removed from the reactor. This was less than 1% of the total volume. The sampling program was started immediately after the polymer addition.

5. Acclimation of bacterial cells to Purifloc C-31

a. Batch unit

Two batch units were started as outlined previously for the standard batch unit and operated a few days before starting the experiment on acclimating to Purifloc C-31. After operating both units on the standard synthetic waste for three days, one unit was "weaned off" glucose and on to Purifloc C-31, starting on the fourth day. This unit was fed the standard concentration of buffer and mineral salts, but the glucose feed was decreased in 50 mg/l increments from 1000 mg/l. At the same time, the C-31 dosage was increased in 50 mg/l increments daily so that the combined concentration of glucose and C-31 was

equal to 1000 mg/l. The control unit was operated under the standard conditions during the entire time. Samples were taken from each unit 23 hours after their daily feeding.

After the treated unit reached a feed concentration of 0 mg/l of glucose and 1000 mg/l of C-31, and its daily sample was taken, the cells from both the control and the C-31 treated units were harvested and resuspended in separate containers in 0.05 M. phosphate buffer. The following day the cells harvested from the control unit were divided into three separate units and fed 1000 mg/l glucose, 500 mg/l glucose + 500 mg/l C-31 and 1000 mg/l C-31 respectively. The standard concentrations of buffer and mineral salts were also added to each unit. The air was turned on at a rate of 2000 cc/min/l and the sampling program was started. The next day this same experiment was performed on the cells harvested from the C-31 treated unit.

b. Continuous flow units

The continuous flow unit was operated under the conditions described previously. After steady state was reached, the unit was run a few more days before the acclimation test was started. In starting this experiment, 25 mg/l of C-31 was added to the incoming synthetic waste. Each day thereafter the C-31 concentration was increased by 25 mg/l until the concentration reached 200 mg/l.

At that point, the test was terminated. Samples were taken from the effluent three times during each day.

CHAPTER IV

RESULTS

Analytical Tests on Puriflocs A-21 and C-31

The results for all analysis run on the polyelectrolytes are given in tables 1 through 6. Chemical oxygen demand was determined on Puriflocs C-31 and A-21 and 1 mg/l of polymer exerted a COD of approximately 0.796 and 0.853 mg/l respectively. These figures represent a statistical average of 16 values. Purifloc C-31 did not interfere with the anthrone or the glucostat test; however, A-21 was slightly reactive to these tests. The biuret test was not effected by A-21, while C-31 exhibited a slight interference. In the BOD tests oxygen usage was not retarded by Purifloc A-21; however, Purifloc C-31 did retard oxygen usage. This result gave indication of possible inhibitory effects due to the polymer.

Flocculation of Biological Solids

1. Dosage requirements

Flocculation of biological solids grown under batch and continuous flow steady state conditions are shown in

TABLE I

COD TEST ON PURIFLOC C-31

Bottle Number	Sample Size mls.	Polymer mg.	Titration	(a-b)	COD
Standard			11.65		
Standard			11.65		
Blank			11.40		
Blank			11.40		
1	2	1.0	10.95	.45	386
2	2	1.0	10.95	.45	386
3	5	2.5	10.30	1.10	412
4	5	2.5	10.25	1.15	395
5	7	3.5	9.80	1.60	392
6	7	3.5	9.85	1.55	380
7	10	5.0	9.00	2.40	412
8	10	5.0	8.95	2.45	421
9	12	6.0	8.65	2.75	393
10	12	6.0	8.60	2.80	401
11	15	7.5	7.85	3.55	406
12	15	7.5	7.90	3.50	401
13	17	8.5	7.45	3.95	399
14	17	8.5	7.45	3.95	399
15	20	10.0	6.80	4.60	395
16	20	10.0	6.80	4.60	395
				TOTAL	6373

1 mg/l of Purifloc C-31 = $\frac{6373}{16} \times \frac{1}{500} = 0.796$ mg/l COD

Note: Purifloc C-31 at 500 mg/l Concentration

TABLE II

COD TEST ON PURIFLOC A-21

Bottle Number	Sample Size mls. (@ 1000 mg/l)	Polymer mg.	Titration	(a-b)	COD
Standard			11.60		
Standard			11.65		
Blank			11.50		
Blank			11.50		
1	2	2	10.50	1.00	858
2	2	2	10.50	1.00	858
3	5	5	9.00	2.50	858
4	5	5	9.00	2.50	858
5	7	7	8.00	3.50	858
6	7	7	8.00	3.50	858
7	10	10	6.55	4.95	850
8	10	10	6.55	4.95	850
9	12	12	5.55	5.95	851
10	12	12	5.55	5.95	851
11	15	15	4.05	7.45	853
12	15	15	4.05	7.45	853
13	17	17	3.10	8.40	848
14	17	17	3.10	8.40	848
15	20	20	1.55	9.95	854
16	20	20	1.60	9.90	850
				TOTAL	13656

1 mg/l of Purifloc A-21 = $\frac{13656}{16} \times \frac{1}{1000}$ = 0.853 mg/l of COD

Note: Purifloc A-21 at 1000 mg/l Concentration

TABLE III

GLUCOSTAT TEST ON PURIFLOCS A-21 AND C-31

Tube Number	Polymer Used	Sample mg.	Dist. Water mls.	% T	O.D.	Glucose mg.	Glucose mg/l
Standard	Glucose	0	1.00	100.0	0	0	
Standard	Glucose	.015	.95	85.0	.0706	.015	
Standard	Glucose	.03	.90	68.5	.1643	.030	
Standard	Glucose	.09	.70	35.5	.4500	.090	
Standard	Glucose	.15	.50	18.5	.7330	.150	
Standard	Glucose	.30	---	5.0	1.3010	.300	
1	C-31	.03	.9	100.0	0	0	0
2	C-31	.09	.7	100.0	0	0	0
3	C-31	.15	.5	100.0	0	0	0
4	C-31	.18	.4	100.0	0	0	0
5	C-31	.24	.2	100.0	0	0	0
6	C-31	.30	0	100.0	0	0	0
7	A-21	.03	.9	92.5	.0339	.006	60
8	A-21	.09	.7	87.5	.0580	.011	37
9	A-21	.15	.5	84.5	.0731	.014	28
10	A-21	.18	.4	80.5	.0942	.018	30
11	A-21	.24	.2	75.0	.1249	.024	30
12	A-21	.30	0	67.5	.1707	.033	33

TABLE IV

ANTHRONE TEST ON PURIFLOCS A-21 AND C-31

Polymer	Sample Conc. mg/l	mls.	mg.	D.W. mls.	% T	O.D.	Carbohydrate mg.	mg/l
Glucose	300	0	0	2.5	100	0	0	
Glucose	300	.1	.03	2.4	81.0	.0915	.03	
Glucose	300	.3	.09	2.2	50.25	.2990	.09	
Glucose	300	.5	.15	2.0	31.0	.5090	.15	
Glucose	300	.7	.21	1.8	18.5	.7330	.21	
C-31	300	.1	.03	2.4	100.0	0	0	0
C-31	300	.3	.09	2.2	100.0	0	0	0
C-31	300	.5	.15	2.0	100.0	0	0	0
C-31	300	.7	.21	1.8	100.0	0	0	0
C-31	300	1.0	.30	1.5	100.0	0	0	0
C-31	1000	.5	.50	2.0	100.0	0	0	0
C-31	1000	1.0	1.00	1.5	100.0	0	0	0
C-31	1000	1.5	1.5	1.0	100.0	0	0	0
A-21	300	.1	.03	2.4	100.0	0	0	0
A-21	300	.3	.09	2.2	97.5	.0110	.003	10
A-21	300	.5	.15	2.0	94.75	.0235	.008	16
A-21	300	.7	.21	1.8	92.0	.0362	.012	17
A-21	300	1.0	.30	1.5	93.0	.0315	.010	10
A-21	1000	.5	.50	2.0	91.5	.0386	.013	26
A-21	1000	1.0	1.00	1.5	85.0	.0706	.023	23
A-21	1000	1.5	1.50	1.0	81.5	.0888	.028	18

TABLE V

BIURET TEST ON PURIFLOCS A-21 AND C-31

Polymer	Sample Concentration mg/l	mls.	mg.	D.W. mls.	% T	O.D.	Protein mg.	Protein mg/l
Standard	6000	--	0	2.5	100.00	0	0	
Standard	6000	.5	3	2.0	80.00	.0969	3	
Standard	6000	1.0	6	1.5	60.25	.2200	6	
Standard	6000	1.5	9	1.0	45.00	.3470	9	
Standard	6000	2.0	12	.5	34.25	.4650	12	
Standard	6000	2.5	15	0	23.50	.629	15	
C-31	300	.5	.15	2.0	99.75	.0011	0	0
C-31	300	1.0	.30	1.5	99.75	.0011	0	0
C-31	300	1.5	.45	1.0	99.50	.0022	.1	67
C-31	300	2.0	.60	.5	99.50	.0022	.1	50
C-31	300	2.5	.75	0	99.75	.0011	0	0
C-31	1000	.5	.50	2.0	99.50	.0022	.2	400
C-31	1000	1.5	1.50	1.0	99.00	.0044	.2	133
C-31	1000	2.5	2.50	0	98.25	.0077	.3	120
A-21	300	.5	.15	2.0	100.0	0	0	0
A-21	300	1.0	.30	1.5	100.0	0	0	0
A-21	300	1.5	.45	1.0	100.0	0	0	0
A-21	300	2.0	.60	.5	100.0	0	0	0
A-21	300	2.5	.75	0	100.0	0	0	0
A-21	1000	.5	.5	2.0	100.0	0	0	0
A-21	1000	1.5	1.5	1.0	100.0	0	0	0
A-21	1000	2.5	2.5	0	100.0	0	0	0

TABLE VI

BOD TEST ON PURIFLOCS A-21 AND C-31

Incubation Time, Days	Sample				Titration
	Polymer	Conc., mg/l	mls.	mg.	
0					7.8
5					5.1
5	C-31	300	.5	.15	5.1
5	C-31	300	1.0	.30	5.7
5	C-31	300	1.5	.45	5.8
5	C-31	300	2.0	.60	5.9
5	C-31	1000	1.0	1.00	6.2
5	C-31	1000	5.0	5.00	6.2
5	C-31	1000	10.0	10.00	6.1
5	C-31	1000	20.0	20.00	6.1
5	A-21	300	.5	.15	5.3
5	A-21	300	1.0	.30	5.3
5	A-21	300	1.5	.45	5.3
5	A-21	300	2.0	.60	5.3
5	A-21	1000	1.0	1.00	5.2
5	A-21	1000	5.0	5.00	5.2
5	A-21	1000	10.0	10.00	5.2
5	A-21	1000	20.0	20.00	5.2

Note: Seed taken from continuous flow reactor effluent and diluted to O.D. of .14. One ml/liter taken for seed.

Figures 4 and 5 respectively. In both of these experiments, the standard flocculation test procedure was used. In Figure 4, the plotted points labeled "settled control" represent the optical density of the supernatant for cell suspensions to which no polymer was added. In the system dosed with Purifloc C-31, the degree of clarification was increased starting with a dosage of 5 mg/l and it continued to increase up to a dosage of 50 mg/l. Dosages between 50 and 200 mg/l of C-31 did not show any increase over the 50 mg/l dosage. Addition of Purifloc A-21 caused an increase in clarification at a dosage of only 0.1 mg/l and was increasingly beneficial up to a dosage of 1 mg/l. A slight decrease in efficiency was noted from 3 to 50 mg/l, with a large decrease coming between 50 and 200 mg/l. This data was plotted on semi-log paper so that the wide range in data could be easily plotted.

A considerable degree of autoflocculation was evidenced for cell suspensions taken from the continuous flow steady state units (Figure 5) when placed under the slow mix conditions of the standard flocculation procedure. Autoflocculation was not evident in the reactor or when the cells were removed from the reactor and placed under quiescent settling conditions. Upon flocculation and settling in the absence of polymer approximately 45 to 50% removal of the solids from the mixed liquor was observed. The A-21 in this experiment (as for results shown in

Figure 5) indicated increased removal over the settled control for dosages up to 1.2 mg/l. Dosages from 1.2 to 8 mg/l, while still more efficient than the settled control, were not as efficient as a dosage of 1.2 mg/l. Purifloc A-21 dosages above 8 mg/l resulted in decreased clarification in comparison to the settled control. The cationic polymer showed increased clarification over the settled control when the dosage was 15 mg/l or greater. Up to this dosage, the polymer did not increase settling over the control. Increasing the C-31 dosage from 15 mg/l up to approximately 200 mg/l increased the removal efficiency. From 200 to 500 mg/l, there was a slight decrease in efficiency.

2. Effect of solids concentration on flocculation

An experiment to determine how the addition of cationic polymer effected the flocculation of biological solids in comparison to a settled control, while varying the solids concentration and growth time in a batch unit, was performed. This experiment is shown in Figure 6. The solids were grown through a number of feeding cycles without solids wasting and the solids concentration was allowed to increase to 3400 mg/l. This procedure produced solids that would flocculate well in the range of 3 to 5 mg/l of C-31. The solids concentration was varied by making specific dilutions of the mixed liquor with 0.5 molar buffered distilled water. Dosage was expressed on

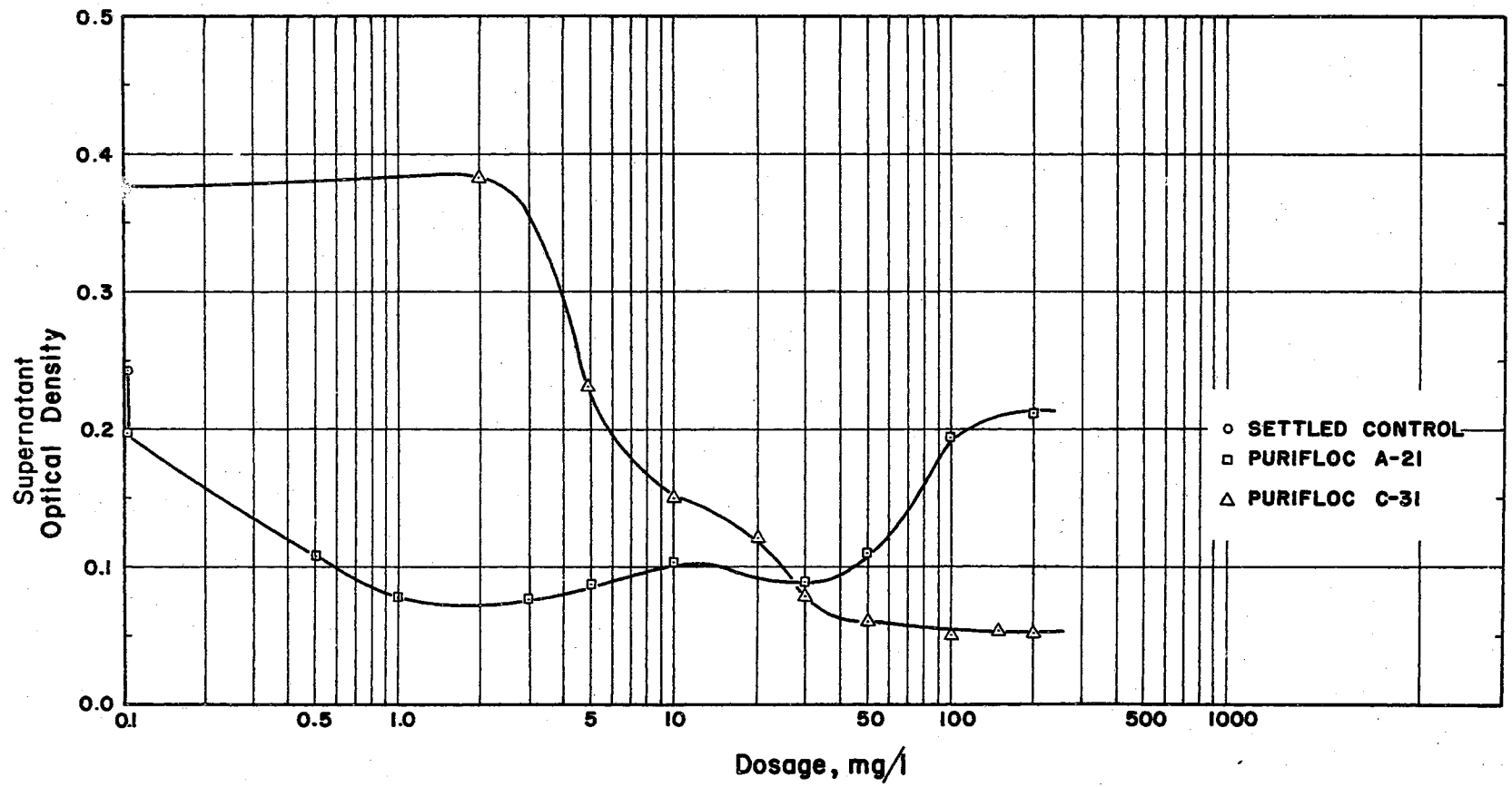


Fig. 4 - Flocculation of Biological Solids Grown Under Standard Batch Growth Conditions

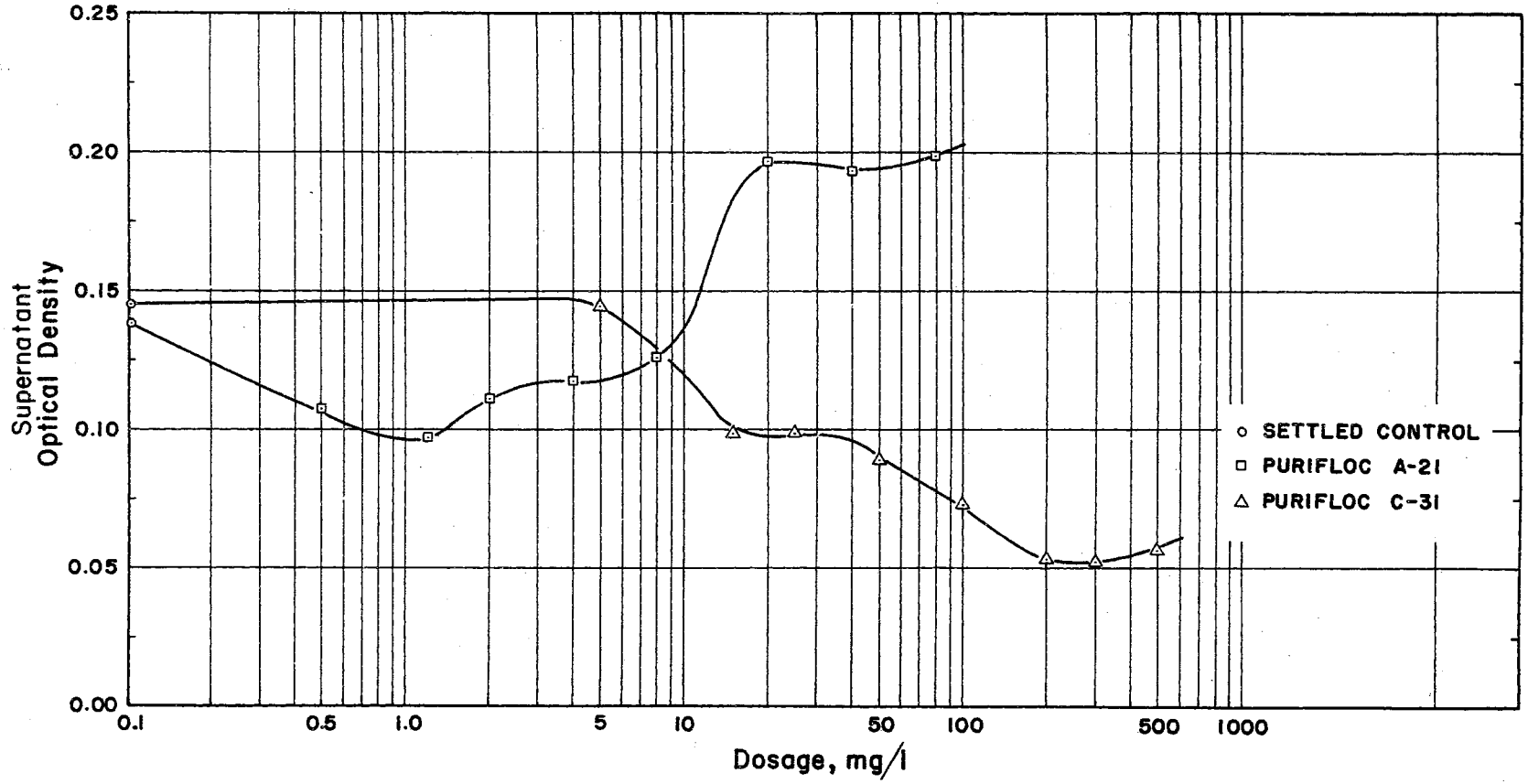


Fig. 5 - Flocculation of Biological Solids From a Continuous Flow Unit Under Steady State Conditions

the basis of mg polymer per gm of solids. The percent relative flocculation efficiency was determined as the difference between the optical density of the control and the treated sample divided by the optical density of the control. This ratio was expressed as a percent. It is seen in Figure 6 that the percent relative flocculation efficiency was increased with a small addition (1.5 mg polymer/gm of solids) of C-31. Slightly higher dosages (1.5 to 2.5 mg polymer/gm of solids) showed a tendency to decrease the removal efficiency. Increasing the dosage above 2.5 mg polymer/gm of solids again established an increase in efficiency.

The data shown in Figure 6 is replotted in Figure 7 to show how the percent relative flocculation efficiency varied with the solids concentration. The relative efficiency for both dosages appeared to be the greatest at the highest and lowest solids concentrations used. As the solids concentration was decreased from the high value and increased from the low value, the relative efficiency decreased to its lowest value at a solids concentration of 2000 mg/l.

The procedure used to collect the data shown in Figure 8 was similar to the procedure used for Figure 6; however, instead of using Purifloc C-31, the anionic polymer, Purifloc A-21 was used. Biological solids were grown under batch conditions without solids wasting to a

concentration of approximately 2500 mg/l. Flocculation appeared to be efficient with A-21 at dosages of 0.5 and 1.0 mg/l. It is seen that the percent relative flocculation efficiency was increased with a very small dosage of 0.2 mg polymer/gm of solids. Increasing the dosage (by decreasing the solids concentration) resulted in a reduction of efficiency.

The data plotted in Figure 9 was taken from Figure 8 and shows how the percent flocculation efficiency varied with the solids concentration. The dosages of A-21 used in this experiment were 0.5 and 1.0 mg/l and the dotted lines represent the average efficiencies obtained when using these dosages. The average efficiencies for both A-21 dosages were decreasing at approximately the same rate in the solids concentration range of 2500 to 1000 mg/l. Below the level of 1000 mg/l solids, efficiencies at both dosages were essentially equal and decreasing.

3. Settling characteristics of biological solids

It would have been desirable to perform each flocculation and settling rate experiment with identical concentrations of cells which were in the same physiological condition; however, this was almost impossible to attain. In an effort to determine if cells at different physiological states exhibited different settling characteristics, the data shown in Figure 10 were collected for various settling times using the standard flocculation

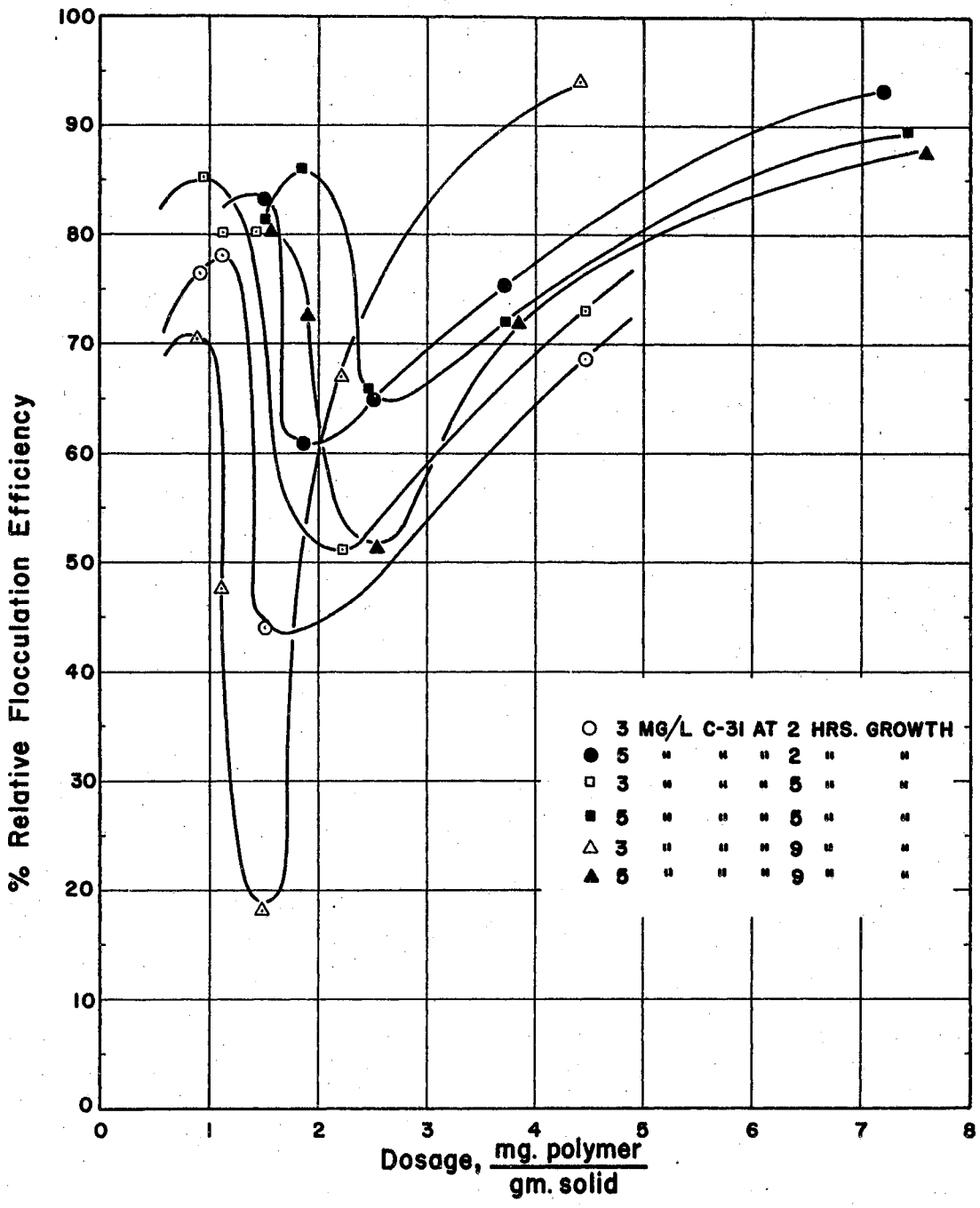


Fig. 6 - Flocculation Efficiency as Effected by Solids Concentration and Purifloc C-31 Dosage

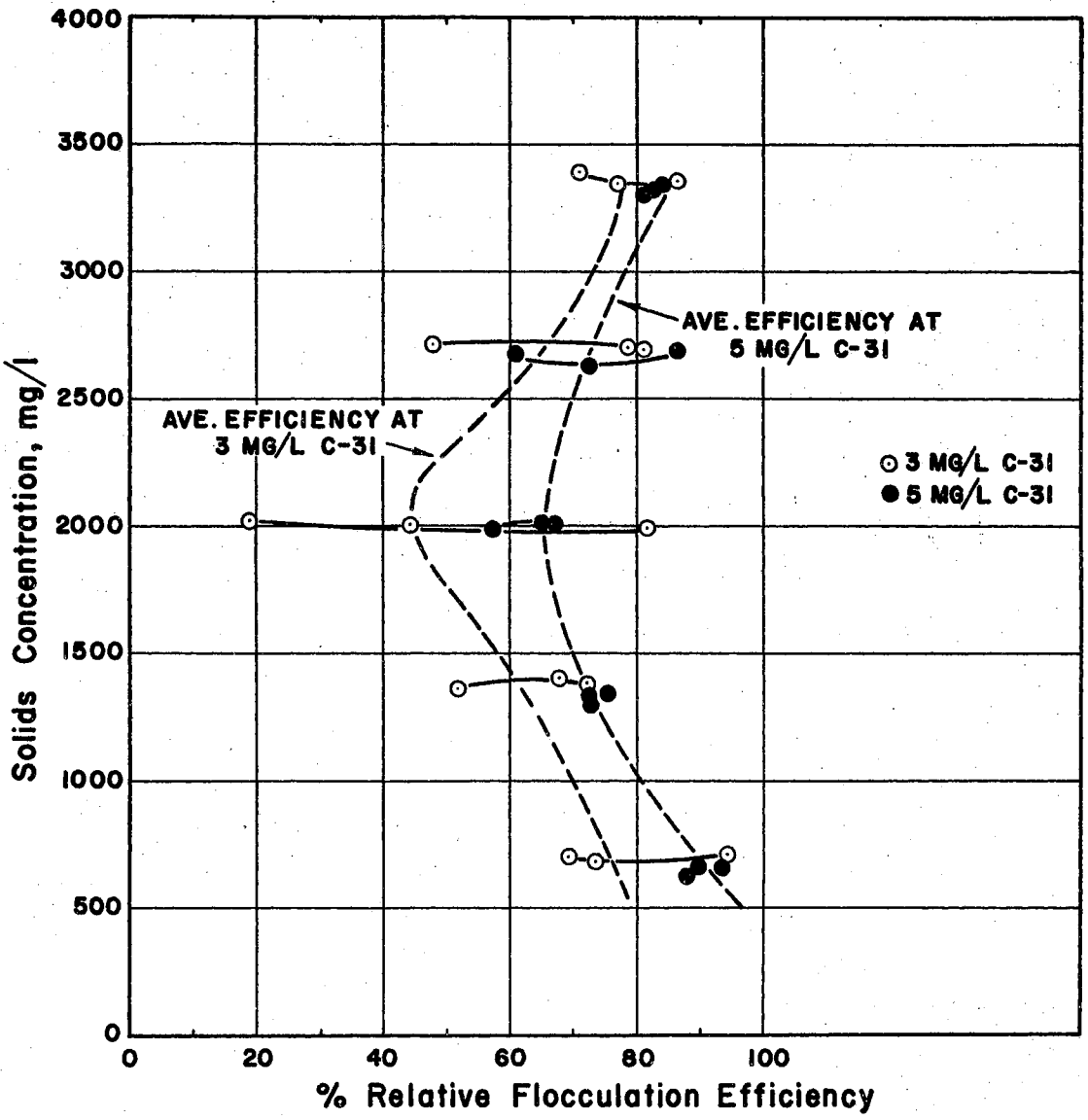


Fig. 7 - Effect of Solids Concentration on Flocculation Efficiency with Purifloc C-31

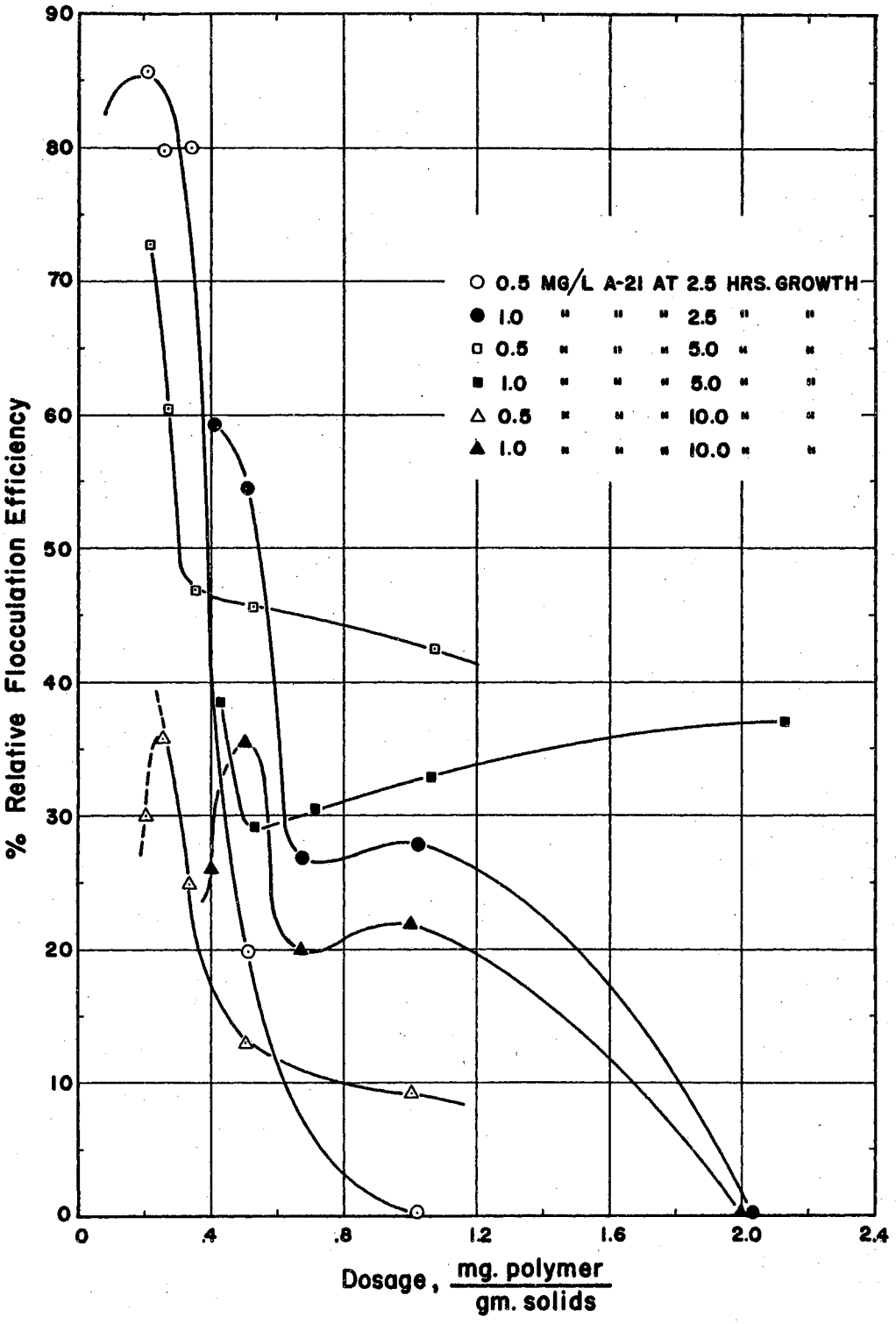


Fig. 8 - Flocculation Efficiency as Effected by Solids Concentration and Purifloc A-21 Dosage

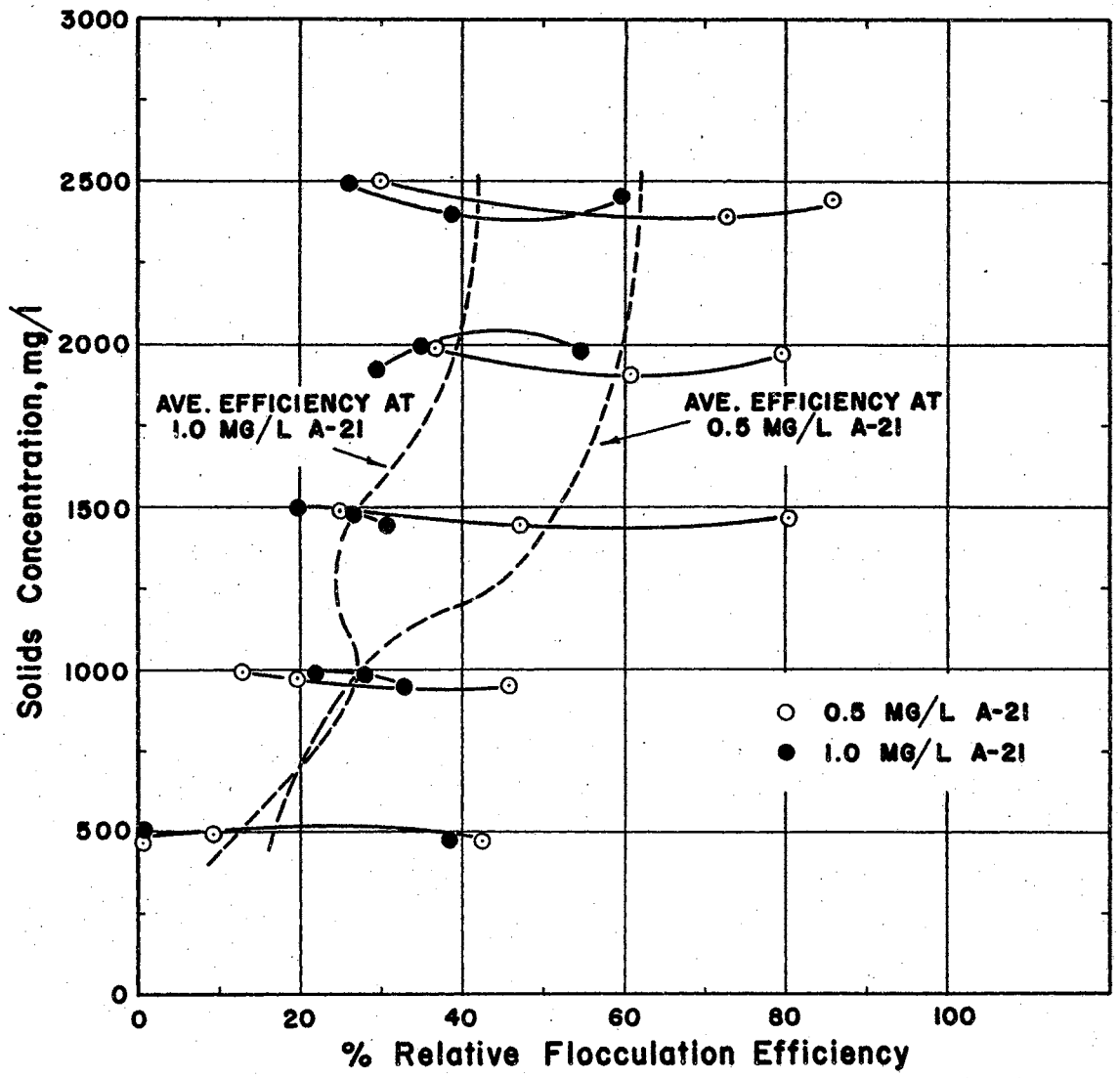


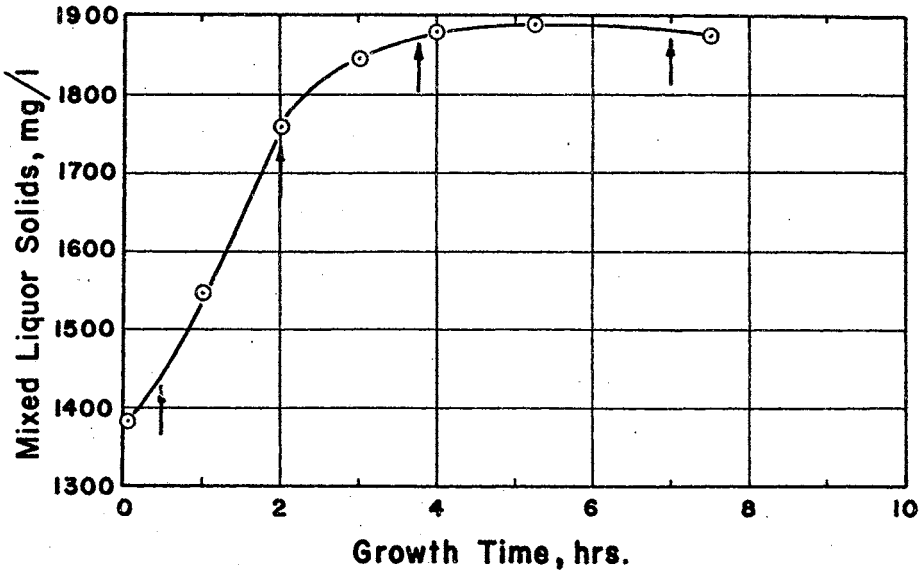
Fig. 9 - Effect of Solids Concentration on Flocculation Efficiency with Purifloc A-21

procedure. No polymer addition was made in this experiment so that any increase in clarification was the result of auto-flocculation. All tests performed at various stages of growth indicate a rapid rate of settling and approximately the same concentration of solids remaining in the supernatant; however, in view of the increase in biological solids during growth, the greatest amount of removal occurred during the endogenous phase. It is interesting to note that the solids concentration at zero time on the settling curve is higher in suspended solids concentration than at the corresponding time on the growth curve. It is emphasized that optical density measurements were used for obtaining solids concentration for the settling tests; at high solids concentration, optical density provides only a rough measure of suspended solids.

The effect of settling time on the clarification efficiency obtained with Puriflocs C-31 and A-21 in comparison to settled control are shown in Figures 11 and 12 respectively. The batch growth curve for the biological solids used in each experiment is given. In Figure 11, the tests were run at the following stages of growth and C-31 dosages: settled control, 2.25 hours; 5 mg/l, 3.5 hours; 10 mg/l, 4.5 hours; and 3 mg/l at 5.25 hours. The settled control required 1.0 minute under the dynamic settling conditions to begin settling and 15 minutes before maximum removal was reached. Three, 5 and 10 mg/l of C-31

however, reached maximum settling in 10, 4 and 1.5 minutes, respectively. In Figure 12, the stage of bacterial growth at which cells were taken for settling tests and the A-21 dosage used is noted; these tests were run in the following order: settled control, 2 hours; 1.0 mg/l, 3 hours; and 0.5 mg/l, 4.25 hours. Maximum removal was obtained with both 0.5 and 1.0 mg/l of Purifloc A-21 at 5 minutes dynamic settling. The settled control from this biological system took 8 to 9 minutes for the same degree of clarification.

Settling rate tests for biological solids grown at steady state conditions are shown in Figure 13. Each test was run on biological solids taken from different steady state reactors which were started from an identical seed and operated until a mixed liquor density of 0.29 was obtained. The settled control, one A-21 treated and two C-31 treated tests all show maximum settling in 5 to 7 minutes; however, the degrees of removal were the striking factor in this experiment. The settled control with no polymer addition produced the least removal; however, there was still 50% clarification from the mixed liquor concentration after subjecting the cells to the standard flocculation procedure. The anionic polymer gave the best removal in the shortest settling time and then had a decrease in removal with the longer settling times. Purifloc C-31 improved clarification by



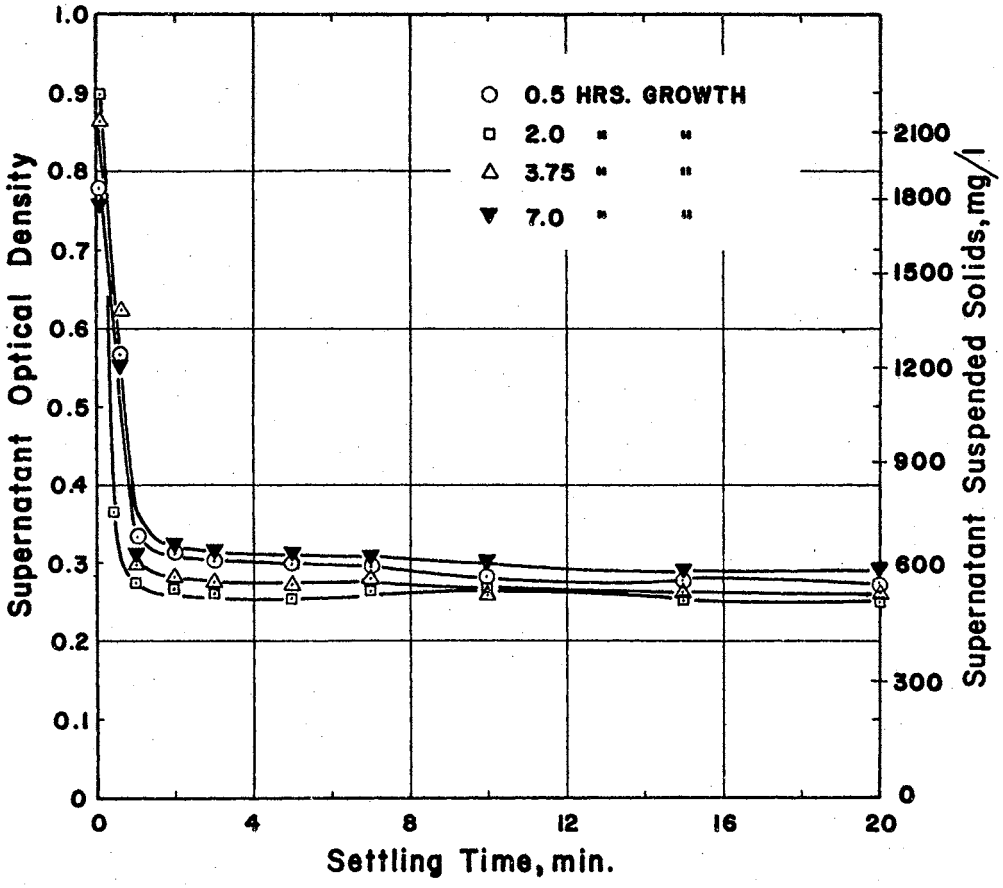
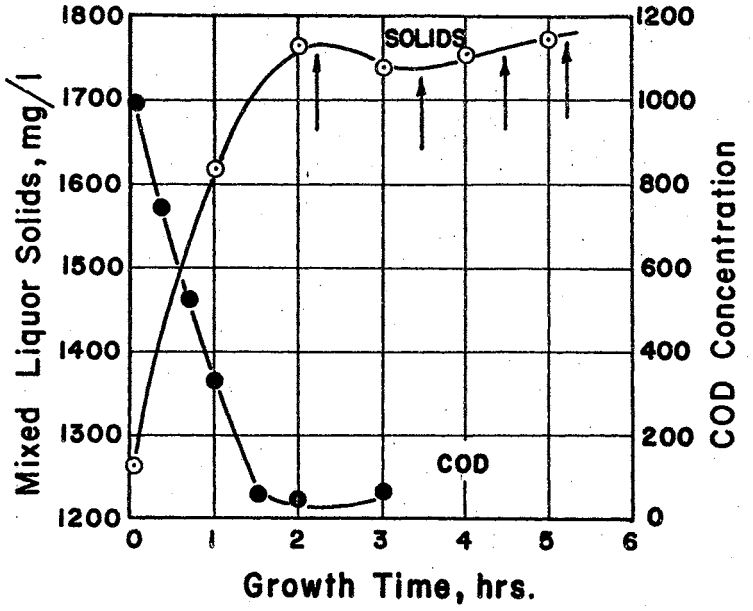


Fig. 10 - Settling Rate of Solids From a Batch Unit at Various Growth Times



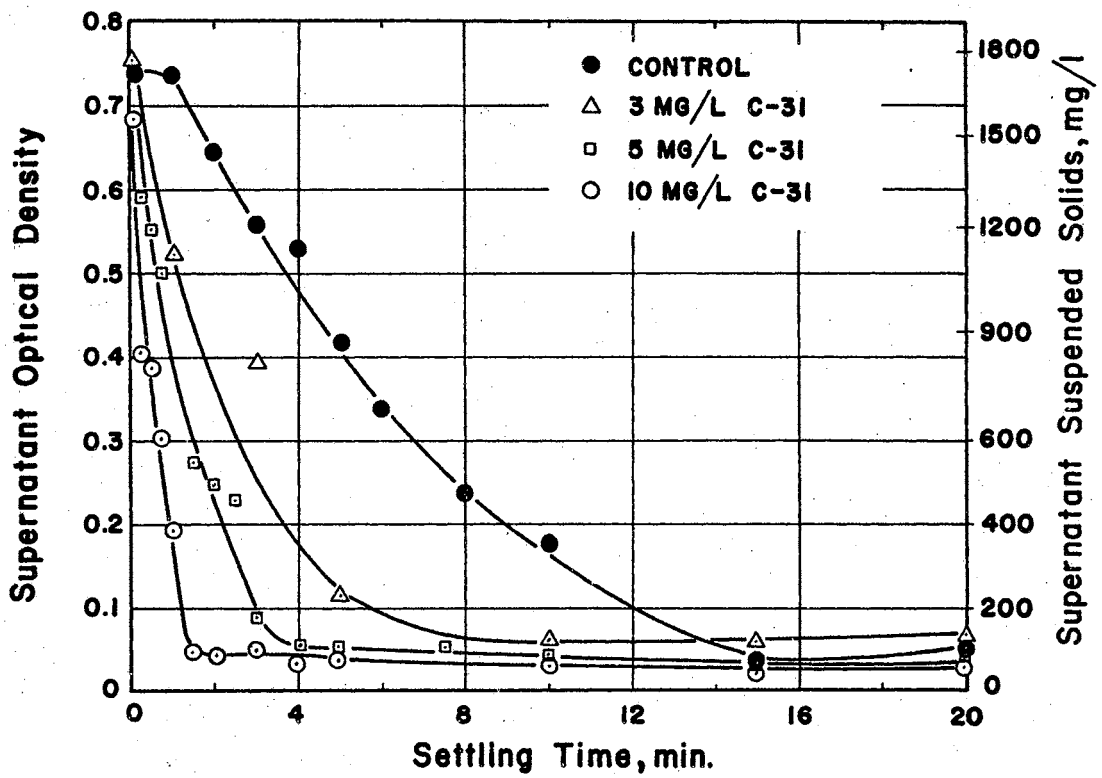


Fig. 11 - Settling Rates of Solids From a Batch Unit With Various Purifloc C-31 Dosages

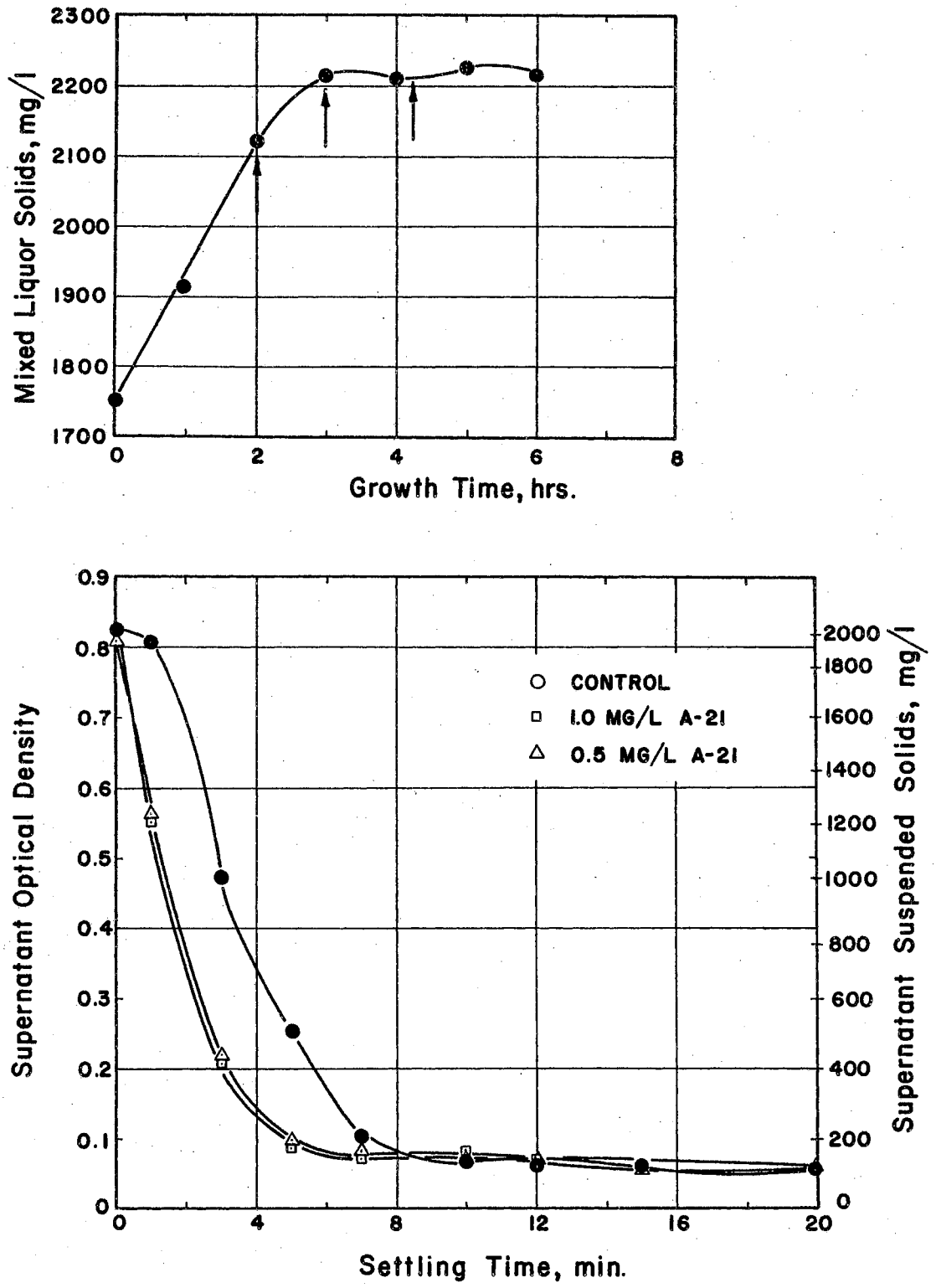


Fig. 12 - Settling Rates of Solids From a Batch Unit With Various Purifloc A-21 Dosages

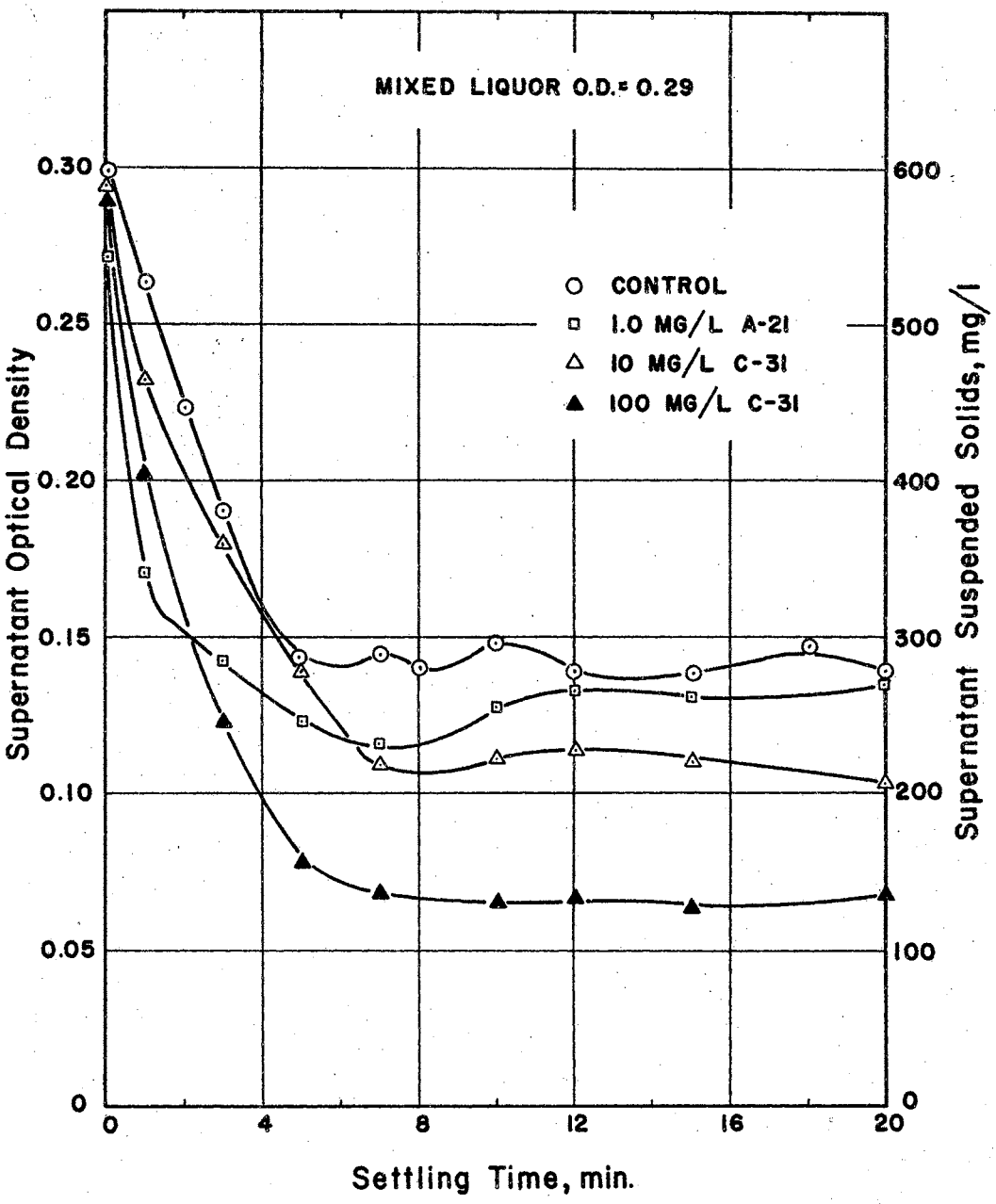


Fig. 13 - Settling Rate of Solids Grown in Continuous Flow Units at Steady State

increasing the dosage from 10 to 100 mg/l and both of these dosages produced better clarification than the settled control or the A-21 treated unit.

Inhibitory Effects on Biological Solids From Puriflocs A-21 and C-31

1. Batch units

Deleterous effects due to Purifloc C-31 addition to bacterial cells are shown in Figures 14, 15 and 16 for cells grown under batch conditions. The control unit was run under identical conditions as the treated units except that the polymer was not added. A toxic effect was shown at the 200 mg/l dosage of C-31 while none was shown at 150 mg/l or less.

Figures 17 and 18 show the effect of Purifloc A-21 on the metabolic capacity of biological solids. The dosages in these experiments were varied up to 200 mg/l. In all the treated units, there were no deleterous effects exhibited. In Figure 18, the COD concentration in the samples treated with A-21 were higher than the control unit. This can possibly be accounted for by the passage of A-21 through the membrane filter. In this experiment the solids concentration and glucose removal for the A-21 treated units were very similar to the control.

2. Continuous flow unit

Shock loadings of Purifloc C-31 were applied to a

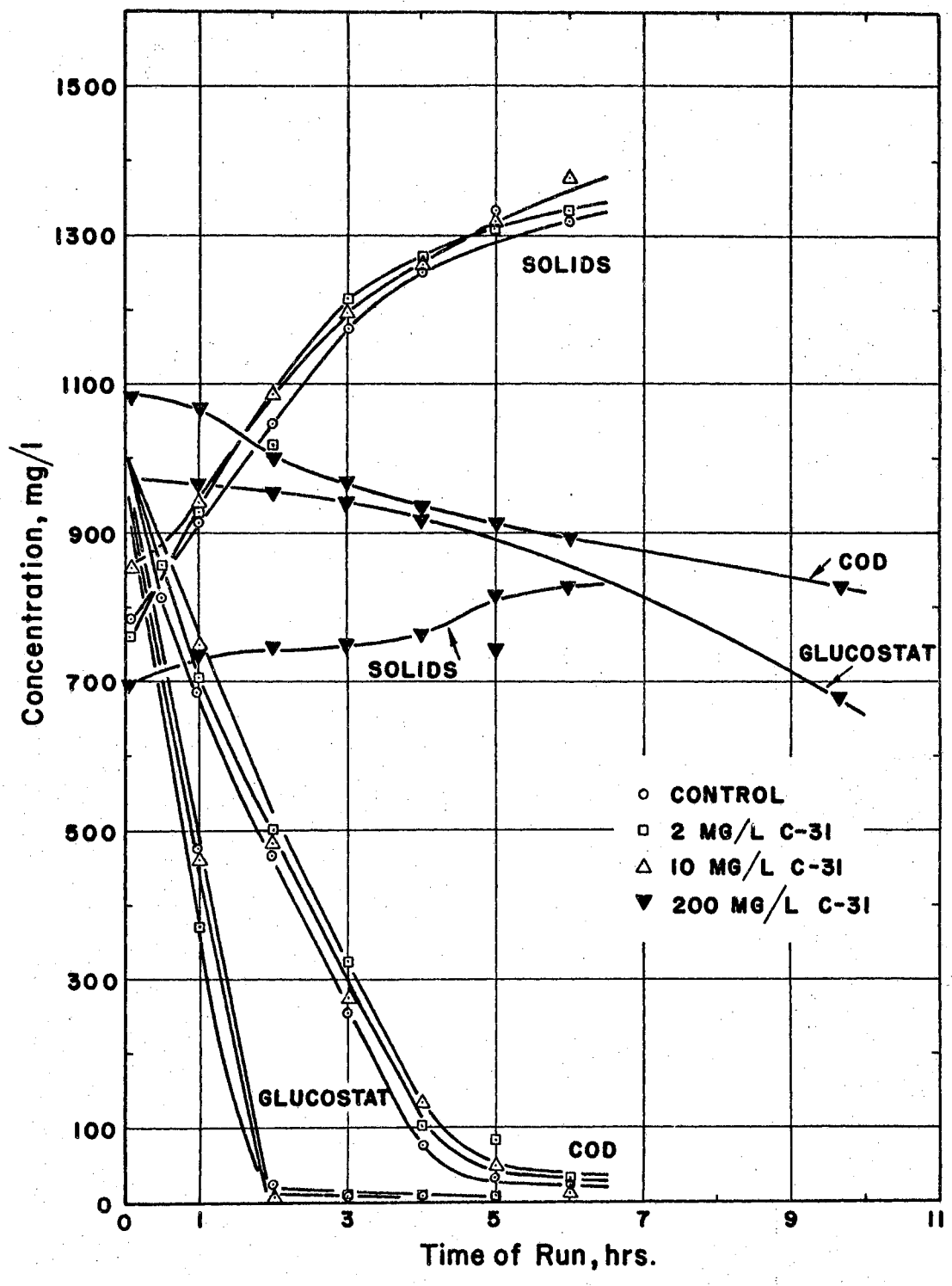


Fig. 14 - Biochemical Effect of Purifloc C-31 at 0, 2, 10 and 200 mg/l Under Batch Growth Conditions

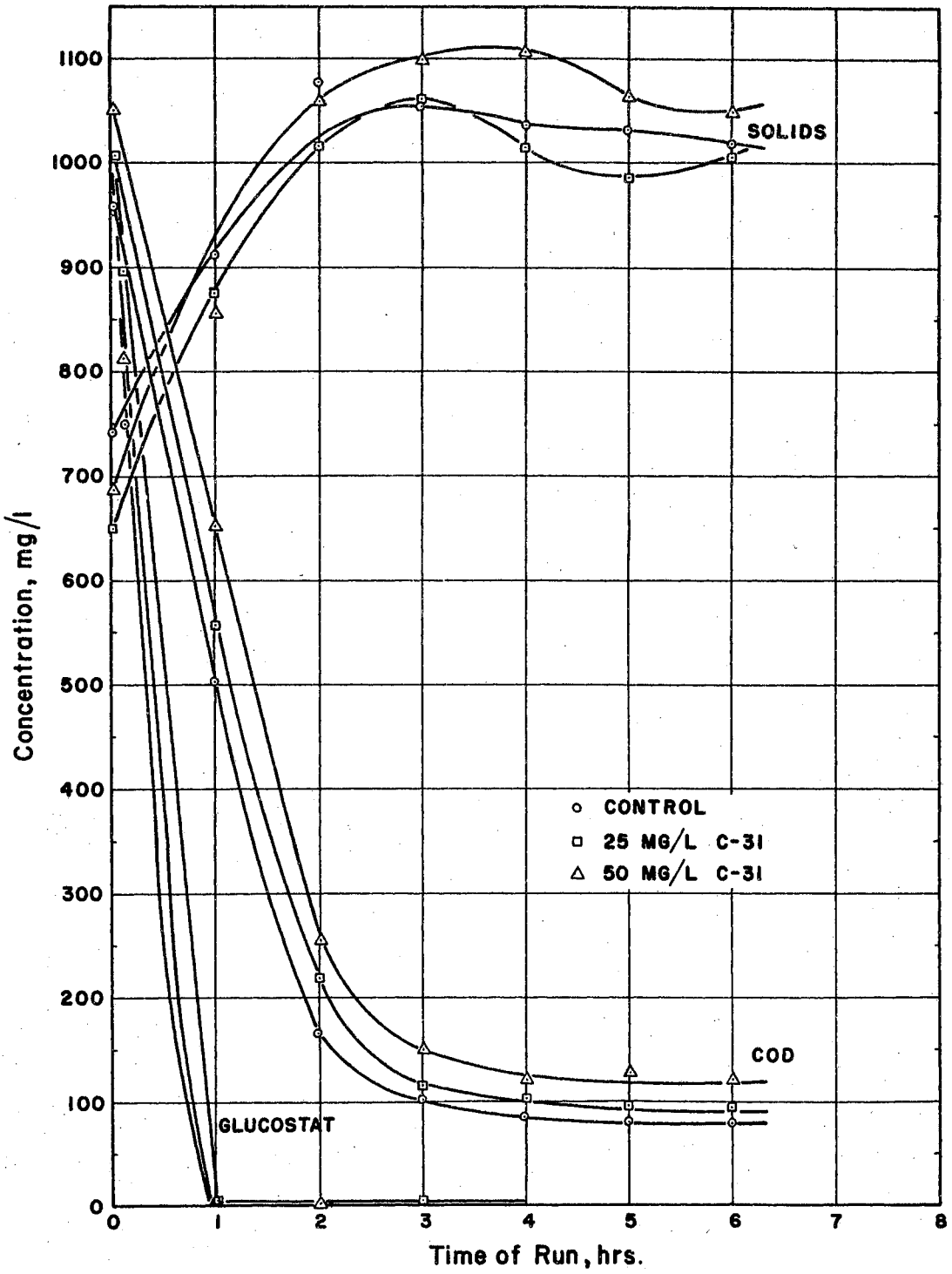


Fig. 15 - Biochemical Effect of Purifloc C-31 at 0, 25 and 50 mg/l Under Batch Growth Conditions

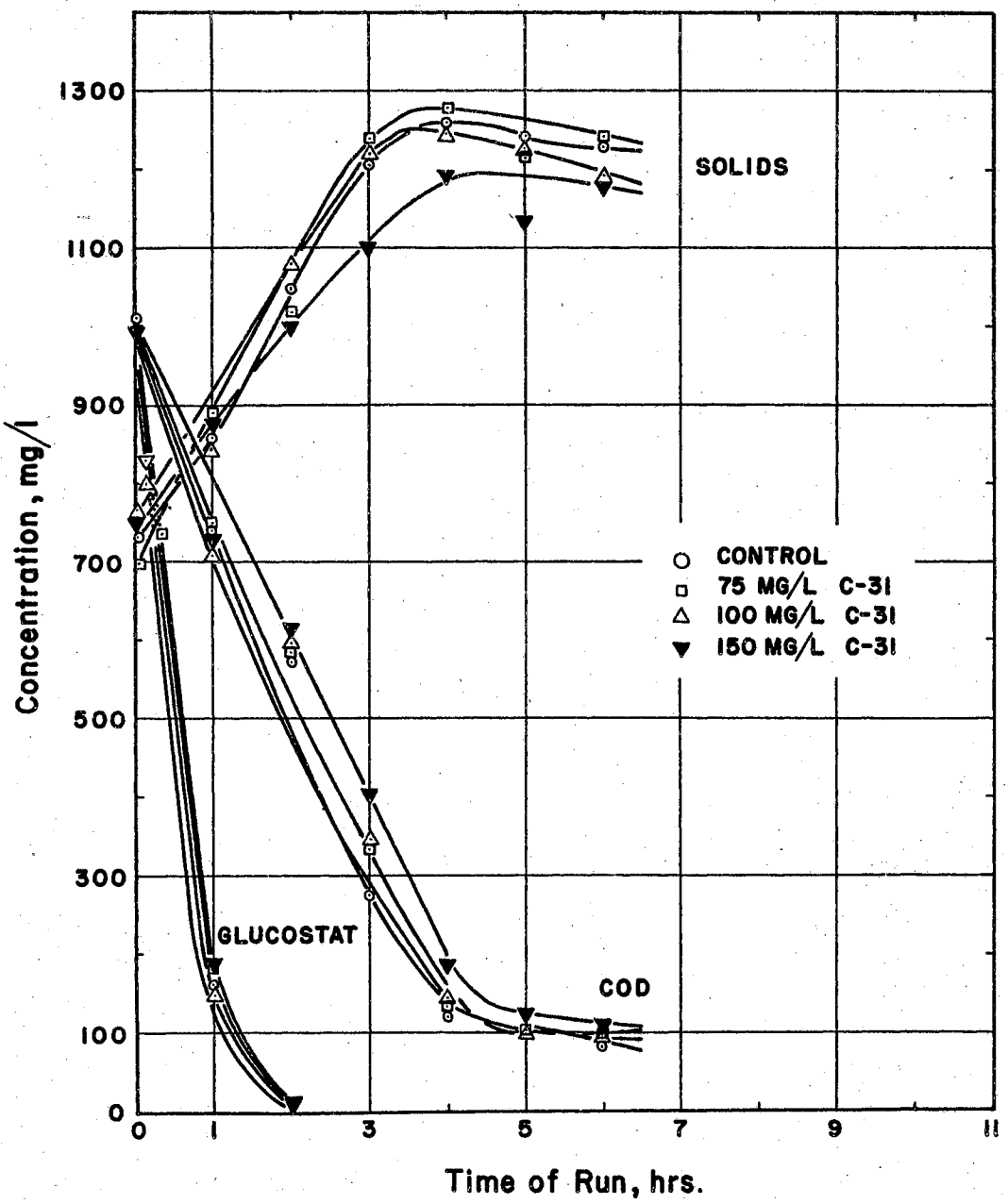


Fig. 16 - Biochemical Effect of Purifloc C-31 at 0, 75, 100 and 150 mg/l Under Batch Growth Conditions

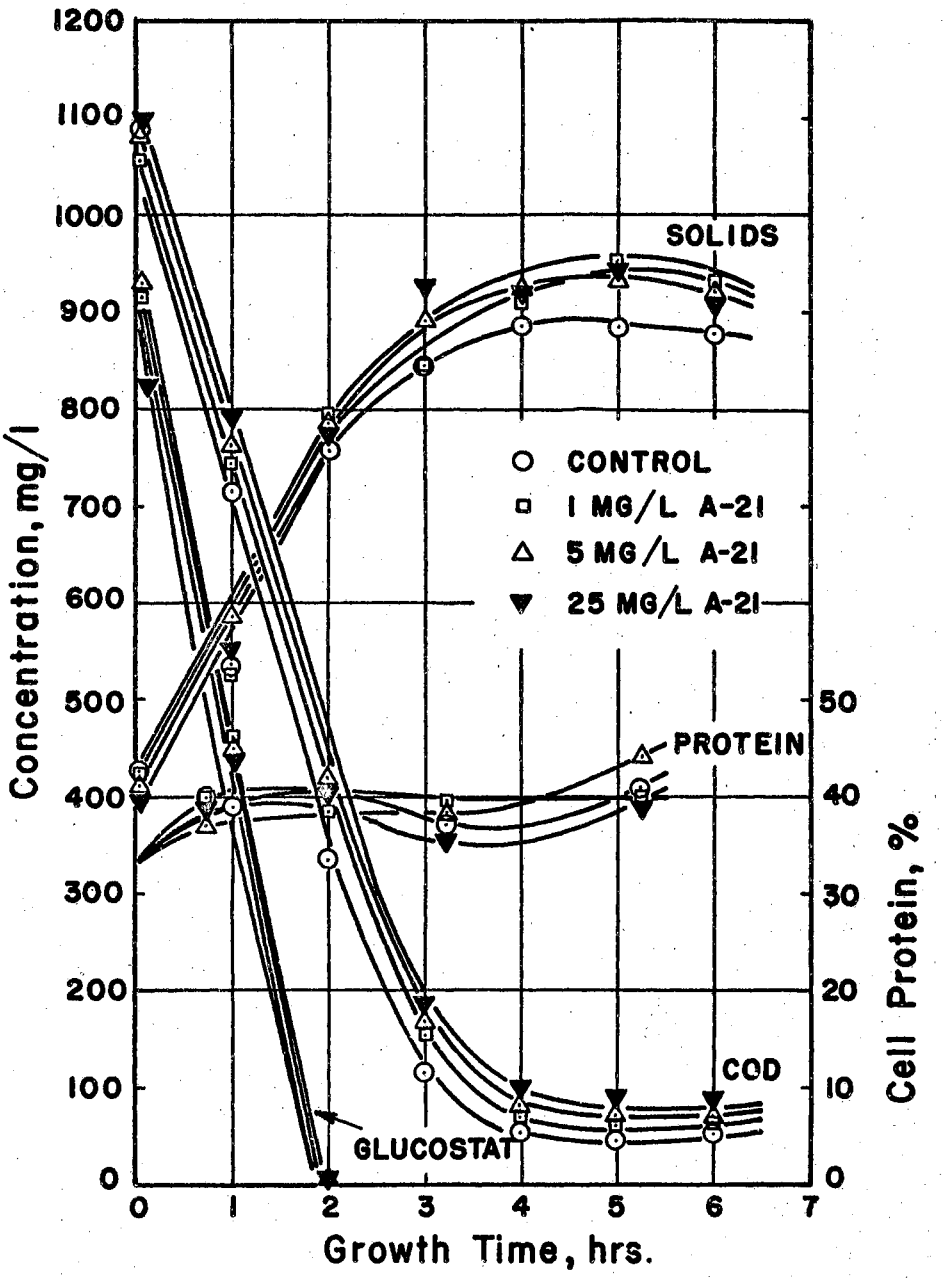


Fig. 17 - Biochemical Effect of Purifloc A-21 at 0, 1, 5 and 25 mg/l Under Batch Growth Conditions

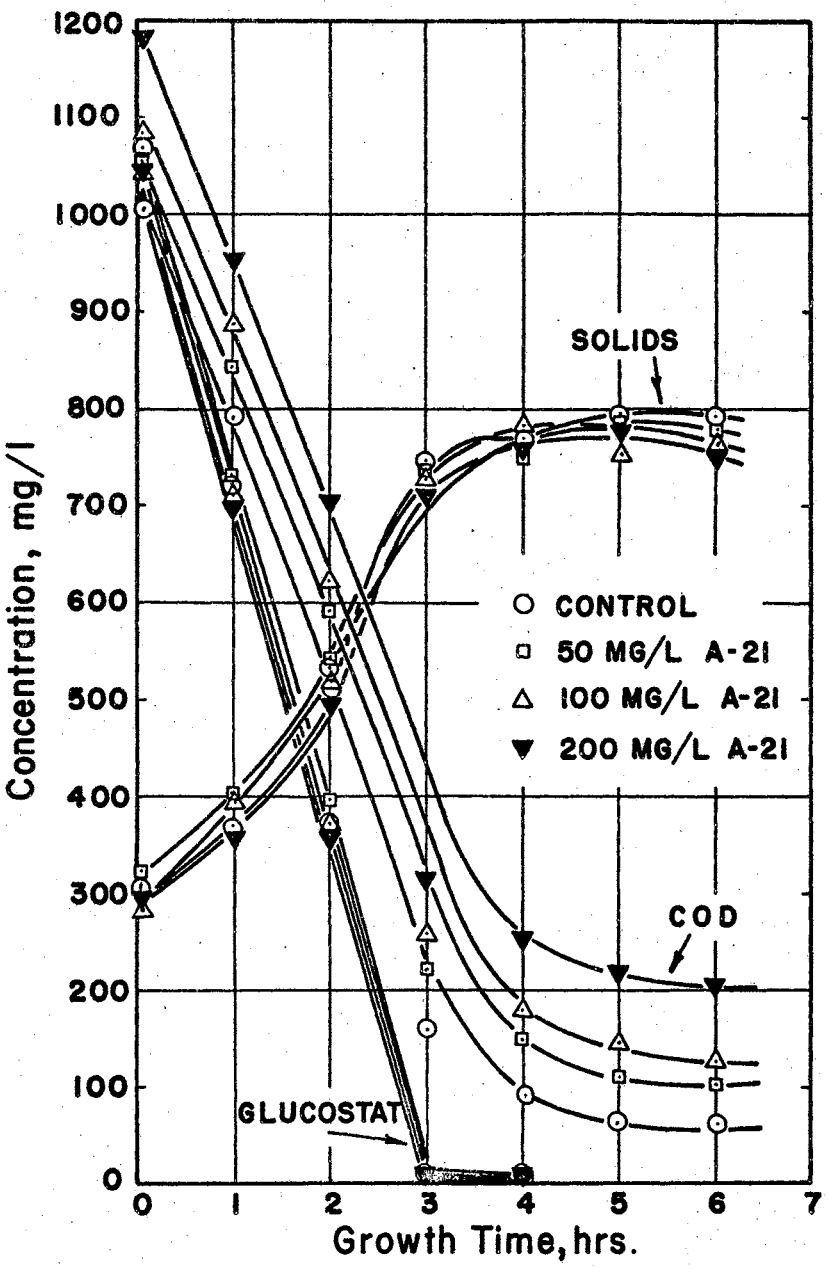


Fig. 18 - Biochemical Effect of Purifloc A-21 at 0, 50, 100 and 200 mg/l Under Batch Growth Conditions

continuous flow unit run under steady state conditions prior to the shock. In all figures showing the response to shock loading of polymer in the continuous flow units, the time axis is given in days prior to the shock and in hours after the shock. In all cases a sample was taken one-half hour before applying the shock loading and immediately thereafter. The theoretical dilute-out curve for Purifloc C-31 is shown for each continuous flow experiment; this curve represents the theoretical C-31 concentration at any given time if it were not retained on the solids. The other theoretical dilute-in or dilute-out curves shown in Figures 22, 23 and 24 are labled; concentrations are given on the left hand scale. If no bacterial metabolism had taken place, these curves would represent the concentration of various constituents in the system. The gross COD (Figures 22 and 24) and gross anthrone concentrations (Figure 23) is the summation of glucose dilute-in plus COD or anthrone dilute-out.

Figures 19, 20 and 21 show shock loadings (as slug doses) of 10, 25 and 50 mg/l of C-31 respectively. In all three cases, no deleterious or inhibitory effects on bacterial metabolism were evident.

The response to a shock loading of 100 mg/l of Purifloc C-31 is shown in Figure 22. The dosage was administered after three days of steady state operation. There was a sudden decrease (125 mg/l) in solids

concentration and a corresponding increase (70 mg/l) in COD. Carbohydrate concentration in the effluent remained stable for two hours then suddenly increased at a rate slightly less than the rate at which it would have increased had it not been metabolized by the cells. After the initial drop in solids concentration, the solids curve followed the theoretical solids dilute-out for a period of two hours and then appeared to attain a new steady state value. It may have been possible that given a longer growth time, the solids would have recovered and returned to the previous steady state concentration. The COD and biological solids curve indicate that substrate was being converted to cellular material.

The effect of shock loading with 150 mg/l of Purifloc C-31 is shown in Figure 23. An immediate decrease (270 mg/l) in solids concentration and a corresponding increase (380 mg/l) in COD and anthrone (175 mg/l) resulted. After the initial shock, the solids attained a new steady state but operated at a reduced overall efficiency. The COD and anthrone values continued to rise gradually at the same rate but at a rate much less then the glucose dilute-in curve. The theoretical dilute-out curve for solids and anthrone are plotted on the identical curve; they should follow the same curve since the concentrations immediately after the shock were approximately the same.

The response to shock loading with 200 mg/l of

Purifloc C-31 is shown in Figure 24. The solids were diluted out of the system nearly in accordance with the theoretical dilute-out curve during the first two hours. Since the C-31 was also diluted out of the system, the solids concentration did increase slightly over the dilute-out curve values, indicating a slight synthesis of cellular material. The COD and anthrone values increased at essentially a parallel rate which was less than the theoretical glucose dilute-in values. There was a slight initial increase in COD (60 mg/l) immediately after the shock load which may be due to a portion of C-31 passing through the membrane filter. Purifloc C-31 does not react to the anthrone test and the slight rise in COD cannot be attributed to cell lysis since it was not accompanied by a corresponding decrease in solids concentration. It should be noted that the solids dilute-out and C-31 dilute-out values are plotted on one curve but the corresponding concentrations are given on different scales.

Acclimation of Bacterial Cells to Purifloc C-31

1. Batch unit

Figure 25 shows the result of adding 50 mg/l of C-31 in daily increments to a batch system while decreasing the glucose concentration by the same amount. The combined loading of glucose and C-31 were maintained constant

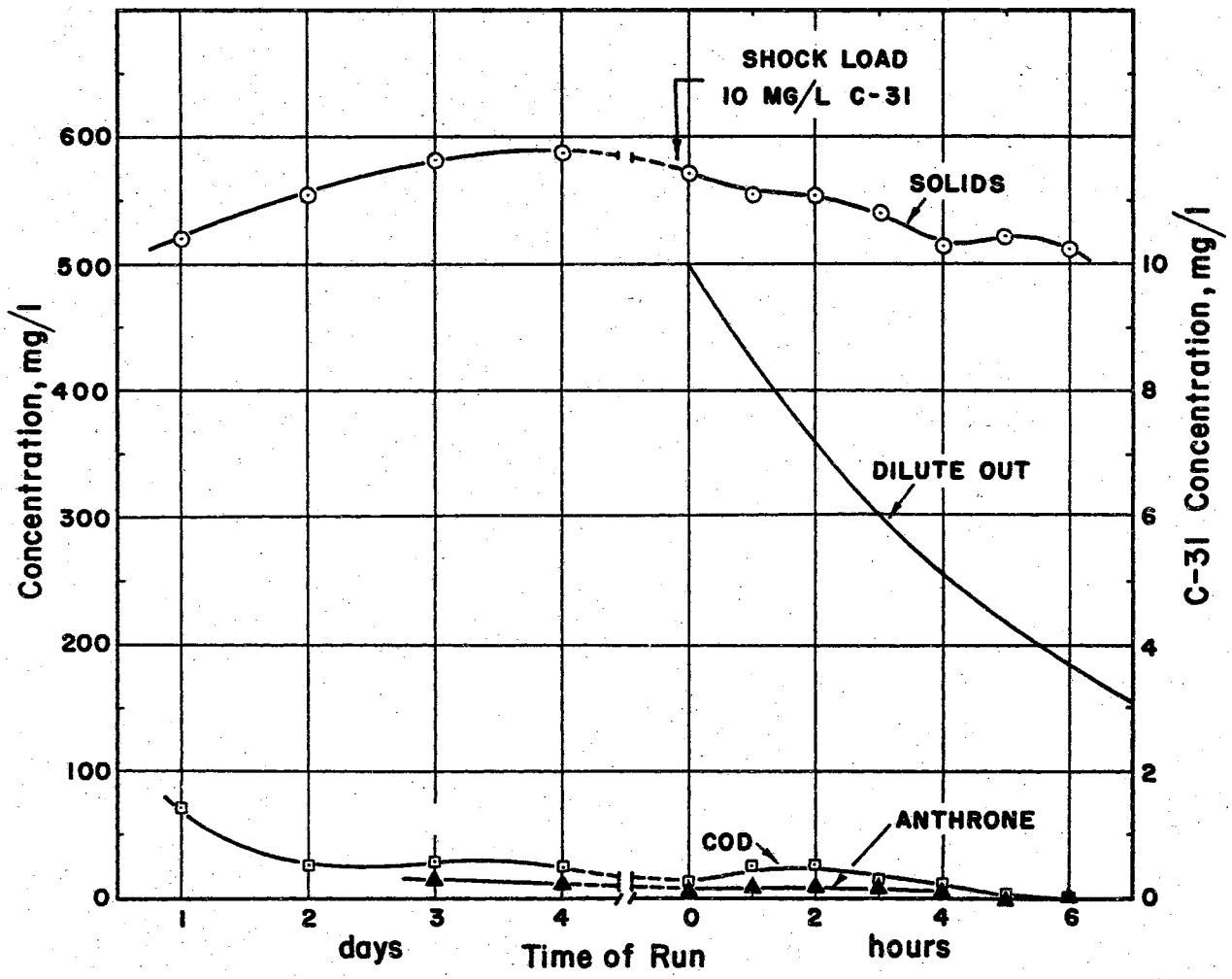


Fig. 19 - Shock Load of 10 mg/l Purifloc C-31 at Steady State

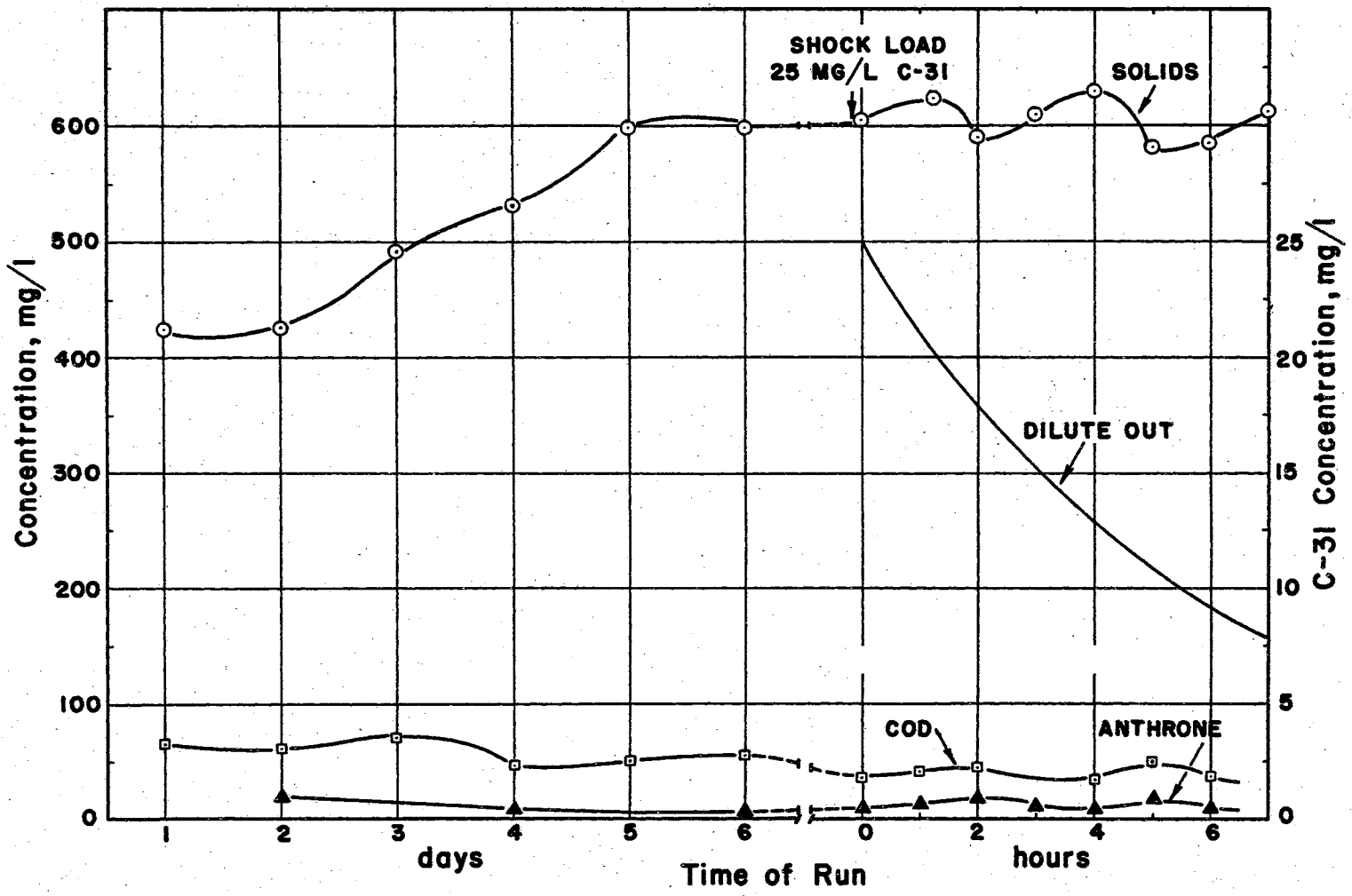


Fig. 20 - Shock Load of 25 mg/l Purifloc C-31 at Steady State

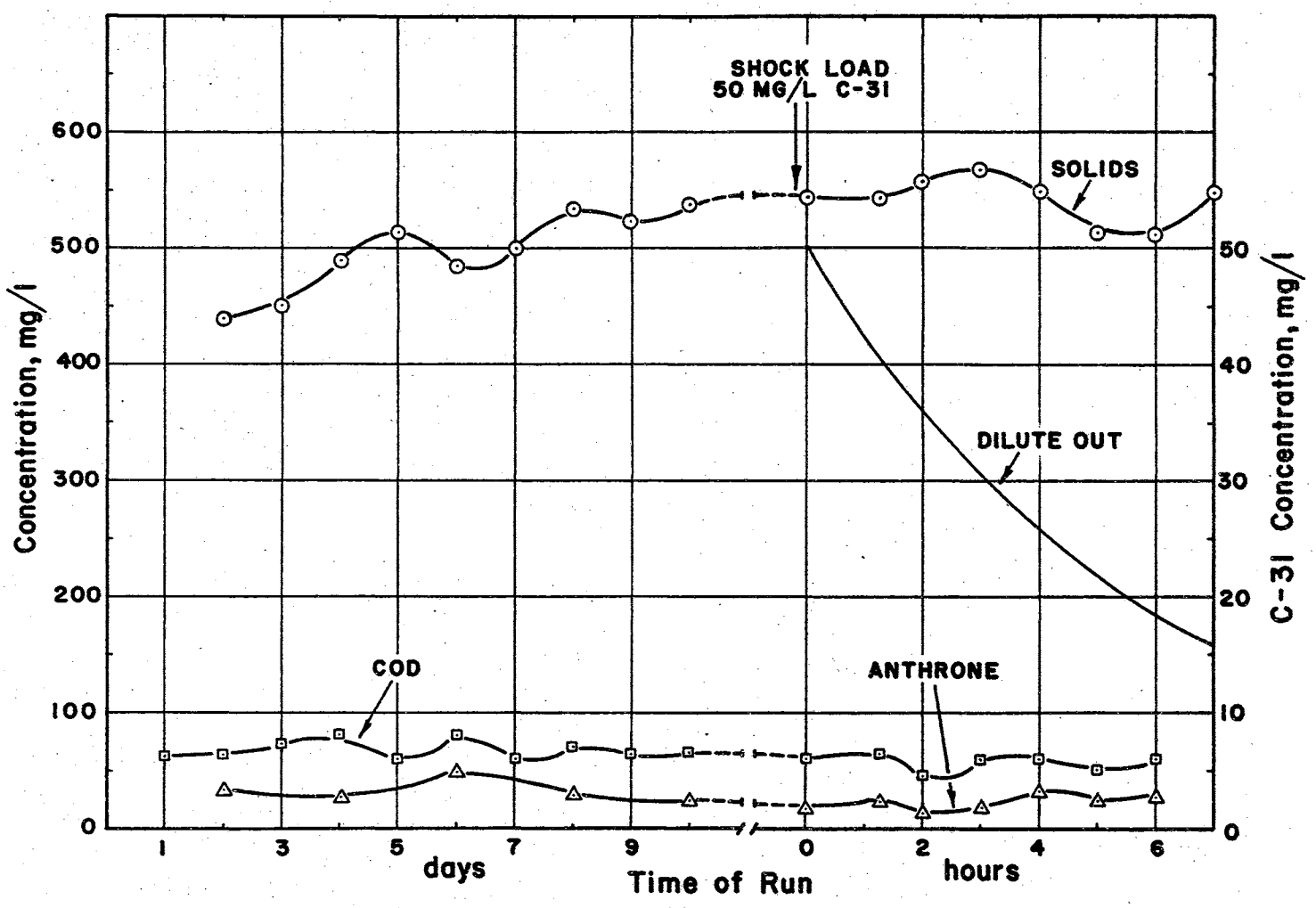


Fig. 21 - Shock Load of 50 mg/l Purifloc C-31 at Steady State

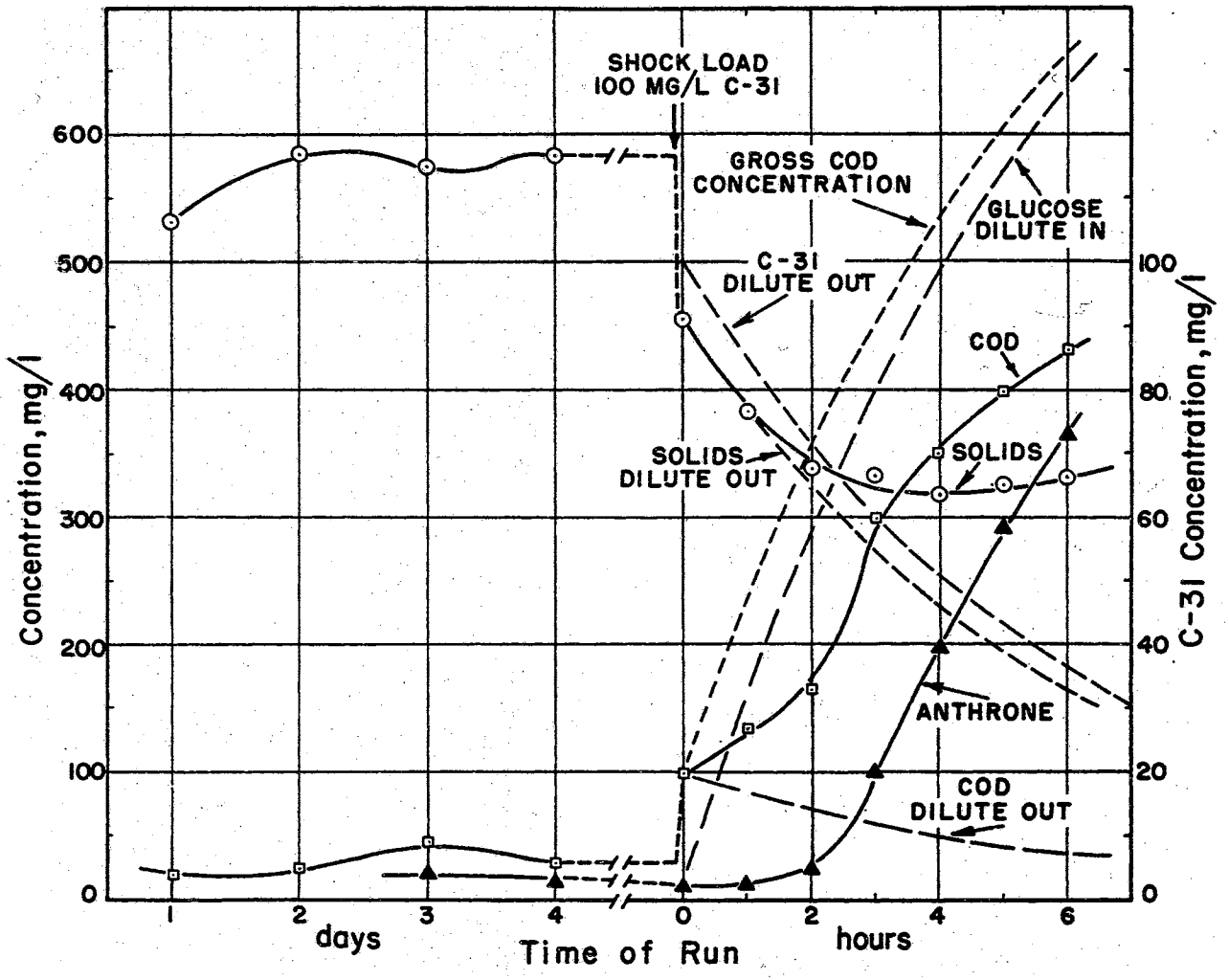


Fig. 22 - Shock Load of 100 mg/l Purifloc C-31 at Steady State

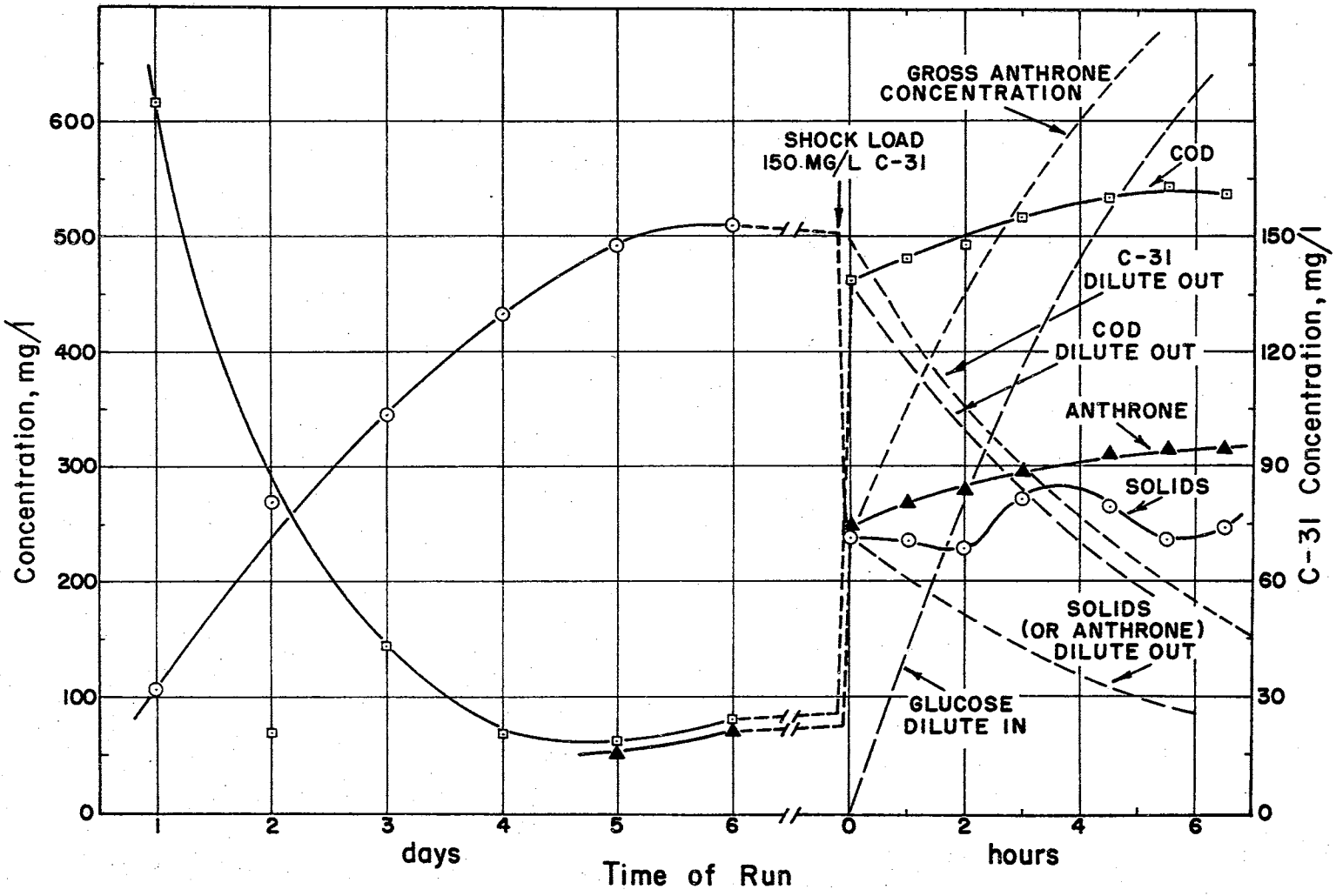


Fig. 23 - Shock Load of 150 mg/l Purifloc C-31 at Steady State

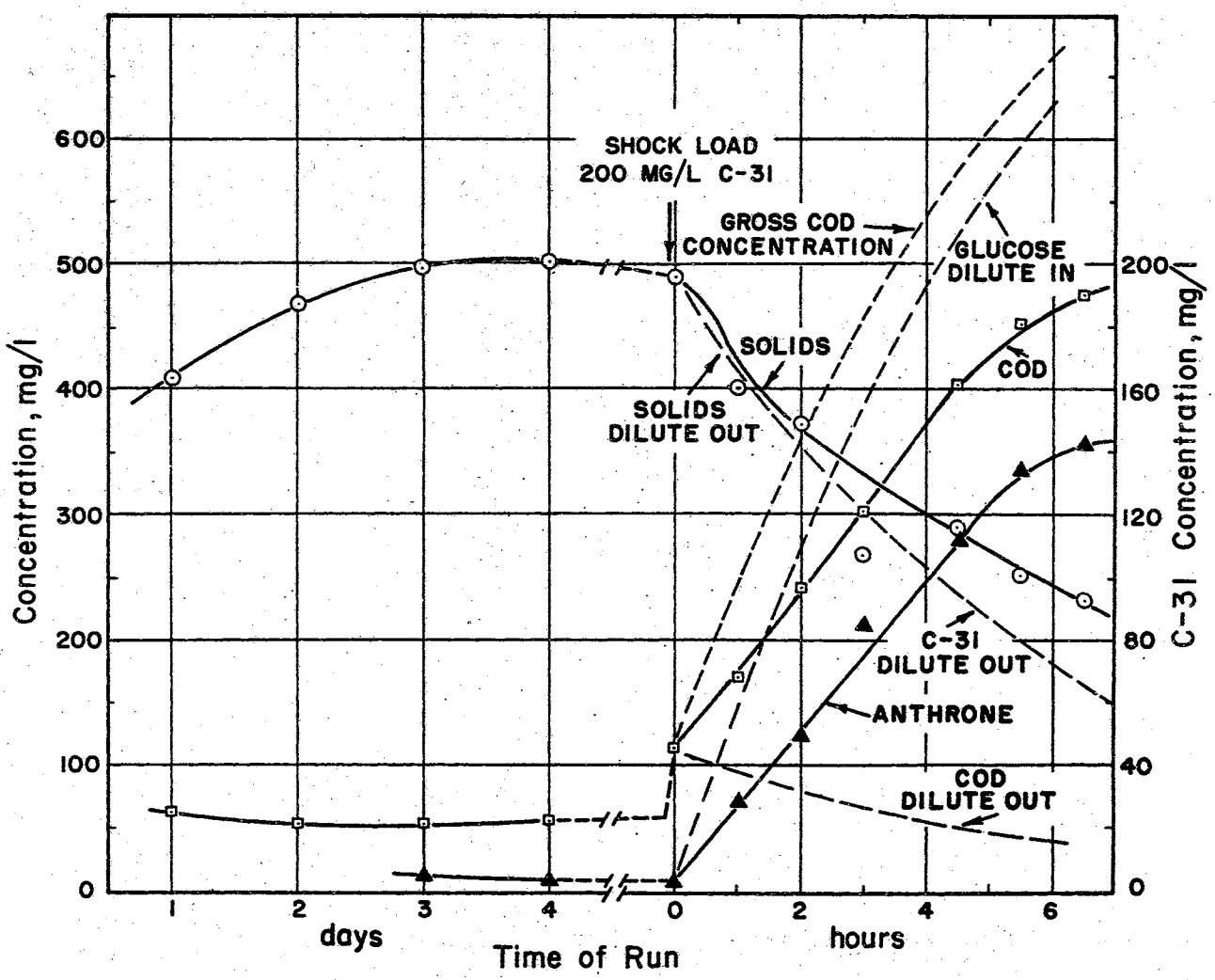


Fig. 24 - Shock Load of 200 mg/l Purifloc C-31 at Steady State

at 1000 mg/l. On the upper part of the figure are shown the daily C-31 dosage and the theoretical concentration assuming one-third of the concentration from the previous day was carried over. The biological solids concentration in the treated unit continued to increase until the C-31 reached a theoretical concentration of 860 mg/l and then they decreased sharply. The COD values in the treated unit began to rise when the theoretical concentration of C-31 reached 490 mg/l. The anthrone values during this time remained fairly constant in the treated unit; however, it must be remembered that the glucose concentration was being decreased daily. After a decline in solids concentration on the fifth day in the control unit, it performed as expected for the remainder of the experiment.

Figures 26 and 27 show the metabolic capacity of sludge taken from the control and the Purifloc C-31 treated unit respectively at the end (24 days) of the acclimation experiment. The control and C-31 treated sludges were each divided into three equal portions and placed in separate units. The three portions of each sludge were then fed as follows:

1000 mg/l glucose

500 mg/l glucose + 500 mg/l C-31 or

1000 mg/l of C-31

The remaining composition of the synthetic waste was kept the same in all the units. In Figure 26, it is seen

that the sludge harvested from the control unit metabolized 1000 mg/l of glucose in a five-hour period. When fed 500 mg/l of glucose + 500 mg/l of C-31, the control sludge was severly hampered in its ability to use glucose. This unit removed 250 mg/l of glucose in a seven-hour period. The unit which received 1000 mg/l of C-31 was unable to use the polymer as a substrate.

The response of the Purifloc C-31 treated sludge harvested from the acclimation experiment is shown in Figure 27. The sludge fed glucose at 1000 mg/l removed approximately 300 mg/l of COD and glucose over a seven-hour period; the solids concentration increased 225 mg/l over the same period. In the unit treated with 500 mg/l glucose + 500 mg/l C-31, the COD and anthrone values were reduced approximately 300 and 350 mg/l respectively, while the solids concentration increased approximately 200 mg/l in the seven hours. The unit treated with 1000 mg/l of C-31 showed almost identical concentrations in all three parameters over the seven-hour period.

2. Continuous flow unit

The results of an attempt to acclimate bacteria to Purifloc C-31 in a completely mixed continuous flow unit is shown in Figure 28. After four days of steady state operation, 25 mg/l of C-31 was added to the incoming synthetic waste. Each day thereafter, the C-31 concentration in the synthetic waste was increased by 25 mg/l

until a concentration of 200 mg/l was reached. The solids concentration appeared to be very unstable after the start of the C-31 addition; however, there were only two major upsets in the system while gradually increasing the dosage of C-31 to 200 mg/l. The first upset occurred between 5.5 and 7 days. During this upset, the solids concentration decreased 225 mg/l while the COD increased 250 mg/l. The C-31 dosage at this time was between 50 and 75 mg/l. The second upset occurred between 9 and 10 days. This resulted in a solids reduction of 250 mg/l and a COD increase of 140 mg/l. The anthrone value during this time increased approximately 95 mg/l indicating that the organisms present during this upset did not use glucose like they did during the first upset. The solids concentration during the second upset dropped approximately 100 mg/l below the level during the first upset while the COD values stayed 150 mg/l lower during the second upset.

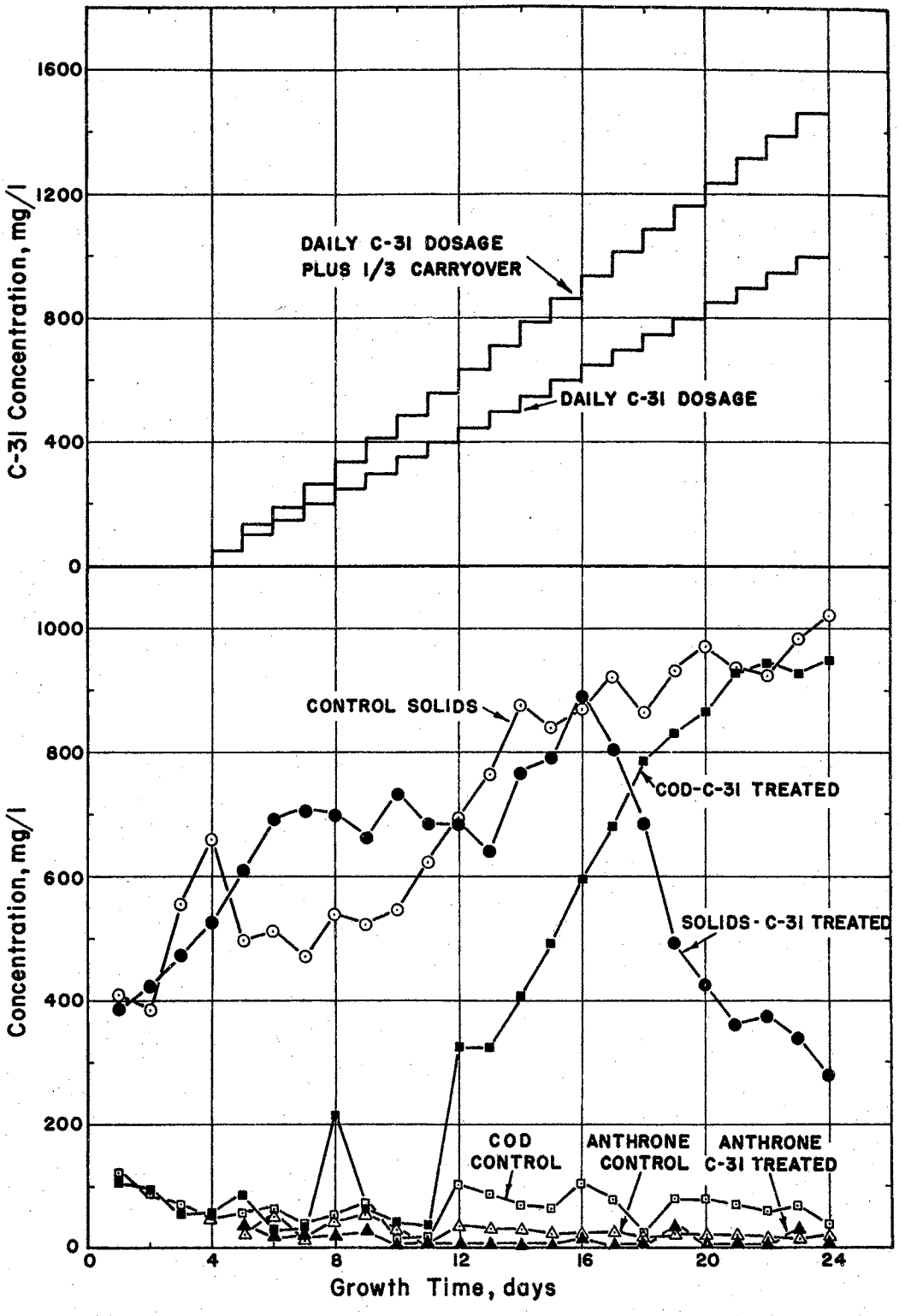


Fig. 25 - Acclimation to Purifloc C-31 Under Batch Growth Conditions

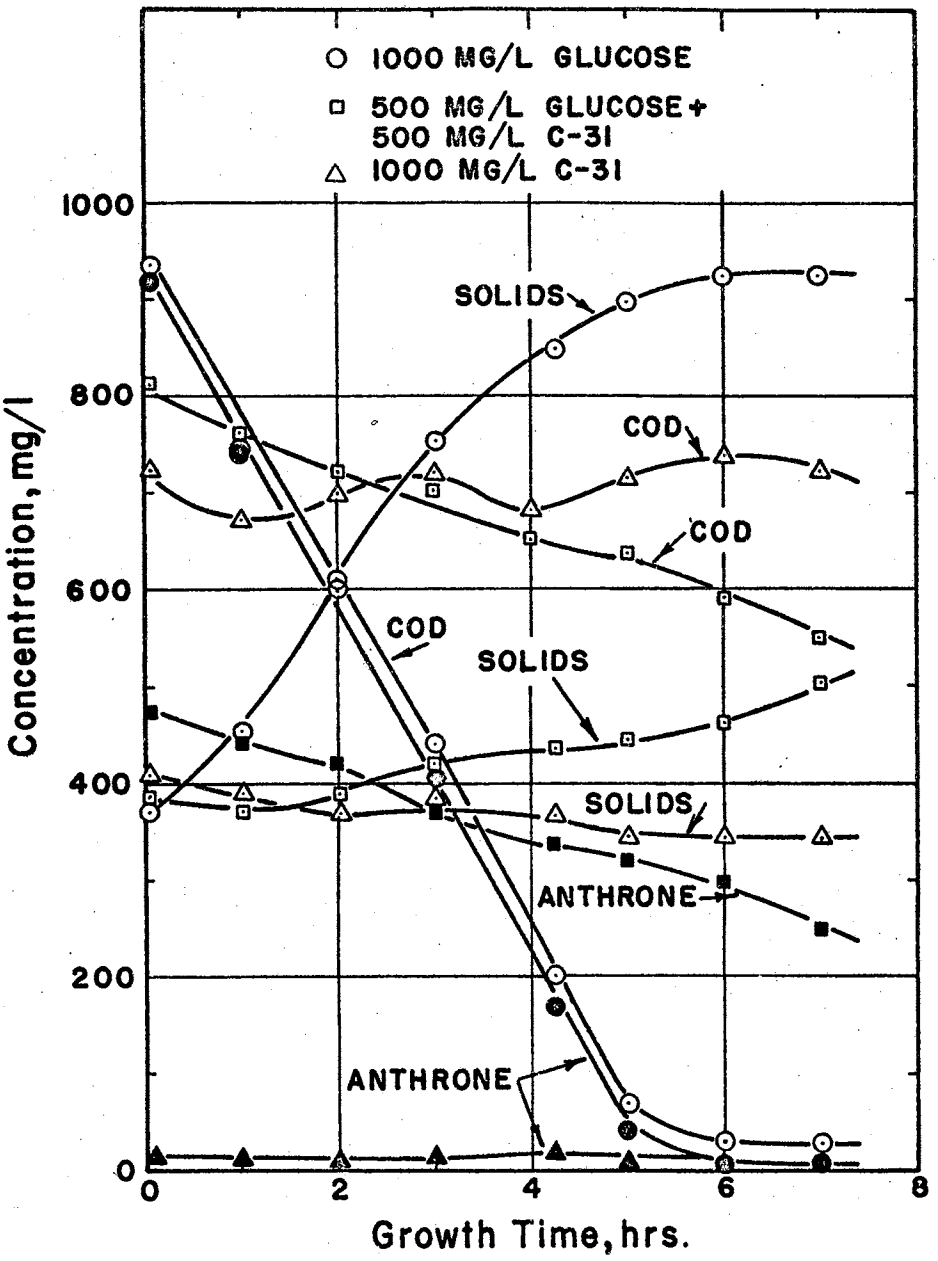


Fig. 26 - Metabolic Capacity
of Control Sludge

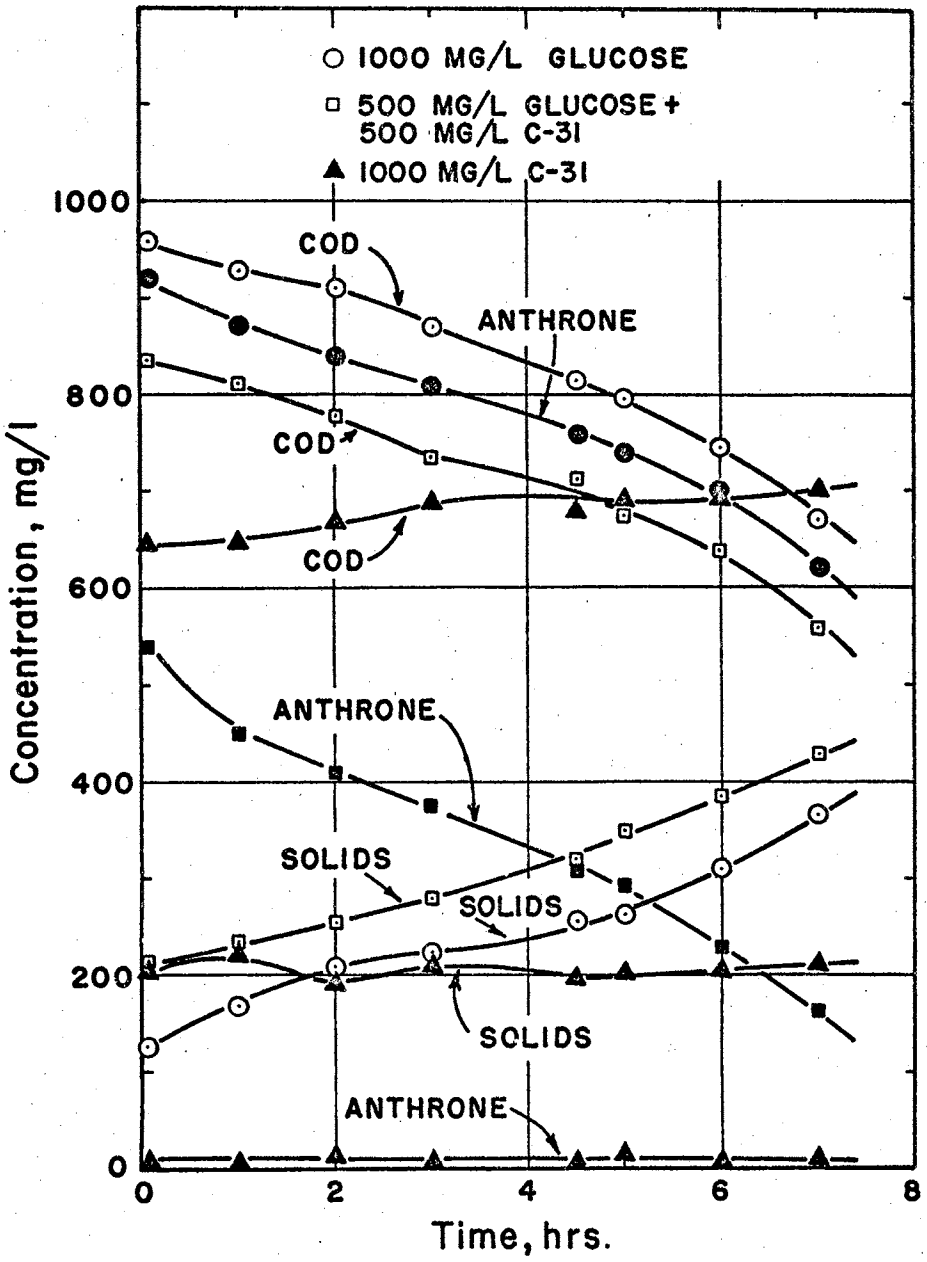


Fig. 27 - Metabolic Capacity of Purifloc C-31 Acclimated Sludge

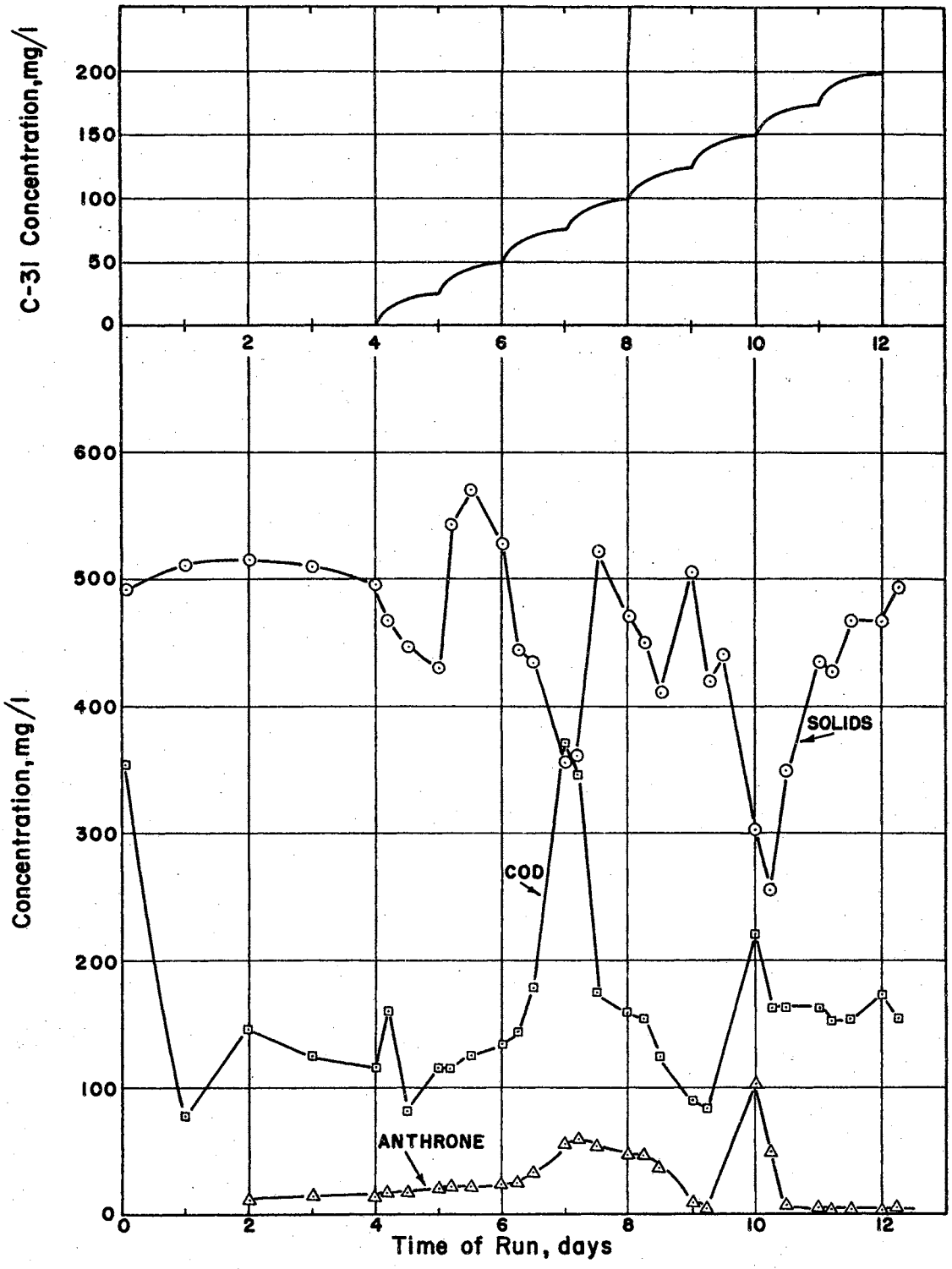


Fig. 28 - Acclimation to Purifloc C-31 in Continuous Flow Unit Under Steady State Conditions

CHAPTER V

DISCUSSION

Analytical Tests on Puriflocs A-21 and C-31

Both the cationic and anionic polymers exhibited a chemical oxygen demand (COD). Purifloc A-21 gave a positive reaction in the anthrone and the Glucostat tests while Purifloc C-31 reacted to the biuret test. In each test, the reaction was very slight and the presence of polymer is of no real analytical significance when using the filtrate; however, the polymers do have a significant chemical oxygen demand. Purifloc A-21 did not exhibit any inhibitory effect on the BOD test and this was also confirmed in the batch activated sludge studies. Purifloc C-31 on the other hand exhibited a deleterious effect on the BOD (table 6); this effect was also observed in both batch and continuous flow experiments. The batch and continuous flow experiments gave more of a quantitative measure of inhibitory dosage, whereas inhibition occuring in the BOD test was a qualitative measure of inhibition. The BOD test was used by Walker and Dougherty (39) to indicate possible inhibitory effects from the polymers.

Flocculation of Biological Solids

It would be expected that biological solids grown under standard batch conditions would be different in their physiological and surface characteristics than cells grown under steady state conditions in continuous culture. Surface characteristics were more important in flocculation studies than the internal composition of the bacterial cells because the charge characteristics may be expected to play a major roll in flocculation. Cells grown under the standard batch conditions tended to flocculate better than cells grown in continuous culture which were in a younger condition. This could be due to predominating species of organism in the solids which tend toward auto-flocculation, according to Butterfield's (19) observations. In previous studies (15) as well as in the present investigation, it has been observed that over a number of wasting cycles in a batch unit, dispersed cells are discarded with the turbid overhead while the flocculated cells settle and remain in the system. This occurance could promote a build up of flocculated cells which were amenable to increased flocculation by polymer addition.

During the endogenous respiration phase, bacterial cells excrete complex polysaccharides and polyamino acids, according to Tenney and Stumm (32), which form a slime layer on the cell. This slime layer was thought

to be composed of natural polymeric materials. Such polymeric materials could cause flocculation in this phase by a sticking together of the slime material from different cell surfaces; however, this slime layer may also serve to reduce the surface charge of the cell. With a pure culture of Aerobacter, flocculation has been observed during active bacterial metabolism while india ink staining methods with the same cells has not shown any evidence of a slime layer in the studies accomplished in the Oklahoma State University Bio-Engineering Laboratories. This last observation would lead one to believe that the slime layer is not necessary for flocculation to occur and that cells do not have to be in the endogenous phase or deprived of an energy-producing substrate in order for flocculation to proceed. The settling rate data in Figure 10 shows approximately the same rate of settling and total removal at various stages of bacterial growth. This finding is not in accord with McKinney's (13) conclusion that flocculation does not occur until after the energy-producing substrate is exhausted.

Biological solids grown at steady state conditions appeared to be more difficult to flocculate than cells grown in batch conditions. During this continuous condition of logarithmic growth, new surface area was continually being formed which may not allow the bacteria time to produce an appreciable slime layer. Riddick (9) found

that cell suspensions in the active growth phase (high degree of viability) possessed a more negative charge than resting cells. It is generally observed that resting cells possess a greater amount of capsular material than actively growing cells (13). It seems possible that lack of a slime layer may enhance a higher negative charge on the cells and therefore establishment of an increased repelling force.

Flocculation of negatively charged bacterial cells with a cationic polymer should occur by either charge neutralization, bridging or a combination of the two mechanisms. Flocculation by the addition of an anionic polymer should work by polymer bridging. In attemping to explain flocculation with polyelectrolytes, experiments were performed using both the cationic and anionic polymers on cells grown under batch and continuous flow conditions. These results were shown in Figures 4 and 5 respectively. Flocculation of cells from both units with the anionic polymer indicated an increase in clarification in comparison to a settled control, for dosages up to approximately 1.2 mg/l. Increasing the anionic polymer concentration over the optimum dosage only caused dispersion of the cells. This occurrence can be interpreted as being in agreement with the zeta potential theory of flocculation wherein an anionic polymer added to a negative system would increase its net negative charge

and could result in dispersion; however, flocculation probably occurred with low dosages of A-21 by polymer bridging before dispersion was a factor. The cationic polymer in these experiments required a higher dosage than the anionic polymer in order to start flocculation. One possible explanation for this may be the shorter chain length (molecular weight) of the cationic polymer. Flocculation was increased starting at a low dosage (3 to 15 mg/l) of C-31 and improved with an increase in dosage. This finding is in accordance with the theory of zeta potential in that a small amount of cationic polymer, while not reducing significantly the negative charge, could cause flocculation of the negative cells by bridging (14). As the dosage was increased, the negative charge was being reduced and a greater amount of flocculation and settling resulted. Charge neutralization appeared to be a factor at the higher cationic dosages since more of the dispersed colloidal material was removed. Bridging on the other hand may have been the mechanism at the lower cationic dosages and very small anionic dosages but was not as efficient in removing solids. The increase in removal of biological solids by the larger C-31 dosage was in agreement with Yu (15), Singer (2) and Tenney (32). The observation made in this work that extremely low dosages of anionic polymer were effective in flocculating solids was in agreement with Ruenwein (14) and Michaels (33)

but was in disagreement with Yu (15). This disagreement is explained by the fact that relatively higher dosages of A-21 were used in his work (15). At these dosages, dispersion was shown to occur.

The effect of solids concentration on flocculation efficiency may also be explained in terms of charge neutralization and polymer bridging. In both these experiments, (Figures 7 and 9), solids concentration was plotted against the percent relative flocculation efficiency. At high solids concentrations and with low dosages of cationic polymer, the bridging mechanism may be more operative than charge neutralization. In the Purifloc C-31 treated systems (Figure 7) for dosages of 3 and 5 mg/l, the efficiency tended to decrease as the mixed liquor solids were decreased. The decrease in particle density usually provides less opportunity for bridging to occur. As the number of particle collisions are increased, the cationic polymer may bridge between the particles which will allow entrapment and removal of a greater amount of solid particles (36). As the solids were decreased, the number of particle collisions also decreased and therefore removal efficiency decreased. As the solids were decreased (below 2000 mg/l in this experiment), the flocculation efficiency started to increase which may have been due to charge neutralization since the polymer to solids ratio was higher.

At the higher solids level, small dosages of anionic polymer were able to cause flocculation probably by polymer bridging. In this case, sites on the anionic polymer must be attracted to and stick on certain sites on the negative charge bacterial cell (34). As the solids were decreased in the system, the number of particle collisions were again decreased which could account for the decrease in efficiency. Also, as the solids were decreased, the ratio of Purifloc A-21 to solids was increased which could have caused a repulsive force between the polymer and the solids. This was in agreement with the results shown in Figure 9 where a dosage of 0.5 mg/l of Purifloc A-21 produced higher efficiencies than 1 mg/l Purifloc A-21 for the same solids concentration. As the solids concentration continued to decrease, average efficiencies at both dosages of A-21 decreased which may indicate that bridging was not efficient at the lower solids concentrations.

Inhibitory Effects on Biological Solids From Purifloc A-21 and C-31

The addition of an organic polyelectrolyte to a biological system could result in bridging, charge neutralization or a combination of the two. The polymers were high molecular weight organic compounds which carried an electrical charge. For a given polymer concentration and physiological condition of the bacteria, the addition

of this polymer to the biological system could result in an inhibitory effect to the biological system. This inhibitory effect could be any one of a number of physical, chemical or biochemical mechanisms.

Reduction in the rate of bacterial metabolism may have been the result of flocculation of biological solids in the presence of the polyelectrolyte. As suggested by Yu (15), aggregation of cells in a floc particle might possibly reduce the total available surface area for entry of substrate and thereby reduce the rate of metabolism. The polymer could have covered the cell sufficiently to prevent, for example, a permease from transporting glucose into the cell. If this phenomenon occurred, attractive forces binding the polymer and solid together, along with the amount of surface area covered, might determine the rate at which the substrate could be used. It may also be possible that the polyelectrolyte could cause enzyme inhibition which would reduce the growth of bacterial cells or substrate utilization. In this case, the polymer may adsorb onto one or more sites on an enzyme in competition with the substrate. In addition, a sudden shock load of polymer on the system could result in lysing or leakage of protoplasm from the cell. Immediate reduction of cell concentration and increase in COD, indicitive of lysing, was observed in this study and in the experiments of Yu (15).

In the batch and continuous flow experiments shown in Figures 14 and 22 to 24 respectively, where high dosages of Purifloc C-31 were added to the biological system and disruption of substrate removal efficiency was noted, one or more of the possible mechanisms described above may have been responsible. In the batch unit study (Figure 14), 200 mg/l (approximately 285 mg polymer/gm of solids) of C-31 resulted in an inhibitory effect. The metabolic rate of the bacteria was slowed to such an extent that 700 mg/l of solids were able to remove only 190 mg/l of COD and 140 mg/l of glucose in six hours. During this time the solids concentration increased by approximately 125 mg/l. Lower dosages of C-31 in batch studies did not result in bacterial inhibition.

The inhibitory effect due to shock loading of 200 mg/l (approximately 400 mg polymer/gm of solids) of C-31 in the continuous flow steady state system (Figure 24) reduced the rate of metabolism to such a degree that the biological solids increased by approximately 70 mg/l over the theoretical solids dilute-out value while the COD was reduced by approximately 200 mg/l in the six hours following polymer addition. The reduction in COD was calculated by the difference between the gross COD concentration and the observed COD. Since the cells did not show a high degree of flocculation in either the batch or continuous flow experiment, it is difficult to envision

the disruption of substrate removal capacity as a mechanical blockage of substrate access to the cell; it would appear that the results were due to a metabolic inhibition. The lower initial solids in the continuous flow unit in addition to continual cell dilute-out, may account for the slower rate of metabolism in the continuous flow unit in comparison to the batch unit.

The response to shock loading a steady state system with 100 mg/l of Purifloc C-31 (Figure 22) produced a reduced rate of metabolism; however, the rate was not reduced as severly as that shown with a shock load of 200 mg/l of C-31. The system shocked with 100 mg/l of C-31 showed a reduction of 230 mg/l in COD while the solids concentration increased by 170 mg/l in the six-hour period in comparison to the respective dilute-out and dilute-in curves.

The metabolic response to a shock load of 150 mg/l of Purifloc C-31 in a steady state system would be expected to be similar to that observed for shock loadings with slug doses of 100 and 200 mg/l of C-31, i.e. fall somewhere between these responses; however, the observed results for a slug dose of 150 mg/l of C-31 produced an immediate and quite severe decrease in solids with a corresponding increase in COD and anthrone. This is indicative of lysing or leakage of protoplasm from the cell. After the shock loading, the system appeared to attain a

new steady state but at a much lower cell concentration and higher effluent COD concentration. The cell yield was approximately the same before and after the shock loading with C-31. From the results, it is impossible to determine why the solids could not remove more of the substrate; however, during the experimental period, it is obvious from the results that the cells did not metabolize the lysed material exclusively since the observed anthrone concentration were much lower than those predicted by the dilute-in curves.

The response of activated sludge to Purifloc A-21 in batch units was shown in Figures 17 and 18. For dosages up to 200 mg/l there were no inhibitory effects observed even though the solids concentration in this experiment was approximately half the concentration used in the equivalent Purifloc C-31 experiment. It appeared that A-21 did not exhibit any toxic or inhibitory effects on biological solids up to a dosage of 700 mg polymer/gm of solids. This finding was in agreement with the result of the BOD tests run on Purifloc A-21. Since the dosage of Purifloc A-21 needed to provide effective flocculation was approximately 1 mg/l and no inhibitory effect was evident at 200 mg/l, it was concluded that this polymer was not detremental to activated sludge and experimentation was not performed in the continuous flow unit.

Acclimation of Bacterial Cells to Purifloc C-31

If bacteria could be acclimated to the polymer, or at least develop a tolerance to high concentrations, then the use of polymer in the activated sludge system might be a possibility since it would obviate concern over polymer build up in the sludge. Result of experimentation to determine if the cells could use the polymer (Purifloc C-31) as a source of energy to build new cellular material was shown in Figure 25. During this experiment in which the C-31 concentration was gradually increased by 50 mg/l/day, the bacteria were able to metabolize the substrate until the "theoretical" C-31 concentration reached 860 mg/l or approximately 1000 mg polymer/gm of solids. This level of polymer tolerance was much higher than that found in the continuous flow unit studies at a shock load of 100 mg/l (Figure 22) or 220 mg polymer/gm of solids or the inhibition observed in the batch unit studies at 200 mg/l or 285 mg polymer/gm of solids. It appears that the bacteria can develop a tolerance for C-31 in a system where high concentrations of the polymer are gradually built up; however, it must be remembered that the glucose concentration was decreased daily and the values shown in Figure 25 were obtained from samples taken twenty-three hours after feeding the glucose. In other words, a slowdown in the rate of metabolism would not necessarily

be shown in these studies. In order to determine the metabolic capacity of both the control and the C-31 treated sludge after twenty-four days exposure to polymer, the sludges were harvested and each one was treated at a different level of glucose and/or C-31 as shown in Figures 26 and 27. Results obtained for the control system indicated that the sludge could metabolize glucose alone, but when 500 mg/l of C-31 was present the rate of metabolism was severly hampered. The rate of metabolism at 500 mg/l of both glucose and C-31 (Figure 26) was still slightly faster than the rate of metabolism for 200 mg/l of C-31 plus 1000 mg/l of glucose shown in Figure 14. The solids concentration in the unit dosed with 500 mg/l of C-31 was only 375 mg/l in comparison to solids concentration of 700 mg/l for the experiment shown in Figure 14. Both of these sludges had never been exposed to C-31 before and they were both grown under batch conditions. The only difference observed was that the age of the bacterial cells used in the 200 mg/l C-31 system (Figure 14) were only a few days old while the cells used in Figure 26 were grown up in twenty-four days. If age of the sludge determined the C-31 concentration at which inhibitory effects could be observed in a system, then this might explain why cells grown at steady state were effected at lower dosages of C-31 as evidenced in Figure 22. The control sludge, treated with 1000 mg/l

of C-31 instead of glucose, was unable to use the polymer as a substrate.

The results indicated that the biological solids which were "weaned off" of glucose and on to C-31 had not lost the ability to metabolize glucose. The rate of metabolism of this sludge was much slower than that of the control sludge. The slower rate may have possibly been due to polymer coating of the cells. This same sludge treated with 500 mg/l of both glucose and C-31 metabolized glucose at the same rate as the sludge fed with 1000 mg/l of glucose. These results indicate that once the biological solids were conditioned to C-31 they would withstand a large slug dosage of C-31 without further slowdown in the metabolic rate of the solids. The acclimated solids shown in Figure 27 when treated with 1000 mg/l of C-31 and no glucose were unable to metabolize the polymer indicating this polymer probably could not be used by the bacterial cells as a source of energy.

In the continuous flow unit experiment, the Purifloc C-31 concentration in the synthetic waste was increased by 25 mg/l each day starting on the fourth day. Disruption in the steady state conditions occurred almost immediately after the start of polymer addition. Continual increase in the polymer concentration caused the biological solids and substrate levels to fluctuate; however, it is interesting to note that at the time when the

experiment was terminated (200 mg/l C-31) the biological solids level was approximately the same as that in the steady state before any polymer was added. It is possible that the unstable conditions observed in this continuous flow system were caused by changes in bacterial predominance induced by polymer additions. No readily apparent changes (i.e. color of cell suspension) were noted.

In general, the results of the continuous flow experiments substantiate the batch experiments. Activated sludge can, with proper acclimation, withstand fairly high concentrations of the polymer.

CHAPTER VI

CONCLUSIONS

Based upon the results of these experiments, the following conclusions have been drawn:

1. Purifloes A-21 and C-31 exert a chemical oxygen demand in the COD test. When these polymers were added to the different biological systems, and the mixed liquor solids concentration was determined, a portion of the polymer passed through the membrane filter and contributed to the COD of the sample; this amount varied.

2. Purifloc A-21 does not seriously interfere with the anthrone and Glucostat tests while Purifloc C-31 was only slightly reactive to the biuret test. The reactions observed were at the higher polymer concentrations, but since these tests were normally made on the filtrate samples, there should not be enough polymer present to affect the test results.

3. The BOD test was found to be a good qualitative measure of the effects of metabolic inhibition due to polymer addition to biological systems.

4. Purifloc A-21, an anionic polymer, was shown to flocculate bacterial cells at dosages of 1 mg/l or less. This polymer demonstrates increased removals at the effective dosage (approximately 1 mg/l) when compared to a settled control. Increasing the dosage of this anionic polymer over the effective dosage (above 3 mg/l) results in dispersion of the solids. This polymer does not flocculate the amount of bacterial solids as well as does cationic polymer.

5. Purifloc C-31 is an effective bacterial flocculant and the dosage range has been observed in these studies to be between 3 and 200 mg/l. This polymer will remove almost all of the colloidal material present in a given flocculation experiment at the optimum dosage.

6. Bacterial cells grown in a continuous flow steady state system were observed to flocculate when aggitated at the low mixing speeds in the flocculation test without polymer addition. In these experiments, approximately 50% reduction in solids concentration was observed.

7. Biological solids grown under batch conditions tend to flocculate better than solids grown in the continuous flow unit. The optimum Purifloc C-31 dosage was lower in the batch systems than in the

continuous flow units. The effective Purifloc A-21 dosage was similar for solids from both systems; however, dispersion occurred at lower anionic dosages in the continuous flow unit.

8. Solids concentration has an effect on the flocculation efficiency of the polymer. Purifloc A-21 was more effective at the high solids concentration while Purifloc C-31 was effective at both the high and low solids levels.

9. Biological solids taken at various stages along the growth curve and subjected to the standard flocculation procedure yielded the same amount of solids remaining in suspension after settling; however, when considering the increase in solids due to growth, the greatest amount of removal occurred at the beginning of the endogenous phase.

10. Increasing the Purifloc C-31 dosage increased the rate of settling under the dynamic conditions used in the flocculation test. For the batch grown solids, the control unit showed a slower rate of settling in comparison to the polymer treated samples; however, the total amount of removal was the same.

11. Purifloc A-21 at the optimum flocculation dosage, increased the rate of removal; however, the total amount

of removal in these systems decreased with increasing settling time under the dynamic conditions used. This effect may have been due to the presence of more loosely bound floc particles than those produced by Purifloc C-31. It was noticed that C-31 produced flocs which appeared to be more dense than those found in the presence of A-21.

12. Purifloc A-21 did not inhibit metabolism of biological solids under batch conditions for dosages up to 700 mg polymer/gm of solids. Purifloc C-31 on the other hand, did show deleterious effects on metabolism at a dosage of 285 mg polymer/gm of solids.

13. Purifloc C-31 inhibited biological solids from the continuous flow steady state system at a dosage of 180 mg polymer/gm of solids (100 mg/l) while inhibition under the batch condition for this polymer did not occur until a dosage of 285 mg polymer/gm of solids (200 mg/l).

14. Bacteria did not acclimate to Purifloc C-31 to the point where they could use it as an energy source; however, the population was capable of developing a resistance to fairly high concentrations of this polymer.

SUGGESTIONS FOR FUTURE WORK

The occurrence of bacterial flocculation either by the natural phenomenon of auto-flocculation or induced chemical flocculation by the addition of organic polyelectrolyte is important to the overall efficiency of the biological treatment process. As pointed out in this investigation, there is still disagreement as to the exact mechanism of auto-flocculation. A better understanding of the effects of organic polyelectrolyte on biological systems may help to explain the mechanism of auto-flocculation.

Before proceeding further into this area of investigation, there are some discrepancies in this study that must be resolved. The lysing effect observed in the steady state system at 150 mg/l of Purifloc C-31 does not correspond with the reduction in rates of metabolism observed under similar conditions with 100 and 200 mg/l Purifloc C-31. The rate of metabolism at 200 mg/l Purifloc C-31 under batch conditions was reduced considerably; however, a reduction in the rate of metabolism was not evident at 150 mg/l C-31 or less as might be expected. Acclimation of bacteria to increasing concen-

trations of Purifloc C-31 under steady state systems produced an unstable system; however, the system attempted to return to its original steady state values. It would be interesting to determine the response by continuing this experiment to higher concentrations of C-31 and also the response to a constant dosage (say 200 mg/l) of C-31 after the concentrations had been increased in gradual steps to that value.

In this thesis research, and in previous investigations on flocculation in the pollution control field, investigators have employed many different flocculation and settling procedures so that correlation between all of these studies may be difficult. It would be beneficial to this field of research if a standard investigational procedure would be adopted which incorporated the use of instrumentation to measure precisely the degree of flocculation. For instance, flocculation is the result of increasing the floc size so that a direct measure of floc size would seem to be the best indication of the degree of flocculation (e.g. the use of a Coulter Counter). Also, an instrument that would indicate the net charge in a system may also provide valuble insight into flocculation process (e.g. electrophoretic mobility apparatus).

The addition of a polyelectrolyte to a system will effect the net charge of the system and also flocculation. The amount of polyelectrolyte necessary to change the net

charge of the system to the point of optimum flocculation (0 ± 3 millivolts according to Riddick -9-) would be an indication of the effectiveness of each polymer. Different polymers could be compared as to their effectiveness in this manner.

One of the objectives of this investigation was to determine if the reduced rate of substrate removal in the presence of Purifloc C-31 was due to bacterial floc size or mechanisms of metabolic inhibition. Since no accurate measure of floc size was available, change in floc size was approximated by the settling rate of the biological solids. This did not prove to be an effective correlation since appreciable flocculation was not observed in studies for which substrate removal was examined. Also, there was some indication that floc size varied with the degree of aggitation present in the flocculation studies. In order to study the effect of floc size on the substrate removal rate, an instrument will have to be adapted to determine floc size directly in a reactor without changing the degree of aggitation in the system. The rate of substrate removal will also have to be determined during this experiment.

There is a definite need to study some of the parameters of auto-flocculation. It would be interesting to determine how floc size varies with the age of the bacterial cells both with and without daily additions of

fresh substrate. Floc size should be measured in the reactor under various conditions of aggitation; however, no way of doing this is presently available. It may be possible to create the same degree of aggitation in the counting apparatus as is in the reactor. It would also be interesting to determine how floc size varies with the presence of a slime layer and also how the net charge in the system will vary with the accumulation of the slime layer.

It has been observed in some experiments in this study that cells grown under steady state conditions have flocculated when placed under the lower aggitation conditions of the standard flocculation test. It would be interesting to conduct further studies in this area.

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