EFFECTS OF POLYELECTROLYTE COAGULANT AIDS ON BACTERIAL CELLS

By

TA-SHON YU

Bachelor of Science

Taiwan Provincial Cheng Kung University

Tainan, Taiwan, Republic of China

1963

Submitted to the faculty of the Graduate School of
the Oklahoma State University
in partial fulfillment of the requirements
for the degree of
MASTER OF SCIENCE
May, 1966

EFFECTS OF POLYELECTROLYTE COAGULANT

AIDS ON BACTERIAL CELLS

Thesis Approved:

Anthony F. Gaudy Jr.

Thesis Adviser

Elizabeth T. Gaudy

J. R. Bozzo

Dean of the Graduate School

ACKNOWLEDGEMENT

The author wishes to express his hearty appreciation to Dr. A. F. Gaudy, Jr., for his valuable guidance and constant encouragement. Without his assistance, little would have been accomplished.

The author also wishes to express his hearty appreciation to Dr. E. T. Gaudy of the Microbiology Department for her advice during the writing of the thesis.

The author wishes to express his thanks to Mr. P. Krishnan and the other students in the Bio-engineering Laboratory for their useful assistance.

The author also owes thanks to Mrs. Vera Aktansel for her excellent typing of the manuscript.

I am grateful to my parents for financial support during the entire year of study in the United States. Without their support and encouragement this thesis would never have been brought to completion.

TABLE OF CONTENTS

Chapter Page

Chapter Page

LIST OF TABLES

LIST OF FIGURES

Figure Page

CHAPTER I

INTRODUCTION

A. Importance of bacterial flocculation and its relation to flocculation aids

The development of civilization in recent centuries has led to considerable sociological prosperity; and in the course of this improvement, the living standard has been raised greatly. Quite naturally, people have made efforts to improve environmental sanitation and public health. Thus, control of the aqueous environment has become one of the major responsibilities of the modern bioengineer.

One of the best means of attaining control over the aqueous environment is through the use of waste water treatment processes which provide for the separation of impurities from waste waters. These impurities may be soluble or nonsoluble organic or inorganic matter, bacteria, and other colloids. The nonsoluble material may be suspended in the waste water or may be settleable.

Bacterial flocculation can be classified in three types which may involve physical, chemical, and biological factors. These are natural flocculation, self-flocculation or autoflocculation and chemical flocculation.

Natural flocculation has been attributed to the collision of bacteria with impurities present in the wastes. In 1914, the discovery of activated sludge by Arden and Lockett in the laboratory of the Manchester sewage works gave rise to the idea of natural flocculation (1). The biological slimes naturally developed in the aerated organic wastes provide an ample surface for contact of other organic matter and formation of zoogleal flocs. These flocs are highly active centers

of biological life, composed of living masses of organisms, food, and slime material (2).

Thus far, no one has clarified the apparent mystery of bacterial auto-flocculation. However, various postulations have been made to explain this phenomenon. The mechanism is usually attributed to the colloidal characteristics of the cells; negative charges distributed over the bacterial surface set up a competitive force between electrostatic repulsion and Van der Waal's attraction. When the latter predominates, self-flocculation can take place (3).

Knowledge of the chemical basis of colloidal flocculation has been continuously growing in the past forty years. From 1923 to 1925, a paper by Theriault and Clark and a series of papers by Miller set forth the fundamental concept of chemical flocculation. They said that there must be present a certain minimum quantity of aluminum or ferric cation; there should be present an anion of strong coagulating power; and pH must be carefully adjusted (4), (5),(6), (7), (8).

In 1940, Larson and Buswell (9) found that the charge on $Mg(OH)_2$ is positive throughout the entire pH range; $CaCO_3$ is negatively charged. Alum flocs are always positively charged below pH 7.6 and always negatively charged above pH 8.2; the isoelectric point falls somewhere between the two values.

Synthetic polyelectrolyte coagulant aids were introduced in the year 1952. It has been found that polycations are effective coagulants alone, while polyanions serve as coagulant aids after a flocculating dose of a metal coagulant has been added. In order to explain their results, Ruehrwein and Ward postulated the formation of a polymer bridge between the colloidal particles (10). Michaels suggested that a polymer must become adsorbed on the solid surface of the particles, if it is to contribute to flocculation (11).

B. Scope and purpose of the present research

Due to the complicated environment in which bacteria grow, it seems unlikely that bacterial flocculation is due to one single mechanism. It would seem almost impossible for one variable to exert an absolute or over-riding effect over another because of their interdependent influence on each other. Therefore, bacterial flocculation is envisioned by the author as the resultant of a complicated mechanism influenced by environmental factors and the interrelation between the various bacterial species which may be present. Regardless of the lack of information, bacterial flocculation is one of the most cogent interests of the bioengineer.

With respect to flocculation in the presence of polyelectrolytes, the major factors affecting flocculation are listed below (12):

(1) pH,

(2) degree of agitation,

(3) type and concentration of polymers,

(4) range of molecular weight of polymers used,

(5) presence of other cations and anions in solution,

(6) temperature, and

(7) mode of addition of polymers.

Successful purification of waste waters which contain soluble organic matter depends upon the metabolism of the organic matter by bacteria and the subsequent separation of the bacteria from the waste waters. It is well known that polyelectrolytes are useful in the separation of colloids from the aqueous phase. However, some polyelectrolytes may be toxic to bacteria; therefore, they would be of no use even though they possess good flocculating power.

One of the major purposes of this study was to gain some insight into possible toxic effects of selected polyelectrolytes. Another important aspect of the research was

the determination of optimum dosages required at constant cell concentration for cells harvested during different phases of growth. Relative flocculation efficiencies of the polyelectrolytes at different cell concentrations was also investigated. A possible mechanism of flocculation involving interrelations of capsule and cell wall constituents resulted from this study.

Cationic polyelectrolytes were chosen for investigation on the basis of charge neutralization, since bacteria carry negative surface charges and may be considered as natural anionic polyelectrolytes. The work was conducted at neutral pH since this is optimum for the growth of most bacteria (13). Temperature was maintained at approximately 22°C. to 25°C. All studies to determine optimum dosage were conducted in a water bath shaker apparatus using the same degree of agitation.

CHAPTER II

REVIEW OF LITERATURE

A. Nature of the bacterial surface

1. Constituents of capsule and cell wall

The capsule which is the outermost layer of a bacterium is composed of either polypeptide or polysaccharide, the latter sometimes having proteinaceous and lipoidal material associated with it (14), (15). Clifton stated that,

> "Little is known concerning the structure or chemical nature of the cell wall. Chemical tests indicate that in some species, it is primarily polysaccharide in character, often resembling cellulose of hemicellulose. In other species, it is composed of complex nitrogenous compounds often conjugated with carbohydrates, lipid, or nucleic acids (14)."

The cell wall constituents of Gram-positive bacteria consist of large amounts of polysaccharide and small amounts of lipid and protein; while, for Gram-negative bacteria, the cell walls are composed of large amounts of lipids and proteins and small amounts of polysaccharides (16), (54).

2. Colloidal nature of bacteria

A colloidal system can be defined as one in which one material is stably dispersed in a second and the dispersed material is of greater than molecular size. In general, it is arbitrarily considered that the dispersed phase in a colloidal system is composed of particles with diameters between 1 and 100 millimicrons (53).

There may be some questions as to why a bacterial culture in liquid medium is considered to be a colloidal

system. In 1904, Neisser, Friedmann and Beckhold investigated the nature of the cell surface and reported that the surface of a bacterial cell carries negative charges (17). Clifton stated that the outermost layer of the cell is actually not an integral part of the cell; it is an ionic atmosphere loosely held by electrical charges on the cell (14). With respect to electrical charge carried on the bacterial surface and particle size, the bacterial cell is similar to colloidal particles which also carry a surface charge, either positive or negative, depending on their nature and on their environment.

Another question might be asked: why does the cell surface carry negative charges? Part of the answer may lie in the fact that proteins and lipids are important constituents of the capsule and cell wall. The fundamental chemical structural units of proteins are α-amino acids, i.e., the amino groups are attached to the α-carbon atom; the general chemical structure is: $NH_2CHRCOOH$. It must be noted that amino acids are ampholytes. The amino group and the carboxyl group have characteristic pK values. The monoamino, monocarboxylic acids in aqueous solution exist as dipolar ions, zwitterions, in which both acidic and basic groups are ionized. The molecule may be represented in the form: $^{+}H_3N\text{-}CHR\text{-}COO^{-}$. The molecule is electrically neutral and isoelectric. In acidic conditions, ionization of the carboxyl group is repressed and the molecule acquires a net positive charge. In basic conditions, a proton is removed from the ammonium group, leaving the molecule with a net negative charge (18). The overall equilibrium can be expressed as follows.

$$^{+}H_3N\text{-}CHR\text{-}COOH \overset{H^{+}}{\rightleftarrows} {}^{+}H_3N\text{-}CHR\text{-}COO^{-} \overset{OH^{-}}{\rightleftarrows} H_2N\text{-}CHR\text{-}COO^{-}$$

Since the isoelectric point is generally below pH 7 (19), and the pH value of most bacterial culture is kept at approximate pH 7, the amino acids of protein on the bacterial surface might be expected to carry negative charges.

In general, lipids are esters of fatty acids and various alcohols and may be presented by the following general chemical structure: RCOOR'. Under slightly acidic conditions, they react as follows:

$$RCOOR' + H_2O \overset{H^+}{\rightleftharpoons} RCOOH + R'OH$$

$$RCOOH \rightleftharpoons RCOO^- + H^+$$

Under a slightly basic condition, the hydrolytic equilibrium reaction converts the ester into a salt (20).

$$RCOOR' \overset{OH^-}{\rightleftharpoons} RCOO^- + R'OH$$

Therefore, either under acidic or basic condition, the lipid constituents on bacterial surfaces also might be expected to carry negative charges.

B. Nature of polyelectrolytes

Polyelectrolytes are made by the polymerization of various compounds which form water-soluble resins. The polymerization reaction involves the joining together of many small organic molecules (monomers) to make very large molecules (polymers). All polyelectrolytes are compounds of high molecular weight (21).

Polyelectrolytes can be treated as colloids. These colloids are linear and threadlike having like charges recurring throughout the length of the molecule. Thus, one would exist in the water stretched out to its full length. Similarly, all adjacent colloids would find themselves in a like situation. Furthermore, each of the water-soluble, threadlike colloids would repel one another with full extension (22), (23). The kinetic force of repulsion would protect the polymers against gravitational settlement and make the system well dispersed.

In general, the operating pH range of polyelectrolytes for flocculation is broad enough to fulfill the requirements for various waste water treatment uses (24), (25), (26), (27), (28), (29).

C. Proposed mechanism of flocculation in the presence of polyelectrolytes

From the information which is available the following mechanism for flocculation may be proposed. As mentioned previously, complex polysaccharides, polyamino acids, and lipoidal materials are major constituents of the cell wall and capsule. Such natural polymeric substances might always be excreted from the cytoplasmic membrane under physiological conditions leading to cell wall formation. Bacteria carry negative charges on their surface due to ionization of these natural polymeric substances, and they possess a zeta-potential. A great number of investigators have studied the role of the zeta-potential in the agglutination process. They concluded that in a stable suspension of bacteria, the cells are kept apart by repulsion due to their like charges. Agglutination by electrolytes is due to a lowering of the zeta-potential below a certain value (critical potential) which allows the bacteria to come closer together. If there is cohesion between the bacteria, they remain attached in clumps. If cohesion is destroyed by a high concentration of electrolytes, the bacteria do not agglutinate (26), (30). A preponderant role is played by ions which carry a charge opposite to that of the surface of the bacteria. Bacteria may be considered as natural anionic polyelectrolytes. The added cationic polyelectrolyte provides active sites to attract these dispersed "anionic polyelectrolytes", if any opportunities are given for collision. In the case of low concentration of cationic polyelectrolyte, or if the polyelectrolyte is not very effective, only a few bacteria can be attracted and small flocs are formed. These small flocs may be electrically neutral or may also carry negative charges. They may or may not settle, depending upon the gravitational weight of the flocs as opposed to Brownian movement forces. Such non-settleable flocs are herein designated by the author as "single-bridged flocs". In the presence of

adequate concentrations of cationic polyelectrolytes, the flocs formed may be designated as "multiple-bridged flocs". The formation of multiple-bridged floc is attributed to the combination of multiple single-bridged flocs bridged by cationic polyelectrolyte molecules. The multiple-bridged flocs are heavy enough to settle rapidly.

D. Aspects of bacterial growth

Since the addition of polyelectrolyte coagulant aids may affect bacterial metabolism, it is important to review certain aspects of bacterial growth kinetics and mechanisms.

In 1913, Michaelis and Menten developed the basic kinetic theory for the reaction between substrate and enzymes produced by organisms. The reaction rate at any given substrate concentration can be calculated from their formula (31):

$$V = \frac{V \text{ max. } [S]}{Km + [S]} \qquad (1)$$

where V represents the reaction rate; V max. represents the maximum reaction rate; Km represents the Michaelis-Menten constant; i.e., the substrate concentration required for half-maximal velocity; and S represents substrate concentration.

The equation is derived from the following basic concept: (enzyme) + (substrate) $\underset{K_2}{\overset{K_1}{\rightleftharpoons}}$ (enzyme-substrate) $\xrightarrow{K_3}$ products + enzyme. It can be shown that Km is equal to $\frac{K_2 + K_3}{K_1}$.

From the Michaelis-Menten equation and the basic concept of enzyme-substrate reaction which it expresses, it is apparent that the rate of a biological reaction is a function of substrate concentration and specific enzymes produced by organisms. Even under constant substrate concentration, different amounts or different kinds of enzymes yield different rates of reaction; the rate of growth

for whole cells may be considered as the summation of reactions of the form of the Michaelis-Menten equation. Based upon experimental results of the growth of cells, Monod has obtained a growth equation of the same form as the Michaelis-Menten equation: (55)

$$\mu = \frac{\mu \text{ max. } [S]}{Ks + [S]} \qquad (2)$$

where, μ represents exponential growth rate, μ max. represents the maximum exponential growth rate, Ks represents the saturation constant, and S represents the substrate concentration.

In the logarithmic phase, the increase in cell population can be expressed by the equation

$$\frac{dx}{dt} = \mu x_o$$

and upon integration the following form is obtained:

$$x_t = x_o e^{\mu t} \qquad (3)$$

where x_t = cell population at time t, and x_o = initial cell population.

From equations (1), (2), and (3), it can be concluded that cell population in a batch system depends upon substrate concentration, initial cell concentration, and detention time; and since μ is specific for individual organisms, the predominance of species plays an important role.

According to Gaudy, the selection of species, the change in metabolic pathway, and the induction of required enzymes are three major factors which can govern the response to qualitative shock loading; and these three effects are interdependent (32). The introduction of a polyelectrolyte may in a sense be considered as a qualitative shock load and could conceivably bring these three factors into play. Hess stated that the overall balance and co-ordination of cell metabolism is a function of all its dynamic and static

components (33). In view of energetic equilibrium, living cells are never at rest; they continuously change in one way or another. An organism may alter its structure, it may grow, or it may undergo changes. This perpetual change of the state of the organism or of its component parts requires the expenditure of energy which is ultimately obtained from some source outside the organism, if life is to be maintained.

In the present study, the cell populations were heterogeneous and changes in predominance could occur irregularly because of different substrate concentrations present in the system at various times. In addition metabolic end products of some organisms may be utilized by others, thus enhancing opportunities for changes in predominance. This could cause a shift in the major metabolic pathway through which substrate and intermediate products are utilized. In heterogeneous systems continual changes in predominance may be expected, and the course of growth for populations such as exist in activated sludge is much more complex than for a pure culture. Regardless of the many complicating factors involved in describing and controlling growth and the physical characteristics of heterogeneous cultures, it is these systems with which the pollution control engineer must deal. Bacterial growth is necessary in order to purify wastes using the activated sludge process. It is also necessary to separate the cells which are produced. Natural gravitational force is by far the most economical means; and if addition of polyelectrolytes can enhance settling without being harmful to the bacteria, it may help bring about more effective treatment. The present investigation should add significant information to this area of knowledge.

E. Recent studies using polyelectrolytes

Since the introduction of polyelectrolytes in 1952, only a few papers have been published on their use in the

pollution control field. Those in which possible mechanisms of flocculation due to electric bridging between colloids and polyelectrolyte were described have been recently summarized by Day (34). Polyelectrolytes have been employed in the treatment of wastes from such industries as coal and iron mining. Schaffer found that different polyelectrolytes are applicable for different industrial wastes and that the concentration of the waste determines the optimum concentration of polyelectrolytes. For some wastes, polyelectrolytes were forced to be effective only in conjunction with various inorganic coagulating chemicals (35).

Katchalsky (36), Ives (37), Cohen, Rourke and Woodward (38), and Tenney and Stumm (12) found that only cationic polyelectrolytes were able to flocculate microorganisms efficiently. Tenny and Stumm also stated that certain cationic polyelectrolytes could flocculate dispersed microorganisms, while some must be used in conjunction with alum to enhance the flocculation. Also, for some systems separation of the flocculated dispersion occurred by subsidence rather than by sedimentation (12). The term "subsidence" used by these authors was meant to describe a uniform compression of the flocs as opposed to true settling.

In a recent paper, Singer, Pipes, and Hermann discussed coagulation of bulked activated sludge using polyelectrolytes. They stated that the addition of cationic polyelectrolytes reduced the sludge volume index of the bulked sludge and enhanced the rate of settling. They also found that doses greater than the optimum dosage did not yield better settling but sometimes decreased the settling efficiency (39). Walker and Dougherty reported that polyelectrolytes did exhibit an inhibitory effect on the BOD reaction. They also stated that polyelectrolytes, in certain cases, enhance entrapment of small gas bubbles in the sludge floc and significantly reduce the settling rate of the sludge; on occasion, sufficient gas entrapment might occur to result in flotation of the sludge flocs (40).

CHAPTER III

MATERIALS AND METHODS

A. Development of heterogeneous populations

1. Basic activated sludge unit

An activated sludge was developed in a laboratory batch unit (1.5 liters) from an initial sewage seed taken from the primary clarifier effluent of the municipal waste water treatment plant at Stillwater, Oklahoma. The batch unit was fed daily with the following synthetic waste: 1000 mg/l of glucose, 10 ml/l 1.0 M potassium phosphate buffer, pH 7.0, 500 mg/l of $(NH_4)_2 SO_4$, 200 mg/l of $MgSO_4 \cdot 7H_2O$, 20 mg/l of $MnSO_4 \cdot H_2O$, 15 mg/l of $CaCl_2 \cdot 2H_2O$, 1.0 mg/l of $FeCl_3 \cdot 6H_2O$, 67 ml/l tap water and distilled water to volume. The following daily feeding procedure was adopted: (1) stop aeration; (2) waste one-third of the mixed liquor (500 ml); (3) settle remainder for 30 minutes; (4) waste 500 ml of supernatant; (5) add concentrated solutions of synthetic growth medium constituents to give the desired final concentrations; (6) make up to the required volume with distilled water; and (7) start aeration.

This unit which was operated through the investigational period is herein designated as the basic unit.

2. Cells from the basic unit

In order to obtain large amounts of cells for experimentation, a new batch culture was started using seed from the basic unit a few days prior to each experiment. This unit was fed 5000 mg/l glucose. The medium contained the same salts as listed above, but the buffer concentration

was tripled and the inorganic salts doubled. The daily feeding procedure was the same as that described above.

3. Cells from a fresh unit

Young cell populations were grown up in the same manner as cells from the basic unit except that the source of seed was different. Instead of obtaining initial seed from the basic unit, a new system was started for each experiment in precisely the same manner as the basic unit was started, i.e., using an initial inoculum of fresh sewage from the Stillwater municipal plant. After undergoing the regular feeding schedule at 1000 mg/l glucose for approximately one week, a portion of cells was placed in the 5000 mg/l glucose medium and grown up for use in flocculation experiments.

B. Nature of selected polyelectrolytes

The polyelectrolytes selected for study were: Purifloc C-31, Purifloc C-32, Hagan 223, Nalco 600, Mogul CO-982, and Purifloc A-21. Purifloc C-31 and Purifloc C-32 are synthetic, water-soluble, high molecular weight, cationic organic polymers. They agglomerate a wide variety of inorganic or organic solids, including colloids which are present in waste waters, and they operate effectively in waters of widely varying pH or chemical content (41). Hagan 223 is a pale amber, clear liquid, cationic polyelectrolyte. Its effective operating pH is from 5.5 to 10. It brings about flocculation by bridging (24), (27). Nalco 600 is a cationic polymer; its operating pH range is from 3 to 12 (24). Mogul CO-982 is a cationic "activated biocolloid" which is composed, according to the manufacturer, of an "aluminate-carbohydrate coordination complex" (28). It has an optimum pH range for flocculation of 4 to 8 (24). Purifloc A-21 is an anionic polyelectrolyte which is effective over a broad pH range in concentrations of 0.1 to 1.0%

by weight. For this polyelectrolyte, it is recommended that dilute laboratory stock solution should be replaced after standing two to three weeks. It is reported that Purifloc A-21 dosages up to 100 ppm do not inhibit aerobic or anaerobic biological oxidation (25).

The five cationic polyelectrolytes are quite water-soluble and stock solutions of 2 gm/l concentration were prepared from the liquid commercial products. The anionic polyelectrolyte, Purifloc A-21, is not so water-soluble as the others, and a stock solution was prepared at a concentration of only 0.5 mg/l.

In the research which is to follow, all polyelectrolyte concentrations are given as weight of the liquid commercial product per liter of water.

C. Analytical techniques

1. Biological solids determination

a. Measurement of optical density

Optical density measurement is one of the means which may be used to evaluate the relative turbidity of colloidal suspensions. The relation between optical density and the percentage of light transmittance can be expressed by the equation $D = -\log_{10} T$, where D represents optical density and T represents the percentage of light transmittance. In the present work optical density was employed to measure biological solids. The instrument used was a Coleman spectrophotometer model 6-D. All measurements were made at a wave length of 540 mμ.

b. Membrane filter technique

Biological solids determinations were made using the membrane filter technique as given in Standard Methods for the Examination of Water and Waste-Water (42).

2. Substrate determination

a. Chemical Oxygen Demand (COD)

The principle of the COD test is based upon the fact that all organic components, with a few exceptions, can be totally oxidized to CO_2 and H_2O by the action of the strong oxidizing agent, potassium dichromate, under acidic conditions. In spite of the fact that the chief limitation of the COD test is its inability to differentiate between biologically oxidizable and biologically inert organic matter, it is widely used in the operation of treatment facilities because of the speed with which results can be obtained and its helpfulness in indicating the presence of biologically resistant organic substances (43). In 1951, Moore and Ruchhoft pointed out that the BOD and COD tests were two distinctly different parameters, but that the ratio between BOD and COD could possibly be used to advantage for treatment plant control purposes on industrial wastes of relatively constant composition (44). Aside from its use in treatment process control, the COD test provides an excellent research tool for measurement of substrate removal in studies on biological treatment of wastes. The detailed procedure for running the COD test is given in Standard Methods (42).

B. Glucostat test (glucose determination)

The conventional procedure for the determination of carbohydrates used in the Oklahoma State University Bioengineering Laboratory is the anthrone test as modified by Gaudy (45). However, polyelectrolytes are synthetic organic compounds, which may contain carbohydrate-like components. Since the "glucostat" test measures only glucose, it was felt that this determination was more suited for the present research. This enzymatic determination was run in accordance with the manufacturer's specifications (Worthington Biochemical Corporation, Freehold, New Jersey)

and the standard glucostat method 1-A was employed (46). The method is as follows: (1) dissolve the contents of a chromogen vial in approximately 60 ml. of distilled water; (2) dissolve contents of a glucostat vial in distilled water and add to the chromogen solution; adjust the final volume to 90 ml.; (3) dilute stock glucose standard with distilled water so that 1.0 ml. contains 0.05 - 0.3 mg. glucose per ml.; (4) dilute sample so that 1.0 ml. contains 0.05 to 0.3 mg. glucose; (5) add 1.0 ml. sample to 9.0 ml. of reagent prepared in (1) and (2) above; (6) include a reagent blank and at least one standard with each set of unknowns; (7) allow reaction to proceed for ten minutes at room temperature, then add one drop of 4M HCL to stop the reaction and stabilize the color; (8) let tubes stand for five minutes after stopping the reaction; (9) read optical density at a wave length of 400 mμ with the reagent blank set at 100% transmittance.

The calculation of glucose concentration was based on a standard curve plotted by measuring three different concentrations of standard glucose covering the range from 0.05 mg. to 0.3 mg. glucose.

3. Measurement of oxygen uptake (Warburg Technique)

The Warburg respirometer is an instrument for direct measurement of biochemical oxygen demand (47). More generally, it is used to measure the oxygen uptake during the respiration of biological samples. In the present study, the oxygen uptake was employed as a measure of bacterial activity.

Oxygen uptake was measured on a Warburg respirometer using 40 ml. of sample and 1.5 ml. of 20% KOH in the center well. The system was maintained at 25°C. and operated at a shaker rate of 104 osc./min. A ten minute equilibrium period was allowed before the manometers were closed. In general, readings were taken at thirty minute intervals

during the period of rapid oxygen uptake and at sixty minute intervals during the remainder of the experimental period. Further details on the techniques and calculations are given in Standard Methods for the Examination of Water and Waste Water (42) and Manometric Techniques (48).

D. Experimental protocol

1. Relation between optical density and sludge concentration

In order to use optical density measurement to estimate the initial cell concentration for each experiment and for measurement of the degree of clarification in the presence of cationic polyelectrolytes, a plot of cell concentration vs optical density was made. In order to plot this curve, glucose-acclimated cells harvested from the batch unit were diluted with synthetic water (daily feeding medium but without glucose) to obtain cell suspensions of various concentrations. Light transmittance of these suspensions was then measured. Forty ml. of each cell suspension was centrifuged, and the cell concentration by weight was measured by the membrane filter technique (42). From these analyses, the relation between optical density and biological solids concentration was determined.

2. Growth curve

Since the growth rate of a bacterial population is a function of substrate concentration, the initial cell population and predominant species; and since it was intended to assess the effect of polyelectrolytes at different phases of growth, it was necessary to gain information on the general shape and kinetic aspects of the growth curve under the experimental conditions employed during the study.

An adequate description of the growth curves was obtained by removing samples for measurement of optical density at one to three hour intervals throughout the growth

period. In all growth curve studies the initial glucose concentration was 5000 mg/l.

3. Studies on flocculation efficiency of various polyelectrolytes

a. Optimum polyelectrolyte dosage at constant cell concentration

(1) Flocculation of cells from the basic unit

At various stages of growth (beginning of log phase, end of log phase, beginning of declining phase, and end of declining phase) suspensions of cells were diluted to 70% transmittance with synthetic water of the same composition as the standard feeding medium but without glucose. Portions of this suspension were placed in 250 ml. flasks, and different concentrations of the various polyelectrolytes were added. The reaction volume was 150 ml. in each case. The suspensions were placed on a reciprocal shaker at room temperature (22°C. to 25°C.). The shaker was operated at a motor speed setting of 6 (110 osc./min.) for thirty minutes. After terminating agitation, portions of each suspension were placed in 100 ml. graduated cylinders and allowed to settle for fifteen minutes. The optical density of the supernatant was then measured at a wave length of 540 mμ. A cell suspension which received no polyelectrolyte was run as a control.

(2) Flocculation of cells from a fresh unit

According to the experiments of Tenney and Stumm, who studied the flocculation of bacterial suspensions taken from the effluent of a continuous flow culture unit, polyelectrolyte doses up to 500 mg/l (at pH 5.0) were required for best flocculation (12). It was felt that these investigators were dealing with young cell suspensions and that the addition of such high dosages of polyelectrolyte (at the stock concentrations herein employed) would lead to unequal dilution of suspension. Therefore, in using cells

from a fresh unit a separate control system was run for each dosage of polyelectrolyte. The control system received a volume of distilled water corresponding to the volume of stock polyelectrolyte solution in the test system. Thus, the percentage transmittance could be directly compared. Preliminary experiments indicated that the polyelectrolytes added to the medium in the absence of bacteria did not exhibit any optical density at concentrations up to 500 mg/l.

b. Effect of cell concentration at constant polyelectrolyte dosage

In this phase of the study, 150 ml. of cell suspensions (both cells from the basic unit and the fresh unit) at 50, 60, 70, and 80 per cent transmittance were prepared in 250 ml. flasks by diluting cells from the respective growth units with synthetic waste devoid of substrate. Each polyelectrolyte was added at the optimum concentration previously determined for the suspension of 70% transmittance. A separate control flask was run for each cell concentration. All flasks were placed on the shaker and the procedure previously described was followed.

4. Effect of selected polyelectrolytes on biochemical efficiency of activated sludge

a. Cells from the basic unit

A batch unit seeded from the basic unit was started in accordance with procedures previously described and polyelectrolyte was added at its optimum flocculating dosage after the system reached a growth corresponding to 70% transmittance .

The effect of the polyelectrolytes on the growth pattern was assessed by making optical density measurements at two hour intervals. A separate control unit to which polyelectrolyte was not added was used for all system studies.

b. Cells from a fresh unit

In order to gain a better insight into possible biochemical effects, cell populations from fresh units were studied in greater detail than for the cells from the basic unit. A fresh sewage seed was used to innoculate a series of new growth units. The substrate (glucose) concentration used was 1000 mg/l. After one week of operation on the standard feeding cycle the cells were harvested, washed in 0.05 M phosphate buffer and used to initiate two new growth units at the same initial biological solids concentration, one to which polyelectrolyte was added and one which served as a control.

During the succeeding aeration period, samples were withdrawn for measurement of biological solids concentration and COD and glucose remaining in solution. At the beginning of the aeration period 40 ml portions of each system were placed on the Warburg apparatus for measurement of oxygen uptake during the experimental period.

CHAPTER IV

RESULTS

A. Relation between optical density and sludge concentration

Figure 1 shows the relation between solids concentration in mg/l and optical density for three separate experiments. It is seen that a straight line relationship is held up to a solids concentration of 700 mg/l. There was some scatter of the data, but the curve does allow a reasonably good estimation of solids concentration from optical density readings.

In Figure 2, the results of growth curve experiments are shown. All were obtained under identical experimental conditions using 100 ml of seed taken on different days from the batch unit. All experiments were run using an initial glucose concentration of 5000 mg/l. It is seen that regardless of the use of different seeding populations (assumed to be different because of changes in predominance), there was general agreement except for one experiment in which the growth rate was considerably retarded. It is interesting to note that even for the experiment in which the growth rate was low the total amount of growth was approximately the same as for the others. Using a final optical density of 0.8, the corresponding sludge concentration would be roughly 2200 mg/l which indicates a sludge yield (mg/l sludge/ mg/l glucose used) of slightly over 40%.

It was felt that with the exception previously noted, the growth curve was sufficiently reproducible to allow estimation of the stage of growth by noting optical density. The optical densities at which cells were harvested in four phases of growth are shown in Figure 2.

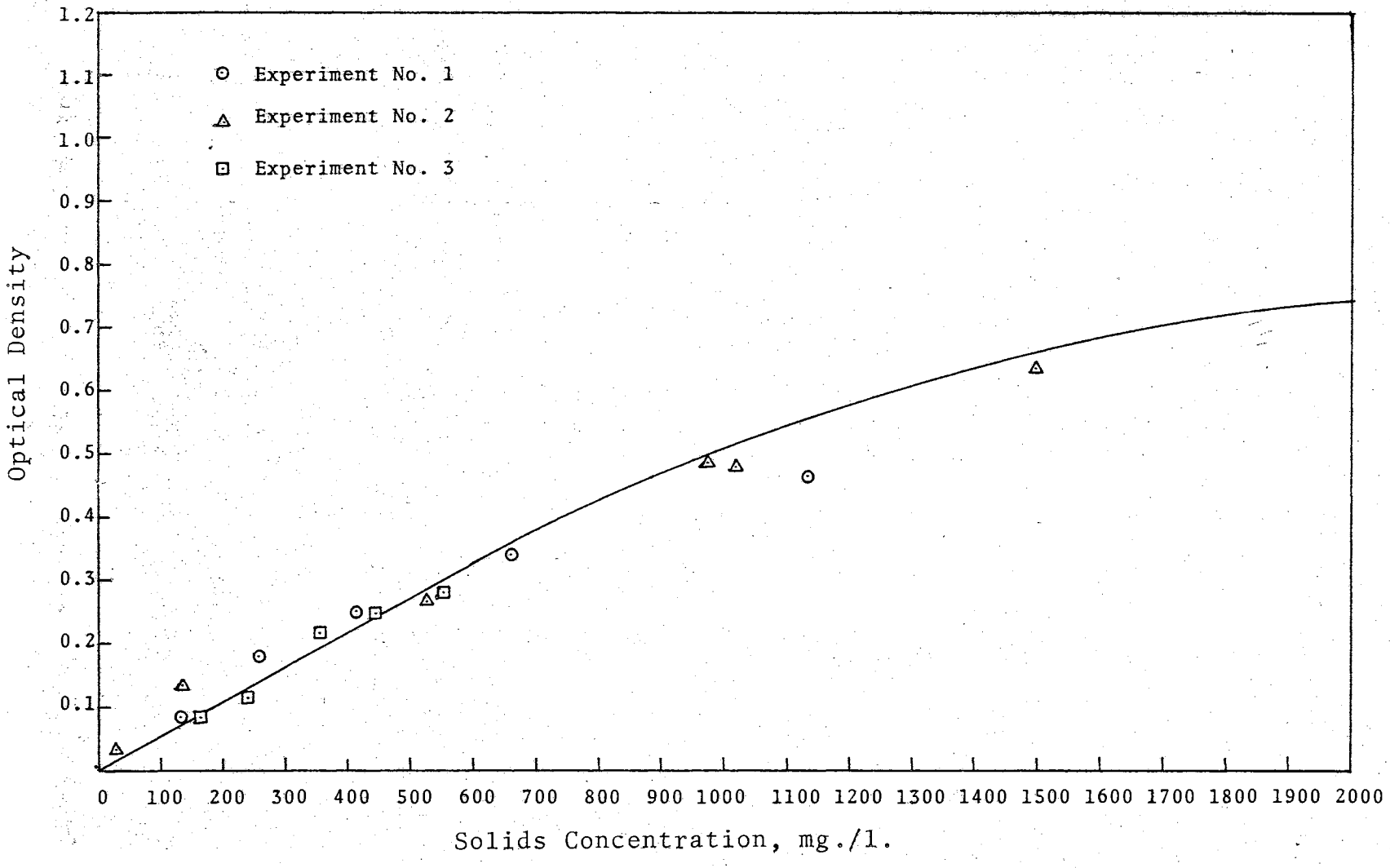

Fig. 1 - Relation between optical density and biological solids concentration.

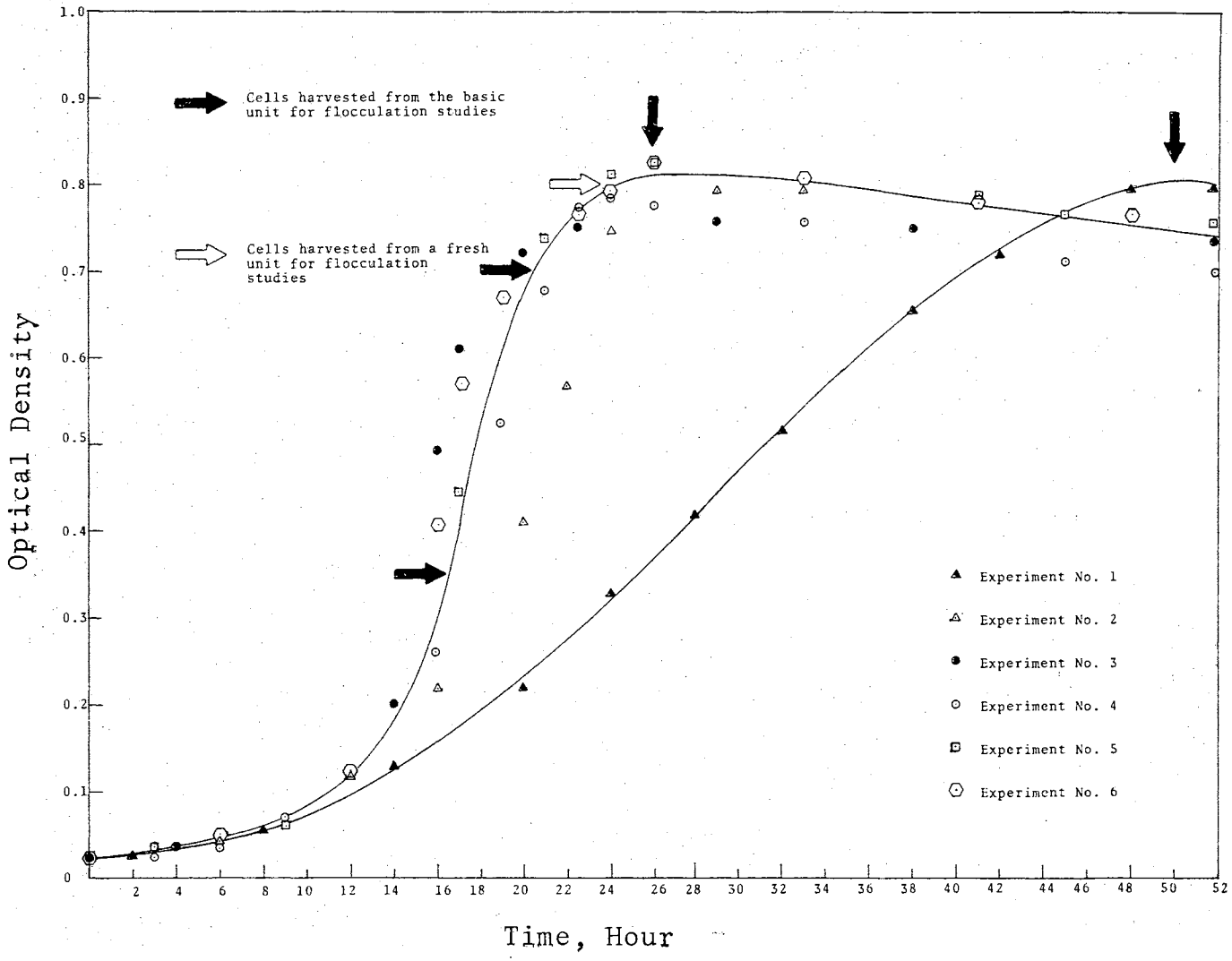

Fig. 2 - Growth curves for cells harvested for flocculation studies.

B. Studies on flocculation efficiency of various polyelectrolytes

1. Optimum polyelectrolyte dosage at constant cell concentration

a. Flocculation of cells from the basic unit

When cells were grown from seed taken from the basic unit, they exhibited, except for one experiment, a very high degree of auto-flocculation when placed in either buffer salts medium or distilled water. Because the cells flocculated rather well without the addition of a polyelectrolyte, the results of this phase of the investigation yield a somewhat conservative estimation of the effectiveness of these coagulants. The effectiveness of the various polyelectrolytes studied at dosages from 30 to 70 mg/l are compared with their corresponding control systems in Tables I through VI for cells harvested at four different stages of growth. The per cent transmittance and the subsequent optical density and sludge concentration of the supernatant after thirty minutes settling are given for each control and experimental system. The reduction in cell concentration of the supernatant brought about by each dosage is also shown (column second from the right). In order to compare the relative effectiveness of the polyelectrolyte dosages, the ratio of reduction in cell concentration to cell concentration of the corresponding control system was computed and is expressed as relative flocculation efficiency (%) in the right hand column. The relative flocculation efficiency is plotted versus polyelectrolyte dosage in Figures 3 through 8.

It is apparent from these results that the optimum dosage for all the polyelectrolytes examined ranged between 40 and 60 mg/l. It may also be noted from Table VI and Figure 8 that the anionic polyelectrolyte Purifloc A-21 did not enhance flocculation but did cause cell dispersion to some extent. It is interesting to note that in general the

relative flocculation efficiency was highest for cells taken in the log phase of growth.

b. Flocculation of cells from fresh units

For cells from fresh units auto-flocculation did not occur when cell suspensions were diluted with synthetic water or distilled water. Therefore, the relative efficiency of polyelectrolytic flocculation was higher than for the previous studies. Preliminary flocculation experiments were made by taking cell suspensions in the log growth phase and the declining growth phase. It was found that there was no measurable difference. Therefore, cells were harvested for study at the end of the log growth phase. The results are shown in Tables VII through XI and Figure 9. The optimum dosage of Purifloc C-31 for flocculation of cells from a fresh unit shown in Table VII, is about 120 mg/l; of Purifloc C-32, shown in Table VIII, 400 mg/l; of Hagan 223, shown in Table IX, 400 mg/l; of Mogul CO-982, shown in Table X, 400 mg/l; of Nalco 600, shown in Table XI, 500 mg/l. These results are somewhat close to those found by Tenney and Stumm (12). They took the culture from the effluent of a continuous flow unit and found that optimum dosage for flocculation was nearly 500 mg/l. In the present study, it was observed that when low concentrations of polyelectrolytes were added, floc particles were visible but remained suspended in the medium.

TABLE I

EFFECTIVENESS OF PURIFLOC C-31 IN FLOCCULATION OF CELLS HARVESTED FROM THE BASIC UNIT

Growth Phase	Mg./l. of Polyelectrolyte	Control Unit After Shaking 30 Min.			Flocculation Unit After Shaking 30 Min.			Reduction In Cell Concentration (mg./l.)	Relative Flocculation Efficiency %
		% T.	O.D.	Cell Conc. (mg./l.)	% T.	O.D.	Cell Conc. (mg./l.)		
Beginning of log growth phase	30	67	0.1739	320	86	0.0655	120	200	62.5
	40	67	0.1739	320	94.50	0.0246	50	270	82.4
	45	67	0.1739	320	95	0.0223	45	275	86
	50	67	0.1739	320	95.25	0.0212	40	280	87.5
	55	67	0.1739	320	96.25	0.0166	30	290	90.7
	60	67	0.1739	320	96	0.0177	35	285	89
	70	67	0.1739	320	95.75	0.0188	35	285	89
End of log growth phase	30	72	0.1427	265	79.5	0.0996	185	80	30.2
	40	72	0.1427	265	89.25	0.0494	90	175	66
	45	72	0.1427	265	90	0.0458	85	180	67.9
	50	72	0.1427	265	93.75	0.0281	50	215	81.2
	55	72	0.1427	265	94	0.0269	45	220	83.2
	60	72	0.1427	265	94	0.0269	45	220	83.2
	70	72	0.1427	265	94	0.0269	45	220	83.2

(Note: Initial cell concentration: 285 mg./l. or 70% T.)

TABLE I (Continued)

Growth Phase	Mg./l. of Polyelectrolyte	Control Unit After Shaking 30 Min.			Flocculation Unit After Shaking 30 Min.			Reduction In Cell Concentration (mg./l.)	Relative Flocculation Efficiency %
		% T.	O.D.	Cell Conc. (mg./l.)	% T.	O.D.	Cell Conc. (mg./l.)		
Beginning of declining growth phase	30	76	0.1192	220	75	0.1269	230	-10	-4.55
	40	76	0.1192	220	77.75	0.1093	200	20	9.10
	45	76	0.1192	220	79.50	0.0996	185	35	15.90
	50	76	0.1192	220	82	0.0862	160	60	27.30
	55	76	0.1192	220	84.75	0.0718	135	85	38.60
	60	76	0.1192	220	86.50	0.0630	120	100	45.50
	70	76	0.1192	220	90	0.0458	85	135	61.40
End of declining growth phase	50	76	0.1192	220	74	0.1308	240	-20	-9.10
	75	76	0.1192	220	83	0.0807	150	70	31.80
	100	76	0.1192	220	89	0.0505	105	115	52.30
	110	76	0.1192	220	89	0.0505	105	115	52.30
	120	76	0.1192	220	89	0.0505	105	115	52.30
	130	76	0.1192	220	90	0.0458	85	135	13.60
	140	76	0.1192	220	90	0.0458	85	135	13.60

(Note: Initial cell concentration: 285 mg./l. or 70% T.)

TABLE II

EFFECTIVENESS OF PURIFLOC C-32 IN FLOCCULATION
OF CELLS HARVESTED FROM THE BASIC UNIT

Growth Phase	Mg./l. of Polyelectrolyte	Control Unit After Shaking 30 Min.			Flocculation Unit After Shaking 30 Min.			Reduction In Cell Concentration (mg./l.)	Relative Flocculation Efficiency %
		% T.	O.D.	Cell Conc. (mg./l.)	% T.	O.D.	Cell Conc. (mg./l.)		
Beginning of log growth phase	30	88.25	0.0543	100	94.25	0.0257	50	50	50
	40	88.25	0.0543	100	90.75	0.0422	80	20	20
	45	88.25	0.0543	100	91	0.0410	70	30	30
	50	88.25	0.0543	100	92	0.0362	75	25	25
	55	88.25	0.0543	100	92	0.0362	70	25	25
	60	88.25	0.0543	100	90	0.0458	85	15	15
	70	88.25	0.0543	100	90	0.0458	85	15	15
End of log growth phase	30	87.5	0.0580	105	94.25	0.0257	50	55	52.4
	40	87.5	0.0580	105	93.50	0.0292	55	50	47.6
	45	87.5	0.0580	105	94	0.0269	50	55	52.4
	50	87.5	0.0580	105	95	0.0223	45	60	57.2
	55	87.5	0.0580	105	95	0.0223	45	60	57.2
	60	87.5	0.0580	105	93	0.0315	60	45	42.8
	70	87.5	0.0580	105	94	0.0269	50	55	52.4

(Note: Initial cell concentration: 285 mg./l. or 70% T.)

TABLE II (Continued)

Growth Phase	Mg./l. of Polyelectrolyte	Control Unit After Shaking 30 Min.			Flocculation Unit After Shaking 30 Min.			Reduction In Cell Concentration (mg./l.)	Relative Flocculation Efficiency %
		% T.	O.D.	Cell Conc. (mg./l.)	% T.	O.D.	Cell Conc. (mg./l.)		
Beginning of declining growth phase	30	89.5	0.0482	95	88.25	0.0543	100	-5	-5.3
	40	89.5	0.0482	95	88.5	0.0531	100	-5	-5.3
	45	89.5	0.0482	95	89	0.0505	95	0	0
	50	89.5	0.0482	95	87.50	0.0580	105	-10	-9.5
	55	89.5	0.0482	95	86.75	0.0617	115	-20	-19.0
	60	89.5	0.0482	95	86.25	0.0642	115	-20	-19.0
	70	89.5	0.0482	95	87.25	0.0593	110	-15	-15.8
End of declining growth phase	30	87.75	0.0568	110	91.5	0.0386	75	35	31.8
	40	87.75	0.0568	110	88.5	0.0531	100	10	11.0
	45	87.75	0.0568	110	88.5	0.0531	100	10	11.0
	50	87.75	0.0568	110	89	0.0505	95	15	13.6
	55	87.75	0.0568	110	86.75	0.0617	115	-5	-4.5
	60	87.75	0.0568	110	85	0.0706	135	-25	-22.8
	70	87.75	0.0568	110	83.5	0.0783	145	-35	-31.8

(Note: Initial cell concentration: 285 mg./l. or 70% T.)

TABLE III

EFFECTIVENESS OF HAGAN 223 IN FLOCCULATION
OF CELLS HARVESTED FROM THE BASIC UNIT

Growth Phase	Mg./l. of Polyelectrolyte	Control Unit After Shaking 30 Min.			Flocculation Unit After Shaking 30 Min.			Reduction In Cell Concentration (mg./l.)	Relative Flocculation Efficiency %
		% T.	O.D.	Cell Conc. (mg./l.)	% T.	O.D.	Cell Conc. (mg./l.)		
Beginning of log growth phase	30	88.5	0.0531	100	94	0.0269	50	50	50
	40	88.5	0.0531	100	94	0.0269	50	50	50
	45	88.5	0.0531	100	93.5	0.0292	55	45	45
	50	88.5	0.0531	100	94.5	0.0246	45	55	55
	55	88.5	0.0531	100	94.5	0.0246	45	55	55
	60	88.5	0.0531	100	94	0.0269	50	50	50
	70	88.5	0.0531	100	93.75	0.0287	55	45	45
End of log growth phase	30	89.5	0.0482	95	95	0.0177	45	50	52.7
	40	89.5	0.0482	95	94.5	0.0246	45	50	52.7
	45	89.5	0.0482	95	94.5	0.0246	45	50	52.7
	50	89.5	0.0482	95	94.5	0.0246	45	50	52.7
	55	89.5	0.0482	95	96	0.0177	30	65	68.4
	60	89.5	0.0482	95	96	0.0177	30	65	68.4
	70	89.5	0.0482	95	96	0.0177	30	65	68.4

(Note: Initial cell concentration: 285 mg./l. or 70% T.)

TABLE III (Continued)

Growth Phase	Mg./l. of Polyelectrolyte	Control Unit After Shaking 30 Min.			Flocculation Unit After Shaking 30 Min.			Reduction In Cell Concentration (mg./l.)	Relative Flocculation Efficiency %
		% T.	O.D.	Cell Conc. (mg./l.)	% T.	O.D.	Cell Conc. (mg./l.)		
Beginning of declining growth phase	30	92.5	0.0482	65	94.5	0.0246	45	20	30.8
	40	92.5	0.0482	65	93.25	0.0304	55	10	15.4
	45	92.5	0.0482	65	95.25	0.0212	40	25	38.5
	50	92.5	0.0482	65	95.25	0.0212	40	25	38.5
	55	92.5	0.0482	65	94	0.0269	50	15	23.1
	60	92.5	0.0482	65	93.5	0.0292	55	10	15.4
	70	92.5	0.0482	65	93.25	0.0304	55	10	15.4
End of declining growth phase	30	92.5	0.0482	65	95	0.0177	45	20	30.8
	40	92.5	0.0482	65	94.5	0.0246	45	20	30.8
	45	92.5	0.0482	65	95	0.0177	45	20	30.8
	50	92.5	0.0482	65	95.5	0.0200	35	30	46.2
	55	92.5	0.0482	65	95.5	0.0200	35	30	46.2
	60	92.5	0.0482	65	94.5	0.0235	45	20	30.8
	70	92.5	0.0482	65	94	0.0235	50	15	23.1

(Note: Initial cell concentration: 285 mg./l. or 70%T.)

TABLE IV

EFFECTIVENESS OF MOGUL CO-982 IN FLOCCULATION OF CELLS HARVESTED FROM THE BASIC UNIT

Growth Phase	Mg./l. of Polyelectrolyte	Control Unit After Shaking 30 Min.			Flocculation Unit After Shaking 30 Min.			Reduction In Cell Concentration (mg./l.)	Relative Flocculation Efficiency %
		% T.	O.D.	Cell Conc. (mg./l.)	% T.	O.D.	Cell Conc. (mg./l.)		
Beginning of log growth phase	30	91	0.0410	75	92.5	0.0327	65	10	13.3
	40	91	0.0410	75	92	0.0362	70	5	6.6
	45	91	0.0410	75	92.5	0.0339	65	10	13.3
	50	91	0.0410	75	92.5	0.0339	65	10	13.3
	55	91	0.0410	75	93	0.0315	60	15	20
	60	91	0.0410	75	93	0.0315	60	15	20
	70	91	0.0410	75	93	0.0315	60	15	20
End of log growth phase	30	92	0.0362	70	94.5	0.0246	50	20	28.6
	40	92	0.0362	70	95.25	0.0212	45	25	35.7
	45	92	0.0362	70	94.5	0.0246	50	20	28.6
	50	92	0.0362	70	95.5	0.0200	40	30	42.8
	55	92	0.0362	70	94.5	0.0246	50	20	28.6
	60	92	0.0362	70	95	0.0223	45	25	35.7
	70	92	0.0362	70	95	0.0223	45	25	35.7

(Note: Initial cell concentration: 285 mg./l. or 70% T.)

TABLE IV (Continued)

Growth Phase	Mg./l. of Polyelectrolyte	Control Unit After Shaking 30 Min.			Flocculation Unit After Shaking 30 Min.			Reduction In Cell Concentration (mg./l.)	Relative Flocculation Efficiency %
		% T.	O.D.	Cell Conc. (mg./l.)	% T.	O.D.	Cell Conc. (mg./l.)		
Beginning of declining growth phase	30	92	0.0362	70	93.25	0.0304	55	15	21.4
	40	92	0.0362	70	94.5	0.0246	50	20	28.6
	45	92	0.0362	70	94	0.0269	50	20	28.6
	50	92	0.0362	70	94.5	0.0246	50	20	28.6
	55	92	0.0362	70	94.5	0.0246	50	20	28.6
	60	92	0.0362	70	94.5	0.0246	50	20	28.6
	70	92	0.0362	70	94.5	0.0246	50	20	28.6
End of declining growth phase	30	92	0.0362	70	93.5	0.0292	55	15	21.4
	40	92	0.0362	70	94	0.0269	50	20	28.6
	45	92	0.0362	70	94.5	0.0246	50	20	28.6
	50	92	0.0362	70	95	0.0223	45	25	35.7
	55	92	0.0362	70	94.5	0.0246	50	20	28.6
	60	92	0.0362	70	94.5	0.0246	50	20	28.6
	70	92	0.0362	70	94.5	0.0246	50	20	28.6

(Note: Initial cell concentration: 285 mg./l. or 70% T.)

TABLE V

EFFECTIVENESS OF NALCO 600 IN FLOCCULATION
OF CELLS HARVESTED FROM THE BASIC UNIT

Growth Phase	Mg./l. of Polyelectrolyte	Control Unit After Shaking 30 Min.			Flocculation Unit After Shaking 30 Min.			Reduction In Cell Concentration (mg./l.)	Relative Flocculation Efficiency %
		% T.	O.D.	Cell Conc. (mg./l.)	% T.	O.D.	Cell Conc. (mg./l.)		
Beginning of log growth phase	30	85.25	0.0693	130	91.25	0.0398	70	60	46.2
	40	85.25	0.0693	130	92.25	0.0351	65	65	50
	45	85.25	0.0693	130	94	0.0269	50	80	61.5
	50	85.25	0.0693	130	94.5	0.0246	45	85	65.3
	55	85.25	0.0693	130	94.5	0.0246	45	85	65.3
	60	85.25	0.0693	130	91.75	0.0374	70	60	46.2
	70	85.25	0.0693	130	91.5	0.0386	75	55	42.3
End of log growth phase	30	88.5	0.0531	100	92.25	0.0327	60	40	40
	40	88.5	0.0531	100	93.5	0.0292	55	45	45
	45	88.5	0.0531	100	94	0.0269	50	50	50
	50	88.5	0.0531	100	93.5	0.0292	55	45	45
	55	88.5	0.0531	100	91.25	0.0398	70	30	30
	60	88.5	0.0531	100	91.25	0.0398	70	30	30
	70	88.5	0.0531	100	92	0.0362	70	30	30

(Note: Initial cell concentration: 285 mg./l. or 70% T.)

TABLE V (Continued

Growth Phase	Mg./l. of Polyelectrolyte	Control Unit After Shaking 30 Min.			Flocculation Unit After Shaking 30 Min.			Reduction In Cell Concentration (mg./l.)	Relative Flocculation Efficiency %
		% T.	O.D.	Cell Conc. (mg./l.)	% T.	O.D.	Cell Conc. (mg./l.)		
Beginning of declining growth phase	30	90.5	0.0434	80	92.5	0.0339	65	15	18.8
	40	90.5	0.0434	80	92.5	0.0339	65	15	18.8
	45	90.5	0.0434	80	92.5	0.0339	65	15	18.8
	50	90.5	0.0434	80	93.25	0.0304	55	25	31.2
	55	90.5	0.0434	80	92	0.0362	70	10	12.5
	60	90.5	0.0434	80	90.5	0.0434	80	0	0
	70	90.5	0.0434	80	90.5	0.0434	80	0	0
End of declining growth phase	30	87.5	0.0580	110	88.5	0.0531	100	10	9.1
	40	87.5	0.0580	110	87.5	0.0580	110	0	0
	45	87.5	0.0580	110	89.75	0.0470	100	10	9.1
	50	87.5	0.0580	110	88.5	0.0531	100	10	9.1
	55	87.5	0.0580	110	90.5	0.0434	80	30	27.2
	60	87.5	0.0580	110	90.5	0.0434	80	30	27.2
	70	87.5	0.0580	110	90.5	0.0434	80	30	27.2

(Note: Initial cell concentration: 285 mg./l. or 70% T.)

TABLE VI

EFFECTIVENESS OF PURIFLOC A-21 IN FLOCCULATION
OF CELLS HARVESTED FROM THE BASIC UNIT

Growth Phase	Mg./l. of Polyelectrolyte	Control Unit After Shaking 30 Min.			Flocculation Unit After Shaking 30 Min.			Reduction In Cell Concentration (mg./l.)	Relative Flocculation Efficiency %
		% T.	O.D.	Cell Conc. (mg./l.)	% T.	O.D.	Cell Conc. (mg./l.)		
Log growth phase	30	90.5	0.0434	80	90.5	0.0434	80	0	0
	40	90.5	0.0434	80	90	0.0458	85	-5	-6.3
	45	90.5	0.0434	80	90	0.0458	85	-5	-6.3
	50	90.5	0.0434	80	90	0.0458	85	-5	-6.3
	55	90.5	0.0434	80	88.5	0.0531	100	-20	-25
	60	90.5	0.0434	80	89.5	0.0482	95	-15	-18.8
	70	90.5	0.0434	80	89.5	0.0505	100	-20	-18.8
Declining growth phase	30	92	0.0362	70	91.5	0.0386	75	-5	-6.3
	40	92	0.0362	70	91	0.0410	80	-10	-12.5
	45	92	0.0362	70	92	0.0362	70	0	0
	50	92	0.0362	70	91.5	0.0386	75	-5	-6.3
	55	92	0.0362	70	90	0.0458	85	-15	-6.3
	60	92	0.0362	70	91	0.0410	80	-10	-12.5
	70	92	0.0362	70	91	0.0410	80	-10	-12.5

(Note: Initial cell concentration: 285 mg./l. or 70% T.)

TABLE VII

EFFECTIVENESS OF PURIFLOC C-31 IN FLOCCULATION OF CELLS FROM A FRESH UNIT

Concentration of Polyelectrolyte (mg./l.)	Control Unit After Shaking 30 Min.		Flocculation Unit After Shaking 30 Min.		Reduction In Cell Concentration (mg./l.)	Relative Flocculation Efficiency (%)
	% T.	Cell Conc. (mg./l.)	% T.	Cell Conc. (mg./l.)		
50	70	285	67.5	315	-30	-10.5
75	70.25	282	87.0	115	167	59.2
100	70.50	280	93.5	55	225	80.5
110	70.50	280	95.0	45	235	84.1
120	70.75	275	95.5	35	240	87.4
130	70.75	275	95.5	35	240	87.4
140	71	272	95.5	35	237	87.2

(Note: Initial cell concentration: 285 mg./l. or 70% T.)

TABLE VIII

EFFECTIVENESS OF PURIFLOC C-32 IN FLOCCULATION OF CELLS FROM A FRESH UNIT

Concentration of Polyelectrolyte (mg./l.)	Control Unit After Shaking 30 Min.		Flocculation Unit After Shaking 30 Min.		Reduction In Cell Concentration (mg./l.)	Relative Flocculation Efficiency (%)
	% T.	Cell Conc. (mg./l.)	% T.	Cell Conc. (mg./l.)		
75	70.25	282	63	370	-88	-31.2
150	71	272	86	120	152	55.8
200	71.50	270	90.5	80	190	70.5
250	71.75	265	92.5	65	200	75.5
300	72	265	92.5	65	200	75.5
400	73	255	93.5	55	200	78.5
500	74	240	91.75	70	170	70.8

(Note: Initial cell concentration: 285 mg./l. or 70% T.)

TABLE IX

EFFECTIVENESS OF HAGAN 223 IN FLOCCULATION OF CELLS FROM A FRESH UNIT

Concentration of Polyelectrolyte (mg./l.)	Control Unit After Shaking 30 Min.		Flocculation Unit After Shaking 30 Min.		Reduction In Cell Concentration (mg./l.)	Relative Flocculation Efficiency (%)
	% T.	Cell Conc. (mg./l.)	% T.	Cell Conc. (mg./l.)		
75	70.25	282	64	360	-78	-27.7
150	71	272	60	410	-138	-50.7
200	71.50	270	60.50	400	-140	-51.8
250	71.75	265	78.50	195	70	26.4
300	72	265	90.50	80	185	69.8
400	73	255	93	60	195	73.6
500	74	240	92.50	65	175	73

(Note: Initial cell concentration: 285 mg./l. or 70% T.)

TABLE X

EFFECTIVENESS OF MOGUL CO-982 IN FLOCCULATION OF CELLS FROM A FRESH UNIT

Concentration of Polyelectrolyte (mg./l.)	Control Unit After Shaking 30 Min.		Flocculation Unit After Shaking 30 Min.		Reduction In Cell Concentration (mg./l.)	Relative Flocculation Efficiency (%)
	% T.	Cell Conc. (mg./l.)	% T.	Cell Conc. (mg./l.)		
75	70.25	282	72	265	17	6.03
150	71	272	72.50	260	12	4.42
200	71.50	270	73.50	250	20	7.40
250	71.-5	265	73.50	250	15	5.67
300	72	265	75	230	35	13.20
400	73	255	76	220	35	13.70
500	74	240	76.50	215	25	10.40

(Note: Initial cell concentration: 285 mg./l. or 70% T.)

TABLE XI

EFFECTIVENESS OF NALCO 600 IN FLOCCULATION
OF CELLS FROM A FRESH UNIT

Concentration of Polyelectrolyte (mg./l.)	Control Unit After Shaking 30 Min.		Flocculation Unit After Shaking 30 Min.		Reduction In Cell Concentration (mg./l.)	Relative Flocculation Efficiency (%)
	% T.	Cell Conc. (mg./l.)	% T.	Cell Conc. (mg./l.)		
75	70.25	282	68	310	-28	-9.9
150	71	272	65	345	-73	-26.8
200	71.50	270	63.50	365	-95	-35.2
250	71.75	265	63.50	365	-100	-37.7
300	72	265	63.50	365	-100	-37.7
400	73	255	67	320	-65	-25.5
500	74	240	69	190	50	20.8

(Note: Initial cell concentration: 285 mg./l. or 70% T.)

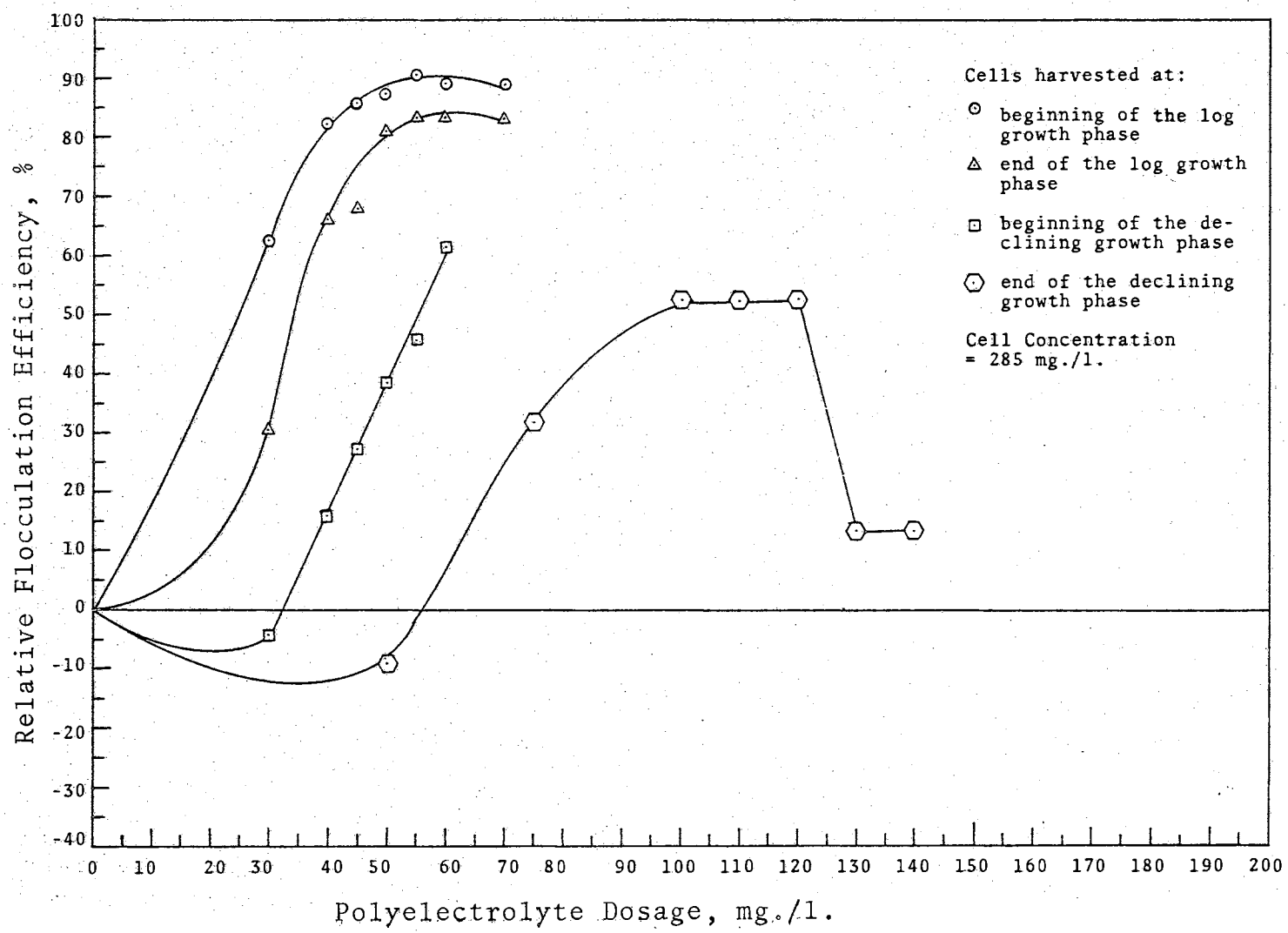

Fig. 3 - Relative efficiency of flocculation for cells from the basic unit with Purifloc C-31.

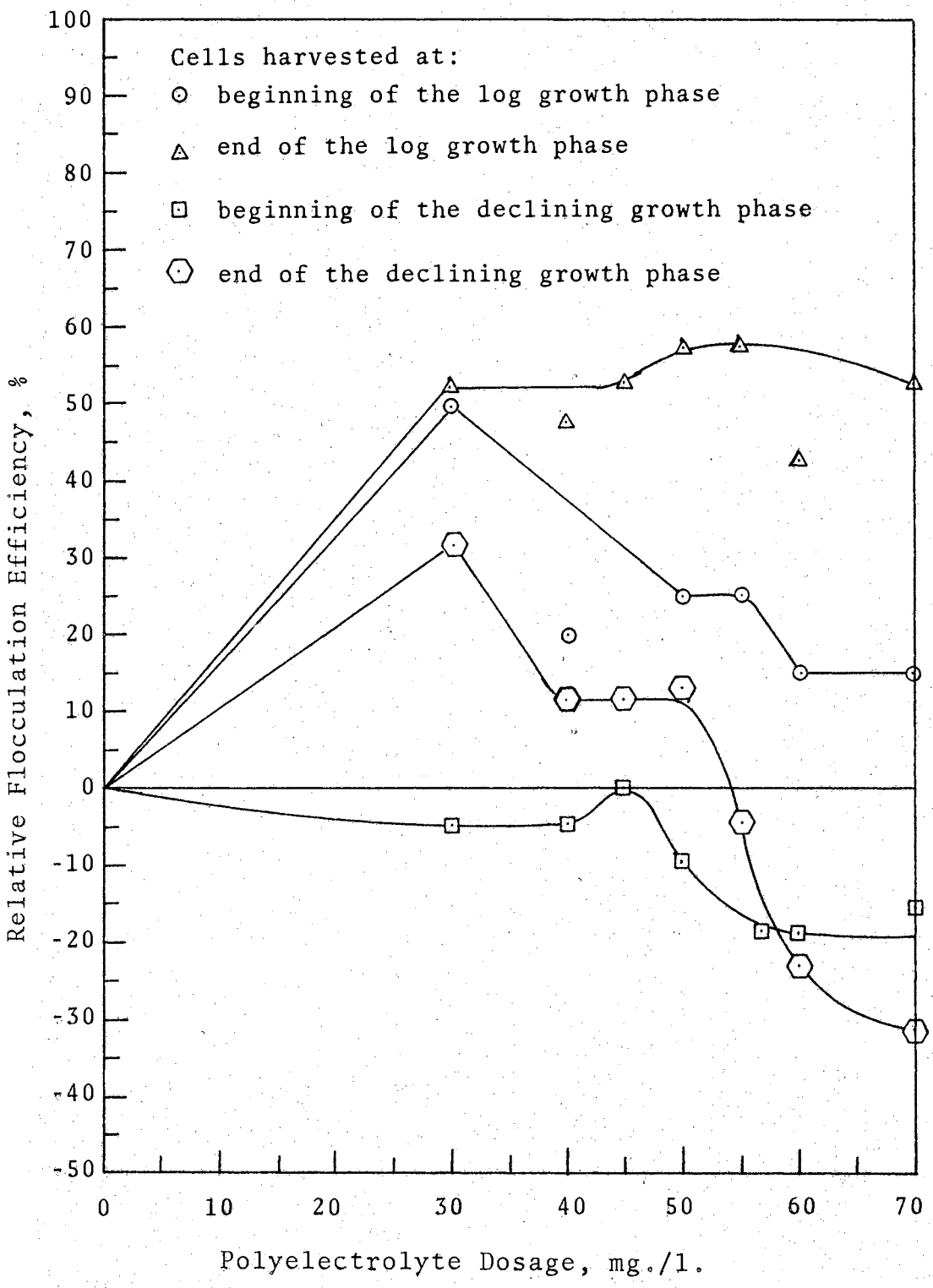

Fig. 4 - Relative efficiency of flocculation for cells from the basic unit with Purifloc C-32.

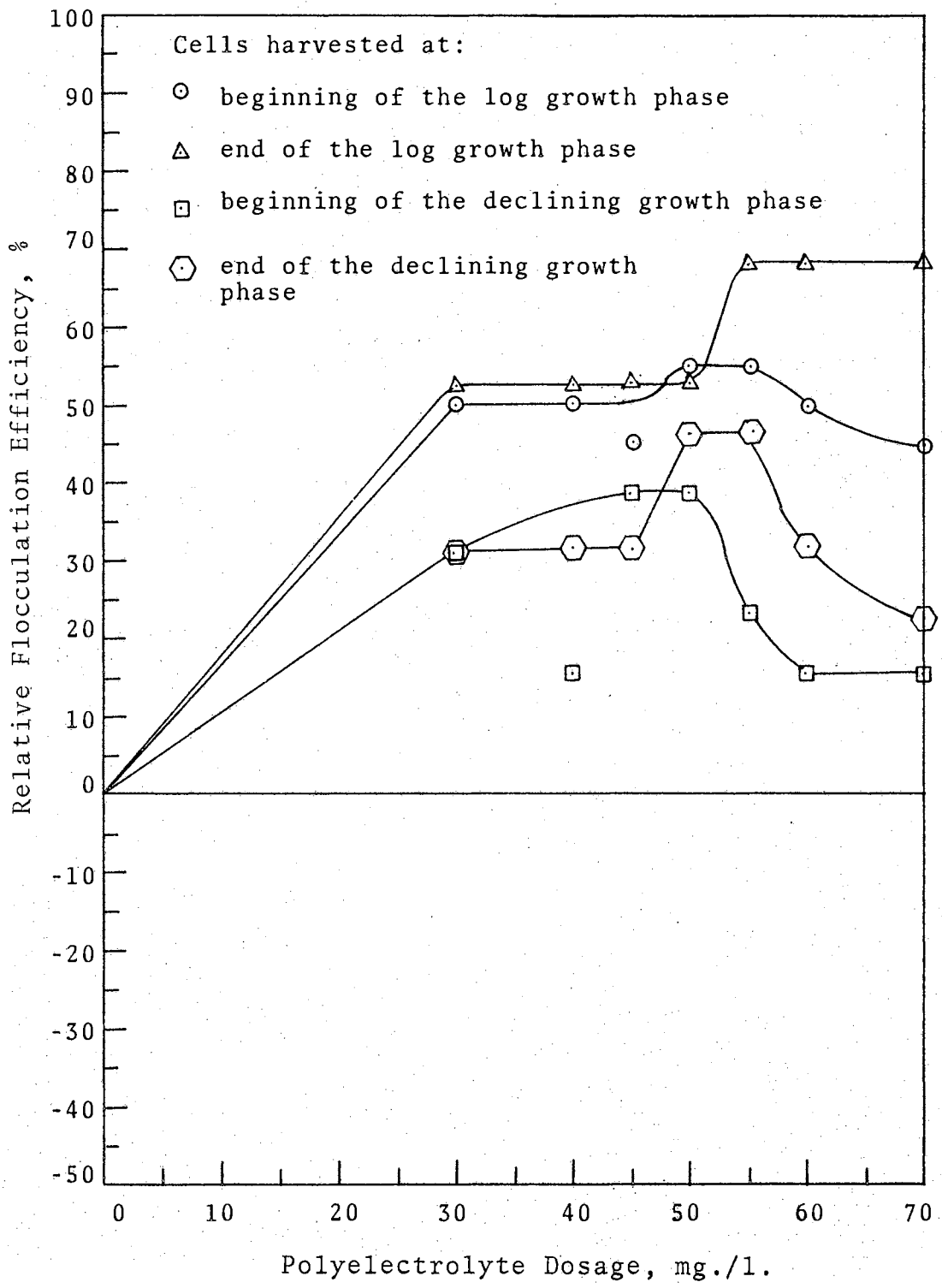

Fig. 5 - Relative efficiency of flocculation for cells from the basic unit with Hagan 223.

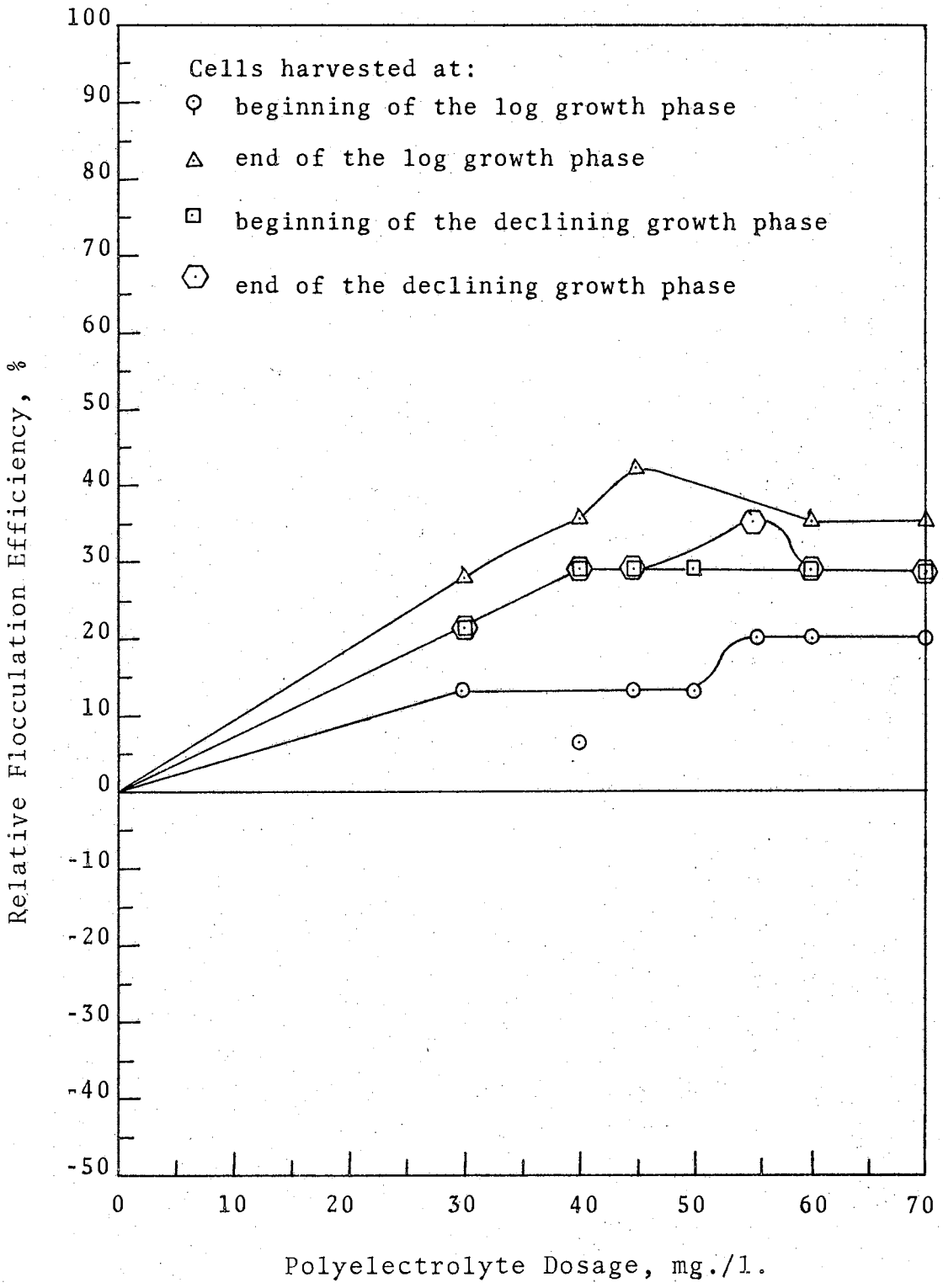

Fig. 6 - Relative efficiency of flocculation for cells from the basic unit with Mogul CO-982.

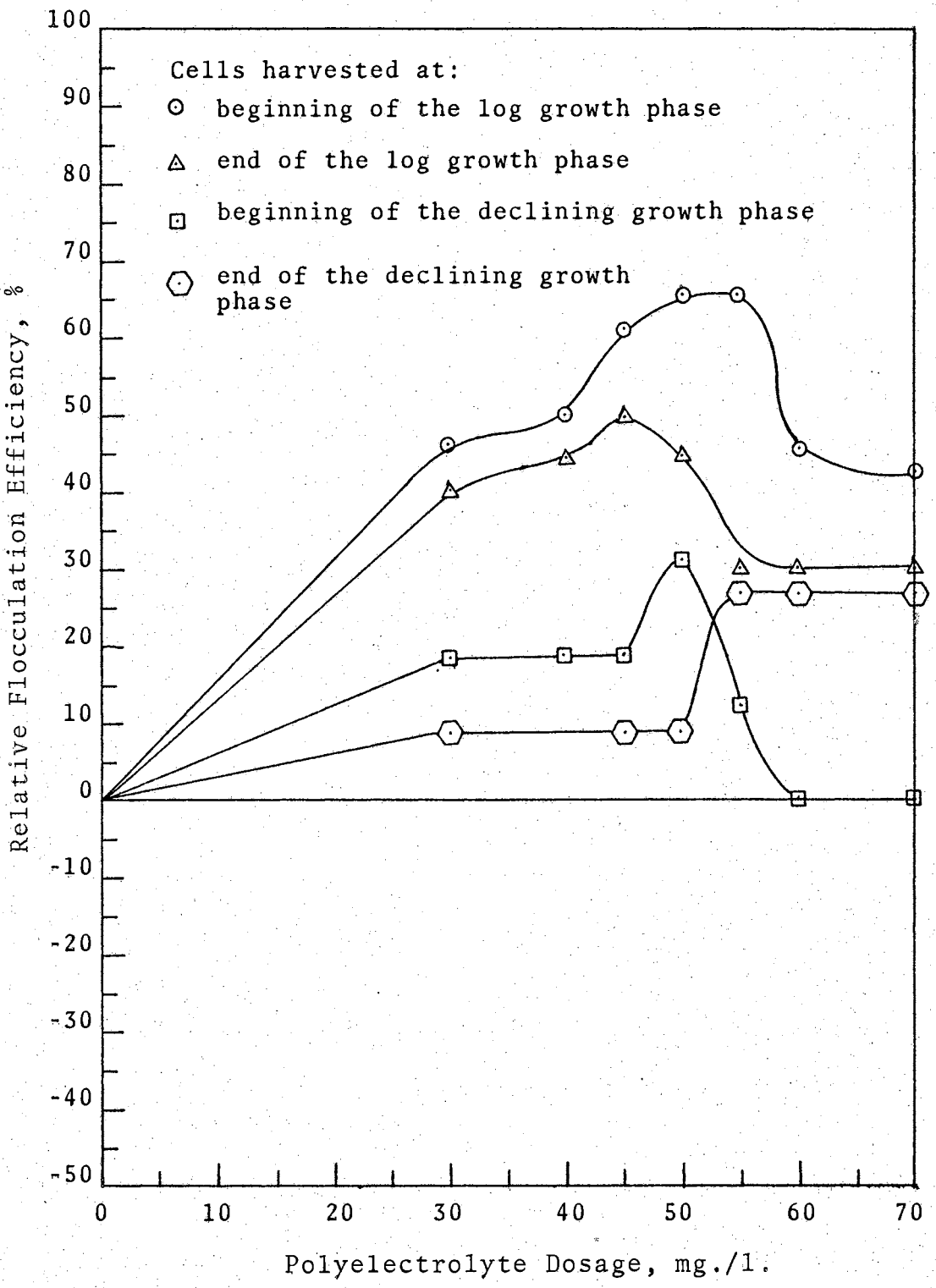

Fig. 7 - Relative efficiency of flocculation for cells from the basic unit with Nalco 600.

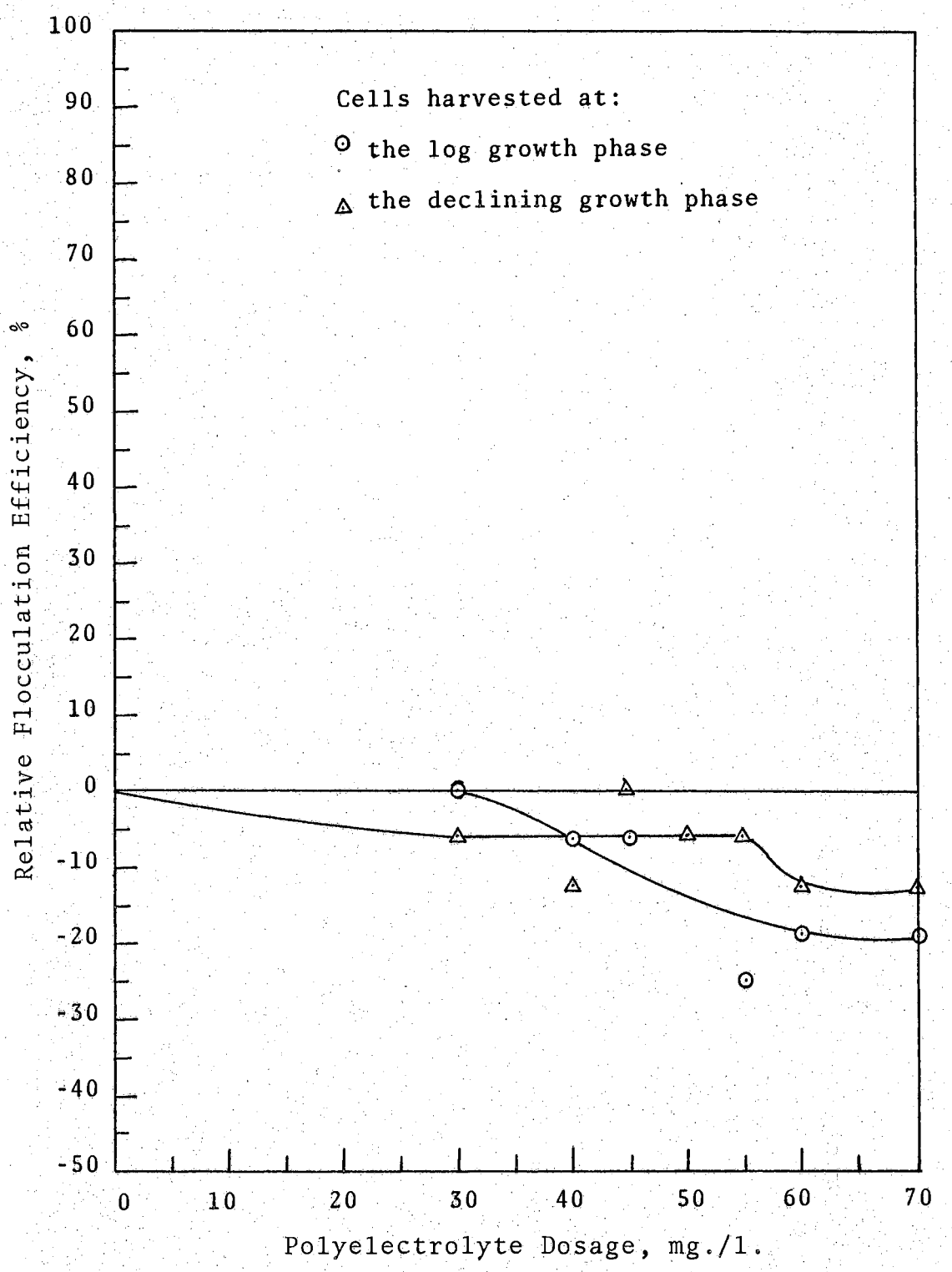

Fig. 8 - Relative efficiency of flocculation for cells from the basic unit with Purifloc A-21.

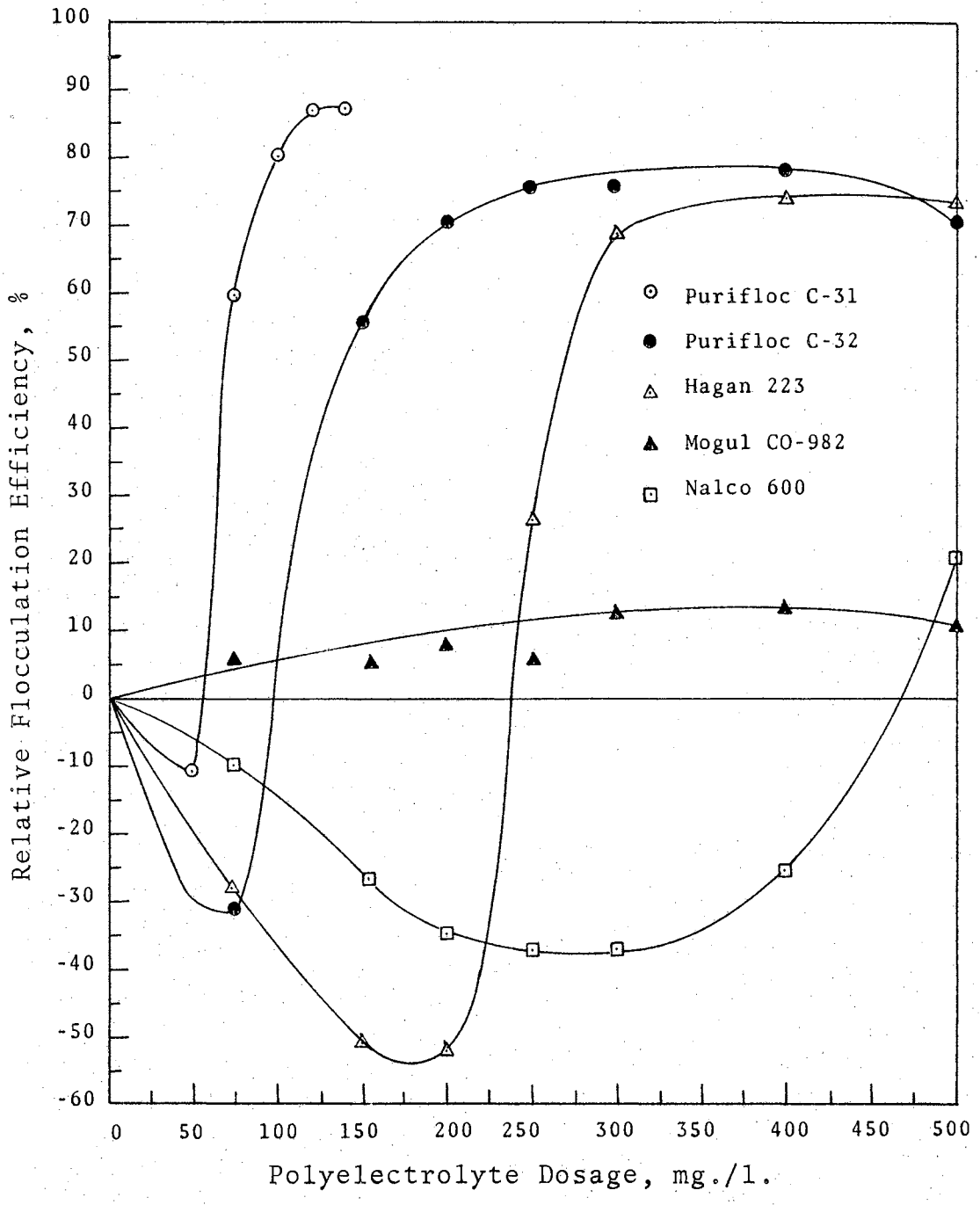

Fig. 9 - Relative efficiency of flocculation for cells from fresh units.

2. Effect of cell concentration at constant polyelectrolyte dosage

The effect of cell concentration on flocculation at constant dosages of each polyelectrolyte for cells from the basic unit is shown in Table XII and Figure 10. It is seen that, in general, the relative flocculation efficiencies for the cationic polyelectrolytes, under the conditions of these experiments, is low at high cell concentrations and high at low cell concentrations. The results at the 70% T. level are not in accordance with this general trend. However, it should be noted that flocculation in the control systems at this solids concentration level was considerably better than in any of the other controls. Such good auto-flocculation causes a relative decrease in the measurable efficiency of the polyelectrolytes and for this reason the 70% T. level cell suspension was not considered in drawing the curves shown in Figure 10.

Relative flocculation efficiencies using cells from a fresh unit are shown in Table XIII and Figure 11. It is seen that in the case of these cells, flocculation efficiency depends somewhat upon the nature of polyelectrolytes employed. For example, Purifloc C-31 and Nalco 600 gave lower efficiencies at higher cell concentrations; whereas for Purifloc C-32, Hagan 223, and Mogul CO-982, cell concentration did not appear to make any difference in the flocculating efficiencies. In general, Hagan 223 and Purifloc C-32 were effective at all the cell concentrations used.

C. Effects of selected polyelectrolytes on biochemical efficiency of activated sludge

1. Studies using cells from the basic unit

Figure 12 shows the effect of adding 60 mg/l of each polyelectrolyte to growing systems during the early log growth phase. All systems were started using the same initial cell concentration (70% T). The effect of polyelectrolyte addition can be measured by noting the length of plateau

TABLE XII

EFFECTIVENESS OF POLYELECTROLYTES IN FLOCCULATION OF CELLS FROM THE BASIC UNIT AT FOUR CELL CONCENTRATIONS

Polyelectrolyte	Cell Conc. % T.	Control Unit After Shaking 30 Min.			Flocculation Unit After Shaking 30 Min.			Reduction In Cell Concentration (mg./l.)	Relative Flocculation Efficiency %
		% T.	O.D.	Cell Conc. (mg./l.)	% T.	O.D.	Cell Conc. (mg./l.)		
Purifloc C-31	50	85.5	0.0680	125	93.5	0.0292	55	70	56
	60	85.5	0.0680	125	96.25	0.0166	30	95	76
	70	92	0.0362	70	96.5	0.0155	30	40	57.2
	80	88	0.0555	105	98.5	0.0066	20	85	81
Purifloc C-32	50	85.5	0.0680	125	88	0.0555	105	20	16
	60	85.5	0.0680	125	91	0.0410	80	45	36
	70	92	0.0362	70	93	0.0292	60	10	14.3
	80	88	0.0555	105	95	0.0223	45	60	57.2
Hagan 223	50	85.5	0.0680	125	90	0.0458	85	40	32
	60	85.5	0.0680	125	95	0.0223	45	80	64
	70	92	0.0362	70	93	0.0315	60	10	14.3
	80	88	0.0585	105	96.5	0.0155	30	75	71.5

(Note: All polyelectrolytes were used at a concentration of 60 mg./l.)

TABLE XII (Continued)

Polyelectrolyte	Cell Conc. % T.	Control Unit After Shaking 30 Min.			Flocculation Unit After Shaking 30 Min.			Reduction In Cell Concentration (mg./l.)	Relative Flocculation Efficiency %
		% T.	O.D.	Cell Conc. (mg./l.)	% T.	O.D.	Cell Conc. (mg./l.)		
Mogul CO-982	50	85.5	0.0680	125	90	0.0458	85	40	32
	60	85.5	0.0680	125	91	0.0410	80	45	36
	70	92	0.0362	70	94	0.0269	50	20	28.5
	80	88	0.0555	105	92	0.0362	70	35	33.3
Nalco 600	50	85.5	0.0680	125	92	0.0362	70	55	44
	60	85.5	0.0680	125	95	0.0223	45	80	64
	70	92	0.0362	70	93	0.0315	60	10	14.3
	80	88	0.0555	105	96.25	0.0166	30	75	60
Purifloc A-21	50	85.5	0.0680	125	84.5	0.0731	140	-15	-12
	60	85.5	0.0680	125	83	0.0809	180	-25	-20
	70	92	0.0362	70	90	0.0458	85	-15	-12
	80	88	0.0555	105	85	0.0706	135	-30	-24

(Note: All polyelectrolytes were used at a concentration of 60 mg./l.)

TABLE XIII

EFFECTIVENESS OF POLYELECTROLYTES IN FLOCCULATION OF CELLS FROM A FRESH UNIT USING PREVIOUSLY DETERMINED OPTIMAL DOSAGES OF EACH POLYELECTROLYTE

Polyelectrolyte	Dosage (mg/l)	Cell Conc. % T.	Control Unit After Shaking 30 Min.		Flocculation Unit After Shaking 30 Min.		Reduction In Cell Concentration (mg./l.)	Relative Flocculation Efficiency %
			% T.	Cell Conc. (mg./l.)	% T.	Cell Conc. (mg./l.)		
Purifloc C-31	120	50	51.75	530	55	475	55	10.4
		60	62.50	375	88.5	175	200	53.3
		70	71.50	270	94	50	220	81.5
		80	80.25	180	97	25	155	86.1
Purifloc C-32	400	50	55.25	470	88	105	365	77.8
		60	66	340	89.5	95	245	72
		70	75	230	92.5	65	165	71.7
		80	84	140	95	45	95	67.8
Hagan 223	400	50	55.25	470	86	120	350	74.5
		60	66	340	88	105	235	69.3
		70	75	230	93	60	170	74.0
		80	84	140	96.5	30	110	78.6

TABLE XIII (Continued)

Polyelectrolyte	Dosage (mg/l)	Cell Conc. % T.	Control Unit After Shaking 30 Min.		Flocculation Unit After Shaking 30 Min.		Reduction In Cell Concentration (mg./l.)	Relative Flocculation Efficiency %
			% T.	Cell Conc. (mg./l.)	% T.	Cell Conc. (mg./l.)		
Mogul CO-982	400	50	55.25	470	60	410	60	12.75
		60	66	340	75	230	10	3.06
		70	75	230	80.5	175	55	23.9
		80	84	140	85	135	5	3.57
Nalco 600	500	50	56.50	455	47	600	-145	-31.9
		60	69	300	75	230	70	23.4
		70	75.5	230	85	135	95	41.3
		80	84	140	91.5	75	65	46.5

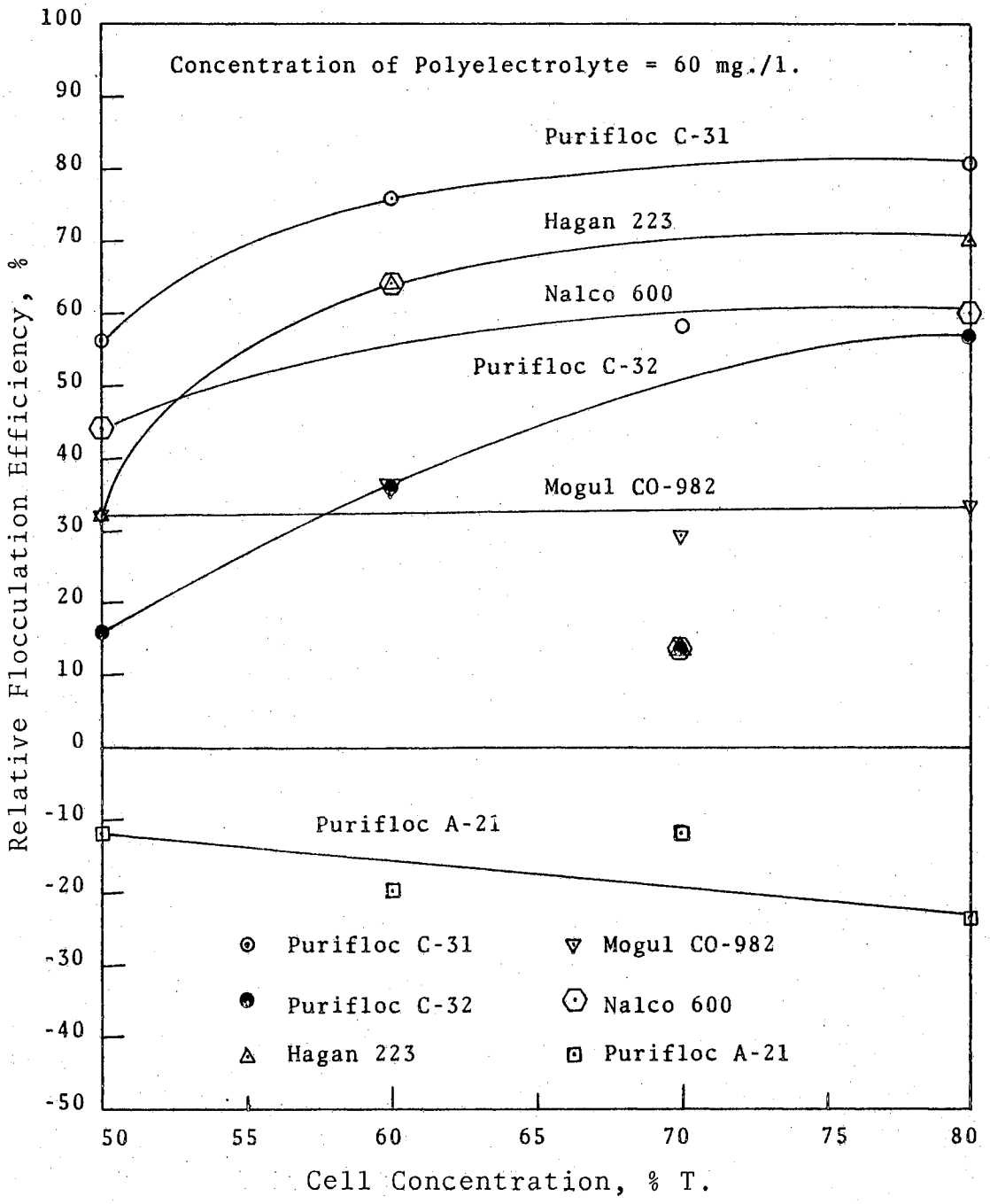

Fig. 10 - Relative efficiency of flocculation for cells from the basic unit at four cell concentrations.

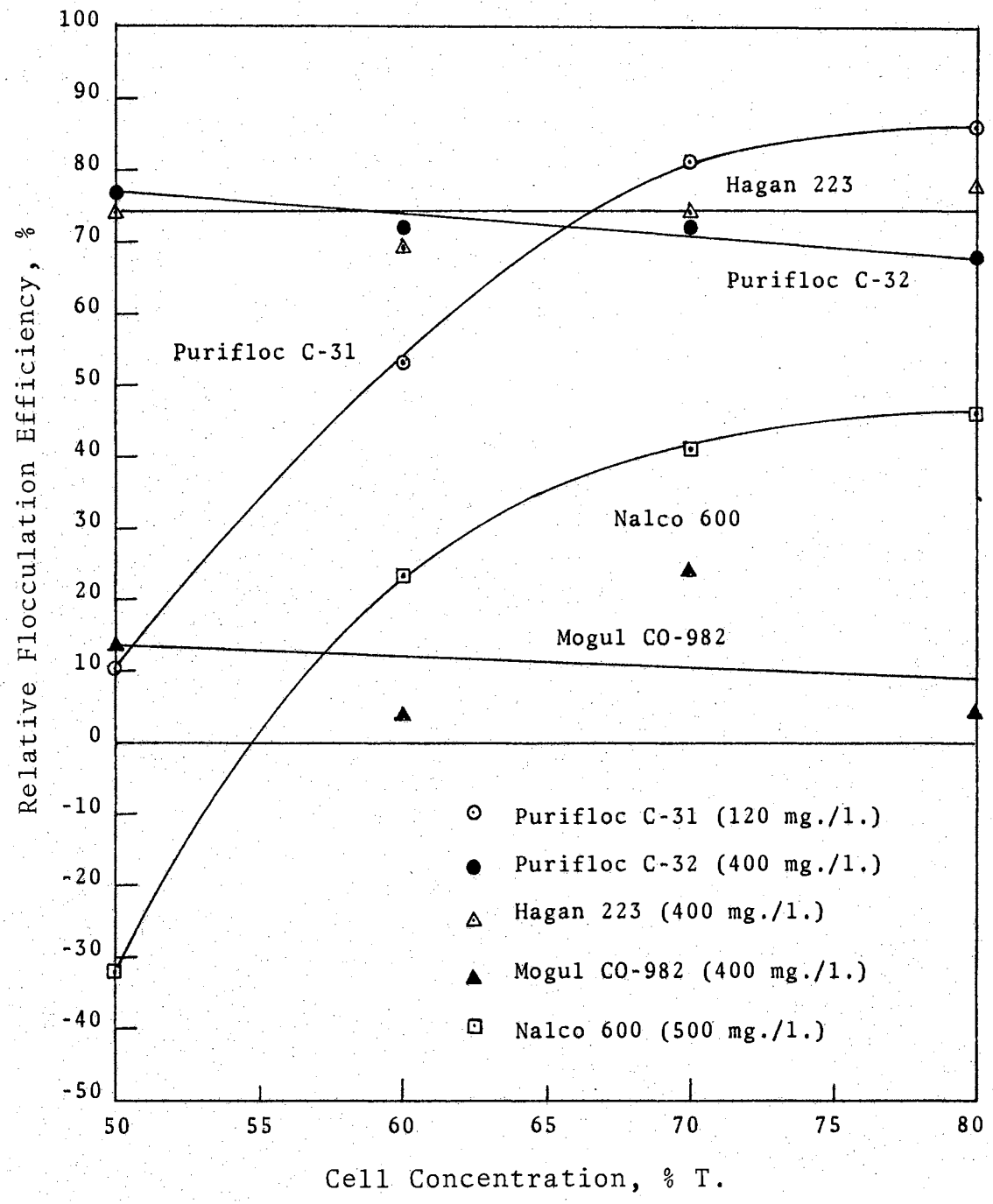

Fig. 11 - Relative efficiency of flocculation for cells from a fresh unit at four cell concentrations with previously determined optimal dosages of each polyelectrolyte.

or pause in the growth curves and, thereafter, comparing the rate of growth in control and dosed systems. For example, Purifloc C-31 exerts a considerable flocculating effect as noted by the cessation of increase in optical density. The plateau lasted approximately eight hours; however, after this period the optical density rose at about the same rate as it did in the control system. It would appear that Purifloc C-31 did not affect the rate of growth. However, it can be noted that it did severely reduce the total cell yield as evidenced by the low final optical density recorded. Purifloc C-32 yielded much the same result as Purifloc C-31 except that it did not severely affect the total amount of growth. Nalco 600 did not affect the rate of growth nor did it exhibit as much of a pause in growth as did either of the Purifloc polyelectrolytes. Hagan 223 caused both a significant pause in the curve and a retarded rate of growth. Mogul CO-982 did not cause a significant pause or flocculation but did retard the rate of growth to approximately the same extent as Hagan 223.

2. Studies using cells from a fresh unit

The biochemical effects on cells from a fresh unit due to the addition of optimal dosages of polyelectrolytes for each individual experimental system are shown in Figures 13-17. Since polyelectrolytes are synthetic organic compounds which might be expected to exert measurable COD in the experimental systems, it seemed necessary to determine the COD of the added polyelectrolyte in each system. These results are shown in Table XIV. It should be re-emphasized that in making solutions of polyelectrolytes of known concentration the weights used were those obtained in weighing a sample of the commercial preparation. Since the commercial products are viscous liquids, it was of interest to determine their water content. Such determinations were made by drying a known weight of the liquid sample. It was not known whether the loss of weight was entirely due to

loss of water since it is possible that the polyelectrolytes may contain materials which will volatilize at the standard 103°C. drying temperature. The weight loss of polyelectrolytes after four days at 103°C. is shown in Table XV.

TABLE XIV

COD OF POLYELECTROLYTES

Polyelectrolytes	Concentration	COD (mg/l)	Mg. COD per Mg. Polyelectrolyte
Purifloc C-31	120	100	0.833
Purifloc C-32	400	250	0.625
Hagan 223	400	235	0.587
Mogul CO-982	400	10	0.025
Nalco 600	500	165	0.330

TABLE XV

WEIGHT LOSS OF POLYELECTROLYTES
AFTER FOUR DAYS AT 103°C.

Polyelectrolytes	Volatile Material and Water Content (%)
Purifloc C-31	43.50
Purifloc C-32	66.44
Hagan 223	54.26
Mogul CO-982	57.78
Nalco 600	76.83

Since 1000 mg/l of glucose was added, the theoretical initial COD of the control units should be 1065 mg/l (1.065 mg/l O_2 is required to oxidize 1 mg/l glucose). The theoretical initial COD of the experimental systems can be estimated by adding 1065 mg/l to the values shown in Table XIV.

For the experiment shown in Figure 13, 120 mg/l of Purifloc C-31 was added to the experimental system. Its measured initial COD was 1090 mg/l, and the final COD after sixteen hours was 110 mg/l; the final COD of the control system was 80 mg/l. With reference to glucose removal, 4.0 hours were required to remove 1000 mg/l in the control system (initial solids concentration of 127 mg/l), while 10.5 hours were required for the Purifloc C-31 system (initial solids concentration of 102 mg/l). During the sixteen hour experiment, the maximum solids concentrations obtained for the Purifloc C-31 system and the control system were 480 mg/l and 530 mg/l, respectively. With respect to oxygen uptake, the control system curve broke at approximately eight hours, whereas, fifteen hours were required for the Purifloc C-31 system.

For the study shown in Figure 14, 400 mg/l of Purifloc C-32 was added to the experimental system. The measured initial COD was 1460 mg/l, and the final COD after sixteen hours was 140 mg/l. The final COD of the control system was 80 mg/l. Approximately twelve hours were required for glucose removal, while in the control system only 4.0 hours were required. The initial solids in the experimental system just after addition of 400 mg/l of Purifloc C-32 was 148 mg/l. This was measurably higher than in the control system which contained an initial solids concentration of 127 mg/l. The maximum solids concentration attained in the control system was 530 mg/l, whereas, solids concentration in the experimental system reached 650 mg/l. The oxygen uptake curve for the Purifloc C-32 system broke at

approximately fourteen hours while only eight hours were required for the control system.

Figure 15 shows the biochemical effects of the addition of 400 mg/l of Hagan 223. The measured initial COD of the experimental system was 1280 mg/l; and the final COD, after sixteen hours of aeration, was 1140 mg/l. This was significantly higher than the 60 mg/l final COD of the control system. Glucose removal in the control system required 3.5 hours. However, after sixteen hours of aeration, 870 mg/l glucose remained in the Hagan 223 system. The initial solids concentration in the control system was 150 mg/l, but in the Hagan 223 system the initial solids, measured immediately after the addition of 400 mg/l Hagan 223, was reduced to only 40 mg/l. The solids concentration in the control system reached 550 mg/l; but in the Hagan 223 system there was very little production of biological solids, and there was a correspondingly small oxygen uptake.

The effect of adding 400 mg/l of Mogul CO-982 to an activated sludge system is shown in Figure 16. It is seen that all of the data obtained for the Mogul CO-982 system (except for solids concentration) were very similar to the control system. From these data it seems apparent that Mogul CO-982, which exhibited no effective flocculating power, also had no deleterious biochemical effects which suppressed cell growth. The measured initial COD in the Mogul CO-982 system was 1085 mg/l. The final COD measured after sixteen hours of aeration was 90 mg/l in the Mogul CO-982 system and 60 mg/l in the control system. The time required for glucose removal was four hours in the Mogul CO-982 system and 3.5 hours in the control system. Oxygen uptake curves for the two systems were essentially identical. Concerning biological solids concentration, it is important to note that the initial solids in the Mogul CO-982 system, measured immediately after the addition of

the polyelectrolyte was 345 mg/l. This value was much higher than the 150 mg/l of initial solids in the control system. The maximum solids in the control system were 550 mg/l; while in the Mogul CO-982 system, a concentration of 740 mg/l was attained.

Figure 17 shows the effect of adding 500 mg/l of the Nalco 600 to an activated sludge system. The measured initial COD in the Nalco 600 system was 1150 mg/l, and its final COD after sixteen hours of aeration was 160 mg/l, which was double that of the control system. Approximately twelve hours were required for glucose removal in the Nalco 600 system, while only 4.0 hours were required for glucose removal in the control system. The initial solids, measured immediately after the addition of 500 mg/l of Nalco 600, was 158 mg/l which was somewhat higher than that of the control system (127 mg/l). The peak solids concentration in the Nalco 600 system was 600 mg/l which was relatively higher than the peak solids concentration (530 mg/l) in the control system. The oxygen uptake curve for the control system leveled off at approximately eight hours, whereas, sixteen hours were required in the Nalco 600 system.

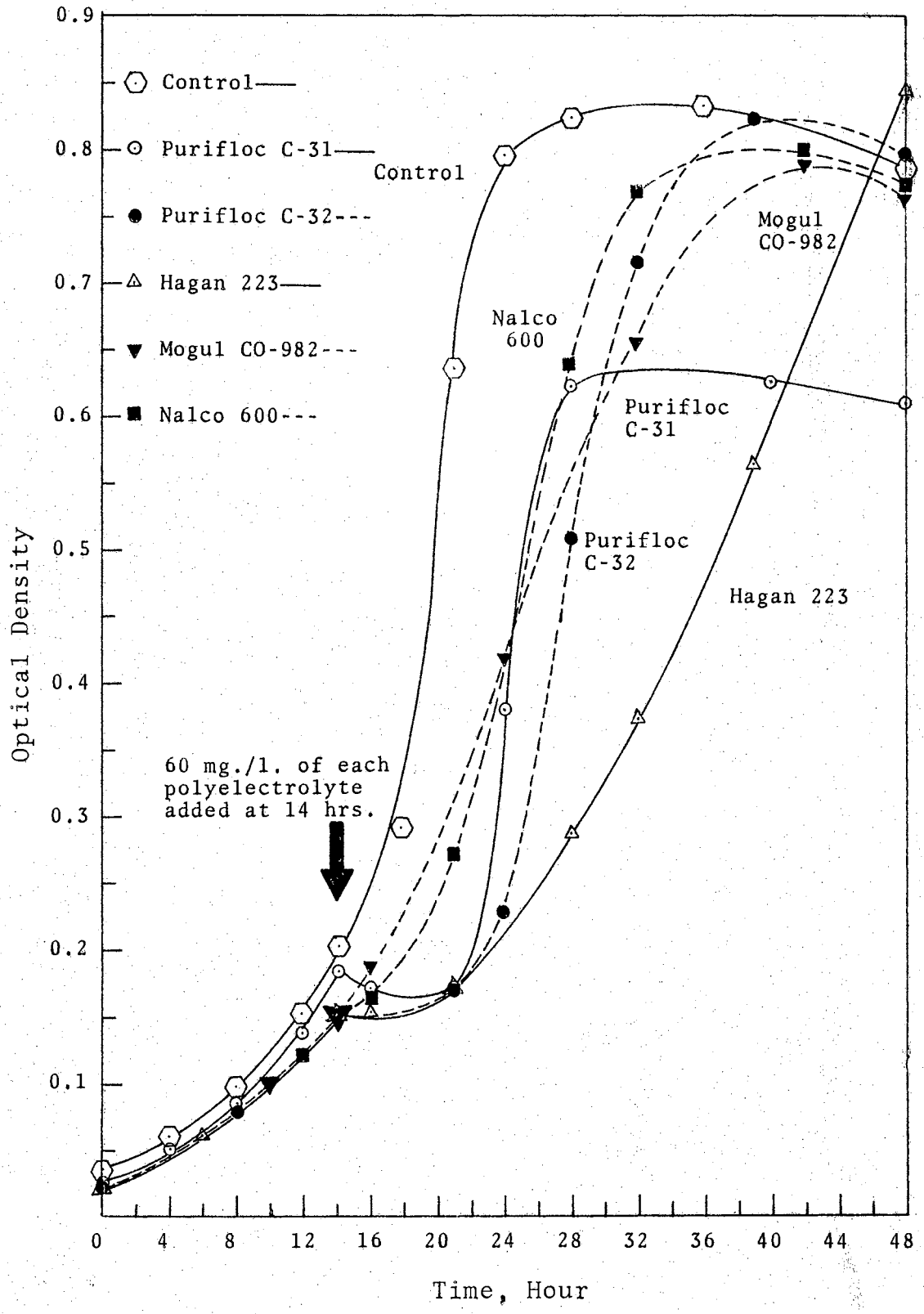

Fig. 12 - Effect of polyelectrolytes on growth of cells from the basic unit.

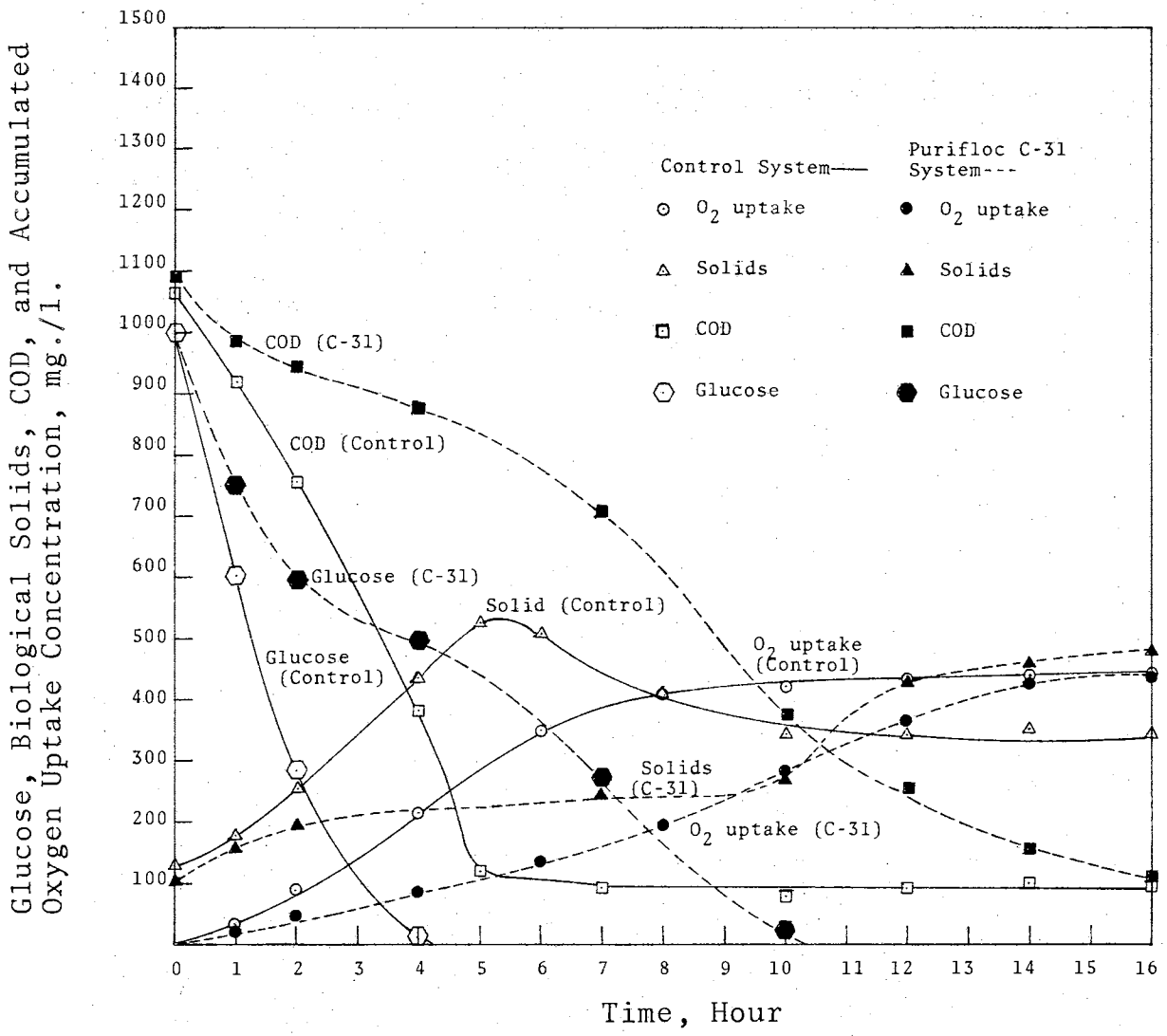

Fig. 13 - Biochemical response of cells from a fresh unit to 120 mg./l. of Purifloc C-31.

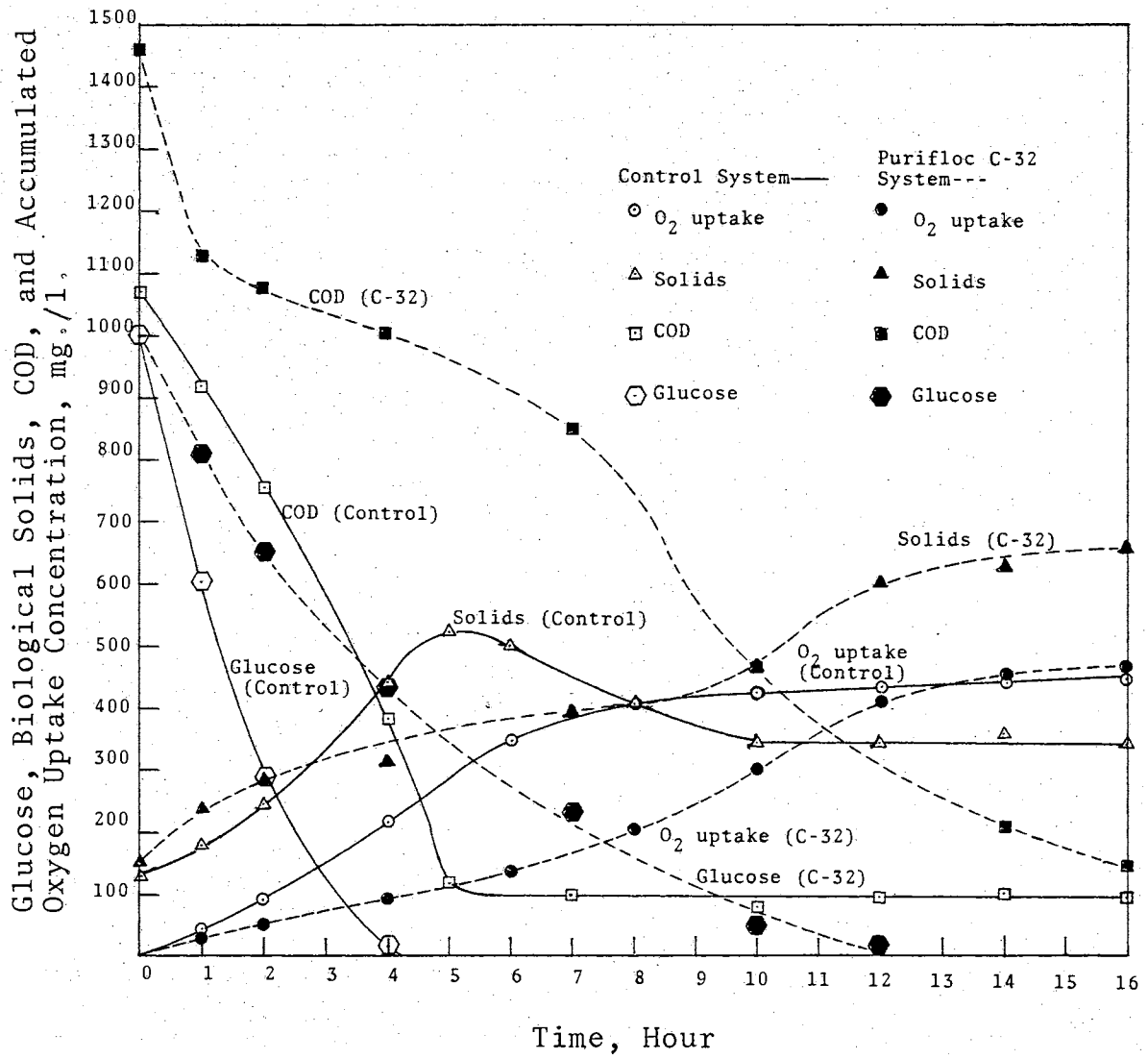

Fig. 14 - Biochemical response of cells from a fresh unit to 400 mg./1. of Purifloc C-32.

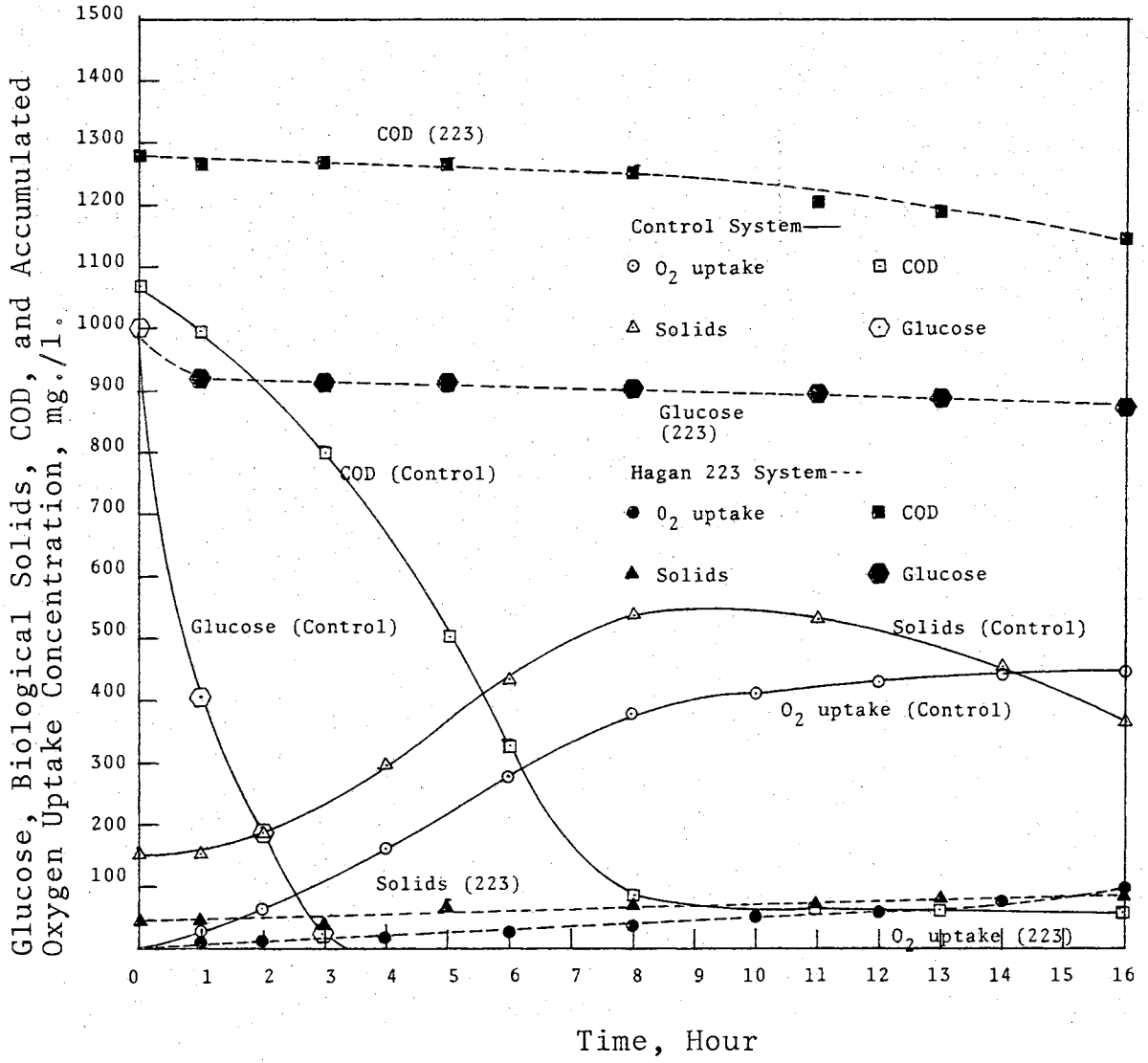

Fig. 15 - Biochemical response of cells from a fresh unit to 400 mg./l. of Hagan 223.

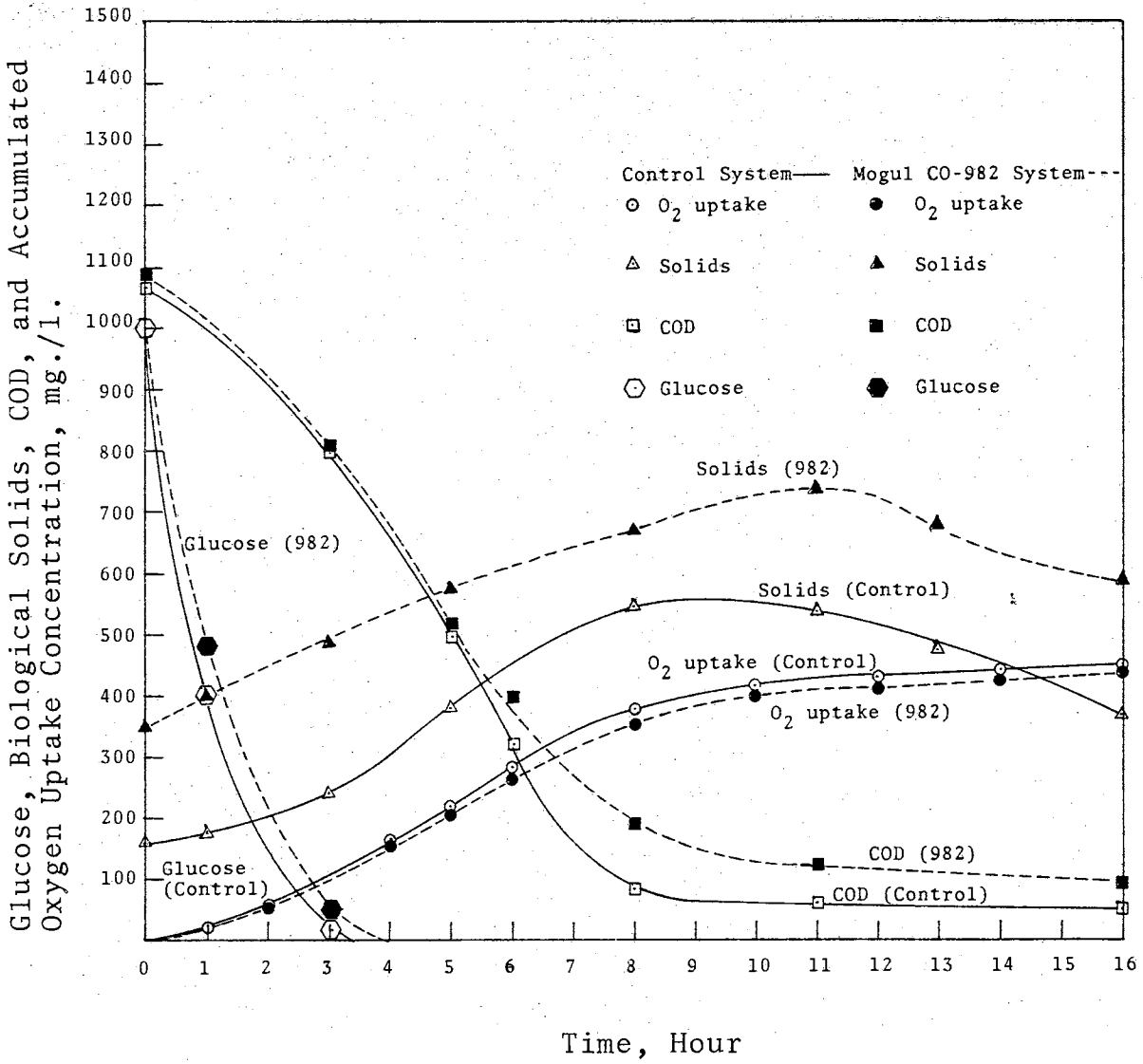

Fig. 16 - Biochemical response of cells from a fresh unit to 400 mg./l. of Mogul CO-982.

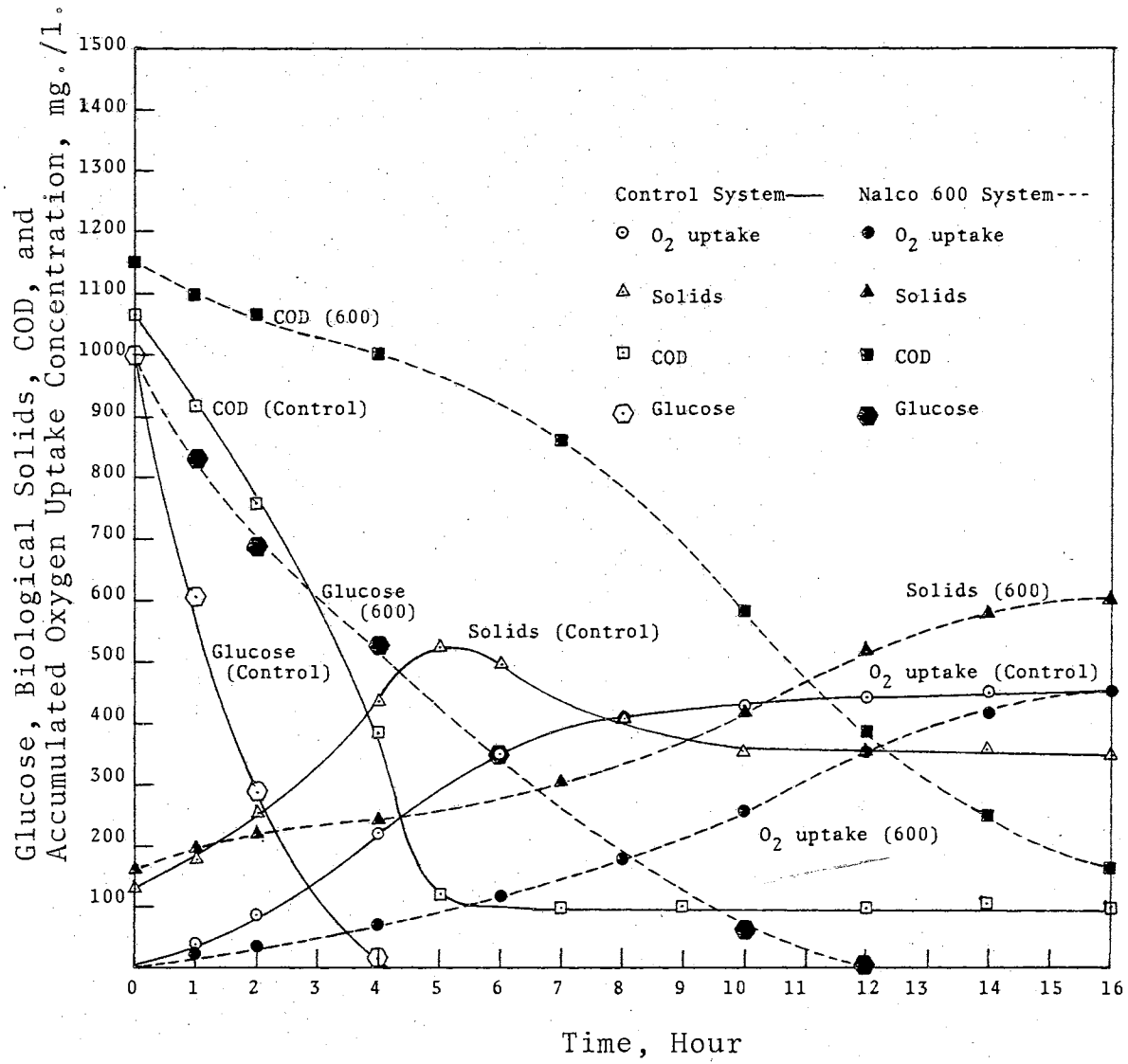

Fig. 17 - Biochemical response of cells from a fresh unit to 500 mg./1. of Nalco 600.

CHAPTER V

DISCUSSION

A. Calibration curve and growth curve studies

1. Calibration curve

The percentage of light transmittance through the spectrophotometer is a function of the wave length of the light and the physical nature of the medium through which the light must pass. The major factor which determines this physical nature is size of the suspended solids or colloids and their color (52). The sizes of bacterial cells are different at different growth phases, and changes in predominance occur randomly. In addition, there are differences in the color of bacteria. Therefore, it was difficult to obtain coincidental or reproducible results in determining the relation between solids concentration and optical density for each experiment. However, within each experiment the data did follow a fairly straight line relation. Therefore, for a single seed population a fairly good correlation between optical density and sludge concentration could be attained. In general, the overall correlation was adequate for the purpose to which it was put in these studies.

2. Growth curve

It was seen that there was some variation in the growth curve data but, as in the case of the correlation curve discussed above, the results were sufficiently reproducible to allow estimation of the phase of growth at which cells were harvested. Since there was nothing to prevent changes in

predominance from occurring and since different species grow at intrinsically different rates, some variation was to be expected.

B. Studies on flocculation efficiency of various polyelectrolytes

The difference in flocculating characteristics for cells of varying ages (log phase versus declining phase) is a very interesting aspect. As early as 1914, Gillespie observed that very young cultures of pneumococci required a longer agglutination period than older cells. Sherman and Albus in 1923 reported that four-hour cultures of Escherichia coli were not agglutinated by an acidity of pH 3.0, whereas, a twenty-four-hour culture was agglutinated at pH 3.8 (49). They did not give any reasonable explanation for this finding, but the fact remains that they found young cultures more difficult to agglutinate than old cultures. In the present study the agglutinating or flocculating agent was a cationic polyelectrolyte, and it seems possible that the reason why more polyelectrolyte was required to flocculate young cells than old cells is that young cells possess more negative charges than old cells.

The difference in the effect of polyelectrolytes upon cells from the basic unit and from fresh units appeared to be related primarily to the tendency of cells obtained from the basic unit to flocculate without addition of a polyelectrolyte. This is probably due to a difference in predominating species in the populations. After a batch unit has been operated for a considerable period of time, it seems reasonable that the population should consist of cells with a greater tendency toward auto-flocculation than would be found in a randomly selected population. This selection of cells with a tendency to flocculate might occur because each time the unit is fed, more non-flocculated than flocculated cells are discarded. Therefore, if there are certain species of bacteria which have a greater tendency toward

auto-flocculation, these should eventually predominate in a prolonged batch operation.

In 1953, Bisset advanced the concept that the cell membrane secretes cell-wall substances at the growing tip of the cell (14). Tenney and Stumm stated that natural polymers, e.g., complex polysaccharides and polyamino acids are excreted or exposed at the surface of the cell predominantly during the endogenous respiration phase after exogenous substrate has been exhausted. They also stated that sewage bacteria in the endogenous phase are capable of excreting substantial amounts of materials which are frequently referred to as polyelectrolytes (12). The above ideas suggest an explanation as to why flocculation by polyelectrolytes was more effective in the log growth phase than in the endogenous phase. If more natural polyamino acids and polysaccharides extrude from the surface in the endogenous growth phase or declining growth phase than in the log growth phase, it would be expected that there could be more negative charges distributed on the surface. Thus, more cationic polyelectrolytes would be required for electrical neutralization in the declining and endogenous phase than in the log growth phase.

The anionic polyelectrolyte, Purifloc A-21, had a negative effect on cell flocculation (Figure 8), owing to mutual repulsion of negative charges carried on Purifloc A-21 and the bacterial surface.

Mutual repulsion can also be cited to explain why dosages greater than optimum result in less effective flocculation. Thus far, the only known theory of polyelectrolyte action is that they form electric bridges between colloids. As the dose is increased beyond that required for neutralization of charge, the remaining positive charges of the cationic polyelectrolyte repel one another and tend to stabilize the suspension.

The flocculating power of an electrolyte depends upon its valence. The influence of valence follows closely a geometrical progression ($1:X:X^2$, where X is the valence number) (50). Knaysi reported that in small concentrations, salts of monovalent cations may increase the zeta-potential. As the concentration increases, a gradual decrease takes place until the zeta-potential equals zero, i.e., the isoelectric point is attained. Beyond this concentration, the cell may take on a positive charge. With electrolytes of divalent or polyvalent cations no initial increase is observed and the zeta-potential decreases continuously and more rapidly than for monovalent cations (17). If the above concepts are correct, the results shown in Figure 9 for the lower concentration of polyelectrolyte could be interpreted as having increased zeta-potential. Actually this is not believed to be the case. At the lower polyelectrolyte concentrations, very minute floc particles were formed, but they remained in suspension. The control system was completely dispersed, and no traces of small floc particles were noted. Therefore, the lower concentrations of polyelectrolyte did not cause an increase in cell dispersion. It seems that minute flocs in suspension can absorb more light than an equivalent concentration of completely dispersed cells.

From Figure 10 and Figure 11, it was seen that at a constant concentration of cationic polyelectrolyte, the relative flocculation efficiency was higher for low cell concentrations than for high cell concentrations. A possible explanation for this phenomenon could be that the higher ratios of polyelectrolyte molecules to bacterial colloids encouraged the formation of multiply-bridged flocs which would be expected to settle more rapidly. In Figure 11, it was seen that Purifloc C-32 and Hagan 223 were effective at either high or low cell concentrations. Although only scant data on the structure of these threadlike molecules are available, it may be that they possess more active

sites (or more positive charges). Therefore, they may attract and bind more cells. From Figure 11, it would appear that Purifloc C-31 was not as effective as Purifloc C-32 or Hagan 223. However, in the experiment shown in Figure 9, it was seen that Purifloc C-31 was more effective than any of the polyelectrolytes studied. It should be noted that for the result reported in Figure 11, the dosage of Purifloc C-31 was 120 mg/1, whereas, 400 mg/1 of Purifloc C-32 and Hagan 223 were used. The results shown in Figures 9 and 11 agree closely when the same concentrations of cells and polyelectrolytes are compared. Therefore, it can be concluded that Purifloc C-31 is more effective with low concentrations of cells, but Purifloc C-32 or Hagan 223 is more effective with higher cell concentrations.

C. Effect of selected polyelectrolytes on biochemical efficiency of activated sludge

1. 120 mg/1 Purifloc C-31 system

The theoretical initial COD in the Purifloc C-31 system (Figure 13) should have been equal to the initial COD in the control unit (1065 mg/1) plus the COD of 120 mg/1 Purifloc C-31 (100 mg/1), i.e., 1165 mg/1. However, the measured initial COD in the system was 1090 mg/1. The solids concentration of Purifloc C-31 unit just after adding 120 mg/1 of the polyelectrolyte was 25 mg/1 (127 mg/1 - 102 mg/1 = 25 mg/1) less than the control system. Although these differences are rather small and could be attributed to experimental error, it is possible that the polyelectrolyte may cause a certain degree of cell lysis. The actual degree of lysis may not be adequately described by the 25 mg/1 decrease in solids concentration because the 102 mg/1 of initial solids measured in the Purifloc C-31 system would also contain part of the added polyelectrolyte dosage, i.e., the portion that was tied up with the cell and retained on the millipore filter. It should also be emphasized that the polyelectrolyte itself loses weight upon drying. This

adds another complicating factor in analyzing the results. The increase in initial COD in the Purifloc C-31 system is 75 mg/l (1165 mg/l - 1090 mg/l = 75 mg/l). Some of this loss is due to the fact that the polyelectrolyte bridges the flocs and is retained on the millipore filter. It would not be expected that this difference in COD would necessarily be balanced by an increase in solids because a portion of the cells may have lysed and because the polyelectolyte loses weight upon drying, whereas, the polyelectrolyte dose was based upon liquid weight.

It was seen that substrate removal as measured by either COD or glucose was slower in the Purifloc C-31 system than in the control system. In this case, it is difficult to say whether Purifloc C-31 inhibited cell growth in a biochemical sense or in a mechanical way. It is seen that substrate removal could have been retarded by purely mechanical factors since when the cells were forcibly flocculated only those cells on the outside layer of the flocs would have maximum opportunity for substrate utilization. It is interesting to compare the result in Figure 12, in which 60 mg/l of Purifloc C-31 was added and growth was measured by optical density, with the growth curve, Figure 13, in which 120 mg/l of Purifloc C-31 was added and growth was measured by the increase in solids concentration. In Figure 12, the polyelectrolyte blocked growth for approximately seven hours, whereas, it caused apparent blockage for nine hours in Figure 13. Although the analytical technique for measuring growth was different for these experiments, it would appear that higher doses of Purifloc C-31 caused a greater hindrance to growth.

Referring again to Figure 13, it was observed that the solids concentration rose sharply after nine hours exposure to the polyelectrolyte. The behavior of the solids concentration curve may also be ascribed to either metabolic inhibition or mechanical factors. If the lag was due to metabolic

inhibition, e.g., enzyme inhibition, it would seem that the inhibition was reversible after nine hours of acclimation. On the other hand, since the cells were flocculated, it is possible that only those cells on the outer surface of the floc were actively multiplying during the lag. The daughter cells may have been released from the floc, thus exposing the next layer. The combination of greater exposure of the cells in the floc and multiplication of the cells in free suspension could have caused an accelerated increase in biological solids concentration.

2. 400 mg/1 Purifloc C-32 system

The theoretical initial COD in the Purifloc C-32 system (Figure 14) should have been equal to the sum of the initial COD in the control system (1065 mg/1) and 400 mg/1 of Purifloc C-32 (250 mg/1), i.e., 1315 mg/1. However, the measured initial COD in the Purifloc C-32 system immediately after adding the polyelectrolyte was 1460 mg/1. The increase of 145 mg/1 of initial COD in the Purifloc C-32 system seems too large to be caused by experimental error and is believed to have been caused by a combination of cell lysis and Purifloc C-32 which passed through the millipore filter.

The biological solids curve is somewhat similar to that shown for the previous experiment (Figure 13). There was a slight rise during the first two hours followed by a long period of slow increase and finally a rapid rise in solids concentration. It would appear from these results that the mechanism of retardation (biochemical or mechanical) is not immediately set into action.

Another interesting aspect which seems worthy of discussion is the fact that the maximum solids concentration in the Purifloc system was higher than that of the control system. It should be noted that the COD of the Purifloc C-32 system was 395 mg/1 higher than that in the control system, while the COD difference between the two systems after sixteen hours of aeration was 60 mg/1. Therefore,

the solids increase (approximately 120 mg/l) was due to the utilization of 335 mg/l (395 mg/l - 60 mg/l = 335 mg/l) of COD. This extra substrate could have come partially from the lysed cell material but is attributable partially to the utilization of the polyelectrolyte as a substrate. A portion of the increase in solids concentration may also be due to the dry weight fraction of the polyelectrolyte tied up in the cells.

3. 400 mg/l Hagan 223 system

The theoretical initial COD in the Hagan 223 system (Figure 15) should have been equal to the sum of the initial COD of the control system (1065 mg/l) and that of 400 mg/l of Hagan 223 (235 mg/l), i.e., 1300 mg/l. The theoretical value of initial COD compares very well with the measured initial COD in the Hagan 223 system (1280 mg/l). However, the initial solids concentration for the Hagan 223 unit immediately after adding 400 mg/l of the polyelectrolyte was only one-third that of the control, indicating that two-thirds of the cells may have lysed. The released cell material should have increased the COD in the Hagan 223 system approximately 140 mg/l above the theoretical value. A possible explanation is that the increase in COD due to the material released by lysis was balanced by the COD of an equivalent dry weight of the Hagan 223 retained on the cells.

From the data of the previous experiment on the flocculating ability of Hagan 223, it was found that this polyelectrolyte possessed excellent flocculating characteristics regardless of cell concentration. It seems possible that, at the low initial cell population used in the study shown in Figure 15, 400 mg/l polyelectrolyte was too great a shock loading, thus causing the initial drop in biological solids concentration. The long lag in glucose and COD removal and in solids production and oxygen uptake might be due to an

inhibitory effect of Hagan 223 or simply to low initial cell concentration. In Figure 12, it was seen that, when 60 mg/l of Hagan 223 was added, growth was blocked for only four hours. While the blockage of growth appears to be related to the polyelectrolyte-solids ratio, it is impossible to determine from the data whether the long lag period shown in Figure 15 is the result of low initial cell concentration, metabolic inhibition due to Hagan 223 or the previously described mechanical interference in substrate removal due to floc formation. If Hagan 223 causes an en masse metabolic inhibition, it would appear that it is a reversible one or the cells would not have grown after four hours lag.

4. 400 mg/l Mogul CO-982 system

Mogul CO-982 (Figure 16) was not an effective polyelectrolyte nor did it have any biochemical effect on bacterial cells. The only striking difference between the control and the polyelectrolyte system was in the solids concentration curves. The curve for the Mogul CO-982 system was consistently higher (approximately 200 mg/l) and roughly parallel to the control curve. Since this polyelectrolyte is carbohydrate in nature and most probably is a starch-like molecule, it seems likely that it was retained on the membrane filter. Since its dry weight is approximately 50% of its wet weight (see Table XV), it would be expected that 400 mg/l would increase the membrane filter weight by approximately 200 mg/l.

This polyelectrolyte formed a jelly like paste on the millipore filter and caused the mixed liquor in the batch aerator to turn cloudy. It is believed that this polyelectrolyte caused a thrixotropic phenomenon. Porter (51) stated that

> "If a suitable amount of electrolyte is added to a sol, it will set to a jelly which is no more cloudy than the original sol. If the resulting gel is then shaken, a sol is again formed which

sets once more when allowed to stand and this phenomenon may be repeated many times with the same system. Such a reversible sol-gel transformation has been called Thrixotropy."

5. 500 mg/l Nalco 600 system

The theoretical initial COD in the Nalco 600 system (Figure 17) should have been equal to the initial COD of the control system (1065 mg/l), plus the COD of 500 mg/l of Nalco 600 (165 mg/l), i.e., 1230 mg/l. The measured initial COD in the Nalco 600 system was 1150 mg/l, and the measured initial solids concentration was 31 mg/l more than that of the control system (158 mg/l in the Nalco 600 system and 127 mg/l in the control system). It seems apparent that the increased initial COD in the Nalco 600 system, i.e., 85 mg/l (1150 mg/l - 1065 mg/l = 85 mg/l), was due to the filtrable portion of the polyelectrolyte, and the increased initial solids concentration was due to the dry weight portion of the Nalco 600 which was tied up with biological solids on membrane filter.

As in the previous cases, it is impossible to say definitely whether the slower rate of growth and substrate removal in the Nalco 600 system was due to biochemical effects of the polyelectrolyte or to mechanical effects caused by flocculation of cells. The results shown in figure 12 indicate a lag of only two hours (using 60 mg/l of Nalco 600) before the cells resumed normal growth. From this, it would seem that the polyelectrolyte was not very inhibitory to growth. Also, the substrate removal curves (COD and glucose) and the biological solids curve for the polyelectrolyte system shown in Figure 17, in which 500 mg/l of Nalco 600 were used, indicated that the system could acclimate to this dosage.

D. Possible biochemical mechanisms of inhibition by polyelectrolyte

As previously mentioned, polyelectrolytes are high

molecular weight, synthetic organic compounds which are water soluble and carry electric charges on specific sites along their thread-like extended structure. Such molecules are too large to penetrate into the cell, but positive charges (for cationic polyelectrolytes) on these stretched-out molecules are strong enough to attract the negatively charged bacterial colloid. The so-called "bridging" action between the positively charged polyelectrolyte and negatively charged colloids which has been referred to by other investigators (23), (24), (34) is based on the attractive force between charges of opposite sign. The author believes that this very charge neutralization leads to three possible mechanisms which can explain the apparent deleterious effect of polyelectrolytes on the waste water purification efficiency of the system studied.

1. Blockage of substrate transport

In the normal course of glucose metabolism, enzymes (permeases) may be needed to transport glucose into the cell. However, after the polyelectrolyte molecules cover the surface of the cell, they may greatly reduce opportunities for formation of a permease-glucose complex or for entry of glucose into the cell. Therefore, the rate of glucose utilization decreases and the population increase is slow. However, as more charges are neutralized the binding force between polyelectrolyte molecules and the cell surface may decrease and this, in combination with the agitation caused by diffused air or shaking, may tend to shear the polyelectrolyte from the cell surface. This would increase the opportunity for glucose utilization. Hence, after a lag period, the population increases more rapidly. The extent of inhibition depends on the strength of the binding force between the cells and the polyelectrolyte.

2. The shock load effect

Any change in the physical or chemical environment in

which microorganisms live can cause metabolic modifications in the cells or even enhance the selection of mutants. The former type of response usually involves a temporary change in cell properties; the latter response indicates a permanent change in the genetic properties of the cell. The addition of organic polyelectrolytes to the heterogeneous populations in the batch units constituted a rather drastic change in the physical and chemical environment. Therefore, the batch systems undoubtedly underwent a shock loading which could lead to various metabolic modifications and changes in predominance. Time may be required before the system can adjust to these environmental changes; indeed, one of the responses may be that, in time, the cells can induce enzymes which enable them to utilize the polyelectrolyte as a substrate. This possibility could explain the slightly greater amount of COD removal in some of the polyelectrolyte systems rather than in their corresponding control systems.

3. Enzyme inhibition

Polyelectrolytes may affect bacteria in accordance with well developed theories of enzyme inhibition. They may compete with the substrate for a site on the enzyme or they may combine with the enzyme at points other than the active site If polyelectrolytes do function as enzyme inhibitors, the data obtained in the present study tend to indicate that the inhibition is reversible.

E. A possible mechanism for mechanical interference with bacterial growth by polyelectrolytes

The retardation of growth and substrate removal in the presence of polyelectrolyte may be due totally to the fact that the cells are flocculated. If the cells are clumped, only those on the outer surface may have optimum opportunity for contact with the substrate. Even those cells would have less opportunity to contact substrate than dispersed cells since only a portion of their surface is exposed to the medium.

Such a hindrance to free access to the substrate could increase the doubling time. Upon dividing the daughter cells may be released from the floc and in time the growth rate may increase. The ability of the cells to break away from the floc particle would depend upon the flocculating power of the polyelectrolyte and its concentration in the system.

F. Evaluation of the use of polyelectrolytes in activated sludge plants

While it is true that some of the polyelectrolytes studied exhibited a high degree of effectiveness in flocculating bacterial cells and would, in all likelihood, also aid in coagulating suspended solids in the primary settling tank, it must be emphasized that the primary function of the activated sludge process is the removal of soluble organic matter. In order to fulfill this function the microorganisms must be capable of metabolizing these soluble organic compounds, and it is necessary that any standard for evaluation of the use of polyelectrolytes include their effect on the biochemical efficiency of the system. In addition, it should be noted that even if the polyelectrolyte is a good coagulating agent and does not interfere with metabolism, the floc which is produced may sometimes be a light fluffy one which does not settle readily.

In the present study, it was seen that some of the effects which polyelectrolyte coagulant aids could bring about in the aeration tank included loss of biological solids, apparently due to cell lysis, and a general retardation of the rate of purification. Both of these effects could constitute serious impairment of system efficiency and warrant a considerable amount of further study. In the present study, the polyelectrolytes were added using experimental procedures which could be termed shock loading conditions. It is possible that after prolonged acclimation some of the deleterious effects noted in the present study would be abated.

Another aspect of the use of polyelectrolytes which should be considered is their possible effect on sludge digestion. The author has not uncovered any published reports in which this aspect has been studied, but it should be emphasized that, since much of the sludge produced in the activated sludge tank is channeled to the sludge digester, the polyelectrolyte will be present also. In the present studies which were conducted under aerobic conditions, it appeared that the system could adjust to some of the polyelectrolytes in a matter of a few hours. Since the retention time in a sludge digester is much greater than in an aerator, it might be anticipated that polyelectrolytes would not seriously hamper digester operation. However, it should be noted that while many of the biochemical reactions of aerobes and anaerobes are the same, there are important metabolic differences and one cannot extrapolate the results of an aerobic study to predict effects on anaerobic digestion. It would be of considerable interest to study the effects of polyelectrolytes on anaerobic digestion. These compounds may act as effluent sludge thickeners and thereby permit reductions in digester sludge holding capacity. Also, in view of the fact that in some plants digester mixed liquor is channeled back to the activated sludge tank, it may be possible to reuse some of the polyelectrolyte.

CHAPTER VI

CONCLUSIONS

Based upon the results of the present study, the following conclusions seem warranted:

(1) Using the same compound and substrate concentration and the same initial cell concentration, growth curves for heterogeneous population may vary. This is believed to be due to changes in the predominant bacterial species. However, in the present study, maximum cell concentrations were approximately equal regardless of difference in the shape of the growth curve.

(2) Cationic polyelectrolytes are effective in bringing about bacterial flocculation. Based upon the present study, in which only one was tested, anionic polyelectrolytes are not effective.

(3) The effectiveness of polyelectrolytes in enhancing flocculation is highly variable depending upon the nature of the cationic polyelectrolyte employed.

(4) At times, sludge bulking may develop as a result of using polyelectrolytes.

(5) No measurable auto-flocculation occurred when cells from fresh units were diluted with synthetic waste devoid of substrate, but cells from the basic unit exhibit a high degree of auto-flocculation under this condition.

(6) Since cells from the basic unit exhibited a fairly high degree of auto-flocculation, the optimum dosage of cationic polyelectrolyte, when the initial cell concentration was 285 mg/1 (70% T. at 540 mμ), was relatively low (40 mg/1 to 60 mg/1).

(7) At a cell concentration of 285 mg/l, the optimum dosage of cationic polyelectrolyte for cells from fresh units was in the range from 100 mg/l to 500 mg/l.

(8) For cells from the basic unit, more cationic polyelectrolyte was required to achieve flocculation in the endogenous phase or declining growth phase than in the log growth phase. However, for cells from fresh units the stage of growth did not make much difference in the dosage required to achieve flocculation.

(9) In general, the relative flocculation efficiency for cells from the basic unit (at constant concentrations of polyelectrolytes) were low at high cell concentrations and high at low cell concentrations; while, for cells from the fresh units, flocculation efficiency depended largely upon the particular polyelectrolytes employed.

(10) Under shock load conditions, most of the polyelectrolytes studied exhibited some deleterious effect. Polyelectrolytes may cause some degree of cell lysis.

(11) With some of the polyelectrolytes the bacterial system recovered rapidly from the initial effect, but with some there was a general retardation of substrate removal. The latter effect could be due to any of the mechanisms cited in the report, but it is believed to be due largely to the fact that the cells are forced to exist in a flocculated state.

(12) Effective polyelectrolytes were not necessarily the ones which caused an apparent lysis of the cells. But the one which showed the greatest flocculating ability were those which retarded substrate removal rate to the largest degree. These results tend to substantiate the belief that the lower rate of substrate removal in the presence of the polyelectrolyte was due to cell flocculation rather than biochemical inhibition.

(13) Since polyelectrolytes are synthetic organic compounds, they can exert a chemical oxygen demand; the COD varies for different polyelectrolytes.

(14) There was some indication that certain polyelectrolytes can be utilized as substrates; thus, they also increase the biochemical oxygen demand.

(15) Polyelectrolytes are effective aids in solid-liquid separators and can provide some sludge thickening.

CHAPTER VII

SUGGESTIONS FOR FUTURE WORK

It would be of value to make studies on the addition of polyelectrolytes to continuous flow activated sludge units. In the present study, relatively short term experiments were performed. Under prolonged steady-state operation some of the deleterious effects might be gradually lessened. On the other hand, it is possible that the retardation of substrate removal might be progressively worsened.

It would be interesting to study the use of polyelectrolyte in the biological treatment of toxic wastes. The higher degree of flocculation they bring about may provide a protective mechanism for cells within the floc particle. Also, since some toxic compounds carry negative charges, the polyelectrolyte may tie up some of the toxic compounds.

It would be of significant interest to design studies to determine if the retardation of substrate removal and sludge growth in the presence of cationic polyelectrolyte is due to biochemical inhibition or to mechanical effects brought about primarily because of the greater degree of flocculation.

BIBLIOGRAPHY

1. Mohlman, F. W., "Twenty-Five Years of Activated Sludges", Modern Sewage Disposal, 62, Federation of Sewage Works Association (1938).

2. Nemerow, N. L., The Theories and Practices of Industrial Waste Treatment, 254, McGraw-Hill Book Company, New York (1960).

3. McKinney, R. E., "Biological Flocculation", Biological Treatment of Sewage and Industrial Wastes, Edited by J. McCabe and W. W. Eckenfelder, 1, 88, Reinhold Publishing Corporation, New York (1956).

4. Theriault, E. J., and W. M. Clark, "An Experimental Study of the Relation of H+ Concentration to the Formation of Floc in Alum Solution", Public Health Reports, 38, 181 (1923).

5. Miller, L. B., "On the Composition of the Precipitate from Partly Alkalinized Solutions", Public Health Reports, 38, 1995 (1923).

6. Miller, L. B., "Adsorption by Aluminum Hydroxide Considered As a Solid Solution Phenomenon", Public Health Reports, 39, 25 (1924).

7. Miller, L. B., "A Study of the Effect of Anions upon the Properties of Alum Floc", Public Health Reports, 40, 351 (1925).

8. Miller, L. B., "Some Properties of Iron Compounds and Their Relation to Waste Clarification", Public Health Reports, 40, 1413 (1925).

9. Larson, T. E., and A. M. Buswell, "Water Softening - Sign of Charge on Colloidal Particles of Hydrous Alumina, Hydrous Magnesia and Calcium Carbonate", Industrial Engineering Chemistry, 32, 132 (1940).

10. Ruehrwein, R. A. and D. W. Ward, "Mechanism of Clay Aggregation by Polyelectrolytes", Soil Society, 73, 485 (1952).

11. Michaelis, A. S., "Aggregation of Suspension by Polyelectrolytes", Industrial Engineering Chemistry, 46, 1485 (1954).

12. Tenney, M. W., and W. Stumm, "Chemical Flocculation of Microorganism in Biological Waste Treatment", Presented at the 19th Annual Purdue Industrial Waste Conference (May, 1964).

13. Pelczar, M. J., Jr., and R. D. Reid, Microbiology, 55, McGraw-Hill Book Company, New York (1958).

14. Clifton, C. E., Introduction to Bacterial Physiology, 52, McGraw-Hill Book Company, New York (1957).

15. Knaysi, G., Elements of Bacterial Cytology, 39, Comstock Publishing Company, Cornell University (1946).

16. Pelczar, M. J., Jr., and R. D. Reid, Microbiology, 43, McGraw-Hill Book Company, New York (1958).

17. Knaysi, G., Elements of Bacterial Cytology, 48, Comstock Publishing Company, Cornell University (1946).

18. White, A., P. Handler, and E. L. Smith, Principles of Biochemistry, 106, McGraw-Hill Book Company, New York (1964).

19. White, A., P. Handler, and E. L. Smith, Principles of Biochemistry, 105, McGraw-Hill Book Company, New York (1964).

20. Morrison, R. T., and R. N. Boyd, Organic Chemistry, 486, Allyn and Bacon, Inc. (1963).

21. Conway, R. A., "Cationic Organic Coagulants in Waste Water Treatment", Water and Sewage Works, 109, 342 (Sept. 1962).

22. Borchardt, J. A., "Coagulant Aids", Water and Sewage Works, 108, R-173 (1961).

23. Black, A. P., "Basic Mechanism of Coagulation", American Water Works Association Journal, 52, 462 (1960).

24. Oliver, R. H., "Guide to the Use of Flocculants in Mineral Processing Plants", Engineering and Mining Journal, 164, 193 (June 1963).

25. "Purifloc A-21 Flocculant", Dow Chemical Company, Midland, Michigan (1964).

26. "Purifloc C-31 Flocculant", Dow Chemical Company, Midland, Michigan (1964).

27. "Hagan Coagulant Aid No. 223", Bulletin HSP-936, The Hagan Chemical & Controls, Inc., Pittsburgh, Penn.

28. "Mogul CO-982", Product Bulletin PB-904, The North American Mogul Product Company, Cleveland, Ohio.

29. "Nalco 600", Nalco Product Bulletin P2-600, The Nalco Chemical Company, Chicago, Illinois.

30. Pilivoch, J. B., A. P. Black, F. A. Eidsness, and T. W. Stearns, "Electrophoretic Studies of Water Coagulant", American Water Works Association Journal, 50, 1467 (1958).

31. Webb, F. C., Biochemical Engineering, 150, D. Van Nostrand Company, London (1964).

32. Gaudy, A. F. Jr., "Biochemical Aspects of Qualitative Shock Loading of Aerobic Waste Treatment Systems", Doctoral Thesis, University of Illinois, Urbana, Illinois (1959).

33. Hess, B., "Control of Metabolic Rate", Control Mechanisms in Respiration and Fermentation, Edited by B. Wright, 323, The Ronald Press Company, New York (1961).

34. Day, R. F., "Use of Organic Polymers in the Treatment of Industrial Wastes", Presented at the 12th Ontario Industrial Waste Conference, Lake of Bays, Ontario Canada (June 1965).

35. Schaffer, R. B., "Polyelectrolytes in Industrial Waste Treatment", Water and Sewage Works 111, R-300 (1964).

36. Kalchalsky, A., "Polyelectrolytes", Endeavour, 12, 90 (1953).

37. Ives, K. J., "Electrokinetic Phenomena of Planktonic Algae", Proceedings of the Society for Water Treatment and Examination, 5, 41 (1956).

38. Cohen, J. M., G. A. Rourke, and R. L. Woodward, "Natural and Synthetic Polyelectrolyte as Coagulant Aids", American Water Works Association Journal, 50, 463 (1958).

39. Singer, P. C., W. O. Pipes, and E. R. Hermann, "Coagulation of Bulked Activated Sludge Using Polyelectrolytes", Proceedings of the Fifth Industrial Water & Waste Conference, Texas Water Pollution Control Association, Dallas, Texas (June 1965).

40. Walker, J. F. H., and J. H. Dougherty, "Use of Polyelectrolyte Coagulants to Enhance Settling Characteristics of Activated Sludge", Presented at Purdue University, 20th Annual Industrial Waste Conference (May 1965).

41. "Sludge Conditioning with Purifloc C-31", Dow Chemical Company, Midland, Michigan (1964).

42. Standard Methods for the Examination of Water and Waste Water, 11th Edition, American Public Health Association, New York (1960).

43. Sawyer, C. N., Chemistry for Sanitary Engineers, 258, McGraw-Hill Book Company, New York (1960).

44. Moore, W. A., and C. C. Ruchhoft, "Chemical Oxygen Consumed and its Relationship to BOD", Sewage and Industrial Wastes, 23, 705 (1951).

45. Gaudy, A. F. Jr., "Colorimetric Methods for the Determination of Protein and Carbohydrate Content of Biological Sludges", Industrial Water and Wastes, 7, 17 (1962).

46. "Glucostat for the Enzymatic Determination of Glucose", Worthington Biochemical Corporation, Freehold, New Jersey (1963).

47. Jaeger, K., and W. Niemitz, "BOD Determination of Sewage by the Direct Method Using a Warburg Apparatus", Sewage and Industrial Wastes, 25, 631 (1953).

48. Umbreit, W. W., R. H. Burris, and J. F. Stauffer, Manometric Techniques, Burgess Publishing Co., Minneapolis, Minnesota (1957).

49. Porter, J. R., Bacterial Chemistry and Physiology, 120, John Wiley & Sons, New York; Chapman & Hall, London (1946).

50. Porter, J. R., Bacterial Chemistry and Physiology, 19, John Wiley & Sons, New York; Chapman & Hall, London (1946).

51. Porter, J. R., Bacterial Chemistry and Physiology, 27, John Wiley & Sons, New York; Chapman & Hall, London (1946).

52. Operation Directions for the Model 6-D Coleman Junior Spectrophotometer, Coleman Instrument Inc., Maywood, Illinois, U.S.A.

53. Daniels, F. and R. A. Alberity, Physical Chemistry, 573, John Wiley and Sons, Inc., New York (1963).

54. Thimann, K. V., The Life of Bacteria, p. 112-115, The Macmillan Company, New York; Collier-Macmillan Limited, London (1964).

55. Herbert, D., R. Elsworth, and K. C. Telling, "The Continuous Culture of Bacteria - A Theoretical and Experimental Study", Journal General Microbiology 14, 601 (1950).

VITA

Ta-shon Yu

Candidate for the Degree of

Master of Science

Thesis: EFFECT OF POLYELECTROLYTE COAGULANT AIDS ON BACTERIAL CELLS

Major Field: Sanitary Engineering and Public Health (School of Civil Engineering)

Biographical:

Personal Data: Born in Chang-Hwa, Taiwan, the Republic of China, October 16, 1940, the son of Hsi-tsung Yu and Shaing Lee.

Education: Attended Taiwan Provincial Cheng-Kung Middle School in Taipei, Taiwan, the Republic of China; received the degree of Bachelor of Science from Taiwan Provincial Cheng Kung University, Tainan, Taiwan, the Republic of China, in June, 1963.

Professional Experience: Served in Chinese Navy as Civil Engineering Officer from July 1963 to July 1964.