

RESEARCH PROJECT TECHNICAL COMPLETION REPORT
OWRR PROJECT A-035-OKLA

METABOLISM OF COMPONENTS OF EXTENDED AERATION
ACTIVATED SLUDGE

Submitted to

The Oklahoma Water Resources Research Institute
Oklahoma State University
Stillwater, Oklahoma

By

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A-035, WRRRI-OKLAHOMA, OWRRRI-USDI
(July 1, 1971 - June 30, 1974)

PREFACE

In accord with the joint goals of the Oklahoma Water Resources Research Institute, the Center for Water Research in Engineering, and the Bioengineering and Water Resources program of the School of Civil Engineering of the Oklahoma State University, this research project was designed with the dual aim of accomplishing useful research and training graduate student researchers in important areas pertinent to the development and use of the nation's water resources.

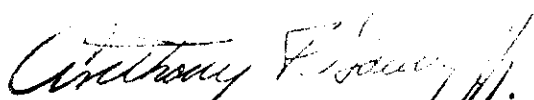
The work has allowed the principal investigator to gain needed information on metabolism of components of extended aeration activated sludge, with particular regard to the utilization of extracellular polysaccharide as a source of carbon for heterogeneous populations, and on the applicability of the "hydrolytic assist" process for control of aeration solids concentration in an extended aeration activated sludge. The conduct of this research project has also allowed examination of the maintenance concept in relation to its utility in a kinetic model for the quantitative description of the performance of activated sludge processes in general.

The work has also contributed to the training of future researchers and investigative engineers. Although not all of the students listed below received financial support from the conduct of this

project, they were in one way or another benefited by its conduct since their thesis investigations had either direct or indirect bearing on the work of this project. PhD students who benefited due to the conduct of this project are Dr. Ping Yi Yang (PhD 1972), Dr. Alan W. Obayashi (PhD 1972), Dr. David Scott, Jr. (PhD 1973), and Mr. R. Shrinivasaraghavan (current PhD candidate). MS students who benefited from the conduct of the work are: James W. Godlove (MS 1973), K. S. N. Murthy (MS 1974), Homayoon Saidi (MS 1974).

The accompanying document represents a detailed report on the completion of the project, and embodies pertinent information suggested in the OWRI "Reporting Guidelines." It consists of an abstract, general conclusions, summary report, and six appendices which serve as the sources of detailed information. The appendices are reprints of papers describing work partially supported by this project.

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ABSTRACT

This project was undertaken to investigate the use of biological cells and/or their individual cell components as substrates by other microorganisms. The utilization of microorganisms as substrates concurrently with performance of their functions as substrate users is the basic concept upon which the extended aeration or total oxidation process is based. From results of a previous project (A-017) it had been concluded that the concept was sound. This conclusion was in opposition to reports of past researchers in the field who had concluded that the extracellular polysaccharide material surrounding some bacteria was a major inert fraction of biological sludges. Thus, in the present study, this aspect was investigated directly by growing, in pure culture, cells which produced copious amounts of extracellular polysaccharide, harvesting polysaccharide and feeding it as sole source of carbon to heterogeneous microbial populations of sewage origin. It was found that, after a period of acclimation, heterogeneous microbial populations could be developed which metabolized this extracellular polysaccharide material very rapidly.

Also in this investigation, long-term pilot plant studies were undertaken in which the "hydrolytic assist" to an extended aeration process was examined. Briefly, the "hydrolytic assist" involves

withdrawing some cells and performing chemical hydrolysis in order to solubilize some of the macromolecules. This portion of the sludge is then recycled as essentially liquefied sludge along with intact cells. Thus, when required, the initial attack, i.e., hydrolysis, is performed for the cells via this engineering control practice. Long-term pilot plant experiments were run to determine if this mode of operation could provide for adequate control of biological solids concentration and permit the system to retain its nitrifying characteristics as well as high substrate removal capability. The pilot plant studies indicated that such a process was indeed feasible. In these studies, aspects of cell maintenance were examined, using data from continuous pilot plant studies. It was found that model equations for biological solids concentration and effluent substrate concentration would be improved with respect to prediction of excess biological sludge or cell output by the inclusion of a term for cell maintenance.

KEY WORDS

total oxidation - model equations for activated sludge - chemical hydrolysis - maintenance energy coefficient - metabolism of extracellular polysaccharide.

INTRODUCTION

This research project was initiated for the primary purpose of investigating the metabolism of components of extended aeration activated sludge, and this purpose was accomplished in three general lines of investigation.

First, in a previous project of the principal investigator it had been established that the concept of total oxidation of biological cell material, which is the basis for the extended aeration activated sludge process, was not theoretically unsound as had been concluded by other researchers. One important cellular constituent which had been cited by other workers as being biologically inert, i.e., incapable of being used as a substrate by microorganisms, was the extracellular polysaccharide slime layer of bacteria. This aspect was investigated and is reported in detail in Appendix I.

Secondly, an important aspect regarding the metabolism of components of activated sludge is the degree to which biological autolysis proceeds concurrently with removal of exogenous substrate. This aspect bears on the so-called maintenance requirement of microorganisms, and since it was of significant interest in the present project and had an important relationship to previous research of the principal investigator on a model for the design and operation of the activated sludge process, work on this aspect was included in the present investigation. These findings are included in Appendices II, III, and IV of this report.

Thirdly, a major purpose of this investigation was to study the

feasibility of engineering control of metabolism of components of extended aeration activated sludge through use of the "hydrolytic assist," which is a process devised by the principal investigator based upon work done on a previous project (A-017). These findings are presented and discussed in considerable detail in Appendices V and VI.

I. METABOLISM OF EXTRACELLULAR MICROBIAL POLYSACCHARIDES

Others had concluded that there would be a gradual buildup of inert biological solids in an extended aeration process and that this would ultimately cause it to undergo biochemical failure, i.e., to reach a point at which it could no longer serve as a remover or purifier of substrates. Extracellular polysaccharide had been cited by researchers as the major inert organic constituent of biological cells. Previous studies by the principal investigator provided evidence which caused serious question regarding that conclusion. Operation of an extended aeration process over a three-year period with positive retention of all cells did not result in a buildup of carbohydrate of the sludge; however, the only direct way to refute or confirm the conclusions that had been made by others regarding the inert nature of extracellular polysaccharide was to devise a straightforward experiment in which extracellular polysaccharide was presented to a heterogeneous population of sewage origin as a sole source of carbon. Accordingly, cells known to produce copious quantities of extracellular polysaccharide were grown in pure culture. After growing the cells, they were put through various separation procedures in which the

extracellular polysaccharide was harvested and purified. These polysaccharides were then used as carbon source for an enrichment culture in which the initial seed consisted of a small sample of municipal sewage obtained from the municipal treatment plant at Stillwater, Oklahoma. After appropriate acclimation time, experiments were run to test the metabolism of the polysaccharide as carbon source. Growth on these carbon sources was determined by biological solids concentration and measurement of the protein and carbohydrate content of the cells. Substrate removal was measured by the chemical oxygen demand (COD) test, and by tests specific for the polysaccharides.

In these studies, the sources of extracellular polysaccharide were the following organisms: Aerobacter aerogenes, Arthrobacter viscosus, Azotobacter vinelandii, Xanthomonas campestris, and Zoogloea ramigera. Typical metabolic patterns for the metabolism of each of these carbohydrates is shown in Figures 1 through 5 in Appendix I. It is obvious from these results that extracellular polysaccharide provides an excellent substrate for heterogeneous populations and it may therefore be concluded that extracellular bacterial polysaccharide cannot be classified as biologically inert material.

II. CONTROL OF BIOLOGICAL GROWTH IN ACTIVATED SLUDGE PROCESSES AND EFFECTS OF CELL MAINTENANCE

In order to provide useful guidelines for design and operation of either extended aeration activated sludge processes or for any activated sludge process, it is important to describe the kinetics of the process with sufficiently quantitative and accurate model equations to

predict the behavior of the system under various conditions of operation. Such a model had been devised by the principal investigator and his student co-workers, and it was important to provide an experimental test of the model and to determine if the model equations should be modified to include a factor for autodigestive metabolism of components of activated sludge.

Laboratory pilot plant experimentation was undertaken in order to determine whether a "steady state" in aerator biological solids concentration and effluent substrate concentration could be approached using the model we had devised. A flow diagram for the model is shown in Figure 1 of Appendix II, and a diagram of the pilot plant is shown in Figure 2. Studies were performed at various organic feed concentrations holding the dilution rate at 0.125 hr^{-1} , the hydraulic recycle ratio at 0.25, and the recycle sludge concentration at 10,000 mg/l. Also, values of maximum specific growth rate and saturation constant were determined. Sample results are shown in Figures 3, 4, and 5 (Appendix II), and it is seen that this mode of operation permitted a rather close approach to a steady state condition for these heterogeneous populations. The model equations are shown in Table I, and they are compared with those of Herbert for a pure culture of bacteria. We had found in previous experimentation that the model of Herbert did not approximate a steady state condition for heterogeneous populations. It may be seen that the model with constant sludge recycle concentration does not include a term for the maintenance or autodigestive coefficient. However, we did devise steady state equations which did include this term. These equations are given in Table V, Appendix III;

the maintenance coefficient is designated as k_d , and it is seen that the extra biological constant does not overly complicate the equations.

It was important to determine whether the inclusion of this term made a significant improvement in the model equations. The determination of the true yield coefficient, Y_t , and the maintenance coefficient, k_d , from the experimental pilot plant data is shown in Figure 6, Appendix III. Using this experimental value as well as experimentally determined values for the other biological constants and comparably selected engineering parameters for both models, the values were substituted into the appropriate equations to determine whether better predictions of the observed biological solids and effluent substrate concentrations as well as cell output could be made with or without inclusion of the cell maintenance factor. The general finding was that the inclusion of the term for autodigestion made little difference in the accuracy of the prediction of residual substrate or biological solids concentration over a rather wide range of specific growth rates. However, the prediction of excess sludge, X_w (i.e., cell output), was considerably enhanced, especially at the low growth rates or high cell retention times, by inclusion of the cell maintenance factor. It was concluded that either set of equations could be employed, but in the interest of refining the prediction of X_w , those including the maintenance coefficient were to be recommended.

The fact that the observed cell yield decreases when cells are grown at decreasing specific growth rates in continuous reactors is usually taken as a manifestation of the cell maintenance energy concept. While in the present investigations it was found that the

observed cell yield decreased as specific growth rate decreased (see Figure 6, Appendix III), we also obtained data which we have concluded is inconsistent with the cell maintenance concept. When cells were taken from the reactor during operation at each value of specific growth rate and employed for determination of cell yield in batch growth at substrate concentrations which allowed much higher values of specific growth rate (those near the maximum specific growth rate attainable), the observed cell yield was found to be the same as that obtained at the lower specific growth rates in the continuous flow system from which the cells were harvested. These results are presented and their important basic and applied ramifications discussed in Appendix IV. Work on these aspects as well as other important avenues of investigation opened up through this research are continuing even though the project support has terminated.

III. USE OF THE "HYDROLYTIC ASSIST" FOR CONTROL OF THE EXTENDED AERATION ACTIVATED SLUDGE PROCESS

Although we were able to prove without fear of contradiction in a previous project that the theory or basic concept of total oxidation as embodied in the extended aeration process was theoretically sound, since biologically inert organic matter from previously synthesized cells did not build up in the system, there were times when the biological solids concentration approached very high values, thus causing sedimentation problems in the clarifier. The so-called "hydrolytic assist" was devised in order to initiate chemically a process which is rather difficult to initiate biologically; namely, the initial attack

on, or hydrolysis of, macromolecules.

A diagram of an extended aeration activated sludge process incorporating the "hydrolytic assist" is shown in Figure 1, Appendix V. Operational data for the pilot plant during the first 200 days of operation are shown in Figures 7 and 8, Appendix V, and the nitrifying characteristics of the system during this period of operation are shown in Figure 9. Operational data over the next 200 days of operation as well as nitrifying characteristics during the total period of operation are shown in Figures 4 and 6, and Figure 5, respectively.

Results obtained in these studies indicate that an extended aeration activated sludge process with total sludge recycle can be operated successfully, i.e., can provide a high degree of treatment, using the "hydrolytic assist." Furthermore, the hydrolytic assist does accomplish its primary objective of providing a means of controlling the biological solids concentration in the system. Also, it is apparent from these results that the desirable nitrification characteristics of an extended aeration process are in no way hampered through use of the hydrolytic assist. Also, as a result of these studies, it can be concluded that the system showed a rather good ability to accept shock loadings. The sludge carbohydrate and protein content were always within ranges indicative of a metabolically-active biomass, and the endogenous oxygen uptake values, or unit respiration activity of the sludge, were slightly higher than the values obtained using total cell recycle systems without the hydrolytic assist.

CONCLUSIONS

The following general conclusions may be made:

1. Direct evidence is provided enabling the unequivocal conclusion that extracellular polysaccharide of microorganisms is not a biologically inert material, as had been concluded by other workers in the field. Results shown in Appendix I clearly indicate that the extracellular polysaccharides of five organisms selected at random were rapidly metabolized after a heterogeneous population of sewage origin had been acclimated to these materials as sole source of carbon.

2. The model system equations for activated sludge processes devised by Ramanathan and Gaudy and by Shrinivasaraghavan and Gaudy provide excellent prediction of the observed values of biological solids concentration and effluent substrate concentration for continuous growth of heterogeneous populations (i.e., activated sludges) (see Appendices II, III, and IV). The equations of Shrinivasaraghavan and Gaudy provide better prediction of excess sludge production particularly at low specific growth rates.

3. An extended aeration activated sludge process with total sludge recycle can be operated successfully using the "hydrolytic assist" to control the mixed liquor biological solids concentration. Under this mode of operation, the system showed a rather good ability to accept shock loading. The "hydrolytic assist" did not hamper the nitrification characteristics of the extended aeration process (see Appendices V and VI).

LIST OF PUBLICATIONS

1. Obayashi, Alan, W., and Gaudy, Anthony F. Jr., "Aerobic Digestion of Extracellular Microbial Polysaccharides." Journal Water Pollution Control Federation, 45, 1584-1594 (1973).
2. Gaudy, A. F. Jr., and Srinivasaraghavan, R., "Experimental Studies on a Kinetic Model for Design and Operation of Activated Sludge Processes." Presented at the 166th ACS National Meeting, Chicago, Ill., August 26-31 (1973).
3. Srinivasaraghavan, R., and Gaudy, A. F. Jr., "Operational Performance of an Activated Sludge Process With Constant Sludge Feedback." Proceedings, Purdue University, Lafayette, Indiana, May 7-9 (1974).
4. Gaudy, A. F. Jr., and Srinivasaraghavan, R., "Effect of Specific Growth Rate on Biomass Yield of Heterogeneous Populations Growing in Both Continuous and Batch Systems." Biotechnology and Bioengineering, XVI, 423-427 (1974).
5. Yang, P. Y., and Gaudy, A. F. Jr., "Control of Biological Solids Concentration in the Extended Aeration Process." Journal Water Pollution Control Federation, 46, No. 3, 543-553 (1974).
6. Yang, P. Y., and Gaudy, A. F. Jr., "Nitrogen Metabolism in Extended Aeration Processes Operated With and Without Hydrolytic Pretreatment of Portions of the Sludge." Biotechnology and Bioengineering, XVI, 1-20 (1974).

APPENDIX I

Obayashi, Alan W., and Gaudy, Anthony F. Jr., "Aerobic
Disgestion of Extracellular Microbial Polysaccharides."
Journal Water Pollution Control Federation, 45, 1584-
1594 (1973)

AEROBIC DIGESTION OF EXTRACELLULAR MICROBIAL POLYSACCHARIDES

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INTRODUCTION

The extended aeration, or total oxidation, process differs from conventional activated sludge processes because it is operated with total sludge recycle, and a longer detention time is provided to allow for autoxidation of biological solids. The theoretical premise upon which the operational concept is based is that the increase in biological solids resulting from metabolism of the incoming waste is balanced by the decrease in biological solids due to their aerobic digestion. The theory requires that all organic constituents of the cell, i.e., those in the cytoplasm, the walls and membrane, and the capsular slime layer, can be metabolized and thus converted to CO₂. It is a concept which has been the subject of much controversy in the water pollution control field during the past two decades.

Porges, et al. (1), on the basis of laboratory studies on the biological treatment of skim milk waste, theorized that total oxidation

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was possible under the proper conditions. The theory was further supported by Forney and Kountz (2), who also concluded that total oxidation was possible. Later, however, Kountz and Forney (3) as well as other researchers (4)(5)(6) concluded that the concept of total oxidation was theoretically unsound. Symons and McKinney (4) studied batch-fed systems in which nitrogen was limiting and theorized that total oxidation was not possible because of a buildup of extracellular polysaccharides which they had noted by microscopic observation of sludge stained with Alcian blue. They pointed out that extracellular polysaccharide often accumulated as a waste product of internal metabolism and that, being a waste product, it would not be subject to further biological action, i.e., it would form an inert portion of the sludge. Washington and Symons (6) also reasoned that the so-called "inert" material which would accumulate in an extended aeration unit would be "the remains of cell capsular and external slime, for it is this material which is least degradable by the organisms themselves." Busch and Myrick (5) also concluded from their continuous flow pilot plant studies that total oxidation was impossible, and they noted that at times appreciable amounts of biological solids were present in the effluent.

On the other hand, Washington, et al. (7) reported a long-term adaptation of microorganisms to an accumulated sludge mass. In their studies, batch reactors were operated for one year with no sludge wasting. Under these conditions, the biological solids did not build up continually, as predicted by other researchers (3)(4)(5)(6). The biological solids level showed a cyclic trend, i.e., a period of increase in solids followed by a decrease in solids. Although positive retention of all biological solids was not provided for in the experimental apparatus, the decrease in solids cannot be attributed to loss

of solids in the effluent, since it was possible for them to make the observation that "there was essentially no loss of volatile solids in the effluent."

Recently, a long-term study in which return of all biological solids was positively controlled has been reported by Gaudy, et al. (8) (9). The experimental apparatus consisted of a continuously fed, completely mixed laboratory extended aeration plant in which the biological solids which did not settle out in the clarifier chamber were returned daily to the unit through use of a Sharples centrifuge. The three-year study demonstrated conclusively that the total oxidation theory was not unsound, and that biological solids would not continually build up in a total oxidation unit. In addition, evidence of total oxidation in closed (batch) systems has been presented by Thabaraj and Gaudy (10). Also, recent studies on the degradation of sludges developed on acetic acid have indicated the occurrence of essentially total oxidation of the sludge (11).

The recent studies cited above do much to remove the onus of theoretical unsoundness which has been attached to the extended aeration process, and the recently proposed modification employing the "hydrolytic assist" (9) offers a possible means of gaining engineering control of biological solids concentration in the system. However, because of the essential importance of the treatment process and the need for greater insight into the nature of the carbon cycle, there is ample reason for undertaking basic studies into the utilizability of microbial cells as carbon source (substrate) for other microbial cells.

One can distinguish three major physical categories of cell substance--the cytoplasm, containing soluble enzymes, nucleic acids, storage products, etc., the cell membrane-wall complex, and the cell capsule

or slime layer. While the work cited above (8)(9) demonstrated rather conclusively that microbial cells, in toto, do serve as usable substrate, little is known regarding the relative availability of the various cell components. Previous work by the authors (9) has shown that the cytoplasmic contents (released upon physical breakage of the cell wall by sonication) serve as a usable source of carbon. Only scant information is available on the complete metabolism of cell walls, although a number of enzymes which break specific bonds have been studied. Isolation of a culture growing in a medium in which cell walls composed the carbon source has been reported, but the rate of growth and extent of degradation of the cell wall material were not determined (12).

Only slightly more information is available regarding metabolism of the extracellular capsule or slime layer, which is the microbial constituent about which much of the controversy regarding the concept of total oxidation of the bio-mass revolves. Although the observation of an increase in extracellular polysaccharide made by Symons and McKinney was essentially a qualitative estimate, it seems a plausible result since, under nitrogen-deficient conditions, organisms which are capable of producing extracellular polysaccharide can be expected to make larger amounts of it (13). Also, cell replication can be expected to be retarded because of the nitrogen limitation and the sludge which accumulates in the system under these conditions could contain a rather substantial polysaccharide fraction. Symons and McKinney found that in the system they studied, which was nitrogen-deficient, extracellular polysaccharide did build up. According to Wilkinson (13), organisms which produce extracellular polysaccharide do not make use of this carbohydrate, i.e., it is not a substrate for the organism which produced it. Thus, if this material were to be used, it would have to become a

substrate for other cells in the ecosystem. Since the slime layer usually consists of a complex heteropolysaccharide, the metabolism of such substances would be expected to require synthesis of a considerable complement of inducible enzymes. Since de novo synthesis of enzyme systems requires a nitrogen source, acclimation to the heteropolysaccharide slime layer would be severely hampered in nitrogen-deficient systems. Therefore, the results of Symons and McKinney would not be entirely unexpected for the type of system they investigated. The problem arises, not so much with the reasonableness of their results, but with the extrapolation of the results for their specific system and the applicability of these results as a test of the total oxidation process. Quite apparently, their results are not generally applicable, since in a study of much longer duration, the data of Gaudy, et al. (8)(9) indicated no buildup of carbohydrate material of any sort (either intra- or extracellular).

However, the only direct way to determine whether extracellular bacterial polysaccharide can be utilized as substrate is to isolate the material and feed it as sole source of carbon to microorganisms. A review of the literature reveals that only scant work has been accomplished regarding the biodegradability of microbial capsules or slimes. Work which has been accomplished relates to isolation of pure cultures growing on specific microbial slime (12)(14)(15). However, the extent and rate of degradation were not determined and, other than providing some indication that cells can grow on extracellular polysaccharide, the work is of little direct aid in predicting whether a sludge can be totally oxidized. Therefore, in order to make a direct determination of the availability and extent of utility of extracellular polysaccharides of bacteria as microbial substrates, the work herein described

was undertaken.

The general approach was to secure organisms known to be rather widely distributed in nature and to produce copious amounts of extracellular heteropolysaccharide material, then to grow these cultures, separate the capsular material from them, harvest it, and determine whether it could serve as sole source of carbon for a natural (heterogeneous) microbial population of municipal sewage origin. If an acclimated microbial population could be developed on the heteropolysaccharide, it was intended to employ a portion of it in detailed batch experiments to determine the course and extent of growth and substrate removal.

Information on the chemical composition and structure of the heteropolysaccharide materials used in this study is by no means complete, but data which are available are given below.

The capsule produced by a strain of Aerobacter (Klebsiella) aerogenes has been chemically and structurally characterized by Sutherland and Wilkinson (16). The polysaccharide consists of glucose, glucuronic acid, fucose, and acetate in the ratio of 4:2:2:1, respectively.

The slime produced by Arthrobacter viscosus has been characterized chemically but not structurally (17). The slime consists of equimolar amounts of glucose, galactose, and mannuronic acid. It was also pointed out that the finding of mannuronic acid in A. viscosus is a rare occurrence, and that this is the only known microbial polymer from a nonpathogenic organism which contains mannuronic acid. Twenty-five percent of the polymer also consists of O-acetyl groups, probably attached to one or all of the three structural components.

The polysaccharide of the Azotobacter vinelandii strain used in

this study has been characterized by Cohen and Johnstone (18), and consists of galacturonic acid, D-glucose, rhamnose, and a hexuronic acid lactone, probably mannuronolactone.

The capsular material of Xanthomonas campestris is the most well-characterized structurally of the five slimes, and consists of D-glucose, D-mannose, and D-glucuronic acid in a ratio of 3:3:2, with 4.7 percent of the total weight appearing as O-acetyl groups and 3 to 3.5 percent of the total weight as pyruvic acid, which is attached to glucose (19). The O-acetyl group can be linked to any one of the three sugar residues. The three sugar residues are arranged in a repeating unit consisting of fifteen residues, with a variety of glycosidic bonds linking the monomers. The types of glycosidic bonds present include some unusual ones such as β -1, 2, or α -1, 2 or 3, as well as more common bonds such as β -1, 4 (as in cellulose) and α -1, 4 (as in starch). Needless to say, the slime of X. campestris is quite complex, and the enzyme regime needed to degrade this polysaccharide would also be complex.

The polysaccharide of the Zoogloea ramigera strain used in this study appears to be the least complicated of the five slimes used. According to Parsons and Dugan (20), the polysaccharide consists of only galactose and glucose in an unknown ratio; however, it was also found (21) that it was susceptible to attack by cellulase, indicating that β -1, 4 glycosidic bonds are present.

MATERIALS AND METHODS

Cultures Used as Sources of Polysaccharides

The sources of the five microorganisms used to produce extracellular heteropolysaccharides in this study were as follows: Aerobacter aerogenes, Strain TW, was obtained from Dr. C. H. Wu, Department

of Microbiology, Oklahoma State University. Azotobacter vinelandii, strain #155 (ATCC 12518), was obtained from the Department of Agricultural Biochemistry, University of Vermont. Arthrobacter viscosus, NRRL B-1973, and Xanthomonas campestris, NRRL B-1459, were obtained from the U. S. Agricultural Research Laboratory, Peoria, Illinois. Zoogloea ramigera, ATCC 25935, was obtained from the American Type Culture Collection.

A. aerogenes, Arth. viscosus, and X. campestris were maintained on Difco nutrient agar slants. Azoto. vinelandii was maintained on agar slants of Burk's nitrogen-free medium (22), and Z. ramigera was maintained on Difco nutrient agar supplemented with 0.2 percent Difco yeast extract and 0.2 percent glucose.

Preparation of Microbial Polysaccharides

The methods used to obtain each of the polysaccharides in a relatively pure form were similar. Initially, a 50-ml liquid culture was prepared as inoculum for a large-scale culture. To initiate growth, a loopful of cells (obtained from a stock slant) was inoculated into a 250-ml cotton-stoppered Erlenmeyer flask containing 50 ml of sterile medium. The medium used depended upon the organism. Nutrient broth was employed for X. campestris and Arth. viscosus, while Z. ramigera was grown in nutrient broth supplemented with 0.2 percent yeast extract and 0.4 percent glucose. A. aerogenes was grown in the medium described by Wilkinson, et al. (23); however, for the small-scale cultures, the medium was modified to include sufficient nitrogen for maximum cell growth. The medium described by Wilkinson, et al. is a nitrogen-limiting one, found to produce a maximum amount of extracellular polysaccharide from a strain of A. aerogenes. Azoto. vinelandii was grown

in Burk's nitrogen-free broth. All flasks were shaken at 120 cycles/min at room temperature ($25^{\circ} \pm 2^{\circ}\text{C}$). Arth. viscosus, X. campestris, and A. aerogenes required one day of shaking before being transferred, while Azoto. vinelandii required two days and Z. ramigera needed four days before sufficient growth developed.

All flasks except those containing A. aerogenes were used to inoculate 4-liter glass kettles containing three liters of sterile medium. Since A. aerogenes gave lower yields of polysaccharide, larger volumes were required. The 50-ml culture of A. aerogenes was transferred to a 2-liter glass kettle containing one liter of the same growth medium used for the initial culture. After one day of growth with sterile air being supplied at a rate of 3 liter/min/liter, the mixed liquor was transferred to a 20-liter Pyrex jug containing the sterile medium described by Wilkinson, et al. (23). Sterile air was supplied to the 4-liter glass-covered kettles and the 20-liter jug through diffuser stones at a rate of 3 liter/min/liter. Incubation was at room temperature ($25 \pm 2^{\circ}\text{C}$). The medium used for large-scale culture of the other four organisms varied. The medium used for Azoto. vinelandii was Burk's nitrogen-free medium, while the medium used for X. campestris was that described by Lilly, et al. (24). The medium of Cadmus, et al. (17) was used for Arth. viscosus, and Z. ramigera was grown in the medium described by Parsons and Dugan (20). Incubation time prior to harvesting the cells varied somewhat, and was as follows: A. aerogenes, 3 days; Arth. viscosus, 3 days; Azoto. vinelandii, 4 days; X. campestris, 5 days; and Z. ramigera, 10 days.

After the aeration period, cells and polysaccharide were harvested. Arth. viscosus, Azoto. vinelandii, and X. campestris produced loose polysaccharide slimes which were easily separated from the cells

by centrifugation. The viscous supernatants containing polysaccharides were then poured off, and the cells were discarded. The extracellular polysaccharides of A. aerogenes and Z. ramigera were capsular slimes and required chemical treatment to remove the capsule from the cell. An alkaline treatment developed by Dudman and Wilkinson (25) was used to remove the capsule from A. aerogenes, while an alkaline method described by Parsons and Dugan (20) was used to remove that of Z. ramigera.

After the capsules were removed, the polysaccharides were separated from the cells by centrifugation, as previously described. All polysaccharides were precipitated with acetone, and air-dried. All of the polysaccharides except that from A. aerogenes were then re-suspended in distilled water, dialyzed against distilled water for 48 hours, and finally stored at 4°C for future experiments and analyses. The polysaccharide from A. aerogenes was re-suspended in an acetate buffer solution (4 percent sodium acetate - 2 percent acetic acid) and deproteinized according to the method described by Wilkinson, et al. (23). After deproteinizing, the slime was again precipitated with acetone and air-dried. The polysaccharide was then re-suspended in distilled water, dialyzed against distilled water for 48 hours, and finally stored at 4°C.

Experimental Protocol

In experiments employing a polysaccharide as carbon source, the growth medium contained the following components: polysaccharide, 1000 mg/l as COD; $(\text{NH}_4)\text{SO}_4$, 500 mg/l; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 100 mg/l; $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 10 mg/l; CaCl_2 , 7.5 mg/l; $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 1 mg/l; 1.0 M potassium phosphate buffer, pH 7.0, 10 ml/l; tap water, 100 ml/l, and distilled water to volume.

All experiments were started by shaking 5 ml of sewage (obtained from the primary clarifier of the Stillwater municipal wastewater

treatment plant, and 45 ml of the minimal medium described above, containing one of the isolated polysaccharides, in a 250-ml flask at a rate of 120 cycles/min. Growth was obtained on each of the polysaccharides after a lag period of two to ten days. Thereafter, daily transfers were made as follows: 5 ml of the acclimated culture were added to a 250-ml flask containing 45 ml of fresh medium. Several daily transfers were made before the start of any detailed growth experiment. On the day before an experiment, the 50 ml from the shaker flask were added to a batch unit containing 100-300 ml of the polysaccharide-minimal salts medium. Air was supplied through diffuser stones. This batch unit served as seed for a growth experiment on the following day.

On the day of any given experiment, cells were harvested from the seed unit by centrifugation. After pouring off the supernatant fluid, the cells were re-suspended in a minimal salts medium devoid of polysaccharide. The experiment was then initiated by adding the cell suspension to a batch unit containing the polysaccharide in minimal medium; reaction liquor volume was 1000 ml. Filtered air was supplied through diffuser stones at a rate of 3 liter/min/liter. All experiments were run at a pH of 7.0 and a temperature of $25 \pm 2^{\circ}\text{C}$.

During the course of all experiments, a number of analyses were performed on mixed liquor samples which were periodically withdrawn from the reactor. Microbial cells were easily separated by high speed centrifugation, and the viscous supernatant containing the polysaccharide was carefully poured off and used for determination of carbohydrate, uronic acids and total COD. The cell pellet was washed from the centrifuge tube with distilled water, and biological solids were measured by passing the resulting suspension through a membrane filter (pore size 0.45μ).

Methods of Analysis

Protein was estimated using the Folin-Ciocalteu method, as described by Ramanathan, et al. (26). Carbohydrate was determined by the anthrone method, as outlined by Ramanathan, et al. (26). The total chemical oxygen demand was determined in accordance with Standard Methods (27). The carbazole reaction was used for uronic acids, as recommended by Ashwell (28). Carbohydrate COD and uronic acid COD were measured using glucose and glucuronic acid, respectively, as standards. Hence, these values are only rough estimations, since in both of these colorimetric determinations the intensity of color developed is dependent upon the specific carbohydrate or hexuronic acid present.

RESULTS

Chemical Characteristics of the Heteropolysaccharide Substrates

In Table I is shown the composition with respect to protein, carbohydrate, and hexuronic acid, of the five microbial heteropolysaccharide slime layers which were employed as substrates. The protein content provides some measure of the degree of purity of the harvested slime or capsular material. The capsule of A. aerogenes was subjected to a deproteinization step in the harvesting procedure, and it can be seen that the resulting protein content of the capsular material was extremely low. The remaining four microbial slimes were not deproteinized, since the harvesting procedure without this step yielded substrates which were of ample purity for the purpose of these experiments.

The carbohydrate and hexuronic acid contents (2nd and 3rd columns, Table I) are given as percentages of the polysaccharide COD and, for purposes of comparison, values shown in the 4th and 5th columns were estimated and recorded as predicted carbohydrate and hexuronic acid.

TABLE 1
 CHEMICAL CHARACTERISTICS OF THE FIVE BACTERIAL
 EXTRACELLULAR POLYSACCHARIDES

Source of Extracellular Polysaccharide	Protein (%)	Carbo-hydrate (%)	Hexuronic Acid (%)	Predicted Carbo-hydrate*(%)	Predicted Hexuronic Acid* (%)
<u>Aerobacter aerogenes</u>	1.0	71	27.2	75.8	24.6
<u>Arthrobacter viscosus</u>	10.0	40.0	5.8	40.0	5.1
<u>Azotobacter vinelandii</u>	5.0	17.5	20.0	-	-
<u>Xanthomonas campestris</u>	8.1	54	20.0	53.5	22.5
<u>Zoogloea ramigera</u>	1.4	85	0	-	-

* See text for explanation.

For example, one mg of galactose yields 0.54 the intensity of color developed by glucose, the standard employed in the anthrone test (26). Therefore, for a heteropolysaccharide consisting of equal portions of glucose and galactose, the carbohydrate content registered by the anthrone test (using glucose as the standard) would be 75 percent of the actual amount.

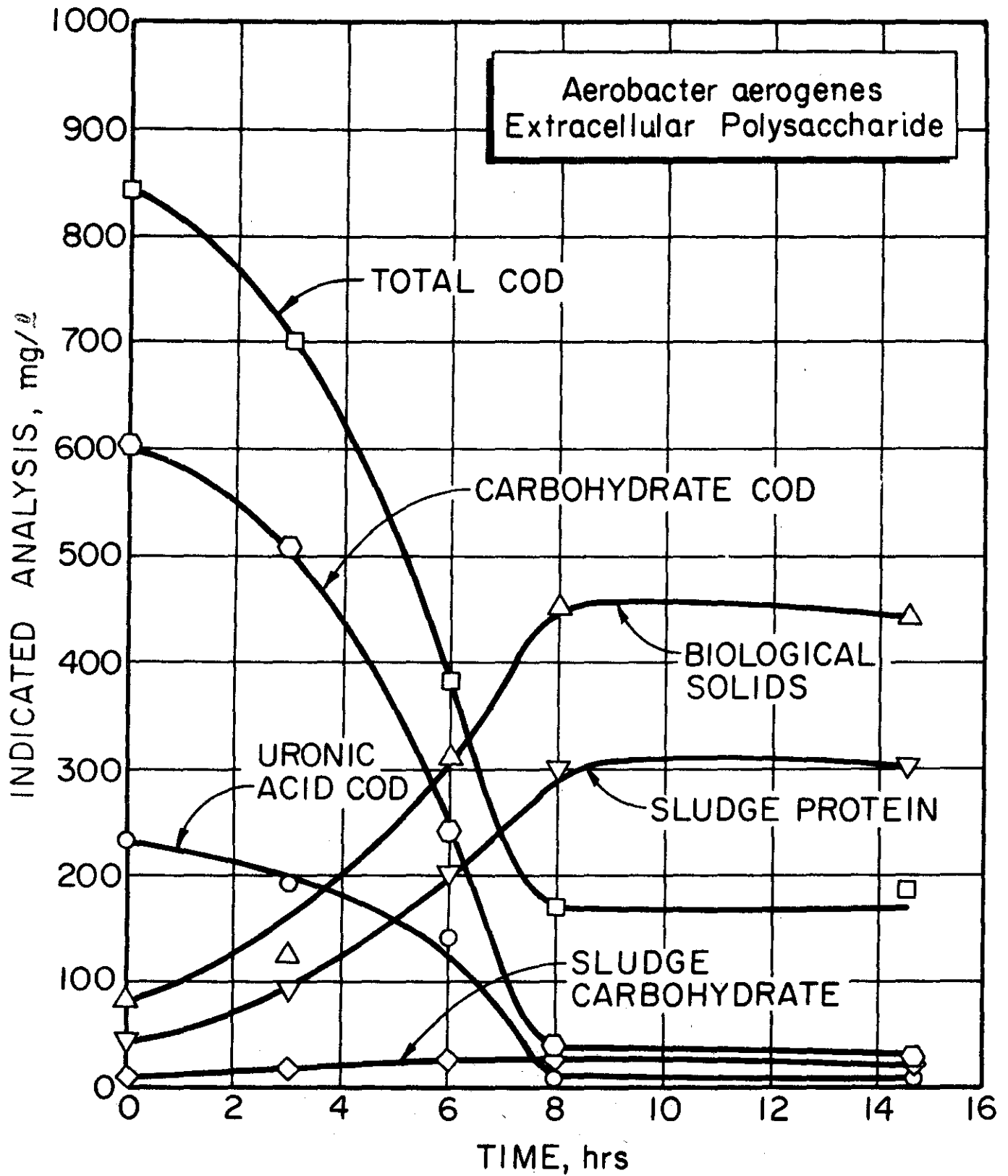
"Predicted" values for carbohydrate and hexuronic acid are based upon amounts of specific carbohydrates and hexuronic acids in these slimes, as reported in the microbiological literature. The predicted values were calculated by multiplying the percentage composition of each known component of the heteropolysaccharide by the ratio of its reactivity and the reactivity of the standard used in the analysis (glucose in the anthrone test and glucuronic acid in the carbazole test). These values were converted to equivalent COD values (e.g., one mg glucose = 1.06 mg glucose COD), and the values for the individual components were summed.

The predicted values and those found by analysis of the polysaccharides agree quite closely in the three cases for which composition has been reported in the literature. Although the strain of A. aerogenes used in the present study was obtained from a source different than that used by Sutherland and Wilkinson (16), the capsules of the two strains are apparently quite similar. The carbohydrate and hexuronic acid content found by analysis in the present study agrees closely with values calculated from the composition reported by Sutherland and Wilkinson for the strain used in their study. There is also good agreement for the slime produced by Arth. viscosus. It should be noted that, although this polysaccharide contains 25 mole percent of hexuronic acid, the content detected by the carbazole reaction and calculated as glucuronic

acid is only 5.8%. This is because mannuronic acid produces much less color in the carbazole reaction than does an equivalent amount of glucuronic acid. Calculated values for the polysaccharide of X. campestris also agreed well with those obtained by analysis of the isolated slime. No quantitative data were available in the literature for the polysaccharides of Azoto. vinelandii or Z. ramigera. The slime of Z. ramigera has been reported to contain only glucose and galactose. From the data obtained in the present study, it would appear that the two sugars are probably present in almost equal amounts since a 1:1 molar ratio of the two would yield a color intensity 75 percent of that produced by glucose alone in the anthrone reaction.

Metabolism of Heteropolysaccharide Substrates

The results obtained when extracellular heteropolysaccharide from A. aerogenes was employed as carbon source for a relatively small initial inoculum of acclimated cells obtained from municipal sewage are shown in Figure 1. These results demonstrate beyond doubt that the capsular material did serve as carbon source for growth of the microbial population. The decrease in COD and concomitant increase in biological solids and cell protein provide direct evidence that the heteropolysaccharide was metabolized by the cells for growth (protein synthesis), i.e., it was by no means biologically inert. The COD removal efficiency was approximately 80 percent. While there was a significant residual COD in this experiment, it is seen that the carbohydrate and uronic acid COD were removed to a much greater extent than the "total" COD. Initially, carbohydrate comprised 71 percent and uronic acid 27 percent of the COD, and together they accounted for 98 percent of the COD; whereas carbohydrate and uronic acid comprised, respectively, 14 and 5 percent



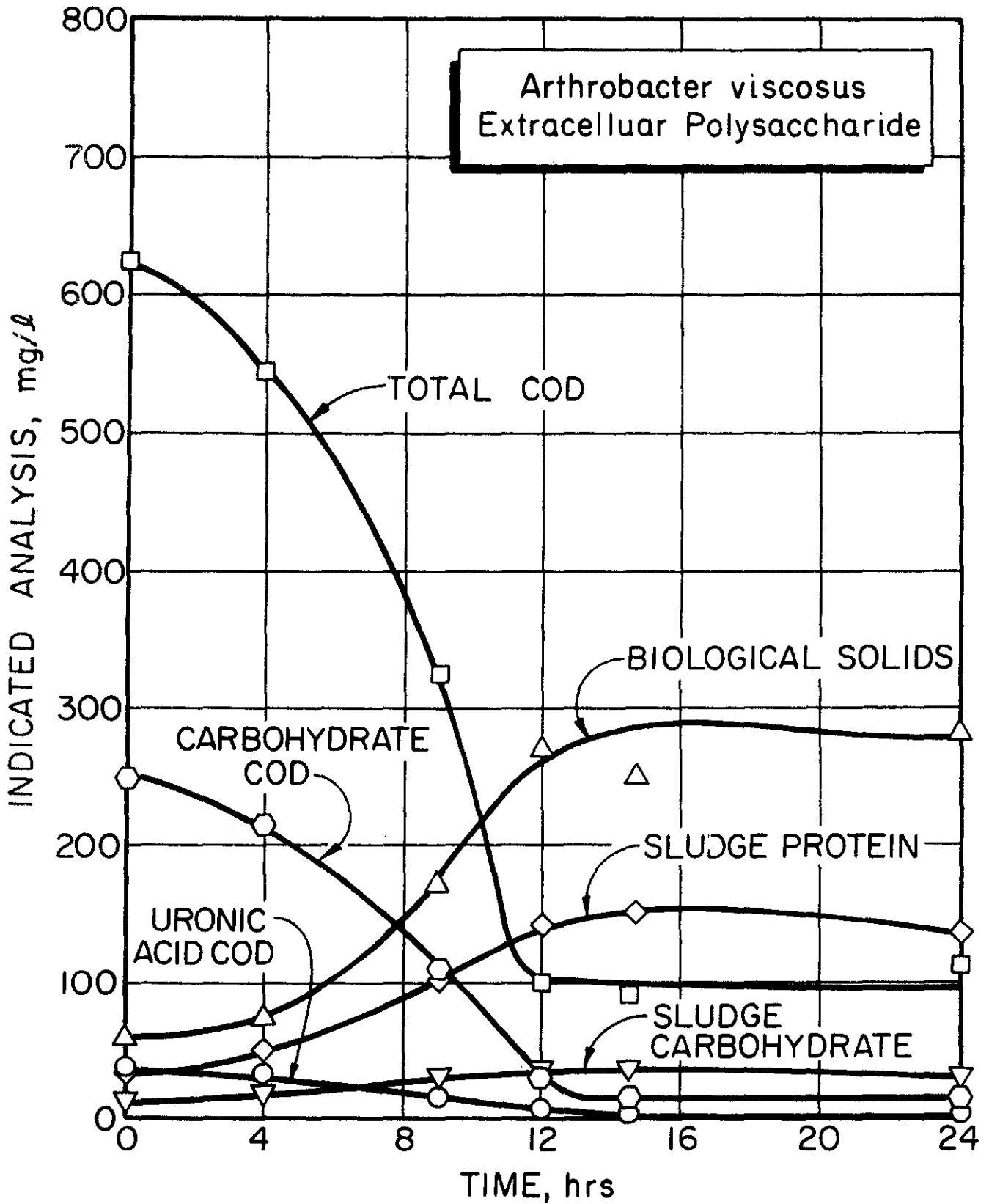
of the residual COD, together accounting for only 19 percent. Thus, there is some indication that the residual COD was comprised, in the main, of newly produced metabolic products resulting from utilization of the A. aerogenes capsular material rather than of biologically inert fragments of the heteropolysaccharide.

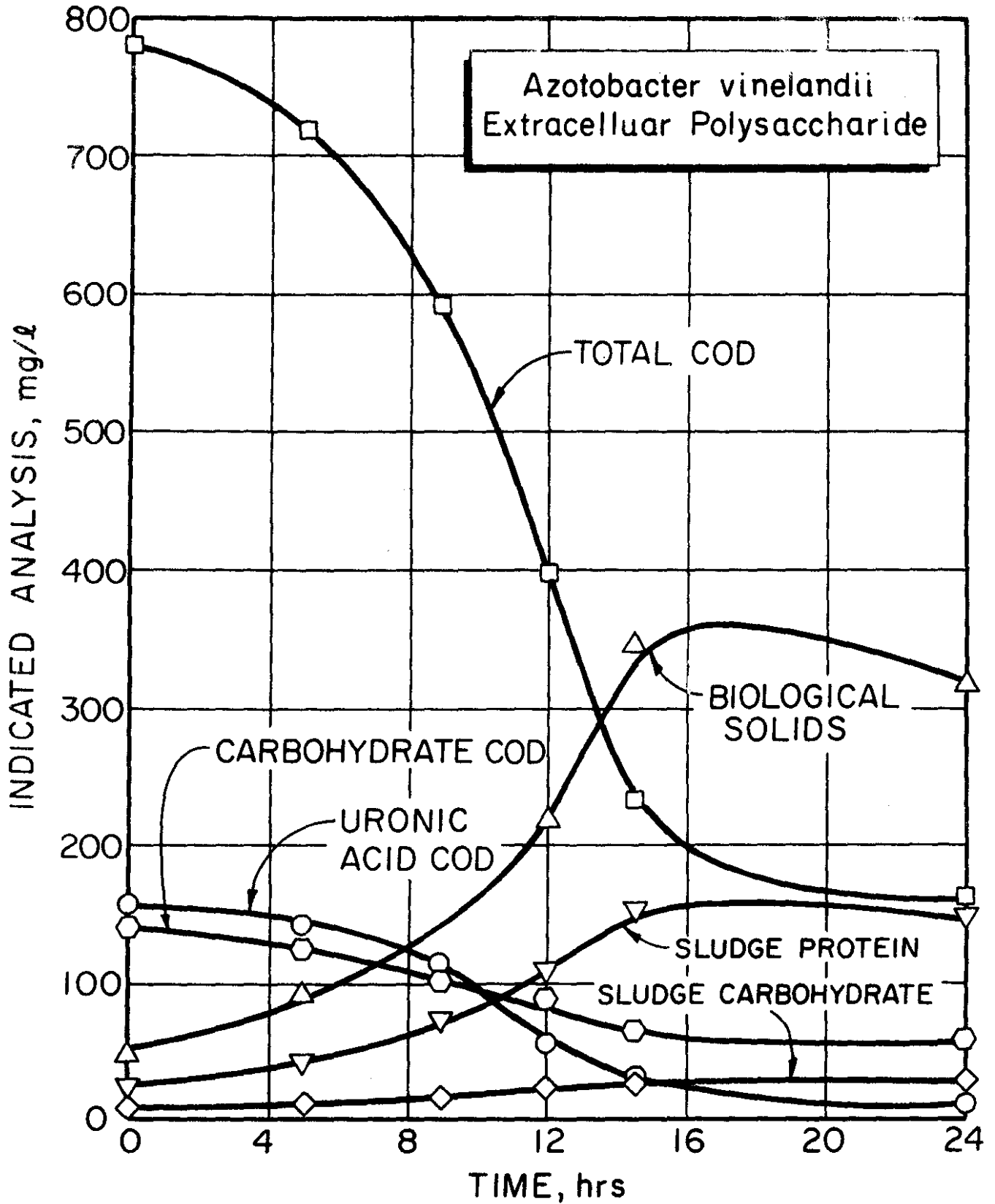
Growth on the extracellular polysaccharide of Arth. viscosus is shown in Figure 2. Again it is seen that this biological waste product was amenable to metabolism by an acclimated heterogeneous microbial population. Approximately 86 percent of the COD was removed in this experiment. In Figure 3, growth on the capsular material of Azoto. vinelandii is shown. Again the course of biological solids and protein accumulation and that of COD removal are typical of a growing system. The COD removal efficiency was approximately 80 percent.

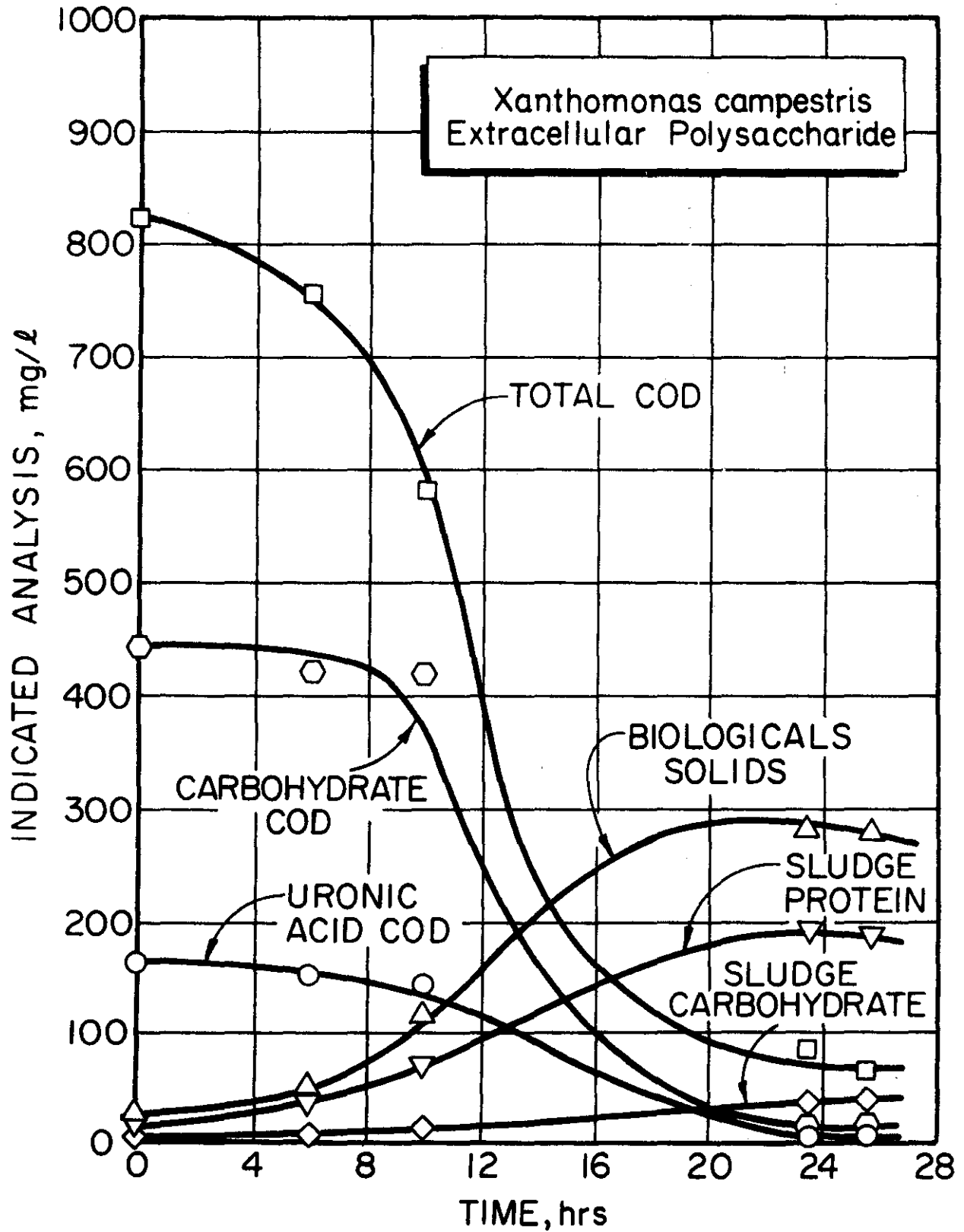
Metabolic utilization of the extracellular polysaccharide material of Xanthomonas campestris is shown in Figure 4. The initial cell inoculum was rather low in this experiment, and metabolism was initiated rather slowly. However, the COD removal efficiency was 92 percent.

Zoogloea ramigera is an organism which has long been of interest and concern in the activated sludge process, and the biological response to its extracellular heteropolysaccharide is of special interest. The growth response on this substrate is shown in Figure 5. It is seen that it was by no means inert to biological attack. The purification efficiency was 93 percent. The growth response is typical of one which might be expected with simple carbohydrate substrates.

Important system parameters for growth of heterogeneous microbial populations on the various extracellular polysaccharides are summarized in Table II. The COD removal efficiency ranged from 80 to 93 percent, and this range is consistent with values one might expect using synthetic







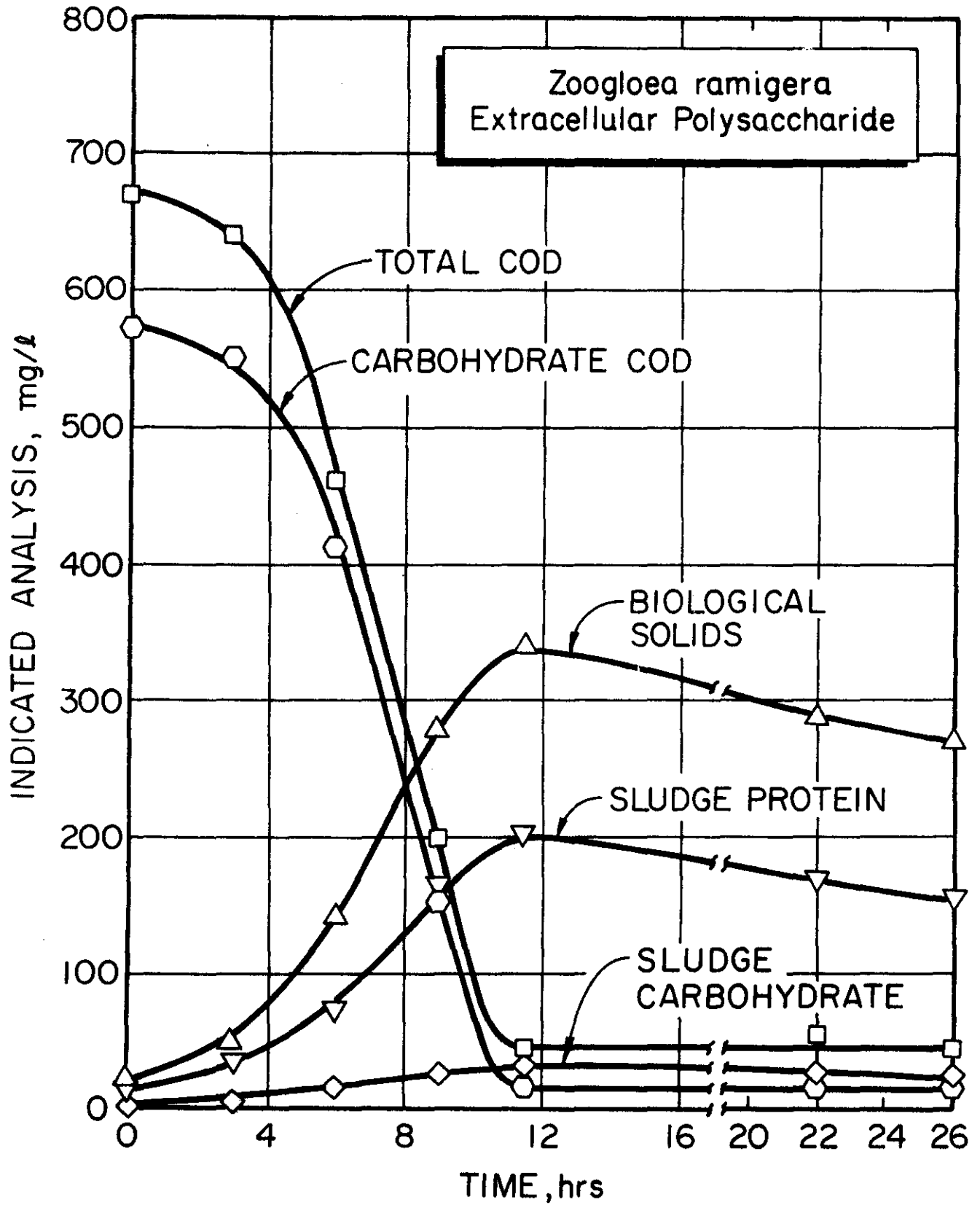


TABLE II
GROWTH YIELD, GROWTH RATE, AND PROTEIN SYNTHESIS DURING
METABOLISM OF BACTERIAL SLIMES

Fig. No.	Source of Extracellular Polysaccharide	% COD Removed	Yield	μ (hr^{-1})	Biological Solids		
					Initial Protein (mg/l)	Peak Protein (mg/l)	Peak Protein %
1	<u>Aerobacter aerogenes</u>	79.5	0.55	0.32	39	302	67
2	<u>Arthrobacter viscosus</u>	85.5	0.42	0.17	32	151	60
3	<u>Azotobacter vinelandii</u>	79.5	0.49	0.14	20	150	43
4	<u>Xanthomonas campestris</u>	92.0	0.35	0.18	15	192	68
5	<u>Zoogloea ramigera</u>	93.2	0.52	0.32	13	201	59

wastes or biologically treatable whole wastes. The values for sludge yield are consistent with values reported by Ramanathan and Gaudy for a variety of organic compounds (29). The values of specific growth rate, μ , estimated from semilogarithmic plots of the biological solids curves, are somewhat lower than those usually observed for more simple carbon sources or more nutritionally complete media such as sewage, but they are within the range of μ_{\max} values on a variety of substrates, including municipal sewage, reported by Peil and Gaudy (30). The last three columns in Table II show the initial protein content, the maximum protein content reached during the experiment, and the percent protein of the accumulated biological solids. These data are very important, because they show irrefutably that the increase in biological solids was due to intracellular biochemical conversion of the polysaccharide substrates to microbial cell materials.

DISCUSSION AND CONCLUSIONS

The heteropolysaccharides employed in this study represent rather complicated natural substrates. A review of the available literature regarding microbial capsules or slimes indicates that most bear some similarity with respect to the monomers they contain. Most of them contain one or more hexoses, hexuronic acid, or deoxyhexoses. A notable exception is the slime layer of Bacillus anthracis, which contains a γ -glutamyl polypeptide (13). The sugar residues are linked by either α or β glycosidic bonds which may involve the substitution of one of several hydroxyl groups of the adjacent sugar residue; thus from similar monomers there is opportunity for formation of a rather wide variety of structural configurations. It was for this reason that several different microbial heteropolysaccharides were studied.

The organisms which produced these slimes are rather widely distributed in nature. A. aerogenes is commonly found in sewage and soil. Both Arth. viscosus and Azoto. vinelandii are soil organisms, the latter playing a role in nitrogen fixation. X. campestris is a plant pathogen, and its extracellular polysaccharide helps prevent dehydration, thus keeping the organism viable. Z. ramigera is an organism found in sewage, and believed by many to play a role in bacterial flocculation. Thus, although the substrates employed in this study are but five of many microbial heteropolysaccharides, they do appear to be representative of a rather wide sample of naturally-occurring extracellular polysaccharides.

This investigation was made for the express purpose of determining whether extracellular heteropolysaccharides of microorganisms can serve as usable sources of organic carbon for growth of other microorganisms. The results which have been presented provide direct evidence that these microbial waste products are readily amenable to biological treatment and, therefore, it may be directly concluded that extracellular polysaccharide cannot be classified as biologically inert material. Consequently, buildup of extracellular polysaccharide cannot be validly cited as evidence against the theory of the total oxidation process. Quite to the contrary, these results as well as other work reported from our laboratory (8)(9)(10) support the conclusion that total oxidation of biological solids can be accomplished.

ACKNOWLEDGMENTS

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APPENDIX II

Gaudy, A. F. Jr., and Srinivasaraghavan, R., "Experimental Studies on a Kinetic Model for Design and Operation of Activated Sludge Processes." Presented at the 166th ACS National Meeting, Chicago, Ill., August 26-31 (1973).

EXPERIMENTAL STUDIES ON A KINETIC MODEL FOR DESIGN AND OPERATION
OF ACTIVATED SLUDGE PROCESSES

By

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Previous experimentation in our laboratory has shown that the classical theory developed for continuous growth of pure cultures in completely mixed aerobic systems in which the recycle cell concentration factor, c , (where $c = X_R/X$) is a selectable system constant, did not provide a suitable model for the heterogeneous (natural) populations of the activated sludge process. Another model was derived in which the recycle cell concentration, X_R , was employed as a system constant instead of c , and computational analysis was performed. Laboratory pilot plant experimentation was undertaken in order to determine whether a "steady state" in aerator biological solids concentration, \bar{X} , and substrate concentration, \bar{S} , could be approached under this mode of operation. Studies were performed at various organic feed concentrations holding dilution rate, D , at 0.125 hr^{-1} , hydraulic recycle ratio, α , at 0.25, and X_R at 10,000 mg/l. Also, values of maximum specific growth rate, μ_{\max} , and saturation constant, K_S , were determined. It was found that the model approached the steady state condition with heterogeneous populations more closely than did the classical model, and the high degree of treatment efficiency predicted by the model was demonstrated experimentally.

The continuous culture of heterogeneous microbial populations in completely mixed reactors has been of long-standing basic and applied research interest in our laboratories. Some time ago, we reported studies (1)(2) in which we sought to determine whether the theory of continuous culture (3)(4)(5)(6) was generally applicable to such

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populations, especially as they are used in biological purification of waste waters employing a process such as activated sludge. These studies were, in the main, affirmative; however, when attempting to employ the model of Herbert including cell recycle, the selection of the cell recycle concentration factor, c , as a system constant militated against successful application of the model. The cell concentration factor, c , is the ratio of sludge concentration in the recycle, X_R , to sludge concentration in the reactor, \bar{X} , i.e., $c = X_R/\bar{X}$. The major problem arose due to the fact that cell yield, Y , for heterogeneous populations varies due to changes in the species present. The biological solids concentration, X , in the aeration tank can vary and it is difficult to operate with c as a system constant because frequent changes in X_R are needed. Also, more importantly, any adjustment of X_R to hold c constant when X has changed, tends to force X further away from the previous steady state value rather than returning it to its former level, thus defeating the aim, i.e., steadiness in X and in effluent substrate concentration, S . Thus, we concluded that a modification of the model was needed in order to provide practical accommodation to the case of heterogeneous populations. It was reasoned that in actual operation of the system, maintenance of X_R as the system constant rather than c would be a more practical aim, and we derived the equations for \bar{S} and \bar{X} by incorporating this change into the mass balance. It is emphasized that this was the only basic change from the derivation of Herbert. We derived equations both on the basis of assuming zero concentration of substrate in the recycle flow, and of assuming that the concentration of substrate in the recycle flow was equal to the concentration of substrate in the effluent, \bar{S} . There was little difference in the final equations

for the two cases; however, the use of X_R as a system constant rather than c , led to equations for steady state concentration of cells, \bar{X} , and substrate, \bar{S} , which were much more complex than the equations of Herbert. These equations are compared in Table I.

A computational program was set up to determine the behavior of the new kinetic equations for \bar{S} and \bar{X} as the biological parameters, maximum specific growth rate, μ_{\max} , saturation constant, K_S , and cell or sludge yield, Y , as well as the engineering constants, hydraulic recycle rate, α , and recycle solids concentration, X_R , were varied. These results have been reported and the kinetic consequences and ramifications of the equations discussed (7). The model using constant X_R as a system parameter for design and operation proved to be less sensitive to high dilution rates than the Herbert model. This is brought about since, if one assumes X_R is a constant, the recycle concentration is made independent of growth in the system. The ramifications of this consequence have been previously discussed (7)(8) and they are not a cause for concern at any but extremely high dilution rates which are beyond a range which would be considered for an activated sludge plant.

A flow diagram for an activated sludge process operated in accordance with the model employing X_R as a system constant is shown in Figure 1. It is basically the same as any activated sludge plant except for the inclusion of a second aeration tank. This type of unit has various possible operational advantages, but insofar as the model is concerned, its prime purpose is to function as a dosing tank which delivers sludge to aeration tank 1 at constant X_R . Returned sludge is thus considered as a prepared biochemical "dosage" to the treatment reactor.

The model seemed to be of possible usefulness in design and

operation of activated sludge processes. While we had data pertinent to continuous growth of heterogeneous microbial populations employing completely mixed systems according to Herbert's recycle model, we had none for the new model and mode of operation shown in Figure 1. It was essential to determine the ease of operation, the steadiness of \bar{X} and \bar{S} , the predictability of \bar{S} and \bar{X} under various operational configurations, the values or variations in value for Y , K_s , μ_{max} , etc. In the present report, results of laboratory pilot plant studies and auxiliary growth experiments for systems operated according to the model, at three loading rates, are presented.

MATERIALS AND METHODS

The general experimental procedure and analyses employed in the present study were the same as those previously reported in other continuous growth studies conducted in our laboratories (1)(2). A diagram of the pilot plant used in this study is shown in Figure 2. The reactor, aerator number 1 (B), and settling tank (C) were the same as those previously employed. Heterogeneous microbial populations were developed in batch operation from an initial sewage seed obtained from the effluent of the primary clarifier at the municipal sewage treatment plant in Stillwater, Oklahoma. When sufficient cells had been accumulated to supply the recycle requirements, the feed solution was pumped to the aeration tank continuously at the desired flow rate. Settled sludge from the clarifier was withdrawn every twelve hours, diluted with effluent to the desired concentration for recycle, placed in the return sludge aerator, and pumped to the aeration tank at the desired flow rate. The ratio of recycle to feed flow, α , was maintained at 0.25.

The solids concentration in the recycle, X_R , was held as close as possible to 10,000 mg/l. The dilution rate, D , was held constant at 0.125 hr^{-1} . The feed substrate concentrations, S_i , for the three experimental runs which were made were 500, 1000, and 2000 mg/l of carbon source (glucose). The composition of the synthetic medium is given in Table II.

The system was checked for complete mixing, using procedures described previously by Komolrit and Gaudy (9). The temperature in the reactor was maintained at $25 \pm 2^\circ\text{C}$; pH was held at 6.9 ± 0.2 , and dissolved oxygen in the aerator, which was measured electrometrically, was always maintained above 90 percent of the saturation value. After it was determined that the system had remained in a pseudo steady-state condition for a period of at least two or three days, the experimental run was initiated.

The biological solids concentrations in the reactor, clarifier effluent, and recycle sludge aerator were measured using the membrane filter technique (10). The feed, effluent membrane filtrate, clarifier supernatant, and recycle sludge filtrate were analyzed for total organic material using the chemical oxygen demand (COD) test (10). Also, ammonia nitrogen concentration (11) in the feed and effluent were measured periodically, and spot checks for nitrite (10) and nitrate-nitrogen (10) were also performed. The protein and carbohydrate contents of the sludge (12) as well as the BOD_5 (10) of the effluent were also assessed from time to time.

At various times during the period of operation at each S_i , cells from aeration tank 1 were used for batch experiments to determine μ_{max} , K_s , and cell yield, Y , using methodologies previously described (1)(8)(13).

RESULTS AND DISCUSSION

The performance characteristics of the system at organic loadings of 500, 1000, and 2000 mg/l of carbon source are presented in Figures 3, 4, and 5. In Figure 3 it is seen that by any parameter of effluent character, total COD, filtrate COD, effluent biological solids, or the relatively few determinations for BOD₅ values, a rather good effluent was produced. The curve labeled "total COD" is a plot of the CODs from the clarifier supernatant; i.e., they include the biological solids in the effluent.

For the purpose of examining the model with respect to \bar{S} , the filtrate COD is the most applicable parameter. Even here, the filtrate COD gives a most conservative estimate of substrate, \bar{S} , since, regardless of how completely the substrate carbon has been metabolized, a residual COD of approximately 25 mg/l, which represents very slowly utilized organic matter, is usually observed. The COD of 500 mg/l glucose is approximately 530 mg/l, and the feed concentration in these studies varied from 440 to 560 mg/l, with an average S_0 COD of 504 mg/l. The observed filtrate COD of the effluent varied from 0 to 50 mg/l, with an average \bar{S} COD of 25.0 mg/l. Thus, the effluent was, on the average, very good, efficiency of substrate removal was 95 percent, and the value of \bar{S} was relatively steady. Similarly, the biological solids concentration, \bar{X} , remained rather steady; it ranged from 2005 to 2250 mg/l, with an average value of 2150 mg/l. It can also be seen in this figure that it was possible to control the sludge recycle concentration at a value of approximately 10,000 mg/l. The range observed was from 9200 to 10,700 mg/l, with an average of 9925 mg/l. It is interesting to note that in preparing the sludge to the desired consistency of 10,000 mg/l,

optical density was used to estimate concentration of sludge. A concentrated sample of sludge was diluted to a range in which optical density is directly proportional to concentration in mg/l, and the OD values were compared to a previously prepared standard curve. The fact that, with experience, this rather simple method for obtaining the desired sludge consistency proved to be applicable, has ramifications for possible future automated procedures for maintaining X_R constant.

It is also important to note in this figure that the COD concentration in the filtrate of the recycle, S_R , was considerably lower than \bar{S} ; the average COD was 11 mg/l. Thus, the assumption that S_R is low enough to be neglected (i.e., $S_R = 0$), made in the derivation of the equations (7), seems justified. Excess sludge, X_W , produced at this loading level varied from 890 to 1200 mg/day, with an average of 1015 mg/day. This value includes not only excess clarifier underflow, but biological solids in the effluent as well as the amount taken for analysis.

The performance of the system when S_i was 1000 mg/l is shown in Figure 4. The effluent filtrate COD varied from 5 to 50 mg/l, with an average of 33 mg/l. It is interesting to point out that the BOD_5 samples indicate extremely low effluent values. It is also interesting to note again that the values for S_R are very much lower than \bar{S} (average $S_R = 11$ mg/l). In addition to substantiating the justification for assuming $S_R = 0$ in the equations, this observation attests to the fact that the residual COD in the effluent is subject to further removal.

Figure 5 shows that this mode of operation can deliver excellent average substrate purification with rather small variation from the average value at a rather high feed concentration of 2000 mg/l.

The mean values for the various operational and performance

parameters for all three experimental runs are given in Table III. It is seen that the average X_R at $S_i = 1000$ mg/l is lower than for the other runs. It is important to note that the values for X_R plotted in Figures 3, 4, and 5 and the average values for X_R shown in Table III are the recycle sludge concentrations which were pumped to aerator number 1. It is recalled that aerator number 2 (the sludge consistency tank) was loaded at approximately 10,000 mg/l return sludge each 12 hours. Thus, portions of the bio-mass being pumped to aerator number 1 could have undergone some amount of aerobic digestion during the 12-hour period. For this reason, biological solids concentration in aerator number 2 was measured at the beginning and end of each 12-hour period (and often at more frequent intervals). Only during this run at $S_i = 1000$ mg/l was there any evidence for an autodigestive decrease in biological solids concentration. The average beginning concentration was 9840 mg/l, and the average ending concentration was 9470 mg/l. Thus, during this run, approximately 370 mg/l, on average, were autodigested in aerator number 2 during each charging period. The mean value for X_R calculated as the average of these values, 9655 mg/l, is shown in Table III.

While the data attest to the utility of this mode of operation in maintaining steadiness in X and S , the utility of the model for predicting performance of the system requires, in addition to control of X_R , α , and relative constancy of S_i , determination of the biological constants μ_{\max} , K_S , and Y , and the substitution of these factors in the equations to predict \bar{S} and \bar{X} . During each run, samples of cells from aeration tank number 1 were employed in batch growth studies to determine μ_{\max} , K_S , and Y . The sludge yield was determined as the ratio of the weight of biological solids produced, ΔX , and substrate removed,

ΔCOD , at the end of the substrate removal period. The utility of this technique for measuring cell or sludge yield has been previously discussed (8)(14). The kinetic growth constants were determined using a straight line form of the Monod equation employing values for exponential growth rates determined at various initial substrate concentrations, i.e., values of S_0 (1)(8). An example of the growth rate data for each run is shown in Figure 6. It is clear from these data that exponential growth was developed at each S_0 value (see semi-logarithmic plots), and that a hyperbolic-type relation between μ and S_0 existed.

The values of the biological constants for each batch experiment during each run, as well as the average values are given in Table IV. Using the average values for the biological constants and the average values of X_R and S_i during each run and using $\alpha = 0.25$, the predicted values of \bar{X} , \bar{S} , and X_W were computed. These are compared with the average observed values in Table V. It is seen that the predicted and observed values of \bar{X} compare rather well and that the predicted value of \bar{S} lies somewhat below the observed value. As mentioned previously, the use of residual COD as a measure of residual microbial substrate is probably a most conservative measure. If one wishes to determine the amount of readily available carbon source in a waste water, he may measure the amount of COD (as a measure of organic matter), which is removed during growth of an acclimated population, i.e., he would measure the " ΔCOD " of the waste, initial COD minus final COD (8)(15). Even for a substrate as readily metabolized as glucose, an apparent residual COD, i.e., final COD, of ± 25 mg/l, usually remains. The exact nature of this residual COD is not known, but we have observed in various experiments that it consists mainly of material other than the original

organic substrate (16). In fact, in experiments in which the carbon source is a carbohydrate, only very small portions, for example, 10 to 15 percent of the residual COD, may register in the anthrone test, which is a specific test for carbohydrates. These materials most likely consist of various cell components which may leak from viable cells, or are components of damaged or dead cells in the population. We have in other studies (unpublished results) shown that this apparent residual COD from secondary treatment is not necessarily permanently residual or inert organic matter, but can consist of material which is more slowly metabolized than the original carbon sources in the waste medium. In any event, these residual materials are not usually substrates in the process of secondary biological treatment; they usually require additional treatment (physical, chemical, or biological) for their removal. Thus, the observed residual COD offers a very conservative estimate of S insofar as testing of the model is concerned.

Both our experimental and analytical research pertaining to the model are continuing. Included in this undertaking are experiments at other concentrations of cell feedback, X_R , and at other substrate loadings, various ways and means of predicting amounts of excess sludge and comparisons of our model with other models, both those derived as fundamental kinetic models for continuous microbial culture and those purported to be uniquely applicable to activated sludge.

From the results which have been reported here, it can be concluded that the proposed model employing X_R as a selectable engineering system constant has been shown to provide rather steady performance employing a bio-mass consisting of a heterogeneous population of sewage origin, thereby justifying the assumption of the approach to steady

state made in the derivation of the equations. Highly effective treatment, i.e., substrate removal over a rather wide range of feed concentration was observed, and the model equations in \bar{X} and \bar{S} were shown to be adequate predictors of the experimental performance.

ACKNOWLEDGMENT

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LIST OF SYMBOLS

- c - Sludge recycle concentration factor, equal to the ratio between the recycle solids concentration, X_R , and the biological solids concentration in the reactor, X .
- ΔCOD - A measure of the amount of biologically available organic matter in a waste sample, mg/l.
- D - Dilution rate. Ratio of the rate of flow, F , and the volume of liquor in the aeration tank, V . It is equal to the reciprocal of the mean hydraulic residence time, \bar{t} , in a completely mixed reactor, hr^{-1} .
- F - Rate of flow of incoming substrate or wastewater, l/hr.
- K_S - A biological "constant" used in the hyperbolic expression relating specific growth rate to substrate concentration. It is known as the saturation constant. It is numerically equal to the substrate concentration at which specific growth rate is $\frac{1}{2}$ the maximum specific growth rate for the system, mg/l.
- S - Substrate concentration, measured as COD, mg/l.
- S_i - Concentration of substrate in the inflowing feed in continuous flow operation, measured as COD, mg/l.
- S_0 - Initial concentration of substrate in batch systems, COD, mg/l.
- \bar{S}_e - Steady state concentration of substrate in the reactor or effluent, filtrate COD, mg/l.
- \bar{S}_t - Steady state concentration of COD in the clarifier effluent, supernatant including non-settled biological solids, mg/l.
- \bar{S}_R - Filtrate COD in the recycle sludge, mg/l.
- X - Biological solids concentration, mg/l.
- \bar{X} - Steady state biological solids concentration in the reactor, mg/l.
- \bar{X}_e - Biological solids concentration in the clarifier effluent, mg/l.
- X_R - Biological solids concentration in the recycle flow to the reactor, mg/l.
- X_W - Excess biological solids (sludge wasted), mg/day.
- Y - Cell yield obtained from batch experiments using cells harvested from the pilot plant during continuous culture.
- α - Recycle flow ratio.
- μ - Specific growth rate in an exponential phase of growth, hr^{-1} .
- μ_{max} - The maximum specific growth rate for a system in exponential growth, hr^{-1} .

FIGURE LEGENDS

- Figure 1. Flow diagram for model employing constant recycle sludge concentration, X_R .
- Figure 2. Activated sludge pilot plant for operation with constant X_R .
- Figure 3. Operational characteristics for an activated sludge process with constant X_R at an S_i of 500 mg/l.
- Figure 4. Operational characteristics for an activated sludge process with constant X_R at an S_i of 1000 mg/l.
- Figure 5. Operational characteristics for an activated sludge process with constant X_R at an S_i of 2000 mg/l.
- Figure 6. Relationships between S_0 and μ for cells harvested from the activated sludge pilot plant

TABLE I

COMPARISON OF STEADY STATE EQUATIONS ACCORDING TO MODELS OF HERBERT AND OF RAMANATHAN & GAUDY

Herbert Constant $c \left(c = \frac{X_R}{X} \right)$	Ramanathan & Gaudy Constant X_R
$X = \frac{Y}{1 + \alpha - \alpha c} (S_i - \bar{S}_e)$	$\bar{X} = \frac{Y [S_i - (1 + \alpha)\bar{S}_e] + \alpha X_R}{1 + \alpha}$
$\bar{S} = \frac{K_S D(1 + \alpha - \alpha c)}{\mu_{\max} - D(1 + \alpha - \alpha c)}$	$\bar{S} = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$ $a = \mu_{\max} - (1 + \alpha)D$ $b = D [S_i - (1 + \alpha)K_S] - \frac{\mu_{\max}}{1 + \alpha} \left[S_i + \frac{\alpha X_R}{Y} \right]$ $c = K_S D S_i$
$\mu = D(1 + \alpha - \alpha c)$	$\mu = D \left(1 + \alpha - \alpha \frac{X_R}{X} \right)$

TABLE II

COMPOSITION OF GROWTH MEDIUM PER 1000 mg/l GLUCOSE

Constituents	Amount
Glucose	1000 mg/l
Ammonium sulfate, $(\text{NH}_4)_2\text{SO}_4$	500 mg/l
Magnesium sulfate, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	100 mg/l
Ferric chloride, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	0.50 mg/l
Manganous sulfate, $\text{MnSO}_4 \cdot \text{H}_2\text{O}$	10.0 mg/l
Calcium chloride, CaCl_2	7.50 mg/l
KH_2PO_4	527.0 mg/l
K_2HPO_4	1070.0 mg/l
Tap water	100 ml/l

TABLE III

MEAN "STEADY STATE" VALUES OF FEED, EFFLUENT, AND BIOLOGICAL SOLIDS FOR ACTIVATED SLUDGE PROCESS
AT D OF 0.125 hr^{-1} WITH CONSTANT X_R

Feed, S_i			Effluent, S								Biological Solids, X					
Nominal		Observed	Filtrate			Supernatant					X mg/l	Recycle		Pro- tein %	Carb. %	X_W mg/day
Glucose mg/l	COD mg/l	COD mg/l	S_e mg/l	COD removed mg/l	%	S_t mg/l	COD removed mg/l	%	BOD mg/l	X_e mg/l		X_R mg/l	S_R mg/l			
500	530	504	25	479	95	46	458	91	11	30	2150	9925	11	47	28	1015
1000	1060	1000	33	967	97	63	937	94	8	34	2275	9655	11	54	38	2600
2000	2120	2006	38	1968	98	61	1945	97	18	22	2885	10140	12	40	33	6280

TABLE IV

VALUES OF THE "BIOLOGICAL CONSTANTS," MAXIMUM SPECIFIC GROWTH RATE, μ_{max} , SATURATION CONSTANT, K_s , AND CELL YIELD, Y , OBTAINED IN BATCH EXPERIMENTS USING CELLS HARVESTED FROM THE COMPLETELY MIXED REACTOR

S_i mg/l	μ_{max} , hr ⁻¹					K_s , mg/l					Y , mg/mg				
	1	2	3	4	Mean	1	2	3	4	Mean	1	2	3	4	Mean
500	0.48	0.51	0.47		0.49	140	110	95		115	0.41	0.39	0.38		0.39
1000	0.25	0.48	0.83	0.63	0.55	205	226	270	438	285	0.49	0.53	0.45	0.52	0.50
2000	0.60	0.56	0.58		0.58	110	100	105		105	0.59	0.53	0.56		0.56

TABLE V
EXPERIMENTALLY OBSERVED AND PREDICTED VALUES OF \bar{X} , \bar{S}_e , AND X_W

S_i	\bar{X} , mg/l		\bar{S}_e , mg/l		X_W , mg/day	
	Observed	Predicted	Observed	Predicted	Observed	Predicted
500	2150	2140	25	3	1015	1158
1000	2275	2320	33	14	2600	2880
2000	2885	2918	38	9	6280	5670

Calculation of \bar{X} and \bar{S}_e based upon average values for Y , μ_{max} , and K_s determined in batch experiments (see Table IV).

Calculation of X_W based upon average Y values from Table IV and predicted values of \bar{S}_e in this table; X_W (predicted) = $F(S_i - S_e)Y$.

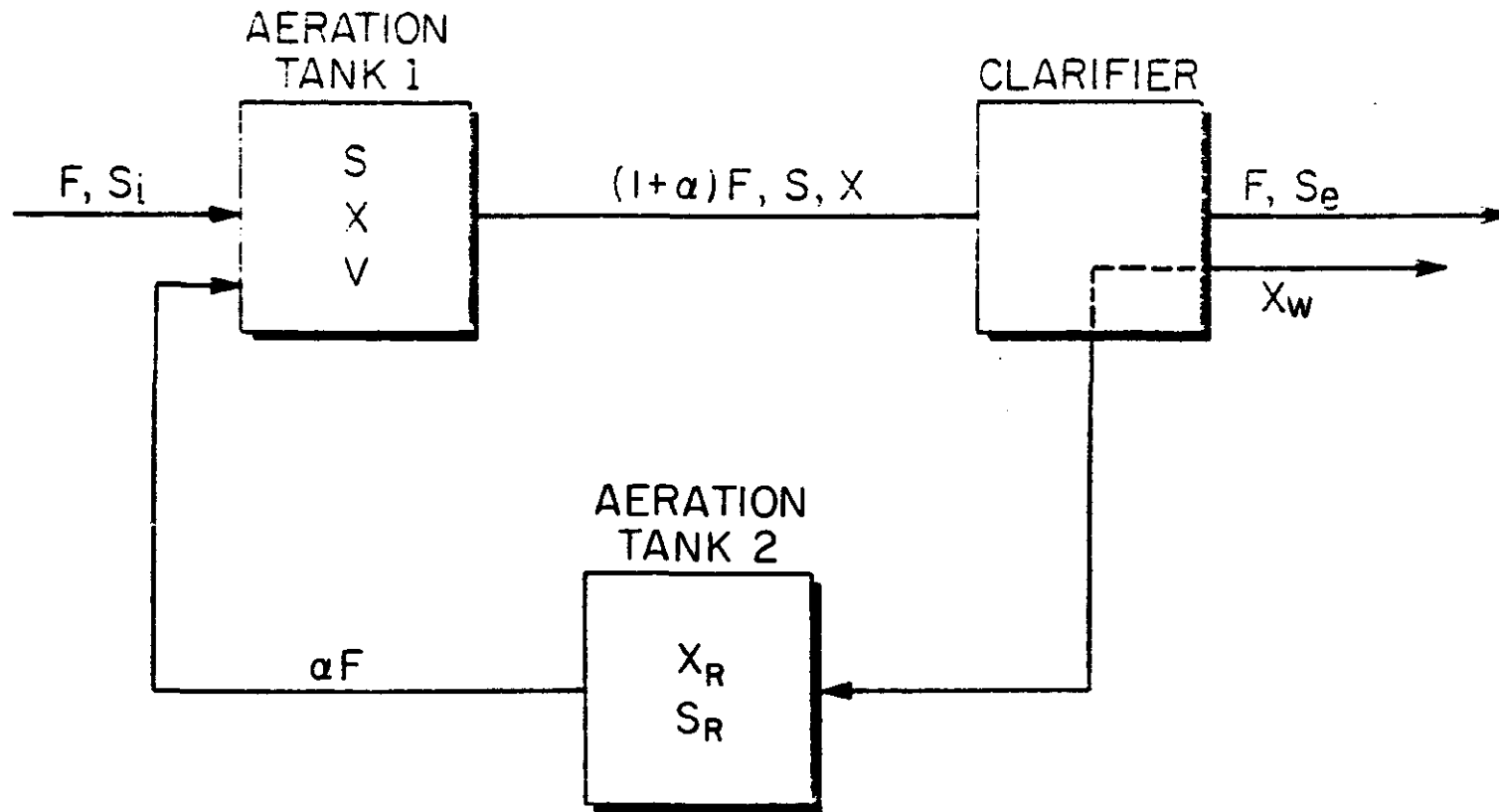


Figure 1. FLOW DIAGRAM FOR MODEL EMPLOYING CONSTANT RECYCLE SLUDGE CONCENTRATION X_R .

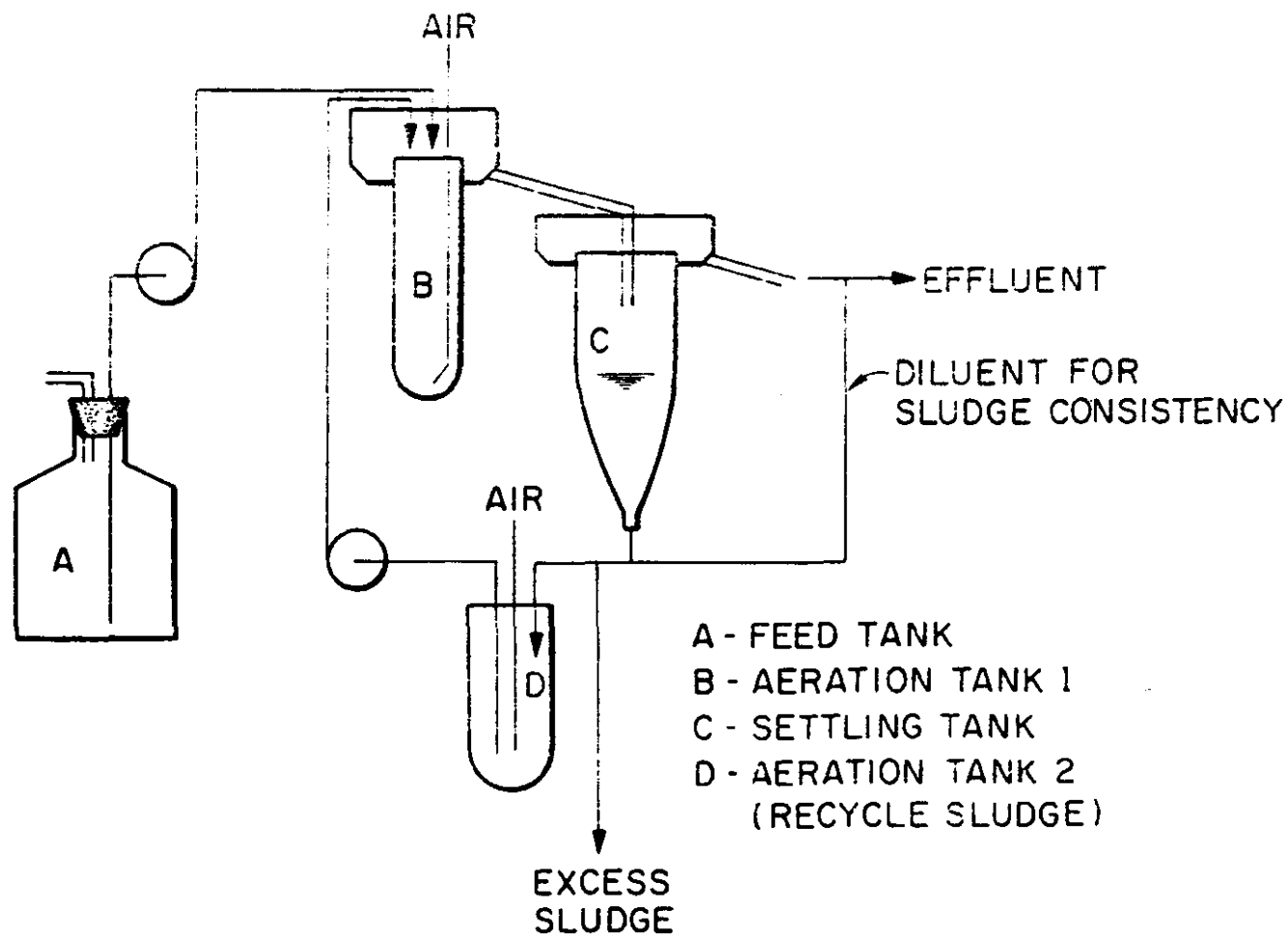


Figure 2. ACTIVATED SLUDGE PILOT PLANT FOR OPERATION WITH CONSTANT X_R .

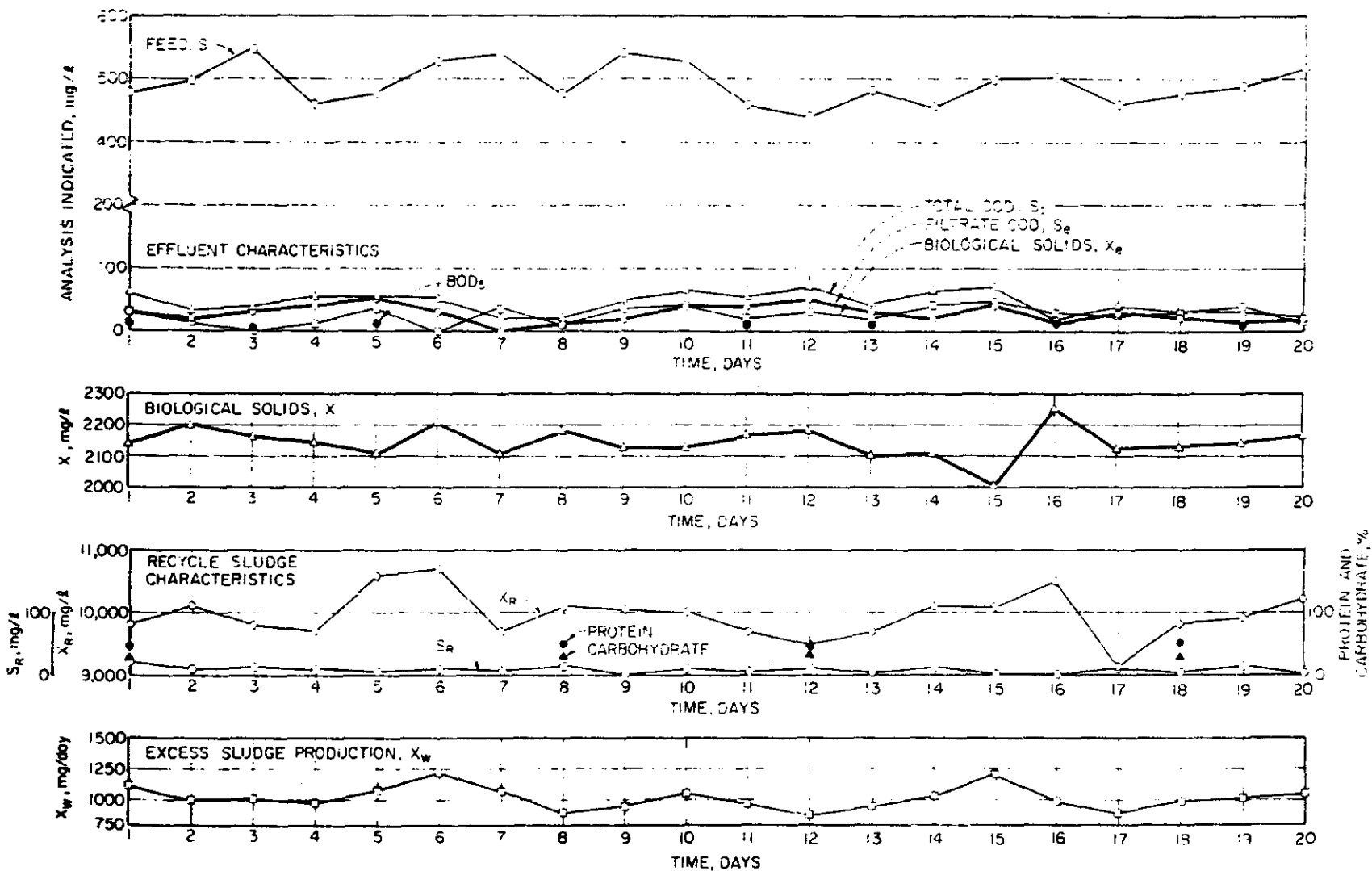


Figure 3. OPERATIONAL CHARACTERISTICS FOR AN ACTIVATED SLUDGE PROCESS WITH CONSTANT X_R AT AN S_i OF 500 mg/l

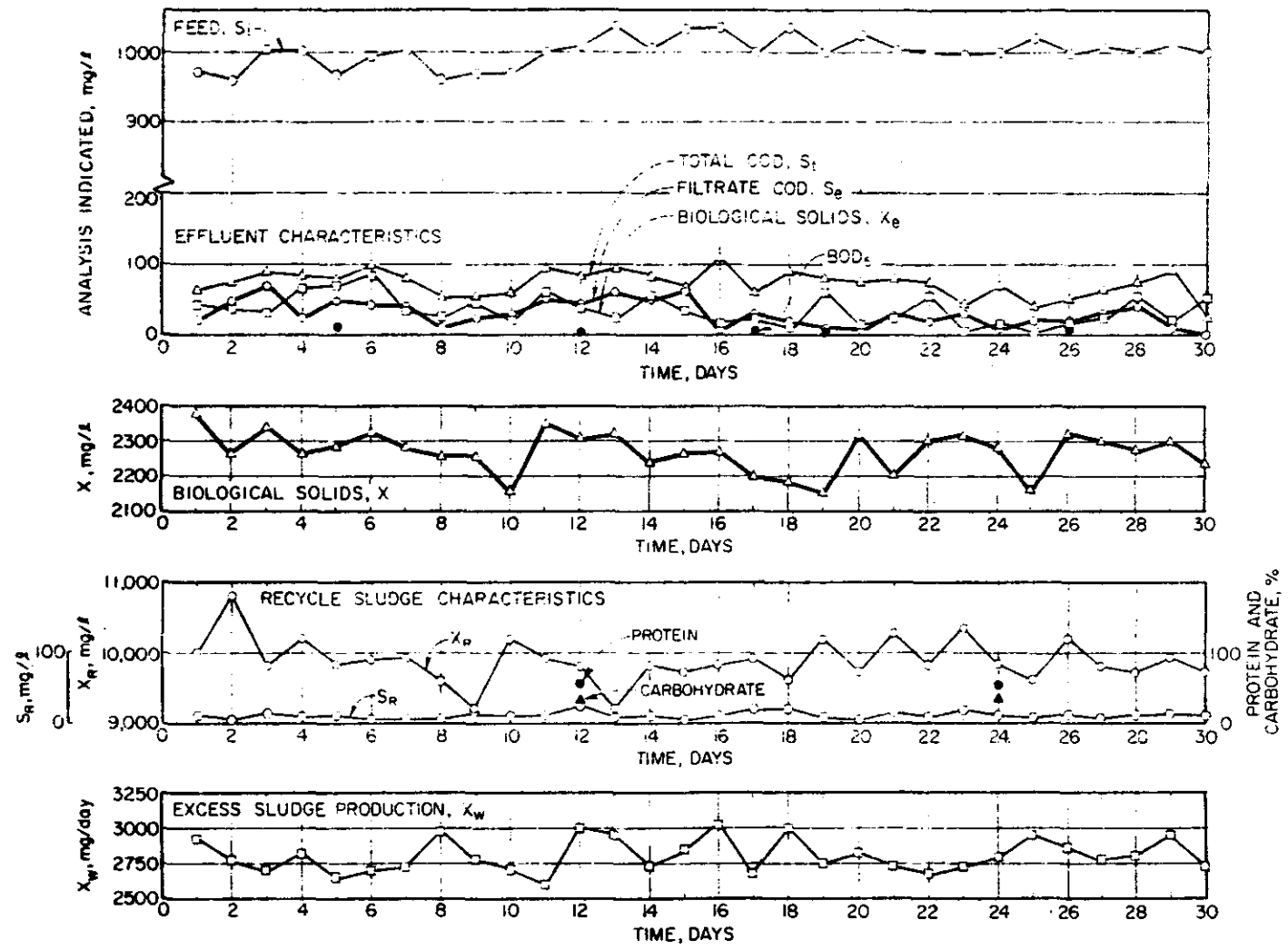


Figure 4. OPERATIONAL CHARACTERISTICS FOR AN ACTIVATED SLUDGE PROCESS WITH CONSTANT X_R AT AN S_i OF 1000 mg/l.

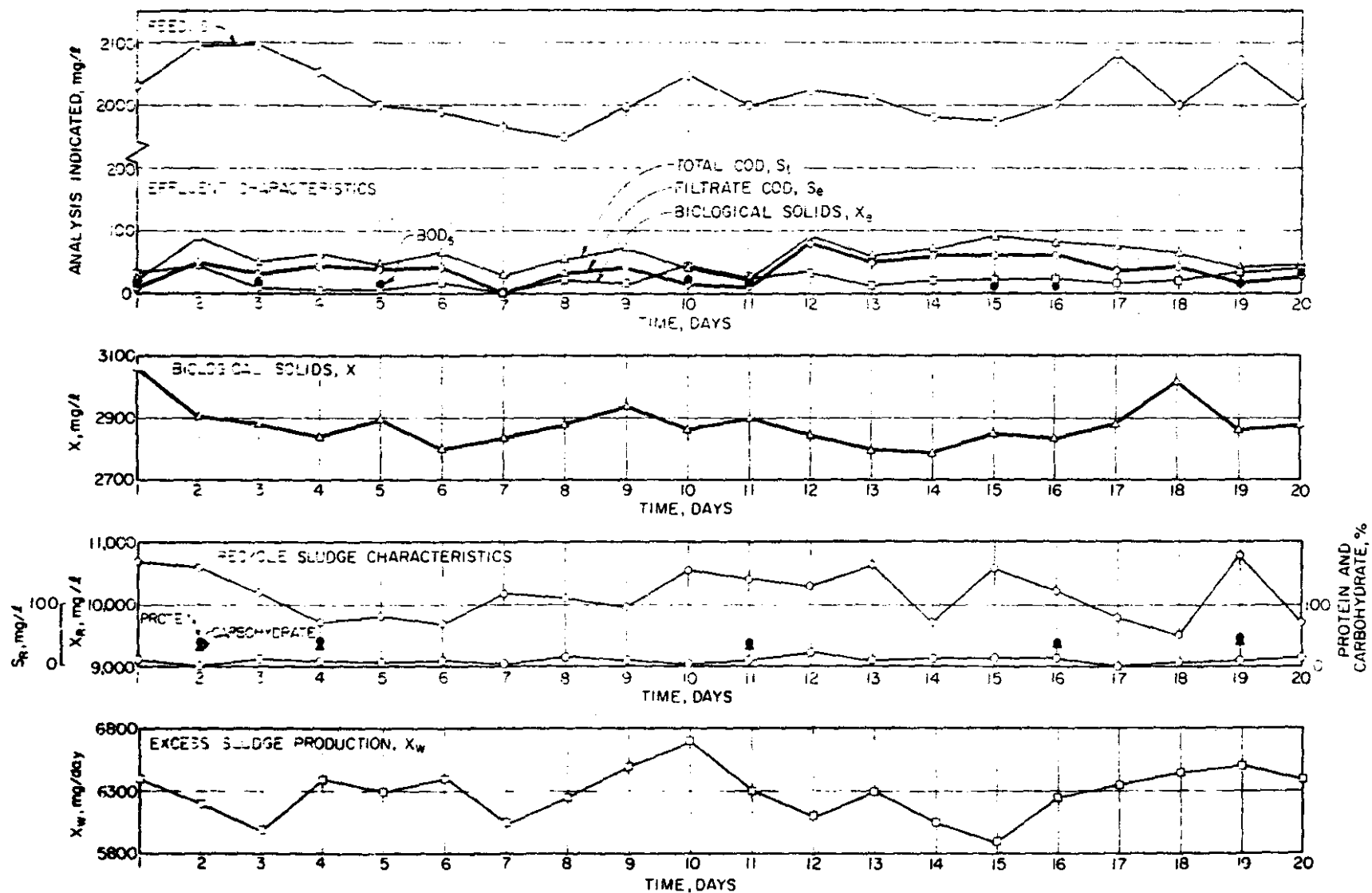


Figure 5 OPERATIONAL CHARACTERISTICS FOR AN ACTIVATED SLUDGE PROCESS WITH CONSTANT X_R AT AN S_1 OF 2000 mg/l

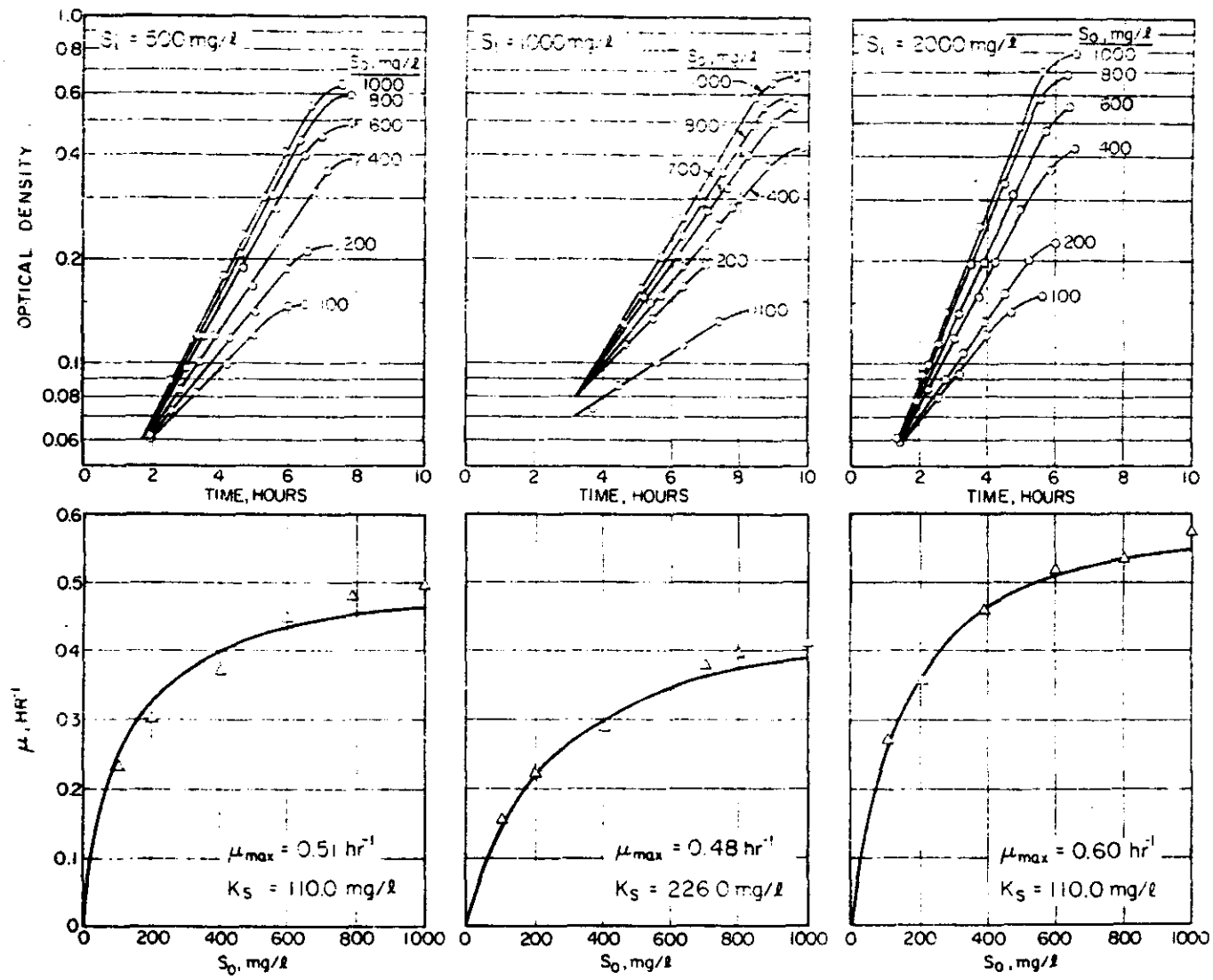


Figure 6. RELATIONSHIP BETWEEN S_0 AND μ FOR CELLS HARVESTED FROM THE ACTIVATED SLUDGE PILOT PLANT.

APPENDIX III

Srinivasaraghavan, R., and Gaudy, A. F. Jr., "Operational Performance of an Activated Sludge Process with Constant Sludge Feedback." Proceedings, 29th Industrial Waste Conference, Purdue University, Lafayette, Indiana, May 7-9 (1974).

OPERATIONAL PERFORMANCE OF AN ACTIVATED SLUDGE PROCESS
WITH CONSTANT SLUDGE FEEDBACK

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INTRODUCTION

There is an ever increasing need for reliable delivery of a high degree of removal of biochemical oxygen demanding organic matter. This has fostered a trend in design of such biological treatment processes as the activated sludge process toward the use of descriptive kinetic models purporting to relate the variables controlling metabolism. Such a trend away from strictly empirical "rules of thumb" is a forward step and has led to many varying approaches to design. All too often these approaches to design neglect to consider operational aspects, and in some cases one might argue that the design models have little communion with theories of continuous culture which have been developed to depict the growth of microorganisms.

Thus, the approach in our laboratories to development of kinetic models has been to seek relationships useful both in design and in operation. Also, much investigative effort has been directed toward determining whether the theory of continuous culture of single species developed by Monod (1) and Novick and Szilard (2), and elaborated upon by Herbert, Elsworth and Telling (3) and by Herbert (4) was applicable

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or could be made applicable to heterogeneous microbial populations such as exist in activated sludge processes. If it were, we would then have the basis for a model which could claim the desired communion with basic concepts of microbial growth. Various research papers on that portion of the research have been published and need not be reviewed in detail here. Briefly, it was found that the kinetic relationships for describing the biomass in pure cultures were in general valid for heterogeneous populations if one would accept reasonable variations in the biological "constants" (5)(6)(7)(8)(9). Also, the continuous growth equations of Herbert for once-through reactors were useful for description of effluent substrate and biomass concentrations (5)(6). However, the equations for "steady state" concentration of effluent substrate and cell or biomass concentration, \bar{S} and \bar{X} , for cell recycle systems were not entirely useful (6). One of the operational or design constants was defined by Herbert as the recycle concentration factor, c , which is the ratio between the concentration of recycle cells (or sludge), X_R , and the aeration tank suspended solids concentration, \bar{X} . Attempts to operate a system using this parameter caused rather severe fluctuation in the "steady state" values of cells, \bar{X} , and substrate, \bar{S} , when heterogeneous populations were employed (6). Thus, it was found necessary to change the equations, and the aim was to do so in such a way as not to lose the basic tie to proven concepts of continuous culture. It was decided to discard the use of c as a system constant and to derive mass balance equations in \bar{X} and \bar{S} , assuming X_R itself to be the system constant. The model equations for design and operation were presented in 1969 (6). The mathematical (or "theoretical") ramifications of maintaining X_R as a system constant have also been discussed and the relationships

between the operational parameters have been presented (10).

In Table I the basic equations as given by Herbert (4) (with c as a selectable system constant) are compared with those of Ramanathan and Gaudy (with X_R as a selectable system constant). In both sets of equations, the effluent substrate concentration, \bar{S} , and the biological solids concentration, \bar{X} , are determined by the descriptive biological "constants," cell or sludge yield, Y , maximum specific growth rate, μ_{\max} , and the saturation constant, K_S . The latter two parameters are recognized as those of the Monod relationship. These three constants are properties of the biological material.

Both sets of equations utilize certain selectable engineering parameters (for design or operation). One of the most important of these is the unit flow rate or dilution rate, D . D is the ratio of the inflow rate, F , and the aeration tank volume, V . Thus, it is the reciprocal of the mean hydraulic retention time, \bar{t} . Another selectable engineering constant used in both sets of equations is the hydraulic recycle rate, α , which is the ratio of the recycle flow and the feed inflow, F ; thus, the recycle flow is αF . The only difference between our equations and those of Herbert is the discarding of c as a selectable engineering constant, and its replacement with X_R . It is seen that in both sets of equations the specific growth rate, μ , is controlled in the main by the selectable constants. However, in the model of Ramanathan and Gaudy, the effect of S_i on μ can readily be seen since S_i affects X . Since in both equations the factor in parentheses must be less than unity in order to employ the equations, the effect of recycle is to reduce the specific growth rate; without recycle, $\mu = D$. Also shown is an equation (4) for calculation of the excess sludge, X_W (weight/time). Combining this

equation with that above for μ , one can obtain the second equation shown for μ (5) in terms of excess sludge and amount of sludge in the aeration tank ($\mu = X_W / V\bar{X}$). The reciprocal of this expression for μ is defined as the sludge retention time (SRT) or sludge age, θ_c , as shown (11)(12). Some workers may prefer to think in terms of sludge age rather than growth rate.

Since developing these equations and testing their behavior mathematically (10), an experimental study has been underway to examine the feasibility of operation with X_R as a system constant and the effect on the stability of the "steady state" as well as the predictive value of the equations. Some of these results have been recently reported (13) and the present study was undertaken to gain insight into the reproducibility of the steady state values \bar{X} and \bar{S} as determined by design and operational conditions with varying heterogeneous populations (sludge). Another aim of the present study was to determine if an additional biological parameter (a bio-mass constant) for autodigestion or energy requirement for cell maintenance would be a meaningful refinement to the design and operation equations.

MATERIALS AND METHODS

A diagram of the continuous flow pilot plant is shown in Figure 1. The reaction liquor volume (aeration tank #1) was two liters. Activated sludge was developed from a seed taken from the effluent of the primary clarifier at the municipal sewage treatment plant in Stillwater, Oklahoma. The composition of the synthetic waste employed is given in Table II. In operating the plant with constant X_R , settled sludge was withdrawn from the clarifier each 12 hours. It was diluted to the selected recycle

concentration ($X_R = 10,000$ mg/l in these studies) and placed in the return sludge aerator (aeration tank #2). Recycle sludge was pumped continuously to aeration tank #1. In these studies, α was 0.25. It is seen that in this system, aeration tank #2 serves as a sludge consistency tank. The flow rate was adjusted to give a dilution rate of 0.125 hr^{-1} (detention time of 8 hours) in the aeration tank. The temperature and pH of the unit were maintained at 25°C and 6.9 to 7.1, respectively. The air supplied was sufficient to keep dissolved oxygen concentration above 90% of the saturation value. The mixed liquor suspended solids concentration, recycle solids concentration, and effluent suspended solids concentration were monitored employing the membrane filter technique (14). The COD test (14) was performed on feed, effluent membrane filtrate, clarifier supernatant, and recycle sludge filtrate. The sludge was analyzed for protein and carbohydrate content (15). The BOD_5 of the effluent was assessed periodically. During the steady state continuous runs at each S_i , cells from aeration tank #1 were employed as initial inoculum for batch experiments to determine μ_{max} , K_S , and Y_B using methodologies described previously (5)(7)(16).

RESULTS AND DISCUSSION

Figure 2 shows the performance of the system operating in accordance with the model (equations and flow diagram) of Ramanathan and Gaudy (10). The inflowing feed concentration was 500 mg/l. Effluent characteristics are shown in the top part of the graph, and it is seen that as measured by filtrate COD in the effluent, suspended solids concentration, total COD in the clarifier supernatant or the few BOD_5 samples taken during the 10-day period of operation, the system provided excellent treatment. It

is seen that the biological solids concentration in the aeration tank varied somewhat but it was very much more "steady" than previous studies have shown X to be for operation with c as a system constant as used in Herbert's model for continuous growth with cell recycle. It may also be seen that it was possible to operate with X_R essentially constant at 10,000 mg/l. Also, it was found that little or no autodigestion of the return sludge took place in aeration tank #2 during each 12-hour pumping period. It is also noted that the substrate concentration in the recycle, S_R was, for all practical purposes, negligible thus justifying the assumption in the derivation that $S_R = 0$ (10). Determinations for protein and carbohydrate content of the sludge indicated values in the expected range, i.e., 50 percent protein, 30 percent carbohydrate. The bottom graph shows the daily production of excess sludge, X_W . It is seen that it remained relatively steady.

Figure 3 shows the results of a longer "steady state" run at the same feed loading (13). All operational parameters were the same as those for the previous run except that the heterogeneous microbial population comprising the sludge was a different one developed from municipal sewage. Again, it is seen that rather steady operational results were achieved, that the system delivered an excellent effluent, and that the values for each parameter are approximately the same as those previously shown.

When the feed loading was increased to 2,000 mg/l, holding all other parameters the same as before, the system performance was as shown in Figure 4. At this high loading, it is seen that the effluent quality was extremely good, and \bar{S} (i.e., S_e) was relatively steady. The aeration tank solids concentration was predictably higher than before, as was the daily production of excess sludge.

It is seen in Figure 5 that the same general result and steadiness of performance could be reproduced. This figure shows the results of a 20-day "steady state" run for which the only difference was that the sludge was developed from a different seed of organisms taken from the municipal sewage treatment plant (13).

Reproducibility and the effectiveness of the design and operational model are assessed in Table III wherein the average values for all four runs are given. Of particular interest is the effluent quality. At the 500-mg/l loading, even the most conservative measure of efficiency (i.e., supernatant COD) yields average values of 91 and 94 percent, and at the high loading, 97 and 98 percent. Of particular interest, insofar as the model is concerned, is the reproducibility of the values of \bar{X} for each loading level. Many steady state runs were made, and during all, separate batch studies were undertaken for determination of the growth constants, μ_{\max} and K_S , and for the cell or sludge yield, Y_B . Having determined these values, it was then possible, using equations 2, 1, and 4, to calculate the values for effluent quality, \bar{S} , aeration tank biological solids concentration, \bar{X} , and excess sludge, X_W . The predicted numerical values will be shown in a later section of this report and compared to the experimentally observed values.

It is interesting now to compare the cell or sludge yield values (Y_B) obtained during batch growth studies using cells harvested from the system with the steady state yields computed directly from the continuous flow pilot plant data (Y_{C_R}). For the runs herein considered, cell or sludge yield was determined yet another way. After completing the data taking period of a steady state run, recycle of sludge was stopped and the unit was allowed to run as a "once-through" growth reactor for a number of days

during which time \bar{S} and \bar{X} were monitored. Thus a cell yield (Y_c) could be determined from the once-through data. It is generally accepted that the cell yield is affected by the specific growth rate, μ , or the sludge age, θ_c , or SRT, i.e., the slower the growth rate or higher the sludge retention time, the less will be the observed cell or sludge yield value. The concept of the utilization of some exogenous substrate to satisfy the maintenance energy requirements of the cells is usually cited to explain this phenomenon. The concept indicates that such requirements are minimal when μ is high. Thus, one obtains the "true" cell yield when cells are growing at or near μ_{max} , and lower yields during growth at lower values of μ . One can also consider the decreased yield values as a manifestation of a higher amount of autodigestion at lower growth rates, but the overall effect is the same; i.e., the observed cell yield decreases as the population is made to grow slower. The value of μ at which Y_{cR} was obtained can be calculated from equation 3, after obtaining \bar{X} from equations 2 and 1. The yield, Y_c , during once-through operation was obtained at $\mu = D = 0.125 \text{ hr}^{-1}$. The Y_B values were obtained in the presence of excess substrate concentration and therefore at high values of μ near μ_{max} . This latter fact was substantiated during the growth studies to determine μ_{max} and K_s . The values of μ during batch growth were determined from a semi-logarithmic plot of growth vs. time. The values for cell yield at the corresponding specific growth rates for both runs at $S_i = 500 \text{ mg/l}$, and at $S_i = 2000 \text{ mg/l}$, are compared in Table IV. It is realized that experimental data do not warrant calculation of μ_{cR} to four places, but this was done in order to show the degree of reproducibility, i.e., if the values were rounded to two decimal places, one would conclude exact reproducibility and this was not the case. A number of interesting observations can be

made regarding this table. First, the effect of cell recycle in diminishing the specific growth rate, μ , is dramatically shown. In continuous culture, there is at the same dilution rate, D , a ten-fold decrease in μ due to recycling of 10,000 mg/l cells at $\alpha = 0.25$ (compare $\mu_{C_R} = 0.0125 \text{ hr}^{-1}$ and $\mu = D = 0.125 \text{ hr}^{-1}$), and the cells were growing at approximately 2.5 percent of their maximum capability (compare $\mu_{C_R} = 0.0125$ and $\mu_{\max} = 0.49 \text{ hr}^{-1}$). It is also apparent that the concentration of inflowing feed, S_i , can exert an effect on the specific growth rate in the steady state (compare $\mu_{C_R} = 0.0125 \text{ hr}^{-1}$ at $S_i = 500 \text{ mg/l}$ and $\mu_{C_R} = 0.046$ at $S_i = 2000 \text{ mg/l}$). It is important to examine the effect μ (or its reciprocal, cell age) has on the observed cell yield. If one compares the Y_{C_R} values at the low and high substrate loadings (or values of μ_{C_R}) it can be concluded that the results are in accord with the maintenance concept, i.e., $Y_{C_R} = 0.36$ at $\mu_{C_R} = 0.0125 \text{ hr}^{-1}$ and $Y_{C_R} = 0.54$ at $\mu_{C_R} = 0.046 \text{ hr}^{-1}$. However, one would surely expect the concept to stand the scrutiny of horizontal comparison, as well. Here there is some cause for caution in embracing the simple concept of cell maintenance. When, for example, the μ and Y values for the run at $S_i = 500 \text{ mg/l}$ are compared, it is apparent that μ had little effect on cell yield over a 50-fold range of specific growth rate. This trend persists for the other runs as well, with the exception of the 10-day run at $S_i = 500 \text{ mg/l}$. Even here, the change in Y for the 50-fold increase in μ , i.e., 0.33 to 0.41 is significantly less than the change in Y_{C_R} from 0.36 or 0.33 to 0.54 for a four-fold increase in μ . It must be emphasized that these were heterogeneous populations. The data suggest that a prolonged period of operation at a low specific growth rate may select cells with a low "true" yield (17). This is a considerably different mechanistic interpretation (i.e., an ecological one) than that

stated in the concept of cell maintenance. It was noted that after prolonged operation as a once-through reactor, subsequent to stopping the recycle operation, the cell yield began to increase somewhat. At least five mean cell retention times and generally more were required before the cell yield increased slightly. In any event, an increasing yield in response to an increase in μ was not rapidly accomplished. We are continuing to study these interesting phenomena in regard to cell yield, because of the fundamental significance of the maintenance concept. Presently we are studying this phenomenon with pure cultures, and we find (unpublished results) that the data show a similar trend (but of lower magnitude) as do these data with heterogeneous populations. Thus, simply ascribing the inability of the cell yield to respond rapidly to a change in μ to ecological causation may not suffice. Our subsequent findings on this topic may cause us to re-evaluate the biomechanic or physiological basis for the cell maintenance concept. It seems possible that the physiological mechanism which may be responsible for the so-called growth rate hysteresis effect (18)(19) may also somehow affect cell yield.

Regardless of possible doubts as to the fundamental basis for the cell maintenance concept, the fact remains that in the steady state, Y_{CR} decreases when the system is operated at lower values of μ . Thus, from a practical engineering point of view, the analytical equations of cell maintenance (or the methods of plotting experimental data) may be employed to assess the relationship between μ and observed values of Y_{CR} to determine the "maintenance coefficient" and the value of the "true" cell yield. Two linearized forms are in common usage. Equation 6 has been employed by Marr, et al. (20), and equation 7 by Schultze and Lipe (21)

$$\frac{1}{Y_0} = \frac{1}{Y_t} + \frac{k_d}{Y_t} \cdot \frac{1}{\mu} \quad (6)$$

$$\mu = Y_t U - k_d \quad (7)$$

The term k_d can be defined as the maintenance coefficient, and U as the specific substrate utilization rate. The terms Y_0 and Y_t are observed and "true" cell yields, respectively. In Figure 6, the data for the four runs herein presented as well as data from other runs at different specific growth rates are plotted in accord with equations 6 and 7. It is seen that the data fit the equations fairly well. The true cell yield and maintenance coefficient according to these data were 0.59 and 0.14 days⁻¹, respectively. The maintenance coefficient is somewhat higher than values previously reported in the literature (21)(22)(23)(24), and the "true" cell yield for these data fall within the range of 0.4-0.6 for true cell yield previously found to be the most probable for heterogeneous populations of sewage origin growing on readily metabolized carbohydrate substrates (8)(9)(16). It is important to note that over a period of more than ten years, studies on cell yield in our laboratories have indicated that the so-called true cell yield obtained in experiments wherein maximum specific growth rate was attained or approached could vary considerably for growth on the same substrate due to the heterogeneity of the population and changes in predominating species which occurred from time to time. Most values fell between 0.4 and 0.6, but cell yields higher and lower than these were also recorded. In the present study, average Y_{CR} values (i.e., observed cell yields during cell recycle operation) over the entire range of specific growth rates ranged from 0.30 to 0.54, which is not much more than the expected range of values for true cell yield. In view of this and the fact that the maintenance coefficient, k_d was somewhat

higher than had been reported previously, there would seem to be doubt that the inclusion of a term for cell maintenance in the steady state design and operation models is entirely warranted.

Notwithstanding, it was of interest to modify the model equations for \bar{X} and \bar{S} (equations 1 and 2) to include the additional biological "constant," the maintenance coefficient, k_d . The boundary conditions and assumptions made in deriving the equations were precisely the same as before, except for the inclusion of the maintenance coefficient. The modified equations are shown in Table V. The inclusion of the maintenance term adds only one factor in the equation for \bar{X} , as can be seen by comparing equations 1 and 8. Also, the inclusion of the maintenance coefficient adds one additional factor to each constant, a, b, and c, in the equation for \bar{S} (as can be seen by comparing equations 2 and 9). Having obtained these equations and the value of k_d for these data, it was of great interest to compare the predictive power of the equations with that of the equations without the terms for cell maintenance. The values of μ_{\max} and K_S were the average of those determined from batch growth studies during each continuous flow run. These were $\mu_{\max} = 0.49 \text{ hr}^{-1}$ and $K_S = 115 \text{ mg/l}$ at 500 mg/l substrate loading, and $\mu_{\max} = 0.58 \text{ hr}^{-1}$ and $K_S = 105 \text{ mg/l}$ at 2000 mg/l substrate loading. The cell yield employed was 0.59, the value obtained for "true" cell yield from Figure 6. This yield value was employed in equations 8 and 9. In the equations without the term for cell maintenance (equations 1 and 2) other values of cell yield covering the expected range of 0.4-0.6 were employed. Predicted daily amounts of excess sludge for both sets of equations were also compared with the observed amounts of excess sludge; the results are shown in Table VI. It is seen that for either set of equations, the observed value of \bar{S} in the effluent (filtrate COD) is somewhat higher than the

predicted values. The use of effluent COD as a measure of \bar{S} remaining in the effluent represents a very conservative estimate of the concentration of soluble substrate remaining. In nearly all experiments on readily metabolized carbon sources, there is a residual COD of the magnitude observed in these studies even though we have found it to consist of very slowly metabolized material with very low biochemical oxygen demand. We have, from time to time, studied this residual material and have concluded that it most probably consists of fragments of cell walls and cell membrane mixed with some very slowly metabolized internal cell components which leak into the medium. We have in other studies shown that residual carbohydrate is much lower than residual COD. Since our substrate was a carbohydrate, this would bring the values closer to those predicted. Also, it should be emphasized that BOD_5 values which were run, not on soluble material in the effluent but on clarifier supernatant which contained small amounts of cells in addition to soluble material, were much lower than the concentration of filtrate COD in the effluent. It is evident that there is little difference in the predicted values of \bar{S} whether equation 2 or 9 is employed.

Comparison of the predicted values of \bar{X} also leads to the conclusion that there is little difference using either equation 1 or 8. Also, the equations provide a rather good prediction of the values of \bar{X} . Regarding excess sludge production, it is seen that at the higher feed level (i.e., higher specific growth rate or lower cell age), the new model provides rather good prediction of excess sludge at a yield value of 0.6, but the original model without the maintenance factor does not predict X_w very closely at this value of cell yield. For the lower S_i (with lower specific growth rate and higher cell age), the model which considers the maintenance factor provides a very good prediction whereas the one which does not

provides an over 50 percent over-estimate of excess sludge (compare 1015 with 1695 mg/day). This result is reasonable, since the cell maintenance factor (or autodigestion factor) exerts a greater effect as cell age increases or as the cells grow at a slower rate. Since low growth rates are those which usually obtain in activated sludge systems and since prediction of excess sludge is an important design parameter, the inclusion of the maintenance term seems a useful refinement to the model.

SUMMARY AND CONCLUSIONS

In summary, these studies show clearly that an activated sludge can be operated without extraordinary operational effort according to the condition that the recycle sludge concentration, X_R , is a selectable constant. In brief, the conditions stipulated in the mathematical model can be carried out in operation. The experimental results also provide evidence that a pseudo steady state in \bar{X} and \bar{S} does ensue, which is another condition stipulated in deriving the model. Comparison of the present and previous results (Figures 2 and 4 with 3 and 5) indicate that the performance with regard to \bar{X} , \bar{S} , and X_W is fairly reproducible. The system follows the general trend of decrease in X_W as growth rate μ is decreased (or as cell age is increased). The data indicate some reason for caution in ascribing this phenomenon to the so-called maintenance energy concept as it is usually stated. However, either of the analytical equations employed to express the kinetics of the concept can be used to handle the data. Equations have been derived which include the addition of the biological "constant," k_d . When these equations were tested against those we had previously used, it was found that there was little difference in the prediction of \bar{S} and \bar{X} in any case. There was also only a slight difference in prediction

of X_W at the higher specific growth rate, but there was a significant difference at the low specific growth rate. Either set of equations can be employed, but in the interest of refining the prediction of X_W , those including the maintenance coefficient are recommended.

Although our studies on this design and operational model are continuing and include other steady state studies as well as examination of the ability of the system to take various types of shock loadings, the results to date warrant the conclusion that we now have a model for the metabolism of activated sludge which has close communion with the general theory of continuous culture. The model includes the biomechanical properties of the sludge, i.e., μ_{max} , K_S , Y , and k_d , the organic substrate concentration, S_i , as well as the selectable (design and operational) engineering constants, F , V (i.e., $D = \frac{1}{t}$), α , and X_R . These are the major factors which determine the behavior of the system, and the model quantitatively relates their interaction in controlling the effluent quality, \bar{S} , the concentration of aeration solids, \bar{X} , and the excess sludge produced, X_W . To operate a plant in accordance with this model, one controls the concentration of X_R , the recycle sludge. This in turn determines, in large measure, the aeration solids concentration, \bar{X} and, consequently, the effluent substrate concentration, \bar{S} .

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LIST OF SYMBOLS

- c - Sludge recycle concentration factor, equal to the ratio between the recycle solids concentration, X_R , and the biological solids concentration in the reactor, X .
- D - Dilution rate. Ratio of the rate of flow, F , and the volume of liquor in the aeration tank, V . It is equal to the reciprocal of the mean hydraulic residence time, \bar{t} , in a completely mixed reactor, hr^{-1} .
- F - Rate of flow of incoming substrate or wastewater, $1/\text{hr}$.
- k_d - Maintenance energy coefficient, day^{-1} .
- K_s - A biological "constant" used in the hyperbolic expression relating specific growth rate to substrate concentration. It is known as the saturation constant. It is numerically equal to the substrate concentration at which specific growth rate is $\frac{1}{2}$ the maximum specific growth rate for the system, mg/l .
- S - Substrate concentration, measured as COD, mg/l .
- S_i - Concentration of substrate in the inflowing feed in continuous flow operation, measured as COD, mg/l .
- S_e - Steady state concentration of substrate in the reactor or effluent, filtrate COD, mg/l .
- \bar{S}_t - Steady state concentration of COD in the clarifier effluent, supernatant including non-settled biological solids, mg/l .
- \bar{S}_R - Filtrate COD in the recycle sludge, mg/l .
- U - Specific substrate utilization rate, day^{-1} .
- X - Biological solids concentration, mg/l .
- \bar{X} - Steady state biological solids concentration in the reactor, mg/l .
- \bar{X}_e - Steady state biological solids concentration in the clarifier effluent, mg/l .
- \bar{X}_R - Biological solids concentration in the recycle flow to the reactor, mg/l .
- \bar{X}_W - Excess biological solids (sludge wasted), mg/day .
- Y_B - Mean cell yield obtained during growth at specific growth rate at or near μ_{max} (batch system).
- Y_C - Mean cell yield obtained during growth at specific growth rate, μ_C (continuous system without cell recycle).

- Y_{C_R} - Mean cell yield obtained during growth at specific growth rate, μ_{C_R} (continuous system with cell recycle).
- Y_o - Observed cell yield.
- Y_t - True cell yield.
- μ - Specific growth rate in an exponential phase of growth, hr^{-1} .
- μ_{C_R} - Specific growth rate in continuous system with cell feedback, hr^{-1} .
- μ_c - Specific growth rate in continuous system without cell feedback, hr^{-1} .
- μ_{max} - The maximum specific growth rate for a system in exponential growth, hr^{-1} .
- α - Recycle flow ratio.
- θ_c - Sludge retention time, days.

TABLE I
COMPARISON OF STEADY STATE EQUATIONS ACCORDING TO MODELS OF
HERBERT AND OF RAMANATHAN AND GAUDY

Herbert Constant $c \left(c = \frac{X_R}{\bar{X}} \right)$	Ramanathan & Gaudy Constant X_R
$\bar{X} = \frac{Y}{1 + \alpha - \alpha c} (S_i - \bar{S}) \quad (1a)$	$\bar{X} = \frac{Y[S_i - (1 + \alpha)\bar{S}] + \alpha X_R}{1 + \alpha} \quad (1)$
$\bar{S} = \frac{K_S D(1 + \alpha - \alpha c)}{\mu_{\max} - D(1 + \alpha - \alpha c)} \quad (2a)$	$\bar{S} = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a} \quad (2)$ $a = \mu_{\max} - (1 + \alpha)D$ $b = D \left[S_i - (1 + \alpha)K_S \right] - \frac{\mu_{\max}}{1 + \alpha} \left[S_i + \frac{\alpha X_R}{Y} \right]$ $c = K_S D S_i$
$\mu = D(1 + \alpha - \alpha c) \quad (3a)$	$\mu = D \left(1 + \alpha - \alpha \frac{X_R}{\bar{X}} \right) \quad (3)$
	$X_W = V\bar{X}D \left(1 + \alpha - \alpha \frac{X_R}{\bar{X}} \right) = V\bar{X}\mu \quad (4)$
	$\mu = X_W / V\bar{X} = \frac{1}{\text{sludge age}} \quad (5)$

TABLE II
COMPOSITION OF FEED SOLUTION

Constituents	Amount
Glucose	500 mg/l
Ammonium sulfate (NH ₄) ₂ SO ₄	250 mg/l
Magnesium sulfate, MgSO ₄ ·7H ₂ O	50 mg/l
Ferric chloride, FeCl ₃ ·6H ₂ O	0.25 mg/l
Manganous sulfate, MnSO ₄ ·H ₂ O	5.0 mg/l
Calcium chloride, CaCl ₂	3.75 mg/l
1 M phosphate buffer solution, pH 7.0	5 ml/l
Tap water	50 ml/l

TABLE III
 REPRODUCIBILITY OF MEAN STEADY STATE VALUES OF EFFLUENT SUBSTRATE AND BIOLOGICAL SOLIDS CONCENTRATION
 AT S_i VALUES OF 500 AND 2000 mg/l

Feed		Effluent								Biological Solids																	
Nominal	Observed	Filtrate				Supernatant				20-day				10-day													
		COD mg/l		% S_e		% S_t		% X_e		% X		% X_R		% X_W		% X_W											
mg/l	mg/l	20	10	S_e	%	S_e	%	S_t	%	BOD	X_e	S_t	%	BOD	X_e	X	X_R	S_R	Pro- tein	Carb.	X_W	X	X_R	S_R	Pro- tein	Carb.	X_W
500	530	504	506	25	95	15	98	46	91	11	30	29	94	10	14	2150	9925	11	47	28	1015	2120	9970	8	49	32	1029
2000	2120	2010	2090	38	98	31	98	61	97	18	22	47	98	15	19	2885	10140	12	40	33	6280	2885	10010	14	53	41	6230

S_e - Steady state concentration of substrate in the reactor or effluent, filtrate COD, mg/l

S_t - Steady state concentration of COD in the clarifier effluent, supernatant including non-settled biological solids, mg/l

X_e - Steady state biological solids concentration in the clarifier effluent, mg/l

X - Steady state biological solids concentration in the reactor, mg/l

X_R - Biological solids concentration in the recycle flow to the reactor, mg/l

S_R - Filtrate COD in the recycle sludge, mg/l

X_W - Excess biological solids (sludge wasted), mg/day

TABLE IV

RELATIONSHIP BETWEEN SPECIFIC GROWTH RATE AND SLUDGE YIELD UNDER
DIFFERENT CONDITIONS OF CONTINUOUS AND BATCH GROWTH

S_i mg/l	Spec. Growth Rate, μ , hr^{-1}			Cell Yield, Y , mg/mg		
	μ_{CR}	μ_c	μ_{max}	Y_{CR}	Y_c	Y_B
<u>500</u>						
20-day run	0.0125	0.125	0.49	0.36	0.35	0.39
10-day run	0.0093	0.125	0.52	0.33	0.36	0.41
<u>2000</u>						
20-day run	0.046	0.125	0.58	0.54	0.53	0.56
10-day run	0.048	0.125	0.56	0.54	0.53	0.54

TABLE V

STEADY STATE EQUATIONS INCLUDING MAINTENANCE ENERGY COEFFICIENT
FOR THE MODEL EMPLOYING CONSTANT X_R

$$\bar{X} = \frac{Y [S_i - (1 + \alpha)\bar{S}] + \alpha X_R}{(1 + \alpha) + k_d/D} \quad (8)$$

$$\bar{S} = \frac{-b + \sqrt{b^2 - 4ac}}{2a}$$

$$a = \mu_{\max} - (1 + \alpha)D + k_d \quad (9)$$

$$b = D [S_i - (1 + \alpha)K_s] - \frac{\mu_{\max}}{1 + \alpha} \left[S_i + \frac{\alpha X_R}{Y} \right] - k_d \left[\frac{S_i}{1 + \alpha} + K_s \right]$$

$$c = K_s D S_i + \frac{k_d}{1 + \alpha} K_s \cdot S_i$$

TABLE VI
EFFECT OF MAINTENANCE COEFFICIENT k_d ON PREDICTED VALUES
OF \bar{S} , \bar{X} , and \bar{X}_W

S_i mg/l	Observed	Predicted under conditions shown			
	mg/l	$Y = 0.59$ $k_d = 0.14$	$Y = 0.4$ $k_d = 0$	$Y = 0.5$ $k_d = 0$	$Y = 0.6$ $k_d = 0$
Effluent Substrate, \bar{S} , mg/l					
500	25	4	3	3	4
2000	38	10	7	9	14
Biological Solids, \bar{X} , mg/l					
500	2150	2140	2136	2174	2211
2000	2885	2862	2654	2811	2968
Excess Sludge, \bar{X}_W , mg/day					
500	1015	1162	1132	1417	1695
2000	6280	6255	4695	5872	7050

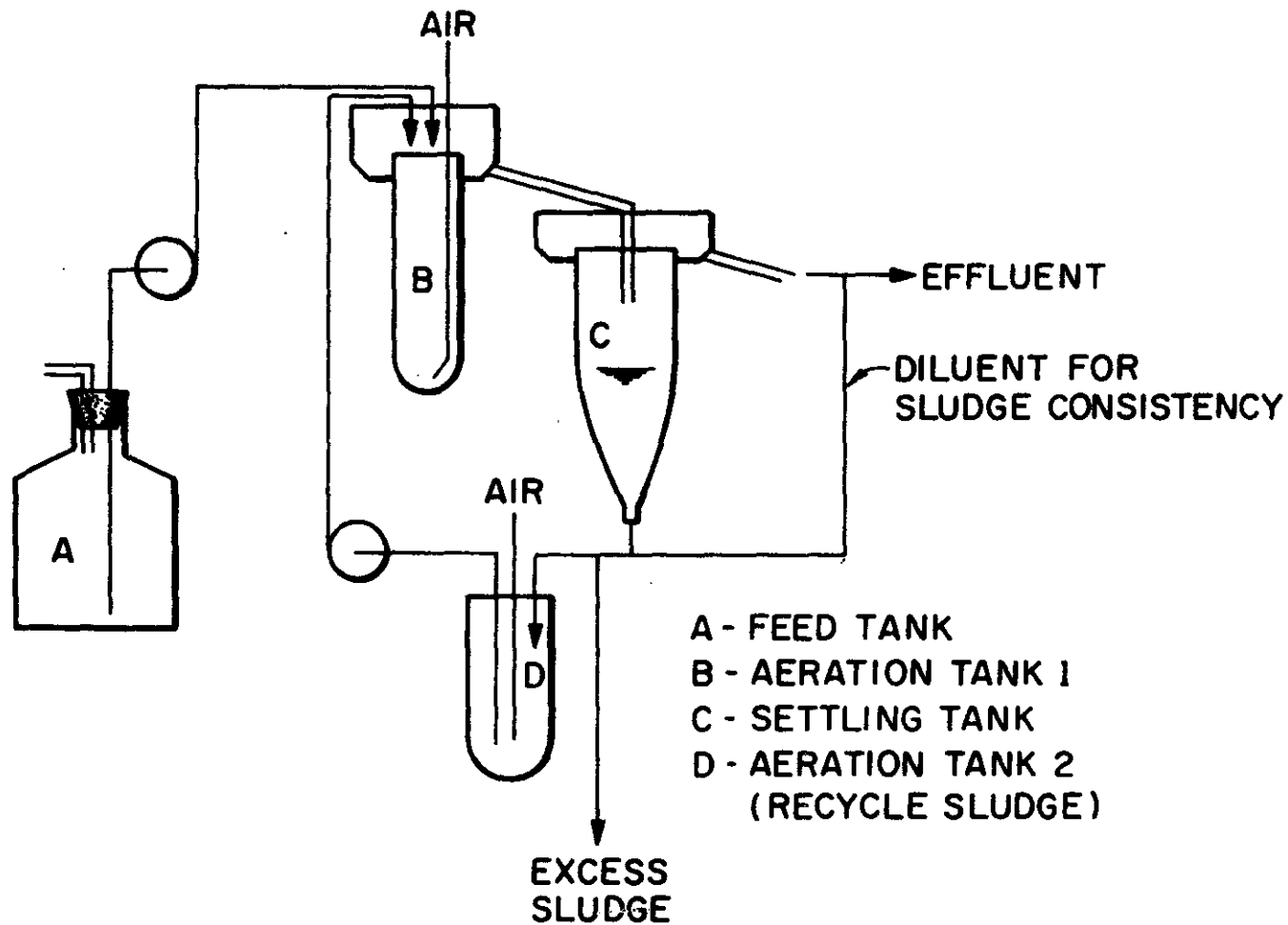


Figure 1. ACTIVATED SLUDGE PILOT PLANT FOR OPERATION WITH CONSTANT X_R .

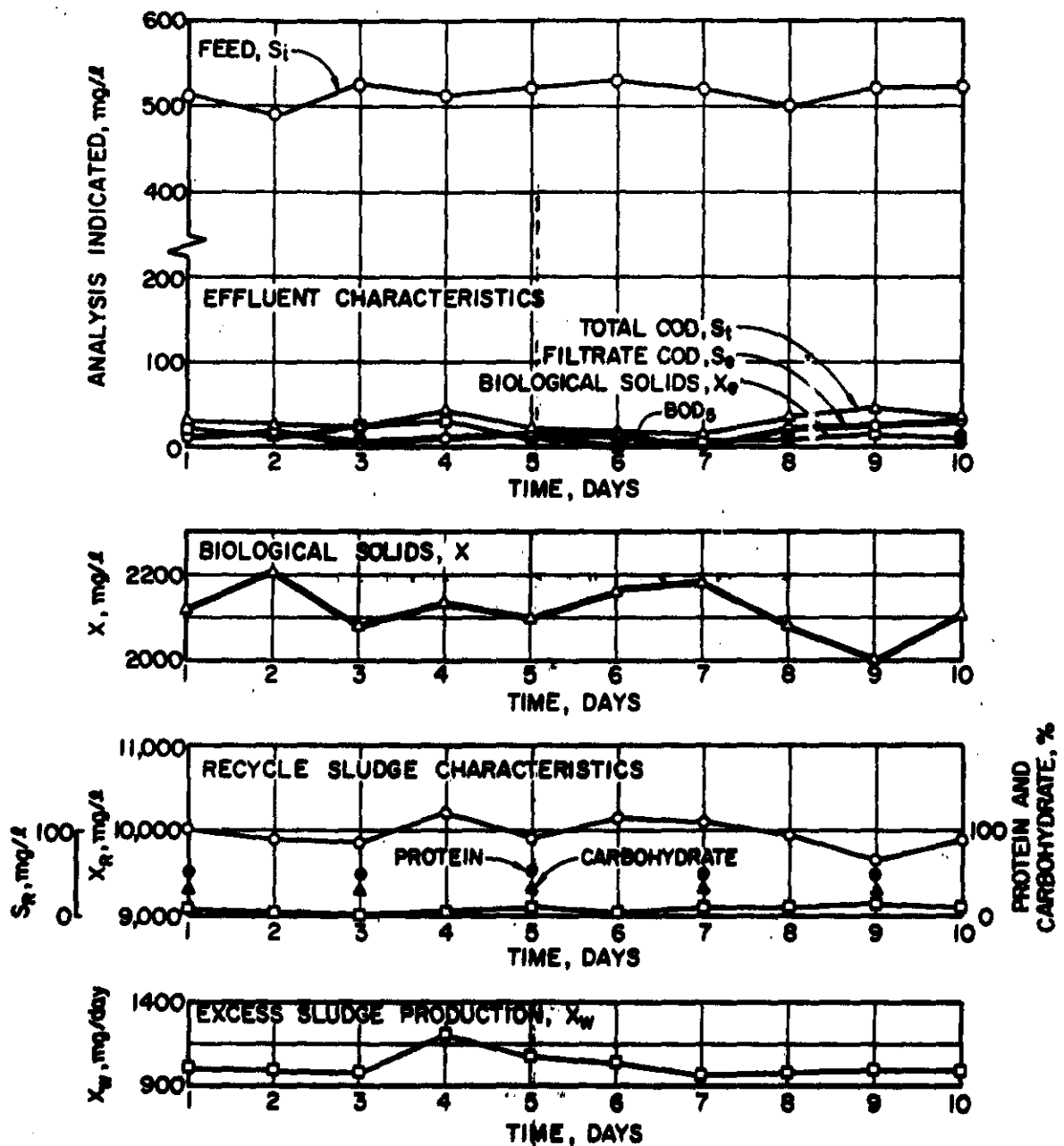


Figure 2. OPERATIONAL CHARACTERISTICS FOR AN ACTIVATED SLUDGE PROCESS WITH CONSTANT X_R AT AN S_i OF 500 mg/l - 10 DAY RUN.

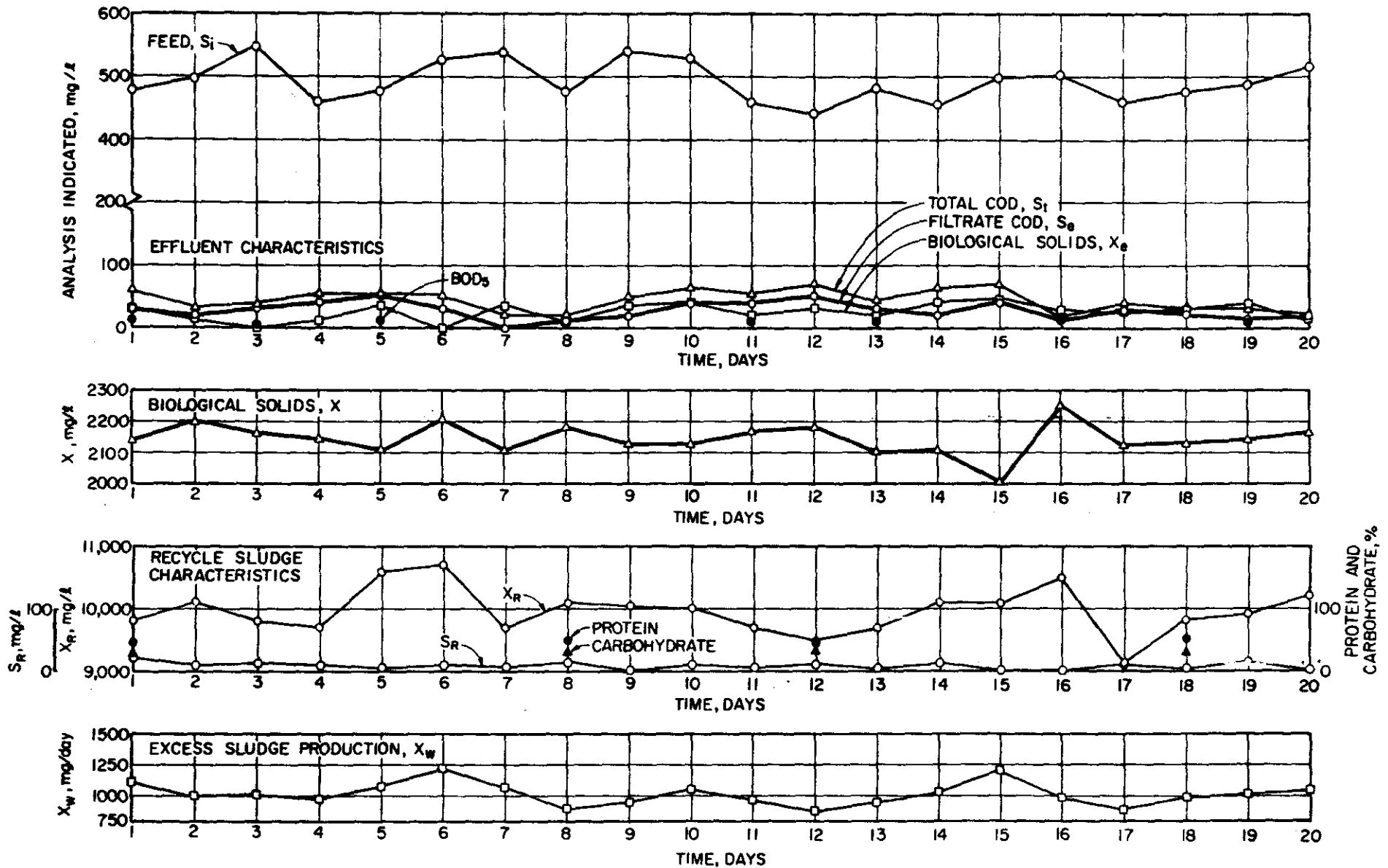


Figure 3. OPERATIONAL CHARACTERISTICS FOR AN ACTIVATED SLUDGE PROCESS WITH CONSTANT X_R AT AN S_i OF 500 mg/l - 20 DAY RUN.

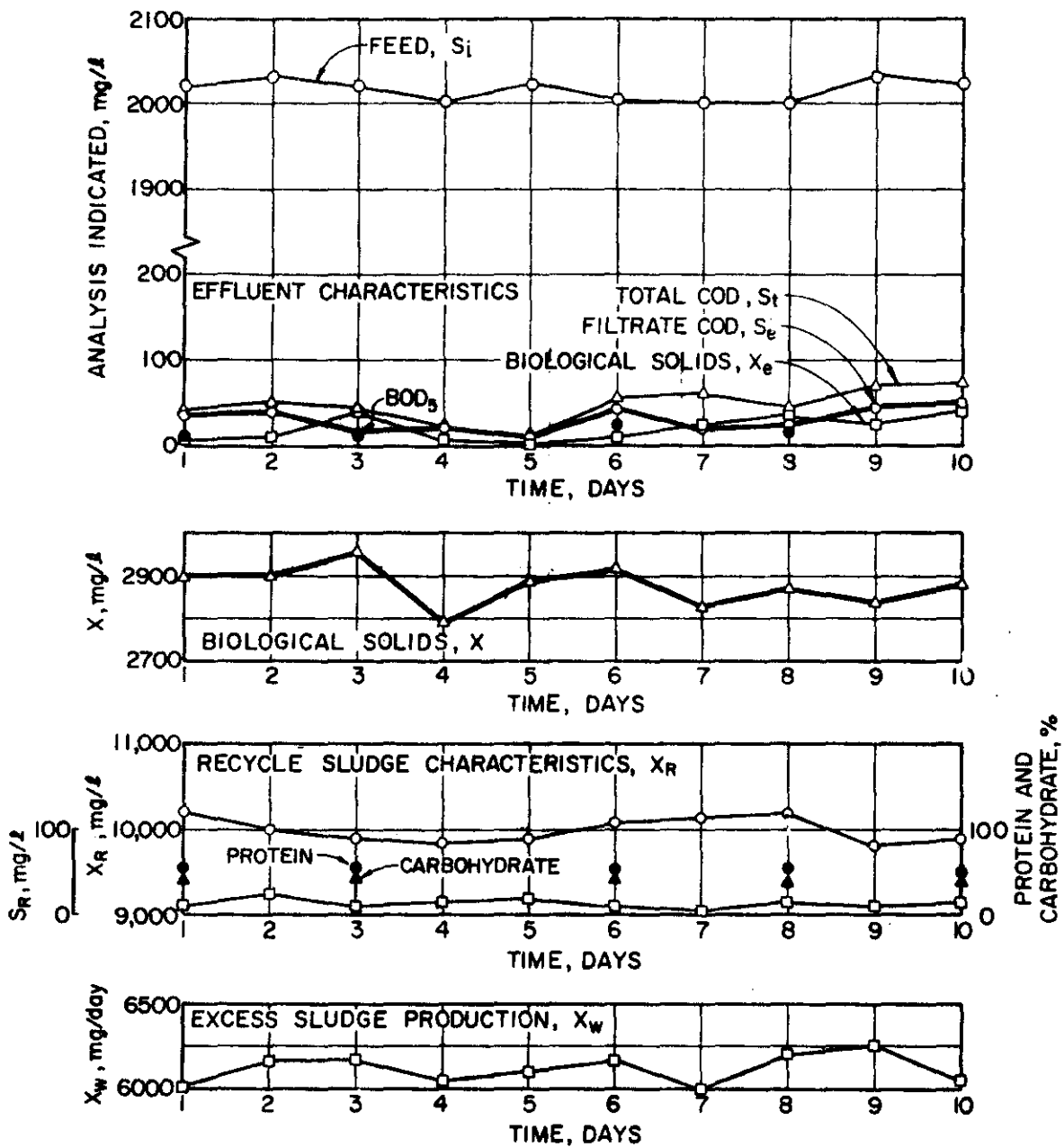


Figure 4. OPERATIONAL CHARACTERISTICS FOR AN ACTIVATED SLUDGE PROCESS WITH CONSTANT X_R AT AN S_i OF 2000 mg/l - 10 DAY RUN.

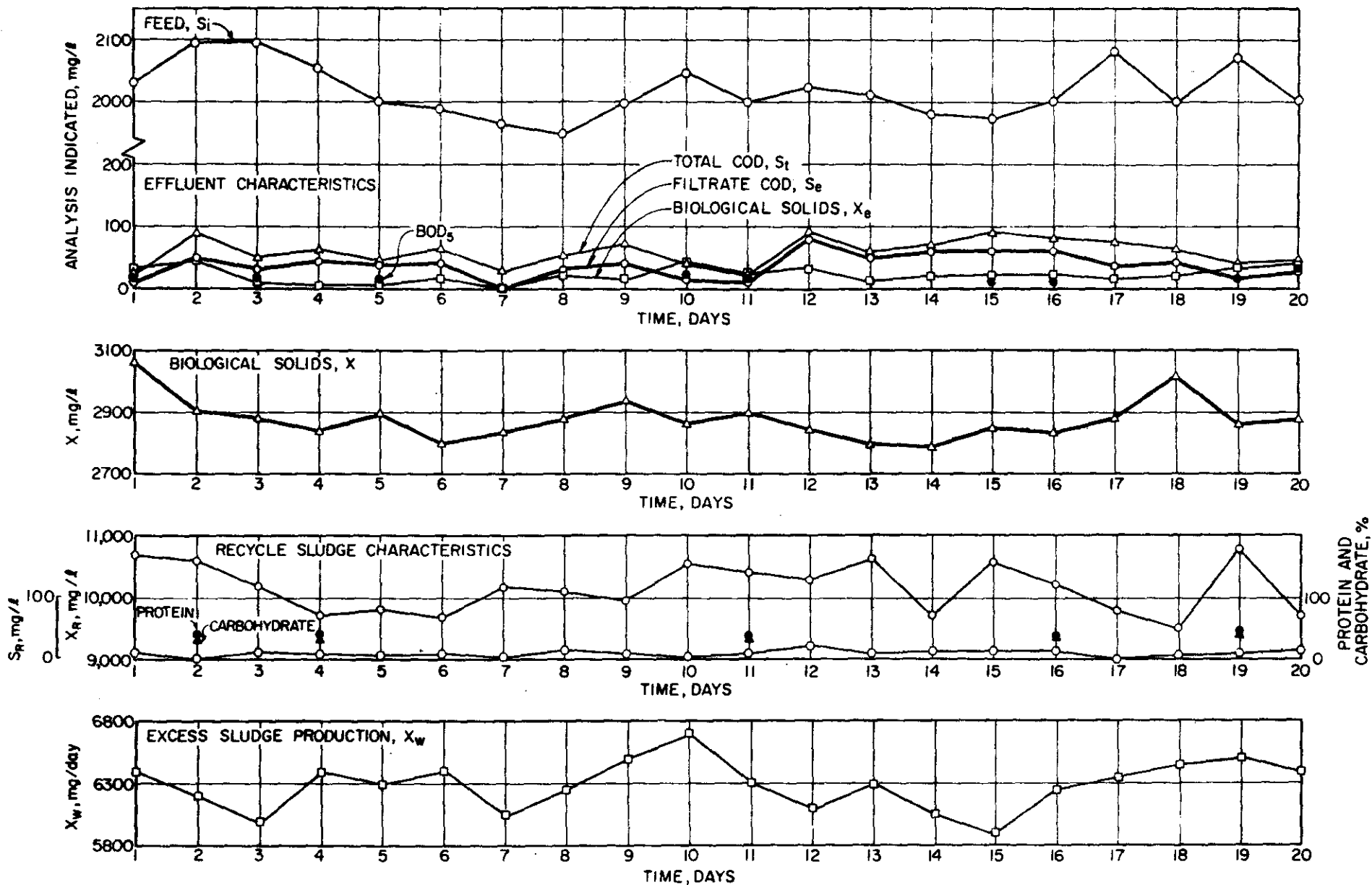


Figure 5. OPERATIONAL CHARACTERISTICS FOR AN ACTIVATED SLUDGE PROCESS WITH CONSTANT X_R AT AN S_i OF 2000 mg/l - 20 DAY RUN

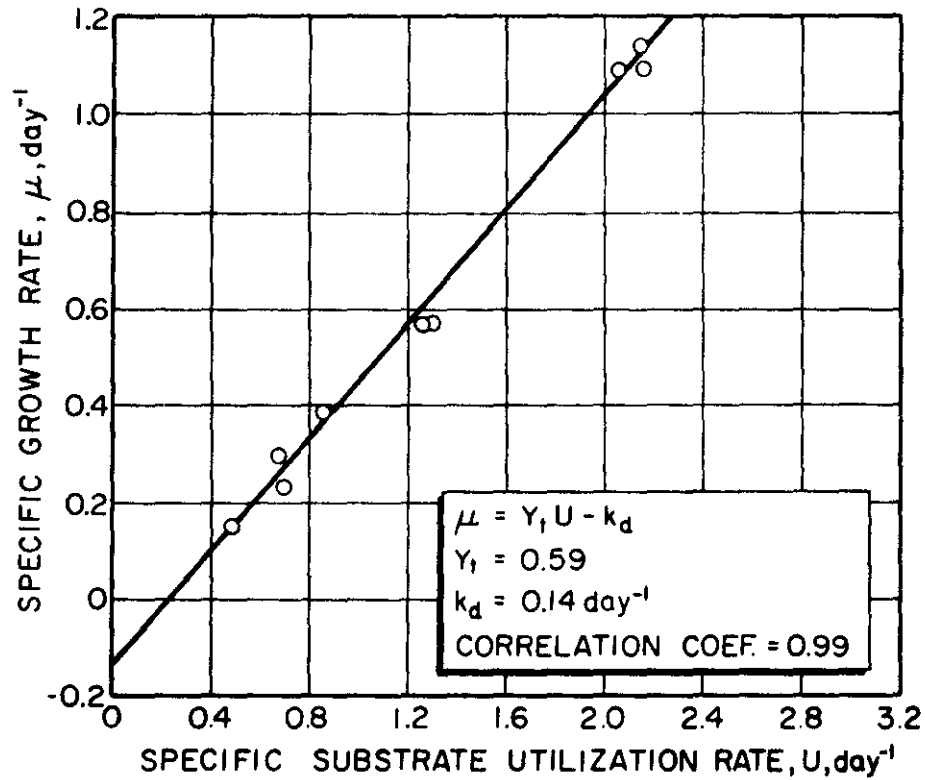
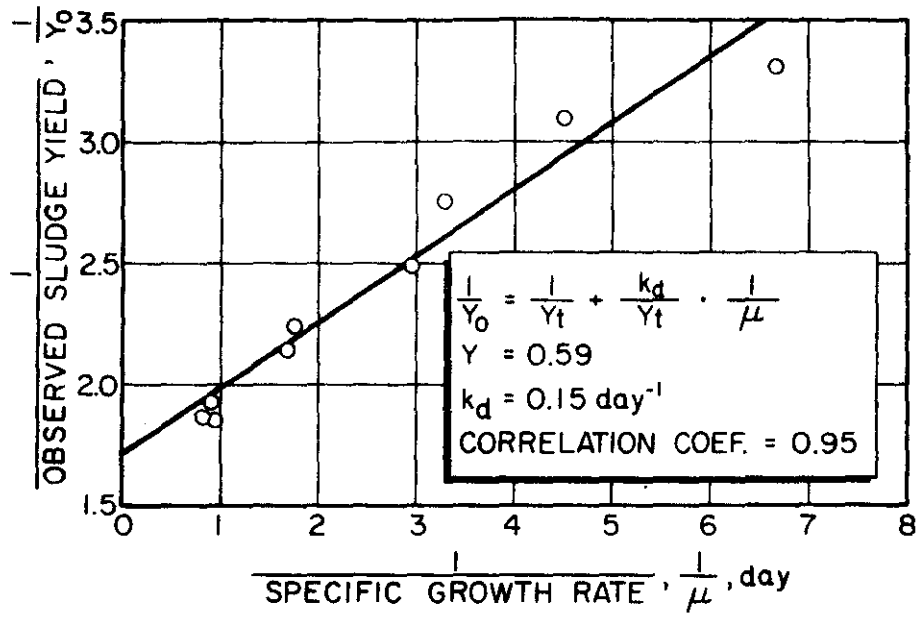


Figure 6. PLOTS OF MAINTENANCE ENERGY EQUATIONS TO DETERMINE TRUE YIELD, Y_t AND MAINTENANCE COEFFICIENT, k_d .

APPENDIX IV

Gaudy, A. F. Jr. and Srinivasaraghavan, R., "Effect of Specific Growth Rate on Biomass Yield of Heterogeneous Populations Growing in Both Continuous and Batch Systems." Biotechnology and Bioengineering, XVI, 423-427 (1974)

EFFECT OF SPECIFIC GROWTH RATE ON BIOMASS YIELD OF HETEROGENEOUS
POPULATIONS GROWING IN BOTH CONTINUOUS AND BATCH SYSTEMS

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ABSTRACT

A decreasing cell yield, Y , when microorganisms are grown in continuous culture at decreasing specific growth rates, μ , is usually thought to be a manifestation of the cell maintenance energy concept. In the present communication, cell yields for heterogeneous populations grown in completely mixed reactors operated with cell feedback were in accord with the maintenance energy concept, i.e., lower for lower values of μ . However, cells taken from the reactor during operation at each value for μ and employed for determinations of Y in batch growth at substrate concentrations which allowed much higher values of μ (near μ_{max}) gave the same value for Y as those obtained at much lower values of μ in the chemostat from which the cells were harvested. Also in the chemostat, when μ was increased by operation as a once-through reactor, the cell yield remained the same. The results are presented and discussed here since they may represent one of the few instances wherein data are available which are both in accord with and in opposition to the maintenance concept. There would appear to be a possibility that continuous growth at a low value of μ may select cells with lower "true" yield.

In a recent study (1), we examined experimentally a mathematical model (2) depicting the continuous culture of heterogeneous microbial cultures with cell recycle. The primary purpose of the study was to determine whether the model equations could adequately predict levels of biological solids, X , and effluent substrate, S_e , under various experimental conditions and to determine the relative steadiness in X and S_e which could be operationally achieved. Based upon those results, we concluded that the model is a useful one from the standpoint of both

design and operation of systems employing heterogeneous populations, e.g., activated sludge processes. In these studies, three inflowing feed concentrations (S_i) were employed: 500, 1000, and 2000 mg/l. Glucose was employed as carbon source in a minimal salts medium with ammonium ion as nitrogen source (1). Dilution rate, D , was maintained at 0.125 hr^{-1} ; the hydraulic feedback rate, α , was 0.25, and cell feedback concentration, X_R , was maintained at 10,000 mg/l. Also during the investigation, small samples of cells were taken for batch experiments to determine the value of the growth "constants," i.e., specific growth rate, μ_{\max} , saturation constant, K_S , and cell or biomass yield, Y . The present communication is addressed primarily to our findings on cell or sludge yield, and to presentation of additional results on sludge yield which, to our knowledge, represent unique findings regarding this important parameter.

The value of specific growth rate, μ , in systems with cell recycle can be expressed as:

$$\mu = D \left(1 + \alpha - \alpha \frac{X_R}{X} \right) \quad (1)$$

Thus, in the recent study, μ varied because S_i was varied and the corresponding change in the value of X caused μ to change. Specific growth rates of 0.0125 , 0.024 , and 0.046 hr^{-1} corresponded to the S_i values of 500, 1000, and 2000 mg/l of glucose COD. It is seen that the rather high concentration of recycle solids, X_R , caused μ to be rather low; for example, compare the μ of 0.0125 hr^{-1} at $S_i = 500 \text{ mg/l}$ with a μ of 0.125 hr^{-1} for operation as a once-through chemostat wherein $\mu = D$.

Operation of a chemostat at low values of μ has been found in various studies with pure cultures to depress the numerical value of cell

yield, and the well-known concept of the maintenance energy requirement has been postulated to explain the decrease in biomass yield with decreasing μ (3). Thus, the observed yield as affected by μ is less than the "true growth yield," which is the fraction of the carbon source channelled into synthesis of new biomass in the absence of substrate utilization for energy requirements of the cells other than for growth. In accordance with the cell maintenance energy concept, "true" yield is the cell yield obtained when the specific growth rate is at or near its maximum value for the system under study (3).

In the study employing heterogeneous populations, previously cited (1), the observed cell yield during the three lab-scale pilot plant runs could be calculated from the pilot plant data by measuring the amount of excess cells, X_W , produced, and dividing this amount by the amount of substrate removed (inflow substrate, S_i - outflow substrate, S_e):

$$Y = X_W / (S_i - S_e) \quad (2)$$

These observed values of Y during cell feedback operation of the chemostat, i.e., Y_{C_R} , increased for increasing values of μ (or S_i). Thus, this result was reasonably explainable in accordance with the concept of maintenance energy requirement, and would probably have been so interpreted were it not for the fact that in these studies, we determined cell yield by other means as well.

MATERIALS AND METHODS

At various times during continuous operation at each specific growth rate, small samples of the cells were taken for the determination of cell yield in well-aerated batch reactors. The medium was the same as that

employed in the continuous flow reactor (1). This cell yield value, Y_B , was defined as the amount of cells, ΔX , i.e., $X_0 - X_t$, which grew as a result of the removal of substrate, ΔS , i.e., $S_0 - S_t$, at the end of the substrate removal phase. In previous studies we have shown that Y during batch growth is the same as at the time of peak biological solids concentration, i.e., time of substrate removal or cessation of growth (4). Since these yields were determined at initial substrate concentrations of 1000 mg/l and 800 mg/l, which were concentrations permitting a close approach to maximum specific growth rate, μ_{max} , these yield values could be expected to be relatively unaffected by the maintenance requirement, i.e., they were essentially measures of the "true" yield. During the study, data were obtained which verify the fact that the μ values obtained at the 800 and 1000 mg/l substrate levels were close to the μ_{max} values (1).

Another mode of measurement for cell yield was also performed. At the end of the data-taking period for each pilot plant run at the various values of μ , cell recycle was terminated and the reactor was operated as a once-through system, i.e., at $\mu = D = 0.125 \text{ hr}^{-1}$. The yield values, Y_C , obtained during this period of operation $\left[Y_C = X / (S_i - S_e) \right]$ would, in accordance with the concept of maintenance energy requirement, be expected to be higher than those observed during operation at the lower μ values which obtained during operation of the pilot plant with cell feedback.

RESULTS AND DISCUSSION

Table I shows the results for all three measurements of yield, Y_{C_R} , Y_C , and Y_B , and the values of μ at which they were obtained. Vertical

comparison of the values shows that Y_{C_R} , as well as Y_B and Y_C , increased with increasing specific growth rate, μ_{C_R} . In contrast, horizontal comparison of yields determined for cells obtained during each run but by different measurement procedures and over a wide range of increasing μ values, indicates little or no difference between Y_{C_R} , Y_C , and Y_B . In previous studies (4), we have shown that for heterogeneous populations, cell yield on a given substrate can be expected to vary under the same conditions of measurement, and in analyzing the present mean yield results it is important to inspect the variation in yield values for the same and for different modes of measurement of yield. Such a comparison is shown in Table II. There were not sufficient data to warrant calculation of standard deviation, σ , and coefficient of variation, CV, for the Y_C and Y_B values; however, the range of values is given. It can be seen that the Y_{C_R} values did exhibit an expected range of variation at each value of μ , but the values were rather closely grouped around the mean and the average values appear to provide a realistic representation of the observed yield, Y_{C_R} . Even without the benefit of statistical estimation of the central grouping of values, the values for Y_C and Y_B are seen to be rather closely grouped. Thus the average values shown in Table I do not appear to be solely attributable to random variation in Y due to the heterogeneity of the microbial population.

Horizontal and vertical comparisons of the cell yield values in Table I present a paradoxical situation. The condition of continuous growth at decreasing values of μ (Table I, vertically), which correspond to decreasing values of yield, Y_{C_R} , caused an apparent non-readily reversible change in Y (i.e., Y_C and Y_B ; Table I horizontally viewed) when the cells harvested during each continuous flow run were grown at

increased values of μ . The latter effect is not in accord with the concept of maintenance energy, while the former one is. It seems possible according to results herein presented that growth at low specific growth rates may select cells with low true yield values. It is important to note that the narrow ranges of Y_C values shown in Table II were observed over a period of five mean hydraulic detention times, i.e., 40 hours. In the present study, the periods of once-through operation following the runs with cell recycle were terminated at 40 hours. However, in subsequent studies (unpublished), the periods of once-through operation were carried forward a longer time and there was indication that after 5-7 mean hydraulic detention times the Y_C values began to increase; such a result would appear to be a reasonable consequence of selection of species.

It is known from past studies (5)(6) that growth at increased specific growth rates tends to select cells with higher μ_{max} (also see Table I); it may be that it also tends to select cells with higher "true" yield. If this is the case, it would be interesting to run comparable experiments using a pure culture, since the results could provide clarifying insight to the concept of maintenance energy. If the reduced Y at lower μ is a manifestation of maintenance energy, the effect should be rapidly reversible when cells are harvested and grown at μ near μ_{max} , whereas if the lower yield resulted from selection of mutant strains with low true yield, the values should not be readily reversible. Such studies would also be an aid in the interpretive application of kinetic models to heterogeneous populations, and since we have seen no comparable data with pure cultures, we are currently designing such experiments

with the aim of illuminating this aspect. The present results provide reason for caution in making the usual assumption that the lower cell yields which are obtained under "steady state" growth in "chemostats" at lower specific growth rates are a manifestation of the concept of maintenance energy insofar as heterogeneous microbial populations are concerned.

ACKNOWLEDGMENT

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TABLE II

VARIATION AND RANGE OF VALUES FOR MEAN CELL YIELDS AT VARIOUS SPECIFIC GROWTH RATES

S_i	Y_{CR}					Y_C			Y_B		
	Mean mg/mg	n	σ	CV %	Range	Mean mg/mg	n	Range	Mean mg/mg	n	Range
500	0.36	20	0.010	2.7	0.31-0.43	0.35	7	0.33-0.37	0.39	3	0.38-0.41
1000	0.46	30	0.010	2.1	0.40-0.51	0.46	8	0.43-0.49	0.50	4	0.45-0.53
2000	0.54	20	0.015	2.7	0.51-0.56	0.53	7	0.52-0.54	0.56	3	0.53-0.59

APPENDIX V

Yang, P. Y., and Gaudy, A. F. Jr., "Control of Biological Solids Concentration in the Extended Aeration Process," Journal Water Pollution Control Federation, 46, 543-553 (1974).

CONTROL OF BIOLOGICAL SOLIDS CONCENTRATION IN THE
EXTENDED AERATION PROCESS

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INTRODUCTION

The extended aeration (total oxidation) process which has been known for three decades, has the unique distinction of being one of the most widely used activated sludge processes, although it has been concluded to be theoretically unsound; i.e., the total oxidation (autodigestion) of the biological population responsible for purification has been thought to be an impossibility. Some of the work upon which such conclusions had been based have been reviewed in previous publications from this laboratory (1)(2)(3). Our most recent publication bearing on this process has shown that extracellular bacterial polysaccharide, previously thought to be biologically inert, served as an excellent source of carbon for growth of heterogeneous microbial populations (4).

Also in previous reports (1)(2) we have presented results of long-term laboratory pilot plant studies in which an extended aeration pilot plant

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was operated for over 1000 days with positive, total recycle of sludge. There was no inadvertent wastage of solids, since the clarifier was "backed up" by centrifuging all effluent, with return of all solids to the aeration chamber. Throughout this period, excellent substrate removal efficiency was maintained and the system gave no indication of building up an inert organic fraction in the sludge. There was no indication of imminent biochemical failure, and we were led to the conclusion that the total oxidation principle of the extended aeration process was not theoretically unsound. The periodicity and amplitude of the irregular cycles of solids accumulation and de-accumulation which were observed could not be predicted. There were times when biological solids concentration in the mixed liquor was so high as to impair their separation in the clarifier, and had we not been centrifuging, biological solids would have escaped in the effluent. It was reasoned that de-accumulation periods (i.e., periods of accelerated autodigestion) could be initiated by providing an engineering assist to the process by withdrawing some of the sludge, hydrolyzing it, thus performing chemically the initial stages of breakdown of macromolecules, and recycling these liquefied cells. We termed this proposed process the "hydrolytic assist;" a flow diagram embodying the principle is shown in Figure 1. Preliminary experiments indicated that cell hydrolysate was an excellent substrate for the growth of microorganisms, and it was decided to run the laboratory pilot plant in accordance with Figure 1.

The former mode of operation (centrifugation of cells) was continued for some time beyond the 1000 days of operation previously reported. Finally, after 1202 days, we terminated this phase (herein referred to as Phase A), and initiated operations to examine the feasibility of the "hydrolytic assist" (herein referred to as Phase B). The purpose of the

present report is to present the results of various experiments designed to gain insight into the chemical and biochemical nature of the hydrolyzed cells and to present results of pilot plant operations employing the hydrolytic assist.

MATERIALS AND METHODS

The pilot plant was the same as that employed in Phase A studies. Total operating volume was 9.4 liters (6.2-liter aeration chamber and 3.2-liter settling chamber). The overall detention time was 24 hours (approximately 16 hours aeration and 8 hours settling). The unit was operated in continuous flow, and there were short periods of batch feeding (e.g., during shock loads consisting of a slug dose of substrate) such as have been previously described for Phase A operation (1)(2). The general operational procedures were the same as for Phase A, with the obvious exception of omission of centrifugation and addition of the operation involving periodic withdrawal of sludge, hydrolysis, and recycling of hydrolysate to the aeration chamber, according to Figure 1.

The composition of the inflowing feeds during continuous flow operation is shown in Tables I and II. After the first 32 days of operation, the feed shown in Table II was employed (carbon source composed of glucose + cell hydrolysate). The cell hydrolysate was prepared by partial hydrolysis of the sludge withdrawn from the settling chamber. The sludge was acidified to pH 1.0 and autoclaved for five hours at 15 psi, 121°C. The hydrolyzed sludge was neutralized to pH 7 with NaOH and combined with the glucose synthetic waste in the feed inflow to the aeration chamber.

At two- to three-week intervals, a small sample of sludge was taken for batch experiments to determine patterns of substrate removal and growth for a low initial inoculum of the activated sludge in the system.

TABLE I

CONSTITUENTS IN THE FEED DURING OPERATION AT SUBSTRATE LOADING
OF 500 mg/l GLUCOSE

Glucose	500 mg/l
$(\text{NH}_4)_2\text{SO}_4$	250 mg/l
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	50 mg/l
$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	0.25 mg/l
CaCl_2	3.75 mg/l
$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	5 mg/l
Phosphate buffer, 1.0 M, pH 7.0 ($\text{KH}_2\text{PO}_4 + \text{K}_2\text{HPO}_4$)	10 ml/l
Tap water	50 ml/l

TABLE II

CONSTITUENTS IN THE FEED DURING OPERATION WITH SUBSTRATE LOADING
CONSISTING OF 300 mg/l GLUCOSE AND VARIABLE AMOUNTS
OF SLUDGE HYDROLYSATE

Glucose	300 mg/l
Hydrolysate COD	45-100 mg/l
$(\text{NH}_4)_2\text{SO}_4$	150 mg/l
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	30 mg/l
$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	0.15 mg/l
CaCl_2	2.25 mg/l
$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	3 mg/l
Phosphate buffer, 1.0 M, pH 7.0 ($\text{KH}_2\text{PO}_4 + \text{K}_2\text{HPO}_4$)	3-12 ml/l
Tap water	30 ml/l

Also, at the same time, a small sample of sludge was taken to determine the endogenous O_2 uptake rate of the extended aeration sludge.

Prior to operating the pilot plant employing the hydrolytic assist, some preliminary investigations were made between days 1000 and 1202 of the Phase A operation which were designed to gain insight into physical and biochemical characteristics of the cell hydrolysates. The protocol for these, along with the results, are given in the next section of this report.

Biological solids concentration was determined by the membrane filter technique (Millipore Filter Co., Bedford, Mass., HA 0.45 μ m) as outlined in Standard Methods (5). Chemical oxygen demand (COD) was run on the filtrate and on the effluent from the settling chamber (unfiltered COD)(5). Protein and carbohydrate contents of the sludge were determined by the biuret and anthrone tests (6). In addition to the COD test, the anthrone test was also run on the membrane filtrate. Periodically throughout the experimentation, dissolved oxygen in the aeration chamber mixed liquor and in the settling chamber effluent was measured electrometrically. Also frequent checks on the pH were made. Microscopic observation of the sludge was made periodically. The airflow to the system was maintained at 2000 cc/min/liter. Temperature was maintained at $23 \pm 2^\circ\text{C}$.

RESULTS

Before presenting results pertinent to the operation of the pilot plant, it is appropriate to describe results of experiments undertaken to gain further insight into the chemical-physical nature of the hydrolysate and its utility as a growth substrate.

In previous experiments, cell suspensions were grown and harvested for the purpose of determining conditions of hydrolysis which could be

employed. In these studies, we were guided by biological literature relative to acid and/or alkaline hydrolysis of protein, since protein is the major macromolecular constituent of the cells. It was found that adjustment of a cell suspension (approximately 5,000 mg/l) to pH 1 with concentrated sulfuric acid (final normality approximately 0.35), followed by autoclaving at 15 psi, 121°C for five hours, sufficiently solubilized the bio-mass so that the neutralized hydrolysate became a readily available microbial substrate. This rather mild condition of hydrolysis did not completely hydrolyze cell protein, but complete breakdown to amino acids was not required for metabolism. Sulfuric acid was employed rather than hydrochloric acid, since large amounts of chloride ion militate against use of the COD test. With freshly-grown cells, this condition of hydrolysis provided nearly complete solubilization of the bio-mass, whereas in experiments run on "old" cell suspensions, the bio-mass was not completely solubilized in all cases (75-80% solubilized). In either case, the material was a utilizable growth substrate.

New experiments were run to determine whether increased acid concentration would cause more of the bio-mass to be solubilized (i.e., pass a 0.45 μm membrane filter). After 1202 days of operation of the previous experimentation (Phase A) in which cells were centrifuged from the mixed liquor and returned to the aeration tank, the pilot plant operation was terminated and some experiments were performed on this very old sludge. To equal portions of the bio-mass, sulfuric acid was added to final concentrations of 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 N. These acidified cell suspensions were then autoclaved in the usual manner, and it was found that increasing the acid concentration had essentially no effect on the degree of solubilization. From 75 to 80% of the COD passed the 0.45 μm filter regardless of the acid concentration. High acid concentration destroyed some of the carbohydrate (i.e., decreased the amount of anthrone-reactive

material present). Since this was not desirable from the standpoint of metabolism of the hydrolysate, and since lower acid usage was, in any event, desirable, the procedure of reducing pH to 1.0, which usually required less than 0.5 normal sulfuric acid, was selected for use in the pilot plant operation for the hydrolytically-assisted process.

Since many of the larger as well as smaller molecules in the hydrolysate carry an electrical charge, it was desirable to examine the hydrolysate with regard to its adsorption characteristics. A neutralized sample containing approximately 1300 mg/l COD was treated with various concentrations of powdered activated carbon (Nuchar-C190N, Fisher Labs). The hydrolysate and activated carbon were mixed for two minutes at 45°C and filtered through Whatman No. 1 filter paper. Samples of filtrate were taken for measurement of COD; the results are shown in Figure 2. It is seen that a significant portion of the hydrolysate was readily adsorbed. At the high carbon dosage, 5000 mg/l, the removal of the COD by filtration was increased by approximately 50 percent due to the addition of carbon.

Figure 3 shows results of another such experiment employing hydrolysate obtained from cells during operation of the pilot plant under the present mode of operation, i.e., employing the hydrolytic assist in accordance with the flow sheet shown in Figure 1. It is seen that the degree of adsorption was approximately the same for this sludge as for the 1202-day old sludge hydrolysate from the previous operations.

Various experiments were undertaken to examine the metabolic availability of the hydrolysate as substrate for microorganisms in an extended aeration activated sludge. Figure 4 shows two of many experiments employing an initial inoculum of sludge taken from the extended aeration pilot plant. In these particular experiments, the hydrolysate substrate was obtained from freshly-grown cells. It is seen that the hydrolysate served

quite readily as growth substrate. The residual COD was approximately 50 mg/l, which compares favorably with experiments in which glucose is employed as substrate. Both experiments indicate that a lag or acclimation period is needed before rapid metabolism begins. However, it was observed in previous studies (see Figure 10 of reference 2) that when such a hydrolysate was slug-fed to the extended aeration pilot plant, the higher biological solids concentration contained therein completely masked out the lag and the same concentration of hydrolysate was removed with the same or better COD removal efficiency within 30 minutes.

Studies were also made on hydrolysate obtained from the mature sludge in the extended aeration unit, and experiments were run to compare the course of substrate removal using this hydrolysate and that obtained from freshly-grown cells. Figure 5 shows the course of substrate removal and solids concentration using hydrolyzed "old" cells and an old cell inoculum, i.e., cells taken from the extended aeration unit during the period between days 1000 and 1202. Approximately 80 percent of the hydrolysate was soluble (i.e., passed a 0.45 μm filter). As with the young cell hydrolysate, this mature sludge hydrolysate served readily as a microbial substrate for the cells existing in the extended aeration unit. It is noted that the residual COD at the time of terminating these experiments was somewhat higher than in the experiment using hydrolysate from freshly-grown cells (i.e., 100 mg/l compared with 50 mg/l in Figure 4). It was not possible to test metabolism of this hydrolysate using a very high inoculum of cells taken from the extended aeration pilot plant, since by this time a considerable amount of the original extended aeration activated sludge was being removed for hydrolysate studies. These experiments did provide some indication that the residual COD during subsequent operation of the pilot plant using the hydrolytic assist might be determined in some measure by the frequency of sludge withdrawal for hydrolysis and refeeding.

In preparing to run the pilot plant, it was anticipated that there would be times when problems with sludge settleability might arise. One well-recognized remedial standby procedure is addition of chemical flocculant. Therefore, between days 1000 and 1202 of operation during Phase A, experiments were run using a variety of chemicals to flocculate and settle microorganisms in the pilot plant effluent. Considerable success was obtained with ferric sulfate and aluminum sulfate. We had some concern that if it became necessary to resort to such an expedient for any length of time and if the chemicals were dosed directly to the aeration chamber, a significant amount of flocculant could be retained in the biomass and might inhibit biochemical removal of the substrate, especially when the sludge was hydrolyzed and re-fed to the system. In order to gain some insight into this possibility, settled sludge which had been treated with these chemicals was hydrolyzed and employed as substrate in experiments using cell inocula from the extended aeration pilot plant. Figure 6 shows substrate (COD) removal and accumulated bio-mass during growth on such hydrolysates. These materials were metabolized quite readily, providing some indication that periodic chemical dosage would not interfere with the biochemical efficiency of the system. In the studies shown thus far, hydrolysate comprised the carbon source, not the complete medium. However, cell or sludge hydrolysate is itself a complete growth medium and when the mineral salts were withheld and growth substrate consisted of the hydrolysate alone, the results were essentially the same as those shown here.

With these preparatory studies as a guide, operation of the pilot plant using the hydrolytically-assisted mode of operation (Phase B) was initiated. It is recalled that during operation of the pilot plant without the "hydrolytic assist" there were periods of biological accumulation and de-accumulation (1)(2). Thus, biological solids concentration in an

extended aeration (total solids recycle) system is not controlled, but subjected to the vagaries of natural ecological phenomena. The prime aim of the current study was to provide control over the biological solids concentration by initiating the de-accumulation periods through chemical hydrolysis of portions of the cells prior to their recycle. Thus, in these initial feasibility studies, it was desirable to make an engineering choice of the biological solids concentration we wished to maintain. The range 3000-5000 mg/l was selected, since this concentration is not so high that settling problems due to concentration effects would be expected, and it is high enough to take advantage of the increased rate of substrate removal provided by large numbers of cells in the aeration tank.

The unit was started on July 14, 1970, with an inoculum of microorganisms taken from the supernatant of the primary clarifier of the municipal treatment plant at Stillwater, Oklahoma. After two weeks' batch development of sludge on daily feedings of 1000 mg/l glucose plus mineral salts, the unit was changed over to continuous flow and the substrate concentration reduced to 500 mg/l glucose. After two weeks' operation, the inflowing feed concentration was reduced to the design concentration of 300 mg/l glucose. The first 100 days of operation are shown in Figure 7. The first of a series of sludge withdrawals for hydrolysis and re-feeding was made on day 44. The amounts of sludge withdrawn are noted at the top of the figure. Above this notation, the amount and kind of feed material is also noted. The total COD in the incoming waste is shown in parentheses. The letter "G" is used to designate glucose in the feed, and "H" is used to designate hydrolysate in the feed. From day 32 the glucose concentration in the feed (synthetic waste) was maintained at 300 mg/l. Throughout operation of the pilot plant, the effluent was characterized by determinations of filtrate COD (biochemical efficiency) and effluent COD (i.e., unfiltered clarifier supernatant-overall efficiency). Also, the carbohydrate content of the

filtrate was determined and the biological solids concentration in the effluent was assessed. Sludge characterization (bottom portion of the figure) included biological solids concentration and periodic determinations of protein and carbohydrate content of the biological solids. Also indicated in the lower portion of the figure are the times when small samples of biological solids were taken from the aeration chamber for auxiliary experiments of the type described previously (1)(2) during the 1202-day run without employing the "hydrolytic assist". Triangles indicate seed taken for metabolic studies; circles indicate samples taken for measurement of endogenous respiration rate of the sludge. Periodically, the effluent was also checked for ammonia, nitrite, and nitrate nitrogen (not shown in Figure 7).

It is seen in Figure 7 that this mode of operation provided for rather good and stable operation from day 50 onward. Biological solids concentration in general ranged between 4000 and 5000 mg/l. The carbohydrate content of the sludge was approximately 12 percent, and the protein concentration approximately 44 percent. The biochemical removal efficiency varied from 90 to 97 percent. Biological solids concentration in the effluent ranged from 5 to 50 mg/l, and in most cases it was approximately 20 mg/l. Carbohydrate content in the effluent was approximately 5 mg/l.

Between days 100 and 111 (see Figure 8), the biological solids concentration exhibited some tendency to increase, and the decision was made to increase the volume of mixed liquor withdrawn for hydrolysis from 600 to 900 ml. This increase in the hydrolysis schedule reduced the solids concentration during the ensuing 40 days to a level of approximately 4000 mg/l. It can be seen that throughout the period from approximately day 125 to day 200, solids concentration could be maintained at approximately 4000 mg/l by withdrawal of 900 ml of mixed liquor and its subsequent hydrolysis and recycle to the aeration chamber along with incoming feed

glucose. The biochemical removal efficiency fluctuated between 88 and 98 percent. Biological solids concentration in the effluent fluctuated from 12 to 72 mg/l. Protein and carbohydrate content of the biological solids remained rather steady at 43 and 12 percent, respectively. Carbohydrate concentration in the effluent remained at approximately 5 mg/l. The results shown in Figures 7 and 8 indicate that the proposed modification of the extended aeration process incorporating periodic hydrolysis and recycle of hydrolysate to the aeration tank provided positive control and effective treatment of organic substrates, as well as sludge disposal. The results indicate that such a system can be successfully operated and that the process being proposed could be a very useful one which could do much to alleviate the sludge disposal problem and permit the wider use of the extended aeration process.

It is recalled that these studies were made at a COD:N ratio of 10:1 in the synthetic waste feed. Thus, nitrogen was added in excess and there was an opportunity to examine the nitrifying characteristics of the process. The filtrate was analyzed for $\text{NH}_3\text{-N}$, $\text{NO}_2\text{-N}$, and $\text{NO}_3\text{-N}$. The sum of these is plotted as effluent nitrogen in Figure 9. The dashed line shows the influent concentration of $\text{NH}_3\text{-N}$. The nitrate concentration in the effluent is also plotted. It is seen that after approximately 50 days of operation, the dominant form of effluent nitrogen changed from $\text{NH}_3\text{-N}$ to $\text{NO}_3\text{-N}$. Thus, employment of the hydrolytically assisted process did not interfere with the nitrifying characteristics of the extended aeration process--at least at the substrate loading herein studied. It is also seen that during this period of operation, approximately two-thirds of the influent nitrogen appeared in the effluent stream. Thus a considerable portion of the added nitrogen was reused when recycled in the hydrolysate. It is seen that at times, practically all of the influent nitrogen appeared in the effluent as $\text{NO}_3\text{-N}$.

Over the next 200 days of operation of the pilot plant, studies at other COD:N ratios were also performed; operation at 20:1 and 30:1 did not cause any adverse effect on purification efficiency. These results are reported elsewhere (7) and have useful ramifications to utility of the process for nitrogen-deficient wastes. The main point to be delineated here is that in systems containing excess nitrogen, the return of readily-available organic nitrogen in the hydrolysate did not inhibit nitrification, i.e., the nitrogenous BOD was exerted in the treatment process.

Auxiliary experiments conducted using small inocula of cells from the mixed liquor were of the same type previously accomplished in the Phase A operation. The mean batch sludge yield for these organisms grown on the synthetic waste (glucose) was 46.9 with a 95 percent confidence limit of the mean of ± 3.8 . The cells were characterized by both low growth rate and low endogenous O_2 uptake rates, but these were somewhat higher than those previously observed in Phase A without employment of the hydrolytic assist.

Some shock loading studies were conducted subsequent to the 200-day operational period covered in this report. Figure 10 shows the results of one batch experiment in which neutralized hydrolysate was added as a slug dose to the pilot plant. Approximately 80 percent of the hydrolysate COD was removed in one hour. The remaining organic matter (COD) was not a residual material but was very slowly removed. At 8 hours, nearly 90 percent of the initial dosage had been removed, and 18 hours were required to achieve approximately 95 percent removal. Fortunately, in an extended aeration process the elongated aeration times provided to accommodate "autodigestion" of the solids also permits removal of slowly metabolized substrates. Had the experiment been terminated at 75 minutes, at which point it appeared that the COD removal was terminating, the COD

would have been registered as residual COD, much the same as that of the two experiments shown in Figure 6 in which a small inoculum of cells was employed and about the same residual remained after 30-40 hours' aeration (for approximately the same range of initial COD concentration). Thus, it would appear that the higher concentration of cells and more mature population combine to remove the slowly metabolizable material in a reasonable time.

It is interesting to note that the terminal (residual) COD shown in Figure 6 could be removed by activated carbon to about the same degree as was accomplished by the extended aeration sludge during the period 75 minutes to 1080 minutes, the difference being that with the extended aeration sludge, the organic matter extracted from the water is converted to CO_2 via biological "incineration," whereas it must be removed from the carbon via chemical combustion or other suitable means. The ideal situation with respect to the more slowly metabolizable constituents in the hydrolysate is one which might combine the benefits of activated carbon and biological treatment. This might be accomplished by periodic addition of small amounts of activated carbon to the aerator where it might serve as a concentrator of the biologically-resistant material and be biologically regenerated since it should be retained in the sludge. To regenerate the carbon biologically, the organic substrate would have to be removed from the carbon by the organisms, and this may be more difficult to accomplish than metabolism of the same substrate molecules in solution. However, if one is employing the hydrolytic assist, the acidizing of portions of the sludge during the hydrolysis operation might enhance regeneration of the added carbon. Such speculative possibilities await experimental investigation; they are mentioned here only to emphasize the numerous possibilities for flexibility of operation inherent in the hydrolytically-assisted mode of operation herein reported. The primary aim is to provide man-made

i.e., engineering, control over the natural autodigestive processes and the operational characteristics of the treatment process so as to assure stable and reliable delivery of treatment efficiency. The hydrolytically-assisted extended aeration process would surely seem a potentially useful step in this direction. Also in regard to engineering control of the bio-mass, it is appropriate to mention here that operational experience with the process subsequent to the 200-day period discussed herein provided some indication that the withdrawal, hydrolysis, and refeeding of portions of the sludge could assist in attempts to control species predominance in the system or, in any event, help purge the system of undesirable predominates (7).

SUMMARY AND CONCLUSIONS

In summary, the results shown herein indicate that an extended aeration activated sludge process with total sludge recycle can be operated successfully using the proposed "hydrolytic assist" to control the mixed liquor biological solids concentration. Thus, through a bioengineering expedient, the process is made independent of the natural periods of accumulation and de-accumulation of biological solids. Also, during the 200-day test period of operation herein shown, excess ammonia nitrogen was present in the feed, and the addition of hydrolysate did not interfere with the nitrification process.

Under this mode of operation the system showed a rather good ability to accept shock loading. The biochemical parameters sludge carbohydrate and protein were within ranges indicative of a metabolically-active bio-mass, and the endogenous O_2 uptake values or unit respiration activity of the sludge were slightly higher than those obtained employing total cell recycle without the "hydrolytic assist."

In general, it is concluded that the results do much to establish practical operational feasibility of the "hydrolytically-assisted" extended aeration process, and contribute useful information regarding sludge characteristics under varying modes of operation. Pilot plant operation using the process is still in progress in attempts to provide design and operational guidelines relative to hydrolysis schedules at higher loadings than those herein employed. It is also planned to run the pilot plant using natural wastes other than the synthetic medium herein employed.

ACKNOWLEDGMENTS

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10. Response of a hydrolytically-assisted extended aeration process pilot plant to shock loading consisting of a slug dose of cell hydrolysate.

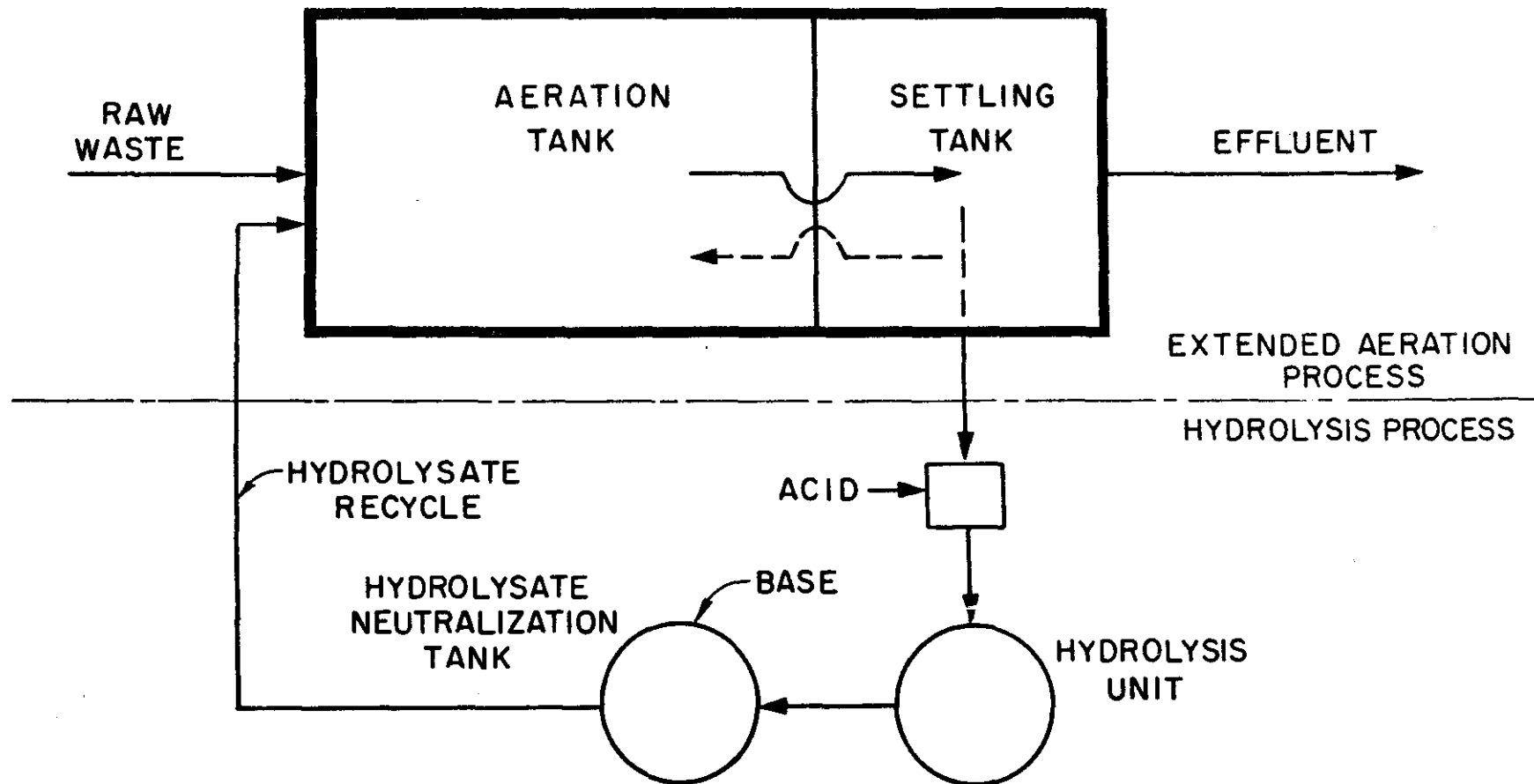


Figure 1. Extended aeration activated sludge process incorporating chemical hydrolysis for control of sludge concentration.

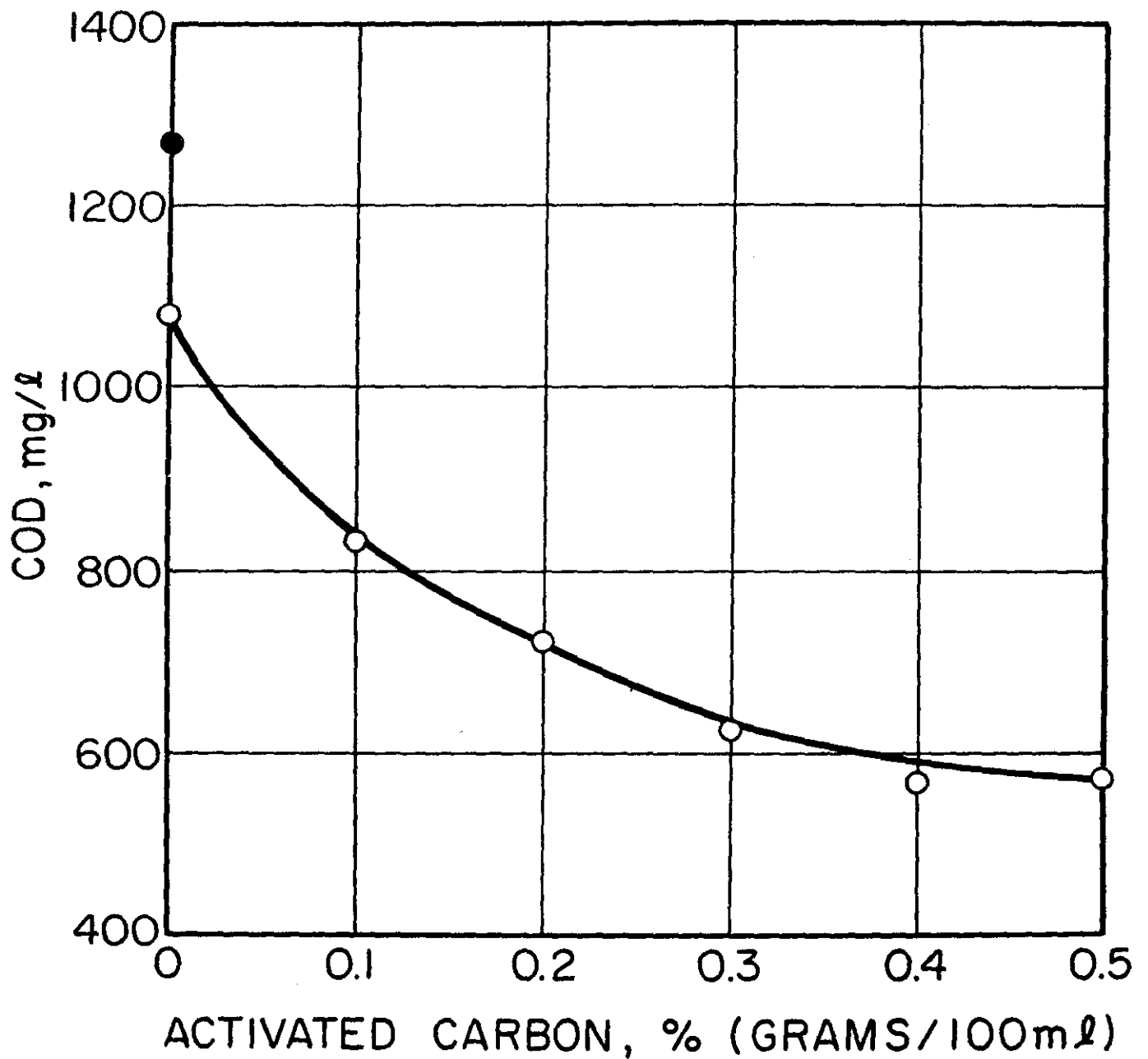


Figure 2. Removal of organic components of sludge hydrolysate of Phase A sludge by activated carbon.

● COD of unfiltered hydrolysate.

○ COD of filtered hydrolysate (Whatman #1) at carbon dosage indicated.

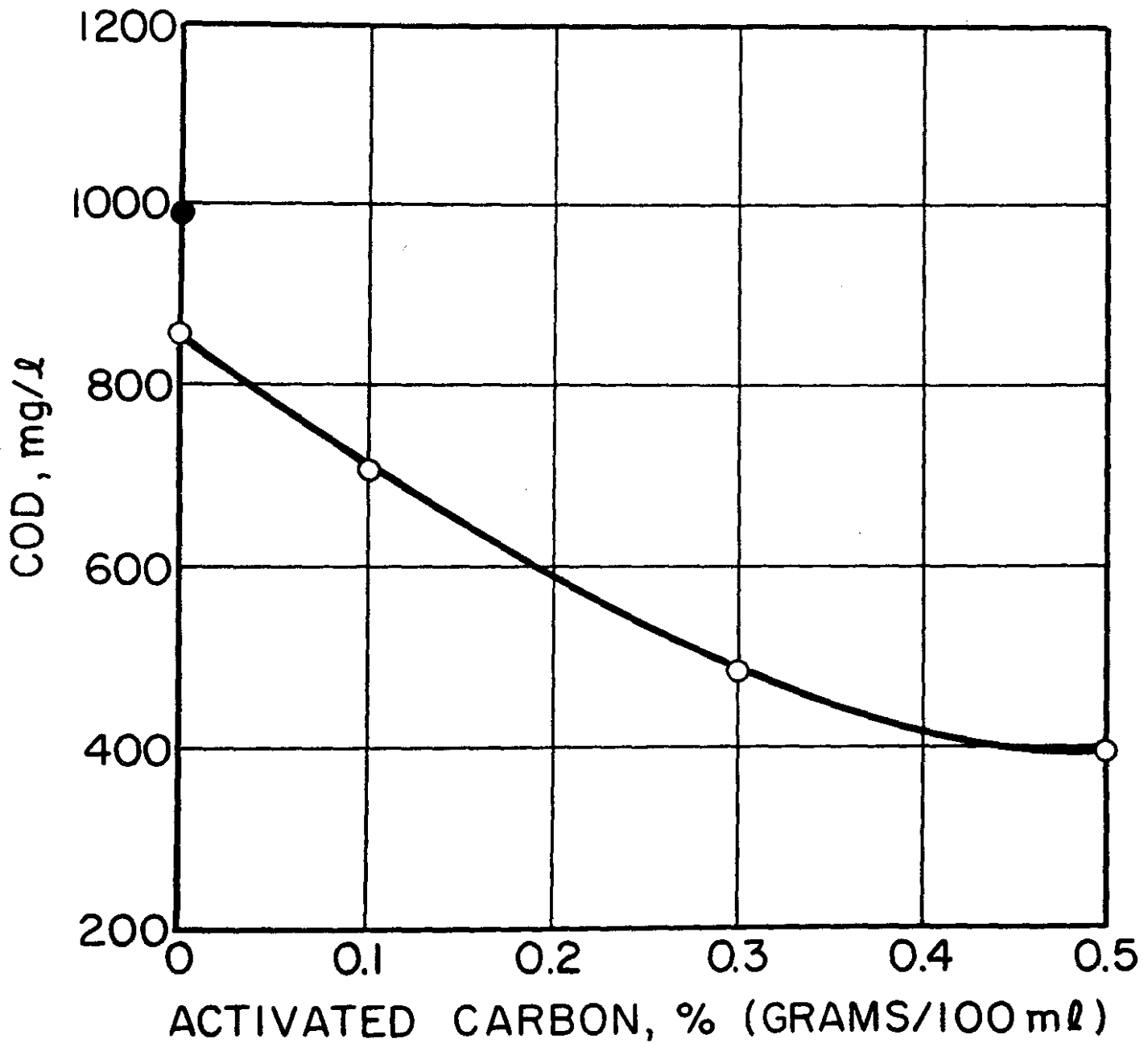


Figure 3. Removal of organic components of sludge hydrolysate of Phase B sludge by activated carbon.

● COD of unfiltered hydrolysate.

○ COD of filtered hydrolysate (Whatman #1) at carbon dosage indicated.

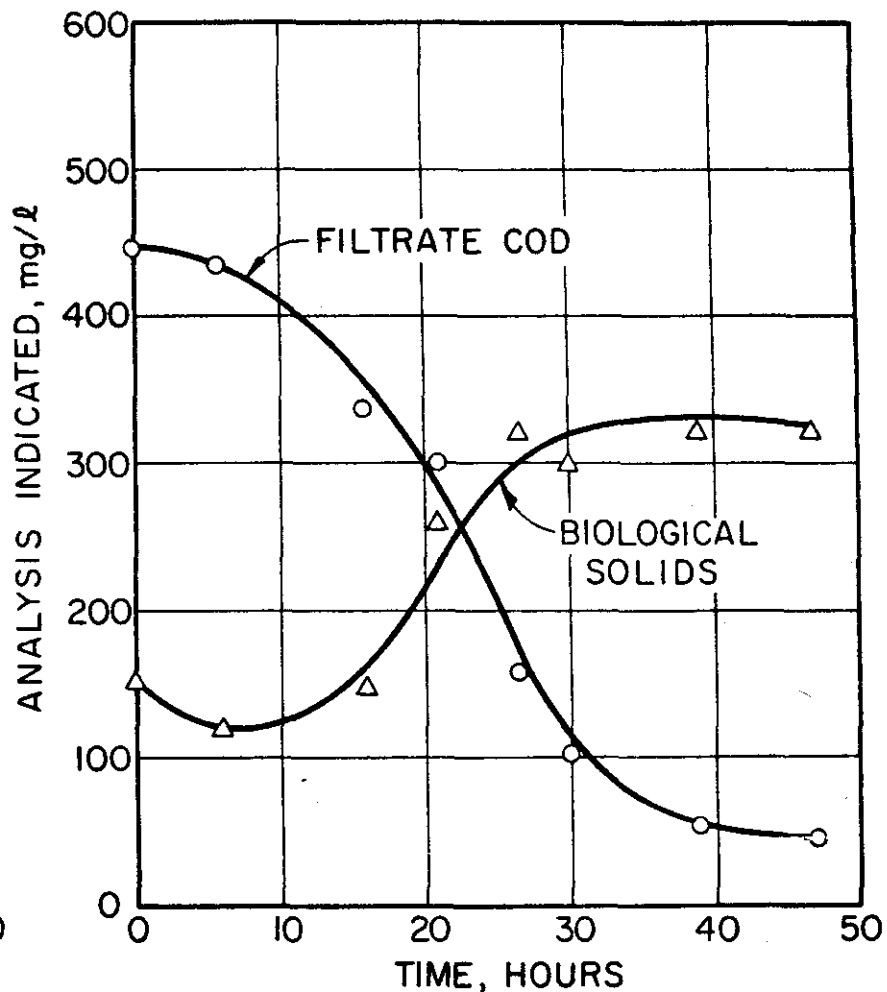
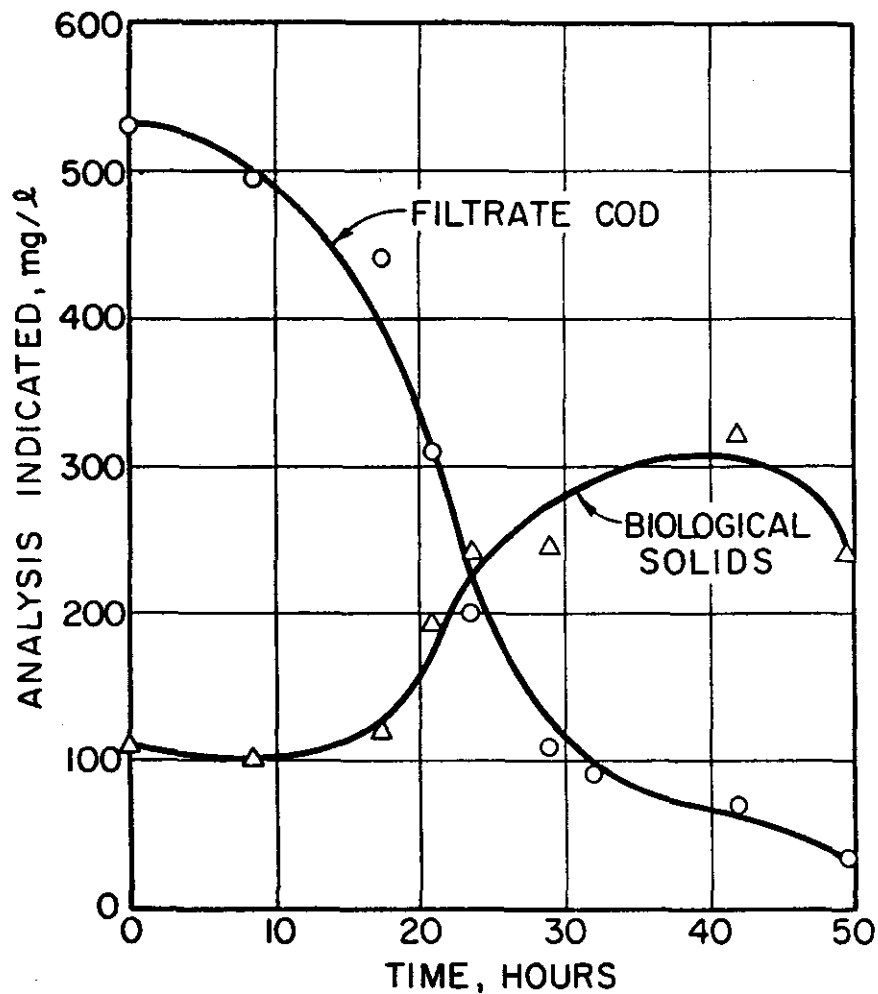


Figure 4. Metabolism of freshly grown and hydrolyzed biological solids by cells taken from an extended aeration activated sludge process after day 907 (left) and day 955 (right) of operation with total cell recycle.

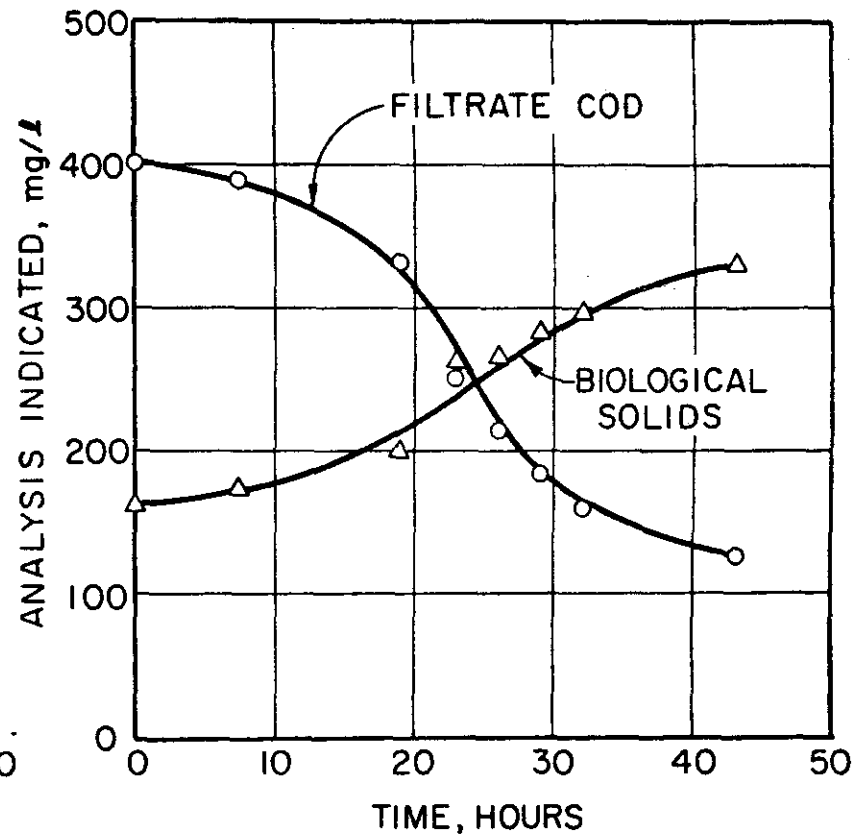
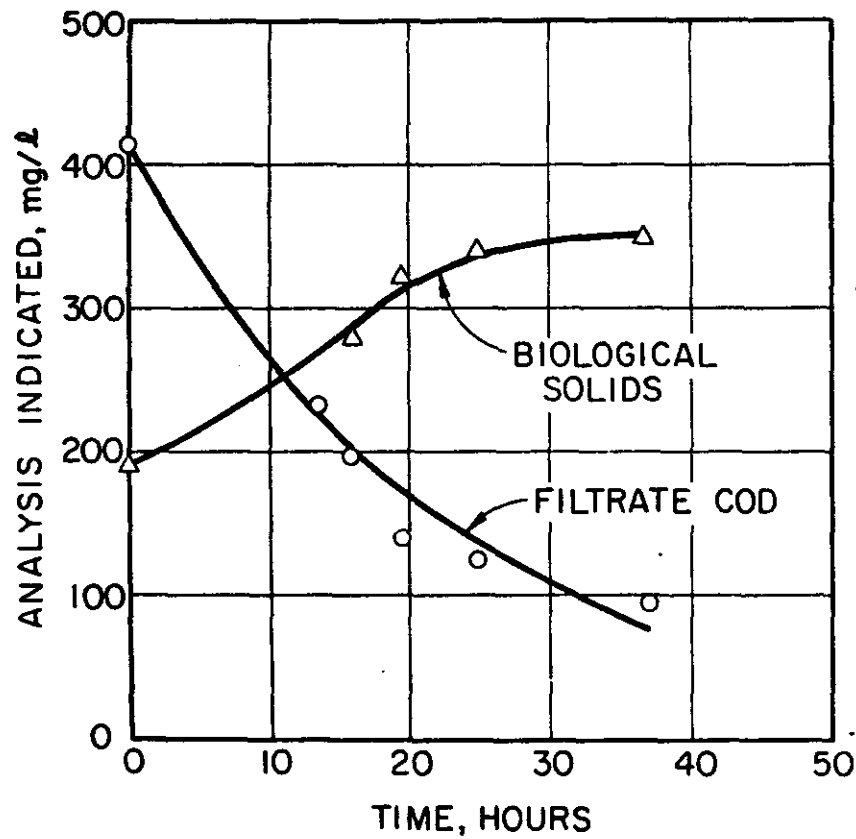


Figure 5. Metabolism of hydrolyzed extended aeration activated sludge (1095 days old) by cells taken from the extended aeration activated sludge pilot plant after 1097 (left) and 1169 (right) days of operation.

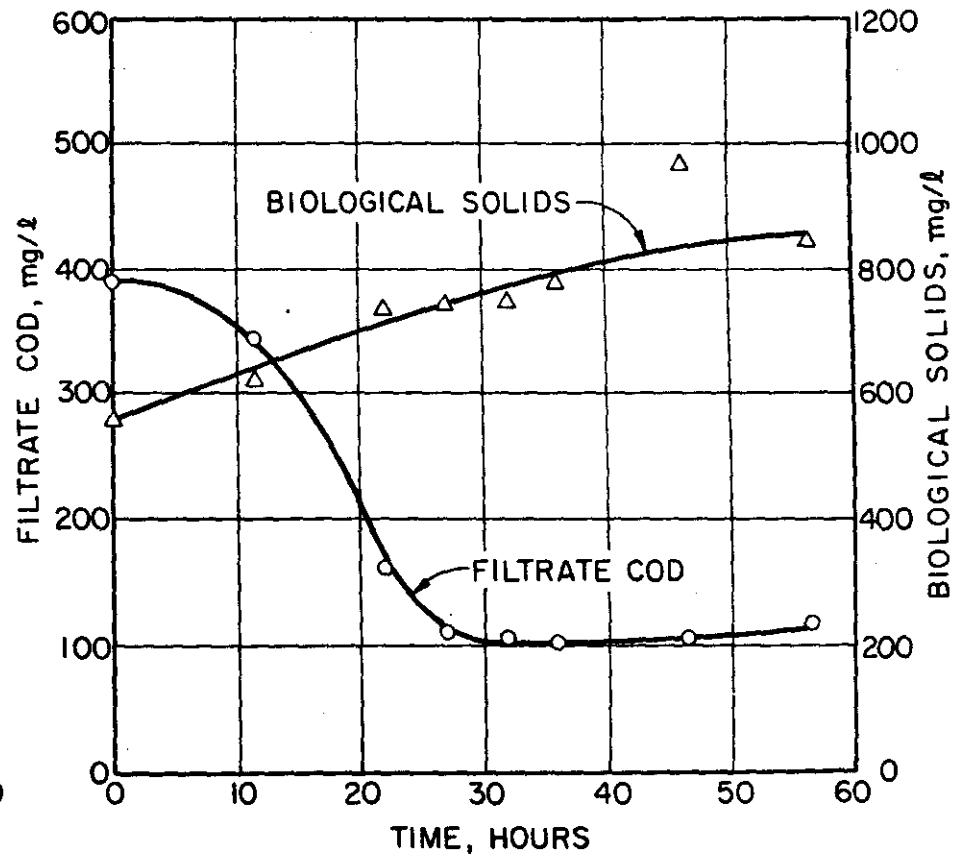
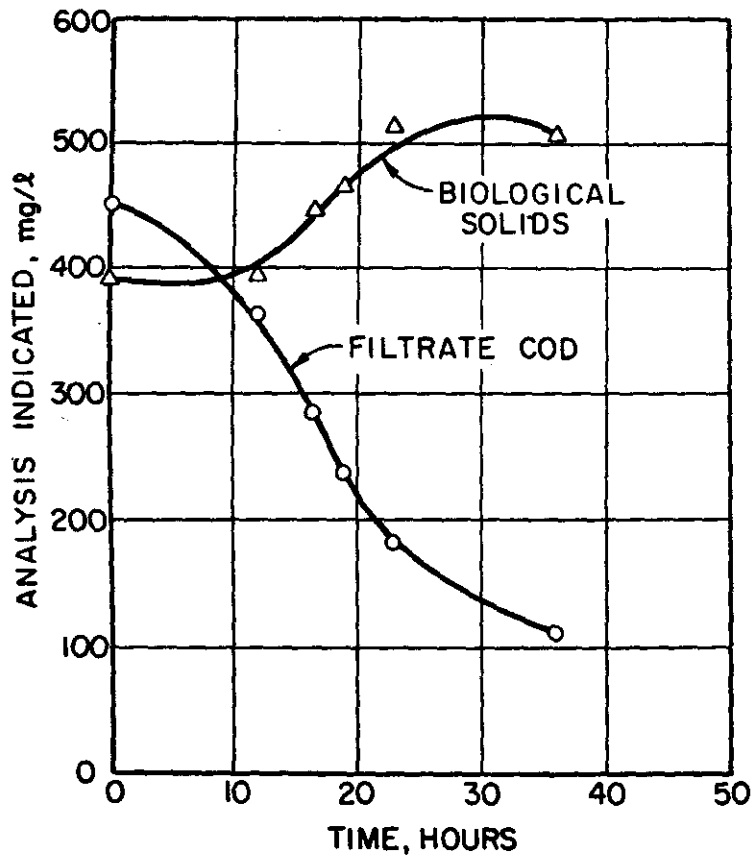


Figure 6. Metabolism of hydrolyzed extended aeration activated sludge which had been previously flocculated using ferric sulfate (left) and aluminum sulfate (right). The hydrolyzed sludge was obtained during a period when bacterial flocculation was enhanced through chemical precipitation. The initial cell inocula for the experiments shown were obtained from the extended aeration pilot plant on day 1182 (left) and day 1188 (right).

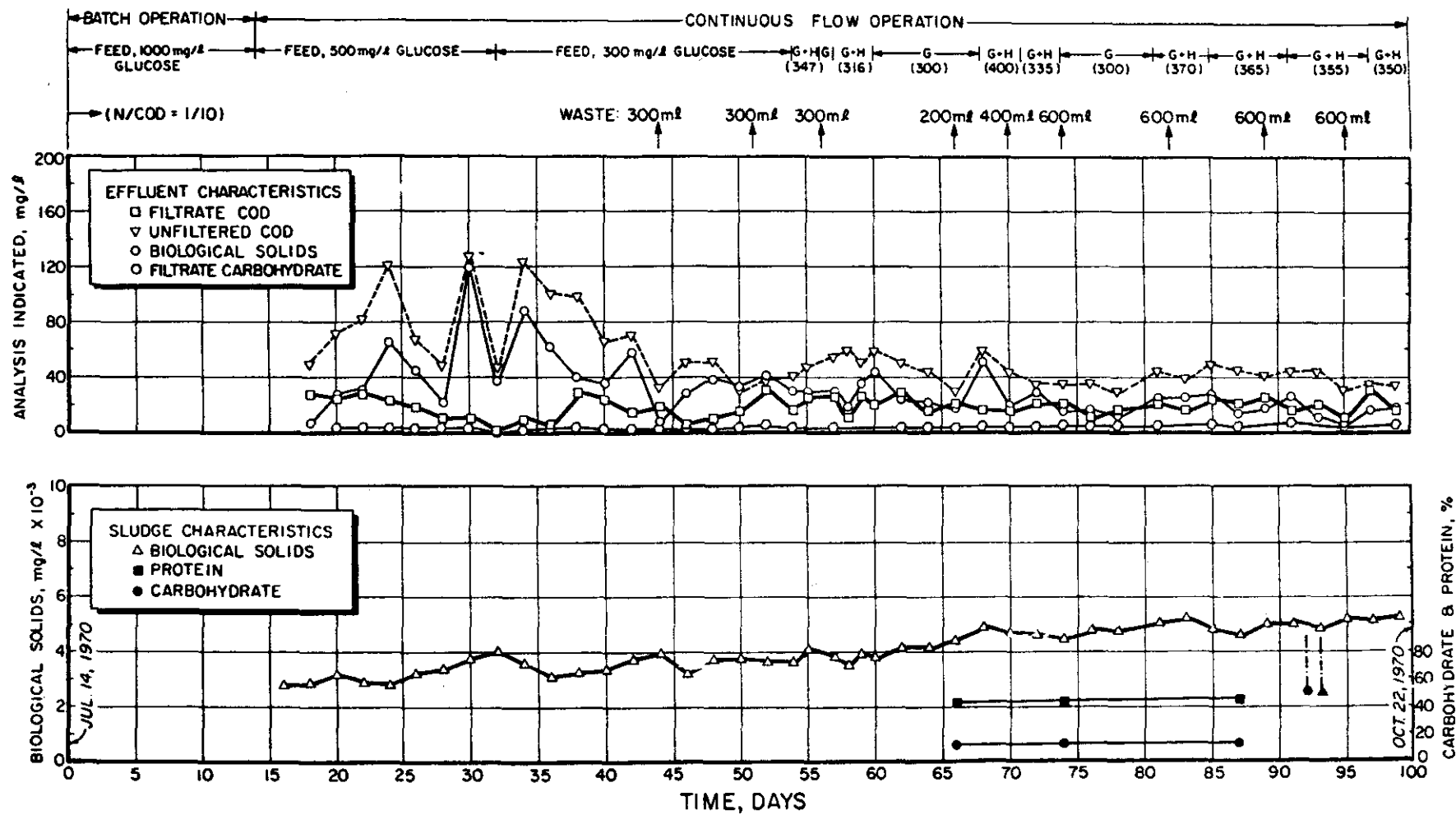


Figure 7. Performance characteristics of a hydrolytically-assisted extended aeration process pilot plant from day zero to day 100 of operation.

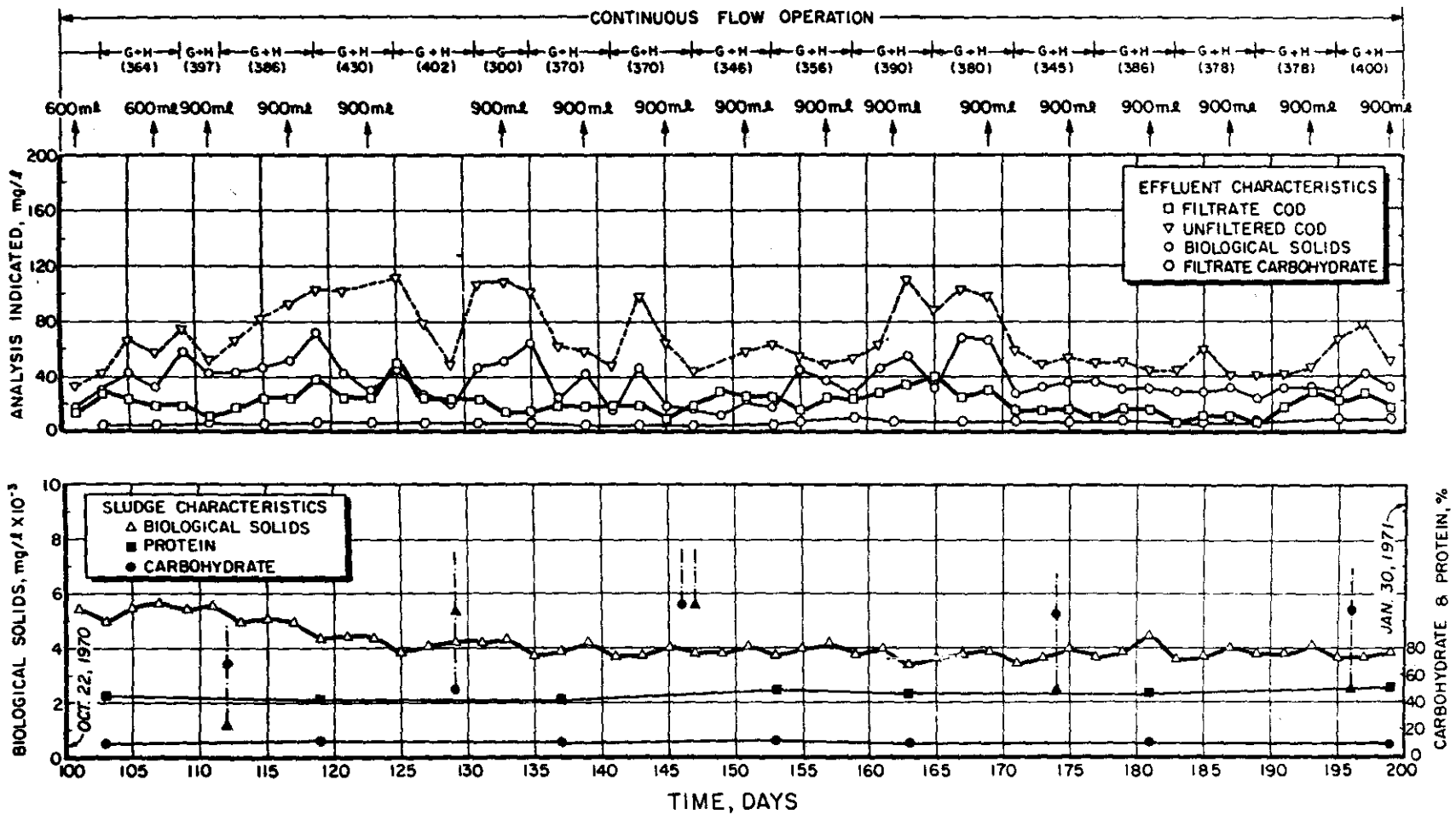


Figure 8. Performance characteristics of a hydrolytically-assisted extended aeration process pilot plant from day 100 to day 200 of operation.

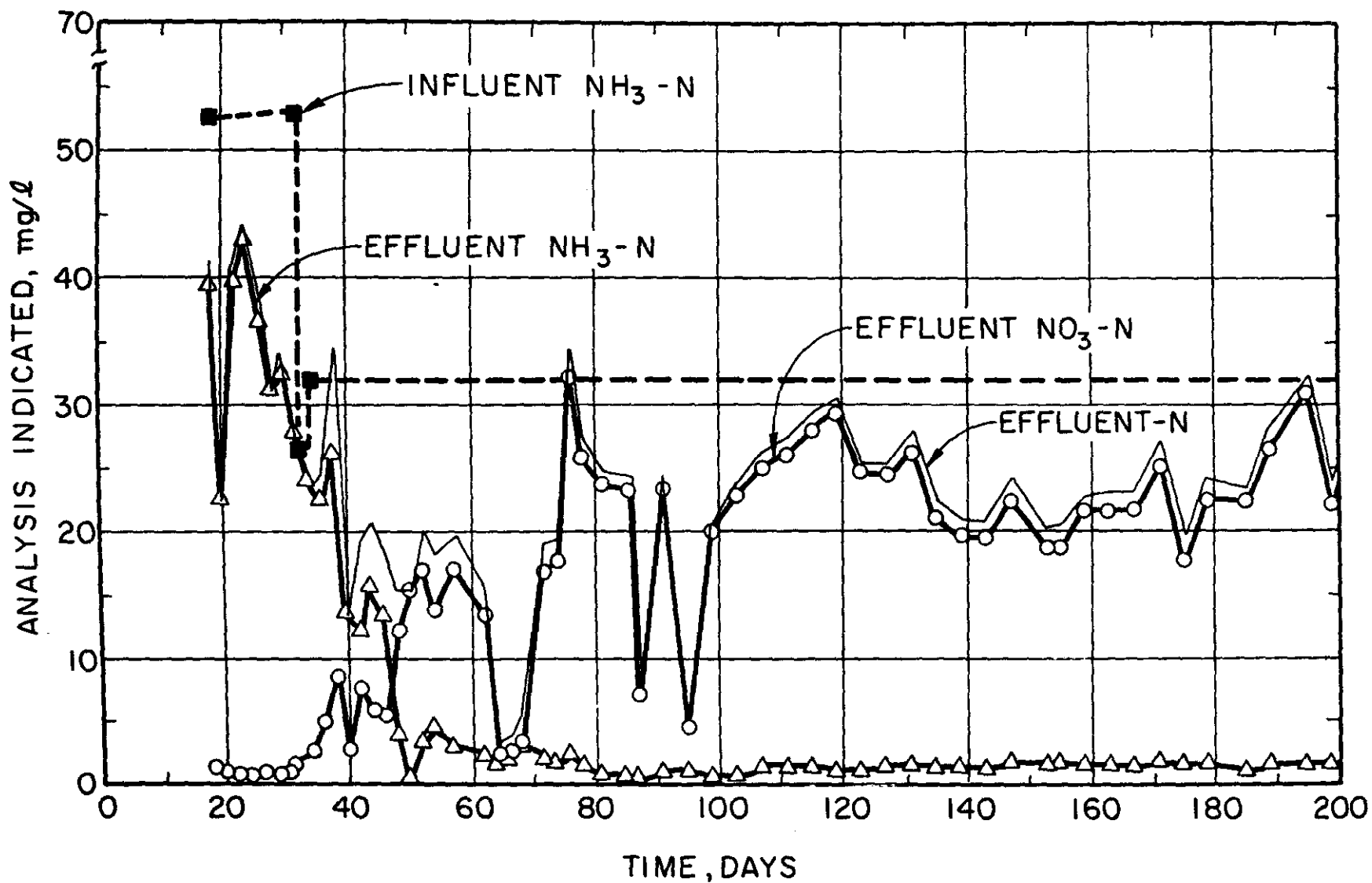


Figure 9. Nitrifying characteristics of a hydrolytically-assisted extended aeration pilot plant during 200-day operational period shown in Figures 7 and 8.

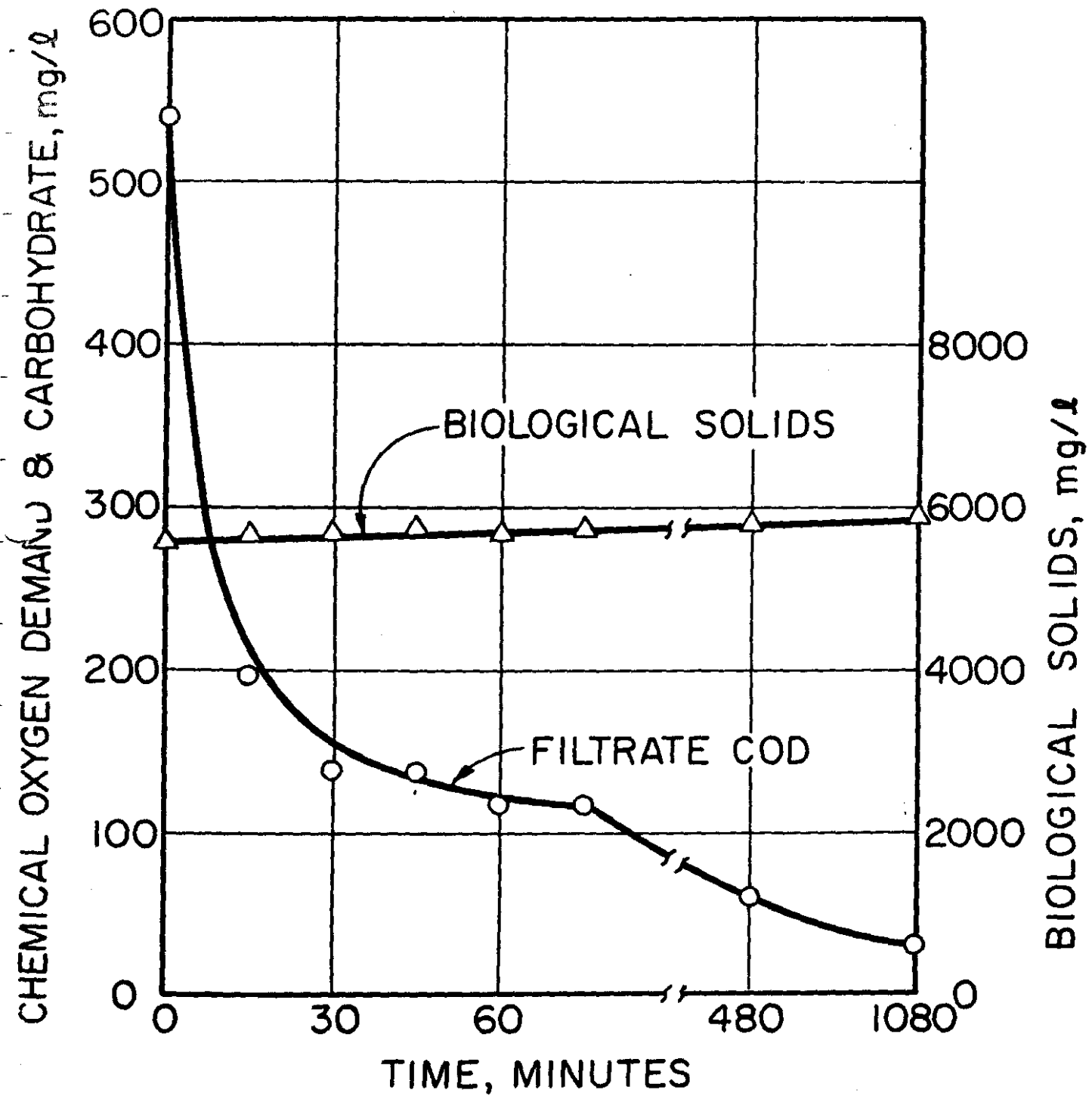


Figure 10. Response of a hydrolytically-assisted extended aeration process pilot plant to shock loading consisting of a slug dose of cell hydrolysate.

APPENDIX VI

Yang, P. Y., and Gaudy, A. F. Jr., "Nitrogen Metabolism in Extended Aeration Processes Operated With and Without Hydrolytic Pretreatment of Portions of the Sludge." Biotechnology and Bioengineering, XVI, 1-20 (1974).

NITROGEN METABOLISM IN EXTENDED AERATION PROCESSES OPERATED WITH AND WITHOUT HYDROLYTIC PRETREATMENT OF PORTIONS OF THE SLUDGE

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SUMMARY

Nitrifying characteristics were compared for an extended aeration (total cell recycle) process and one employing an engineering modification of the process utilizing a "hydrolytic assist" to aid biological autodigestion. Laboratory pilot plants were run over a period of years, and it was found that the recently recommended "hydrolytic assist" did not militate against production of a highly nitrified effluent. Under this mode of operation, the effluent was as nitrified as the effluent from the normal extended aeration process. It was also found that the modified process rapidly recovered its nitrifying capability after a period of deprivation of excess ammonia nitrogen. Throughout the period of operation, substrate removal efficiency remained high.

NITROGEN METABOLISM IN EXTENDED AERATION PROCESSES OPERATED WITH AND WITHOUT HYDROLYTIC PRETREATMENT OF PORTIONS OF THE SLUDGE

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INTRODUCTION

The biological significance of nitrogen is well known in the water pollution control field. Nitrogen, usually as $\text{NH}_3\text{-N}$, is added, at considerable expense, to industrial wastes deficient in a source of biologically available nitrogen in order to effect biological treatment of these wastes. Its leakage in the effluent is, therefore, of economic concern. On the other hand, for municipal wastes and certain industrial wastes containing amounts of nitrogen in excess of that needed for incorporation into the bio-mass, it is becoming increasingly important to remove the excess, since leakage of nitrogen, especially in the form of ammonia nitrogen, can be a cause for concern in the receiving body. In addition to contribution to algal growth, ammonia nitrogen can cause a significant oxygen utilization (exertion of non-carbonaceous biochemical oxygen demand), and it is becoming advisable to exert this demand at the treatment plant site along with removal of carbonaceous substrates.

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Presented at the 24th Annual Pollution Control and Industrial Waste Conference, Oklahoma State University, Stillwater, April 2-3, 1973.

Conditions under which a highly nitrified effluent can be produced in the secondary biological treatment process have been studied by a number of investigators [for example, among others, Wuhrmann (1), Downing, et al. (2), Johnson and Schroepfer (3)]. While there are differences of opinion regarding the governing factors, it may be generally stated that, provided the system has an ample supply of ammonia-N and a favorable pH and temperature and that toxic substances are not present, conditions which have been cited as tending to favor production of highly nitrified effluents are: low organic loading levels, high mixed liquor solids (biological solids) concentrations, long aeration periods, low BOD:N ratios, and high dissolved oxygen content in the system.

Most of these conditions obtain at extended aeration plants, and various investigators have reported production of highly nitrified effluents during operation of such processes [for example, among others, Guiver and Hardy (4), Middlebrooks and Garland (5), Eye, et al. (6), Jenkins and Garrison (7)].

In the authors' laboratories, long term studies on the extended aeration process (total sludge return) have been under way since 1967. These studies have been concerned primarily with examination of the soundness of the concept of total oxidation or autodigestion of the biological solids. It had generally been concluded by various investigators that the concept was biologically unsound. Studies in the authors' laboratories have shown that this is not the case (8)(9)(10)(11). In a long-term study in which an extended aeration pilot plant was operated with total cell recycle, it was found that the system underwent periods of solids accumulation and de-accumulation, i.e., solids did not continually build up in the system. There was no continual accumulation of an inert organic fraction in the sludge; the carbohydrate content did not build up (which would have been

the case if extracellular polysaccharide was accumulating in the bio-mass), and the substrate removal efficiency remained high throughout a period of over 1000 days of operation. Sludge was not inadvertently lost from the system in the effluent, although there were times when sludge concentration was so high that settling in the clarifier was seriously hindered and solids would have escaped in the effluent had all effluent not been centrifuged and the solids returned to the aerator. In other studies, it was shown that extracellular polysaccharides of various microorganisms served, after appropriate acclimation, as excellent growth substrates for heterogeneous microbial populations of sewage origin (12).

In sum, from the results we obtained, we were brought to the conclusion that the concept of total oxidation of the biological sludge produced in the purification stage was not theoretically unsound. However, we did observe that the periods of biological de-accumulation, i.e., periods of accelerated autodigestion, were not predictable and we sought an engineering expedient with which we could control the initiation of a de-accumulation phase.

Our line of reasoning brought us to the flow diagram shown in Figure 1, which depicts the normal extended aeration process and the proposed engineering control--the "hydrolytic assist" (9) (shown below the dashed line). The hydrolytic assist can be employed when biological solids concentration is accumulating to an extent which interferes with settling in the clarifier. Then sludge may be withdrawn from the underflow and the initial step of utilization of biological solids as substrate, i.e., breakdown of macromolecules of the cells, can be accomplished by chemical hydrolysis as shown. The solubilized sludge is then recycled to the aerator along with intact return sludge.

Since suggesting this method of solids control, we have operated an

extended aeration pilot plant in this fashion for over one year. The first 200 days of operation indicated to us that the process was conceptually feasible (13). Purification efficiency remained high, and it was possible to control the concentration of biological solids in the system with total recycle of the sludge, part hydrolyzed, part intact. During the 200-day span, the synthetic waste medium was constituted so that nitrogen ($\text{NH}_3\text{-N}$) was present in excess. In the succeeding 200 days, the COD:N ratio in the feed was varied.

During previous operations employing the centrifuge to retain solids (Phase A studies), nitrogen concentration in the waste was always in excess of that needed to provide a "balanced" C:N or BOD:N ratio for purification. Operational results for the system during the first 1000 days with respect to effluent quality and other parameters has been reported previously (8) (9). The Phase A operation was terminated after 1202 days. Analyses for types of nitrogen in the effluent were made (although not as frequently as for the other parameters), but these data have not yet been reported. It is the purpose of this report to present these results as well as effluent nitrogen data during operations employing the hydrolytic assist (Phase B studies). It is also appropriate to present the performance data for the pilot plant with respect to purification efficiency for the operation from day 200 to day 400 employing the hydrolytic assist.

MATERIALS AND METHODS

The extended aeration activated sludge pilot plant used for these investigations has been described previously in considerable detail (8) (9)(10). Figure 2 is a photograph showing aeration and settling chambers and effluent collection tank. The total volume of the system was 9.4 liters (6.2-liter aeration chamber and 3.2-liter settling chamber). The

movable baffle used to separate the aeration and settling chambers is seen in the photograph. Compressed air provided mixing and oxygen supply to the system. Airflow rate was maintained at 2000 cc/min/l. Temperature was maintained at $23 \pm 2^{\circ}\text{C}$.

Details of the operational procedure have been presented in previous reports (8)(9)(10). In the operation of the extended aeration process without hydrolytic pretreatment of portions of the sludge (Phase A), the effluent collected in the holding tank (plastic bottle, bottom of Figure 2) was periodically (once daily or once every two days when the effluent was very clear) passed through a centrifuge (Sharples Superspeed) and any solids which had been carried over from the settling tank were returned to the aeration chamber.

There was no centrifugation of biological solids suspended in the effluent of the extended aeration process during operations incorporating the "hydrolytic assist" shown in Figure 1 (Phase B studies). The detailed operational procedure and composition of the feed solution during Phase B operations are reported in detail elsewhere (13). Briefly, glucose was used as carbon source, and ammonium sulfate was used as nitrogen source. When hydrolysate was pumped to the aeration tank, the organic nitrogen contained in the sludge hydrolysate could also serve as nitrogen source. During both phases of operation, $\text{NH}_3\text{-N}$ (14), $\text{NO}_2\text{-N}$ (15), and $\text{NO}_3\text{-N}$ (15) of the pilot plant effluent were determined periodically. The dissolved oxygen in the aeration chamber and effluent was measured by a galvanic cell oxygen analyzer (Precision Scientific) in accordance with procedures recommended by the manufacturer (16). The pH was checked periodically with a pH meter (Beckman Zeromatic). Other analyses (e.g., COD, etc.) were also made to assess the performance of the system. These procedures have been delineated in detail in previous reports (8)(9)(10).

RESULTS

Throughout the 1202 days of operation in Phase A, the concentration of $\text{NH}_3\text{-N}$ in the feed was 53 mg/l, i.e., a COD:N ratio of 10:1 was maintained. During the first year of operation, analyses for $\text{NH}_3\text{-N}$, $\text{NO}_2\text{-N}$, and $\text{NO}_3\text{-N}$ in the effluent were not made, but beginning on day 351, such determinations were added to the already rather large number of analytical parameters being monitored in the system. These results are listed in Table I. Influent and effluent COD values on the days for which nitrogen data are available are also shown, since these aid in assessing relationships between organic purification parameters and nitrogen utilization. Column 9 of the table labeled "Total-N" is the total inorganic nitrogen in the effluent and is the sum of columns 6, 7, and 8; i.e., the sum of $\text{NH}_3\text{-N}$, $\text{NO}_2\text{-N}$, and $\text{NO}_3\text{-N}$. Analyses for organic nitrogen were not made because the COD in the effluent was, in general, very low; i.e., the system provided excellent purification efficiency, and therefore only traces of organic nitrogen could be expected. Columns 10 and 11 show the amounts of COD and organic nitrogen removed. Column 12 shows the percentage of the influent nitrogen which passed through the plant. In column 13, the ratio COD removed:nitrogen removed is shown.

Comparison of columns 4 and 9 shows that there were times when the concentration of nitrogen in the effluent approached that in the feed, indicative of periods when much of the nitrogen contained in the sludge was being reused. It is recalled that the protein content of the sludge was also determined during Phase A operation (8)(9). Sludge protein was not low during periods of high nitrogen passage through the system. Thus, the vital need for nitrogen was being supplied during these periods due to the natural autodigestive and resynthesizing characteristics of the total cell recycle system. On average, approximately 2/3 of the influent

nitrogen concentration appeared in the effluent.

The average ratio of COD:N removed (see column 13) was 27.8, a value which compares favorably with results obtained by Goel and Gaudy (17) for continuous growth systems not limited by nitrogen concentration. Goel and Gaudy have also shown that reasonably good COD removal efficiency (86%) can be obtained by activated sludges operating at a COD:N ratio as high as 70 even with no return of sludge (and the nitrogen contained therein) (17); the protein content of the sludge in this instance was low. However, when one returns or recycles biological solids, it can be expected that the nitrogen released through autodigestion of portions of the sludge will be reassimilated and the protein content will remain high even though little of the nitrogen contained in the inflowing waste water is being removed. When one returns all of the sludge, this effect may be magnified. It is because of these effects that workers have found it possible to recommend reduction of the usual supplemental BOD:N ratio for the extended aeration process. Simpson (18), for example, has suggested that the nitrogen supplementation for the extended aeration process might be reduced to one-fifth of the BOD:N ratio normally recommended in the field for activated sludge (e.g., from 20:1 to perhaps as high as 100:1 BOD₅:N).

The system produced a rather well-nitrified effluent during the last 450 days of operation; this is more readily seen in Figure 3, wherein the effluent NH₃-N and NO₃-N are compared. The NO₂-N is not plotted, since as can be seen from Table I, there was normally only a trace amount present. It is recalled that the major purpose for studying the extended aeration system was to determine whether biological solids would continue to build up, and whether the efficiency of treatment would eventually decrease in keeping with the general consensus of opinion in the field regarding the extended aeration process. It is also recalled from previous reports (8)

(9) that such was not the case, and between days 285 and 307, the biological solids concentration in the system decreased from nearly 8500 mg/l to 2400 mg/l. These cells were not lost in the effluent, but were decreased due to a period of accelerated autodigestion (8). It is unfortunate that we were not at that time running nitrogen analyses on the effluent, since it is known that during periods of autodigestion and sludge deaccumulation, leakage of large amounts of ammonia occurs [e.g., see Thabaraj and Gaudy (11)]. The concentration of ammonia in the effluent during this period must have been very high. It is not known whether the system was producing a nitrified effluent prior to this severe period of accelerated autodigestion, but it is amply clear from Figure 3 that it did not produce such an effluent in the immediately ensuing period.

After day 307, the biological solids accumulated in the system, attaining a value of 14,000 mg/l by day 534. During this period, there were only small amounts of nitrate in the effluent and approximately 25 mg/l $\text{NH}_3\text{-N}$. Following this, there was a period of considerable fluctuation in the effluent ammonia nitrogen concentration, and a rising trend in $\text{NO}_3\text{-N}$ concentration. During the period of rising nitrates, the biological solids fluctuated between 14,000 and 18,000. Considering that the feed substrate consisted of 530 mg/l of COD, due to a glucose concentration of 500 mg/l, it can be stated that the organic loading of the system (gram substrate/gram biological solids) was extremely low. This is one of the conditions cited as enhancing nitrification and this effect is consistent with the results shown here. By day 750, the $\text{NH}_3\text{-N}$ concentration had decreased to essentially trace amounts, and a highly nitrified effluent was being produced.

After 1000 days of operating the pilot plant with excellent COD removal efficiency, we felt we had amply demonstrated the validity of the extended aeration or total oxidation principle. On day 1095, biological solids were

removed from the system and the concentration remaining in the system was reduced to approximately 1000 mg/l. The feed concentration was cut in half (to 250 mg/l glucose), but was returned to 500 mg/l on day 1114. From day 1095 to day 1200, the biological solids concentration experienced a rising trend, and the system continued to produce a highly nitrified effluent (see Figure 3) even though the organic feed loading per unit weight of biological sludge in the system was now considerably higher than it had been before removing most of the sludge from the system.

Analyses for $\text{PO}_4\text{-P}$ were also made on these effluent samples, but these data are not shown. It is recalled that large amounts of phosphorus were fed to the system since a phosphate buffer was employed to control pH. The influent $\text{PO}_4\text{-P}$ concentration was 155 mg/l. At times a considerable amount of phosphorus was retained in the sludge, and at times the effluent phosphate concentration was slightly higher than the influent concentration. From the average of 63 samples for effluent PO_4 it was calculated that 93.8 percent of the influent PO_4 appeared in the effluent. The 6.2 percent incorporated into the sludge compares well with the value of 10 percent which can be calculated from the data of Sekikawa, et al. (19).

It was of interest to compare the nitrifying characteristics of the system during Phases A and B of operation of the pilot plant. It is emphasized that the major purpose of the Phase B operation was to determine whether the pilot plant could be operated with good purification efficiency with respect to removal of organic matter employing the hydrolytic assist. The first 200 days under this mode of operation indicated that the process was feasible, and these results are reported elsewhere (13). Before presenting the results pertaining to the nitrification characteristics of the system, it is appropriate to report the plant operational results with respect to organic purification subsequent to

day 200 of Phase B.

The operational results from day 200 to day 300 are shown in Figure 4. The concentration of organic matter (as COD) in the feed is designated at the top of the figure. The feed consisted of glucose, "G" (300 mg/l), plus sludge hydrolysate, "H" (at variable amounts of COD). For example, from day 200 to day 207, the feed consisted of $300 + 64 = 364$ mg/l COD (G + H). Volumes of sludge withdrawn for hydrolysis are also shown. For example, on day 205, 900 ml of sludge was hydrolyzed. The COD:NH₃-N ratio in the feed synthetic waste prior to day 205 was 10:1, i.e., 310 mg/l glucose COD to 31 mg/l NH₃-N.

It should be noted that COD:N ratios given for the feed are based upon the synthetic waste only; the COD and nitrogen in the hydrolysate are not considered in calculating these ratios, since the hydrolysate is considered to be part of the sludge, not the feed. The nitrogen content of the hydrolysate consisted primarily of the nitrogen in amino acids and peptides (organic nitrogen) with some NH₃-N. However, nitrogen analysis on the hydrolysate was not routinely performed.

The four parameters for measuring effluent quality (organic matter) are plotted in the top graph, whereas sludge concentration and composition are plotted in the lower portion of the figure. In the 200 days of previous operation it had been found that the withdrawal (at approximately weekly intervals), hydrolysis and refeeding of 900 ml sludge was sufficient, at the organic loadings in the feed, to control biological solids level in the range 4000-5000 mg/l, and the system provided rather good purification efficiency (13).

Turning attention to the first 200 days of effluent nitrogen analyses (see Figure 5), it can be readily seen that this system also produced a highly nitrified effluent. The dashed line shows the NH₃-N concentration in the feed, and it is seen that the effluent nitrogen amounted

to approximately 2/3 of the influent and consisted almost entirely of $\text{NO}_3\text{-N}$. After 200 days of operation we felt we had shown that the process was operationally feasible and that it was now desirable to experiment with the system with regard to operation under conditions which would not normally be considered in the excess nitrogen range. Thus on day 205, the COD:N ratio was increased from 10:1 to 20:1, the value usually employed for nitrogen-deficient industrial wastes. One week of operation at this level had no drastic effect on the system (as seen in Figure 4), and on day 213 the ratio was increased to 30:1. Since the biological solids were on a decreasing autodigestive trend, the regular withdrawals for hydrolysis were terminated on day 223. It is not known whether it was the change in COD:N ratio which precipitated the slightly rising trend in effluent COD; however, the system did regain excellent purification efficiency by day 227. Microscopic observations of the sludge were made frequently, and it was noted that filamentous organisms were increasing; also, the sludge began to bulk slightly. It was our intent to determine whether decreasing the COD:N ratio would tend to shift the predominance of species away from filamentous forms, and from day 251 to day 287, the COD:N ratio was decreased to 10:1. As seen in Figure 4, changes in COD:N ratio had essentially no effect on effluent quality. The supernatant layer in the clarifier remained rather thin, and filamentous organisms persisted in the system.

Beginning on day 260, the system was subjected to periodic slug doses of ferric sulfate (added directly to the aeration chamber). These had no effect on the purification efficiency, but did have an immediate beneficial effect on sludge settleability and compactness of the sludge in the clarifier, but it was not long-lived. When the dosage was stopped, the supernatant layer in the clarifier again became very thin; the filamentous forms were still present. It did seem, however, that slug dosage

with ferric salts might be effectively employed as a stop-gap remedial measure to concentrate a loose sludge. It is interesting to turn attention again to Figure 5 to examine the effect increasing the COD:N ratio exerted on the concentration of nitrogen in the effluent. Upon increasing the ratio from 10:1 to 20:1 (day 205), the $\text{NO}_3\text{-N}$ decreased rapidly, and a further increase in COD:N to 30:1 all but eliminated nitrates from the effluent. Also, at this ratio there was essentially no leakage of nitrogen in the effluent. When the $\text{NH}_3\text{-N}$ in the feed solution was again increased (COD:N returned to 10:1), there was a rapid but short-lived rise in $\text{NH}_3\text{-N}$ concentration and a rapid but sustained increase in $\text{NO}_3\text{-N}$. These results indicate that the series of quantitative shocks to the nitrifying organisms and the period of starvation for energy source ($\text{NH}_3\text{-N}$) preceding the return to a COD:N ratio of 10:1 did not eliminate them from the ecosystem; when they were needed to supply the desirable nitrifying power to the system, they responded rapidly. Also, during this time the biological solids concentration in the system was not extremely high (± 3000 mg/l, as seen in Figure 4), indicating that high biological solids concentration may not necessarily be a required condition for nitrification.

From day 287 to day 310, the inflowing feed was stopped, and the system was subjected to a period of aerobic digestion. It was of interest to see whether the filamentous forms would be decreased by natural autodigestion during this period. The pH was checked frequently to assure that acid conditions did not develop. Periodic microscopic examination indicated that the filamentous forms persisted--in fact, very little natural autodigestion occurred, and the sludge concentration decreased by only six percent.

Since the filaments were not being successfully decreased by natural autodigestion, it was decided to see if they could be significantly reduced by employing massive chemical hydrolysis. It was reasoned that

hydrolysis would solubilize the filamentous forms. Then these solubilized cells, when fed back to the intact sludge as a substrate or carbon source, might be competed for more successfully by non-filamentous microorganisms, thus helping to purge the system of filaments. Accordingly, 4.7 liters, containing approximately half of the sludge in the system, were withdrawn and hydrolyzed. Microscopic examination of the hydrolysate indicated the absence of filaments. From day 310 through day 322 (see Figure 6), the system was batch-fed the neutralized hydrolysate and glucose. The daily batch feeding schedule is indicated at the top of the figure. Since there was essentially no overflow during the batch operation, the daily residual COD accumulated in the system and was registered as a rise in filtrate COD. By the end of the period of batch operation, there was a significant decrease in the amount of filamentous forms, and those which were present appeared to be considerably shorter than those which existed prior to withdrawal for hydrolysis and refeeding of approximately 50 percent of the sludge.

Continuous flow operation was then reinstated, and a COD:N of 20 was employed. As can be seen in Figure 6, excellent purification efficiency was attained. In Figure 5 it is seen that the nitrogen concentration which had built up in the system during batch operation was rapidly diluted out and in succeeding days there was essentially no leakage (or wastage) of nitrogen in the effluent. It is interesting to compare nitrogen leakage in the effluent during this period at COD:N = 20:1 with that during operation at COD:N = 30:1 for the period day 213 to day 250. It might have been anticipated that after day 323 when operations proceeded at a COD:N of 20:1, some nitrogen would appear in the effluent; in any event, more than at COD:N = 30:1, but there was less, as seen in Figure 5. Examination of the trend in biological solids concentration during these periods provides an explanation of this seemingly anomalous effect. In

Figure 6 it is seen that biological solids concentration was climbing to nearly 6000 mg/l during the period following the reinstatement of continuous flow operation at COD:N = 20:1. Thus, there was a greater retention of nitrogen in the sludge and less reuse of the nitrogen during this period than during the former one at COD:N = 30:1. It can be seen in Figure 4 that biological solids concentration at that time was remaining relatively constant or following a slightly decreasing trend in a slow de-accumulation period. It is also recalled that at the time the system was being fed glucose plus some hydrolysate, thus some organic nitrogen was being fed.

It is seen in Figure 6 that on day 351, 750 ml sludge were removed from the unit for hydrolysis and refeeding. The system was slug-dosed with this neutralized hydrolysate during an ensuing 24-hour batch period of operation of the pilot plant. The response to this shock loading experiment was successful; the slug dose led to an initial COD concentration in the reactor of approximately 550 mg/l. Approximately 80 percent of this material was removed within the first hour of aeration, and the additional aeration time provided for removal of the more slowly metabolized or metabolically resistant organic components in the hydrolysate (13). After resumption of the normal feeding schedule on a continuous flow basis, the biochemical efficiency remained excellent (above 95 percent), and it can be seen from Figure 6 that only small amounts of biological solids appeared in the effluent.

A final experiment in this phase of the study was initiated on day 368 with the removal of a rather large volume of sludge (3.5 liters) for hydrolysis and refeeding to the pilot plant. It was decided to feed this material back to the system over a rather short period of time in order to gain an insight into the ability of the system to accommodate hydrolysate feedback. From day 370 to day 376, the hydrolysate was fed along

with 500 mg/l glucose in the synthetic waste. Biological solids concentration gradually increased from 4000 to 5000 mg/l, and the biochemical efficiency of the system remained above 97 percent. Also, as can be seen in the figure, the biological solids concentration in the effluent was small and the unfiltered COD, i.e., the supernatant COD or effluent COD, remained very low.

From day 376 to day 378, the remainder of the hydrolysate was fed as the sole source of organic matter to the pilot plant. This complete turnover in inflowing feed material caused the biochemical purification efficiency to decrease from 97 to 91 percent. However, this rather massive feeding of hydrolysate did not cause serious disruption of the system. For the next 10 days, from day 378 to day 388, synthetic waste was fed and the biological solids accumulated to approximately 6000 mg/l. Efficiency of COD removal was 97 percent. In Figure 5, it is seen that the forced feeding of hydrolysate did not cause excessive leakage of nitrogen from the system, and that which did appear in the effluent was in the form of nitrate nitrogen. At this point, we terminated the pilot plant operation temporarily, and hydrolyzed all the remaining sludge for possible future experiments.

SUMMARY AND CONCLUSIONS

In general, extended aeration processes can be expected to produce a nitrified effluent, and the results of the Phase A studies during the latter 500 days are consistent with this general expectation. Highly nitrified effluent was produced during the latter part of the Phase A study when the biological solids concentration was rather high. It should be noted that prior to this time, solids concentrations were also very high, and while the effluent contained $\text{NO}_3\text{-N}$, it was not highly nitrified and much more $\text{NH}_3\text{-N}$ than $\text{NO}_3\text{-N}$ appeared in the effluent. Thus, low organic loading

may not be a critical or controlling factor with regard to nitrification. However, since the "hydrolytic assist" is designed to help control biological solids concentration to levels lower than those during which a highly nitrified effluent was produced during Phase A operation, there may have been some question regarding the nitrifying characteristics of the process. But, as was seen in Figure 5, a highly nitrified effluent was produced during the first 200 days of operation when excess nitrogen was present in the inflowing waste, and the biological solids concentration was considerably less (4000 mg/l vs \pm 14,000 mg/l) than during Phase A operation. Thus, it can be concluded that use of the "hydrolytic assist" should not militate against production of a highly nitrified effluent.

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COO AND NITROGEN IN THE INFLUENT AND EFFLUENT DURING OPERATION OF AN EXTENDED AERATION PILOT PLANT WITH TOTAL CELL RECYCLE (Phase A)

Date (Days) Sludge Age (d)	Influent (mg/l)			Effluent (Effluent) (mg/l)			Effluent (Effluent) (mg/l)			Effluent (Effluent) (mg/l)			Effluent (Effluent) (mg/l)		
	COO	NH ₃ -N	COO	COO	NH ₃ -N	NO ₂ -N	COO	NH ₃ -N	NO ₂ -N	COO	NH ₃ -N	NO ₂ -N	COO	NH ₃ -N	NO ₂ -N
1968															
1-15	351	530	53	25	31.5	7.5	10	<0.1	35.0	510	18.0	66.0	20.3	73.5	35.1
3-22	358			38	31.5				39.0	492	14.0	73.5	35.1		
4-8	377			25	26	9.5			35.5	505	17.5	66.9	28.8		
4-10	377			20	25.5	6.3			31.8	510	21.2	60.0	24.0		
4-20	387			20	23	6.3			29.3	510	23.7	55.2	21.5		
4-29	396			20	26	4.3			30.3	510	22.7	57.1	22.4		
5-8	405			20	22	2.8			28.8	510	24.2	54.3	21.0		
5-14	411			20	22	3.2			25.2	510	27.8	47.5	18.3		
5-23	420			20	26	3.2			29.2	510	23.8	55.0	21.4		
6-3	431			20	30.5	3.2			33.7	510	19.3	63.5	26.4		
6-13	441			20	27.5	3.2			30.7	510	22.3	57.8	22.8		
6-21	449			20	25	3.2			28.2	510	24.8	53.2	20.5		
6-29	457			18	25	3.2			28.2	512	24.8	53.2	20.6		
7-12	470			10	25	3.2			28.2	520	24.8	53.2	20.9		
7-19	477			10	48	2.1			51.1	520	1.9	96.4	273.6		
7-26	484			20	25.5	3.2			28.7	510	24.3	54.1	20.9		
8-4	493			20	25.5	3.1			28.6	510	24.4	53.9	20.9		
8-13	502			20	25.5	3.2			28.7	510	24.3	54.1	20.9		
8-24	513			20	25.6	2.2			27.8	510	25.2	52.4	20.2		
9-1	521			20	25	3.2			28.2	510	24.8	53.2	20.5		
9-11	531			10	26.2	3.7			29.9	520	23.1	56.4	22.5		
9-24	544			40	37	8			45.0	490	8.0	84.8	61.1		
10-9	559			60	28.8	1.9			51.2	470	1.8	96.6	261.1		
10-12	562			40	28.2	23			51.2	490	1.8	96.6	272.2		
11-3	584			40	33	3.4			36.4	490	16.6	68.6	29.5		
11-12	593			40	41	1.4			42.4	490	10.6	80.0	46.2		
11-23	604			20	40	2.5			42.5	510	10.5	80.1	48.5		
12-7	618			40	36	3.4			39.5	490	13.5	74.3	36.0		
12-24	635			30	42.6	8.3			51.1	500	1.9	96.4	263.1		
1-10	652			45	40	7.2			47.3	485	5.7	19.0	83.6		
1-22	664				10.5	4			20.5		32.5	8.6			
2-21	694			0	35	10.9			45.9	530	7.1	86.7	74.6		
3-3	704			0	25.4	19.8			45.2	530	7.8	85.3	67.9		
3-11	712			0	26.2	19			45.3	530	7.7	85.4	68.8		
3-26	727			35	38	7.8			45.8	495	7.2	86.5	68.7		
4-8	740			40	23.6	18.2			42.0	490	11.0	79.1	44.1		
4-11	753			20	23.3	0.2			25.8	510	27.2	48.5	18.6		
5-2	764			0	31.1	27.3			30.8	530	22.2	58	27.7		
5-24	785			0	23	24			26.7	530	26.3	50.3	20.0		
6-6	799			0	20.6	0.9			23.5	530	29.5	44.2	17.9		
6-18	811			20	29.3	0.3			33.6	510	19.4	63.3	26.1		
7-1	824			0	20	28.6			30.9	530	22.1	58.2	23.8		
7-14	837			50	1.3	28.6			30.0	480	23.0	56.6	20.8		
8-1	855			0	0.7	29.0			29.8	530	27.2	48.6	19.4		
8-10	864			0	0.7	24.6			25.4	530	27.6	47.8	19.1		
8-27	881			45	1.7	37			38.7	485	14.3	33.9	126.3		
9-10	895			25	1.7	37.5			39.2	505	13.8	36.5	126.3		
9-21	906			50	1.7	47.5			49.2	480	3.8	92.8	126.3		
10-14	929			15	3.0	34.5			41.5	515	11.5	78.1	44.3		
11-3	949			40	1.8	28			30.9	490	22.1	52.1	111.6		
11-22	968			50	1.3	43.5			48.8	480	4.2	92.0	111.6		
12-5	981			40	1.7	41			43.2	490	9.8	81.4	49.4		
12-27	1003			30	1.7	32.5			34.2	500	18.8	64.4	26.4		
1-17	1024			10	0.5	36			38.2	520	14.8	72.1	35.1		
1-30	1037			20	1.7	37.5			38.0	510	23.0	56.4	22.0		
2-11	1049			20	0.4	34.5			36.5	510	16.5	64.8	30.7		
3-9	1075			48	0.5	31			33.6	482	19.4	63.4	24.8		
4-10	1107	265		28	0.1	21			21.6	530	27.6	47.9	19.1		
5-1	1137	530		40	0.1	47.5			43.0	490	10.0	81.0	48.5		
5-14	1145			47	0.4	34.5			34.9	443	10.1	66.0	24.4		
6-7	1164			44	0.5	39.5			40	446	13.0	75.6	37.3		
6-16	1185			40	0.1	41			43.5	490	9.5	82.0	51.0		
7-13	1202			39	0.3	28.5			28.9	491	23.1	56.4	20.7		

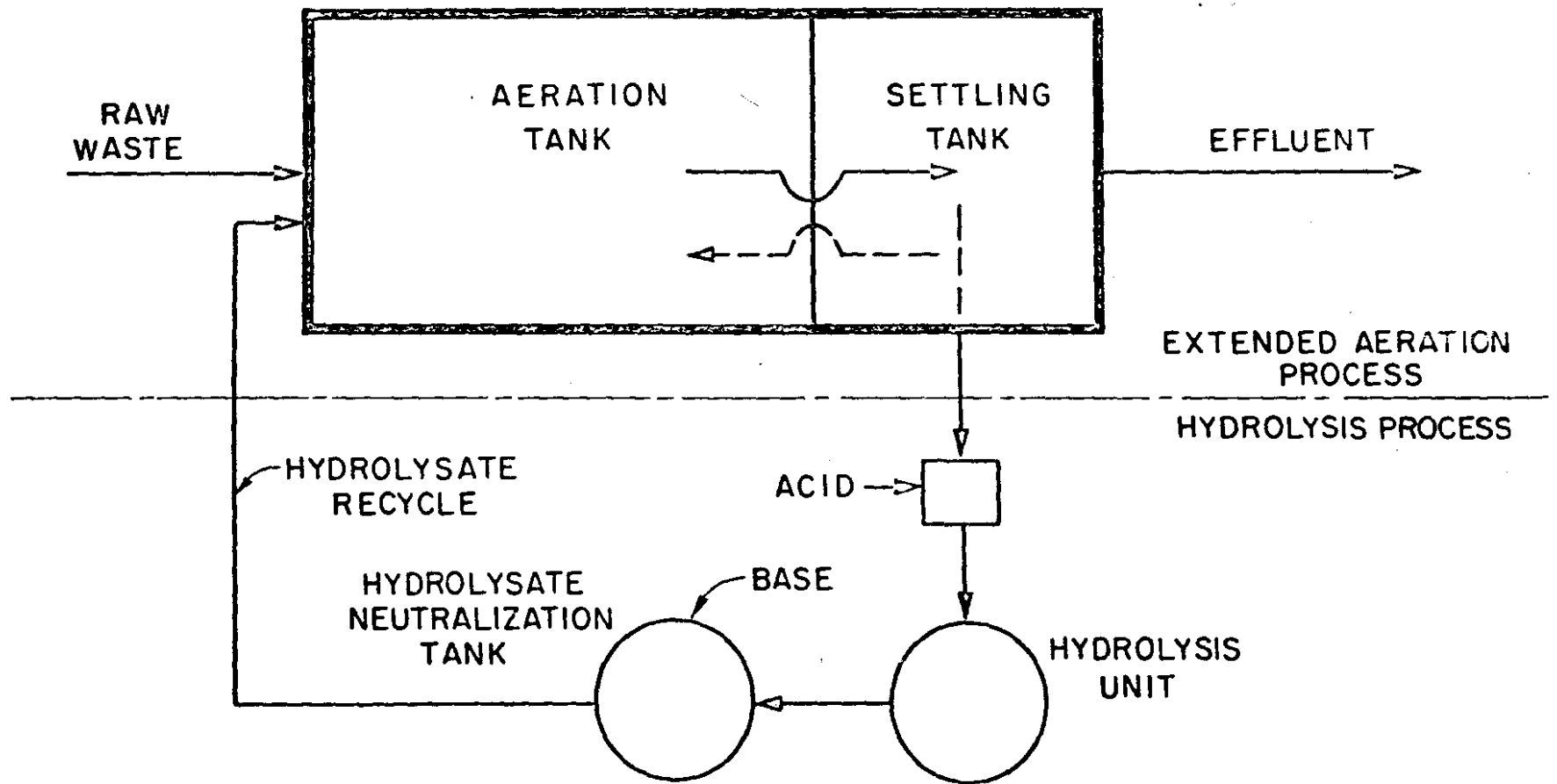
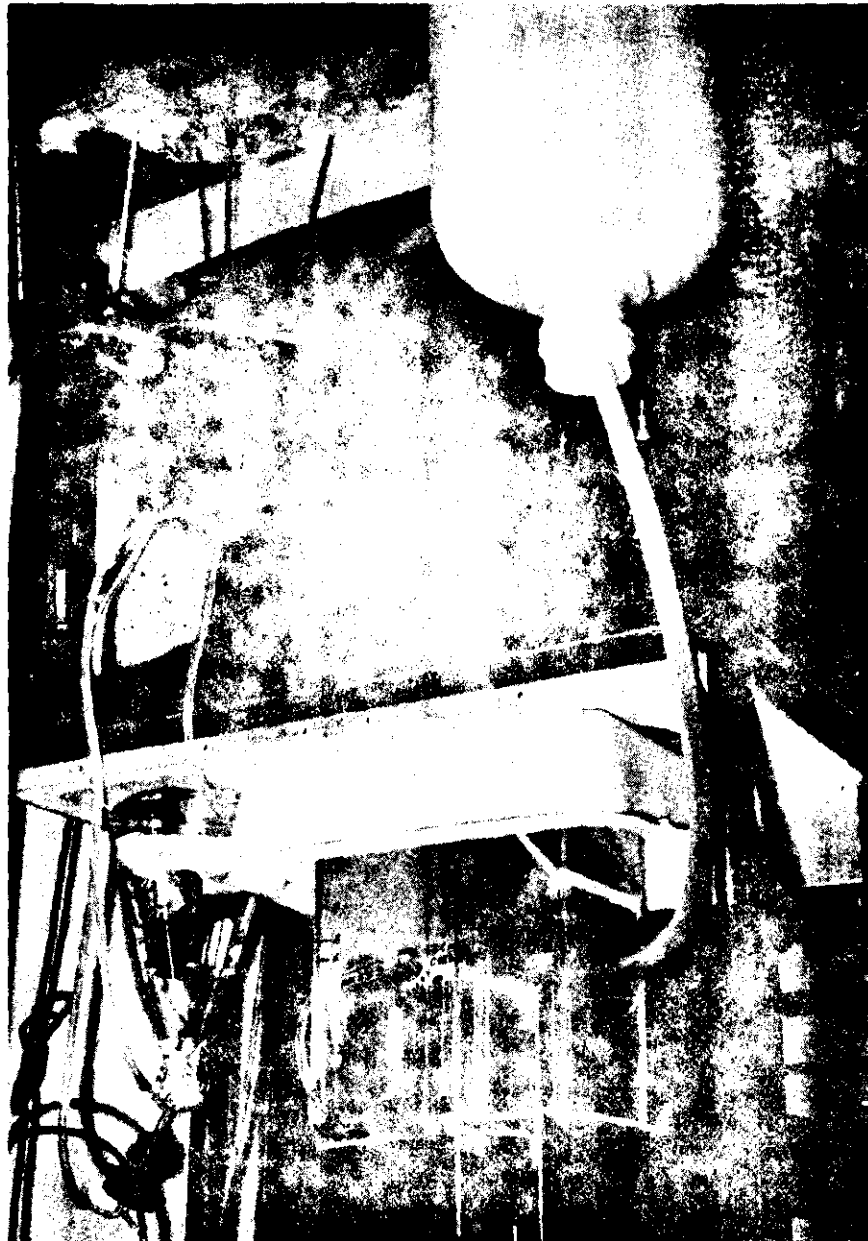


Figure 1. Flow diagram for extended aeration pilot plant employing "hydrolytic assist."



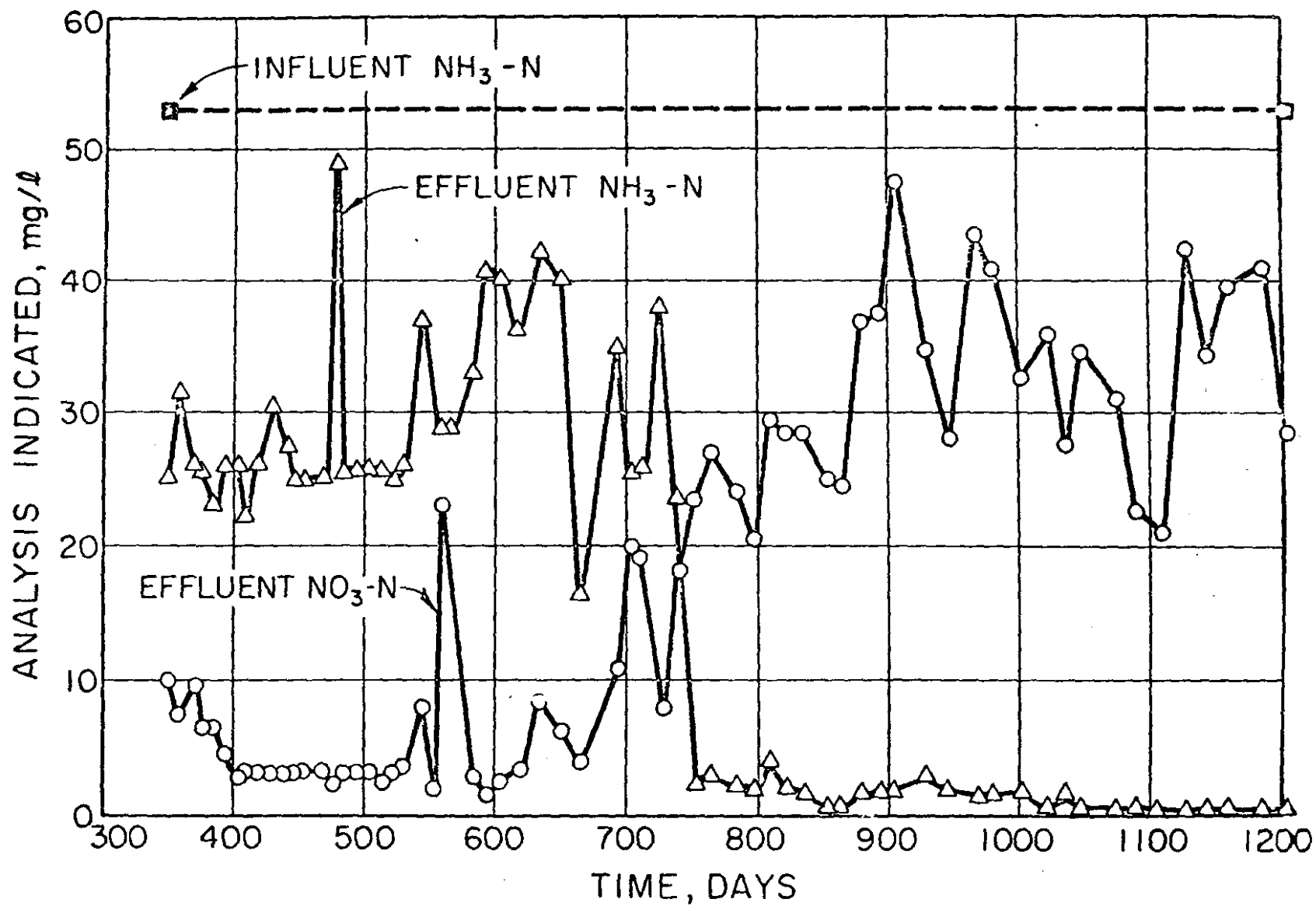


Figure 3. Influent and effluent nitrogen during operation of extended aeration pilot plant without "hydrolytic assist" (Phase A).

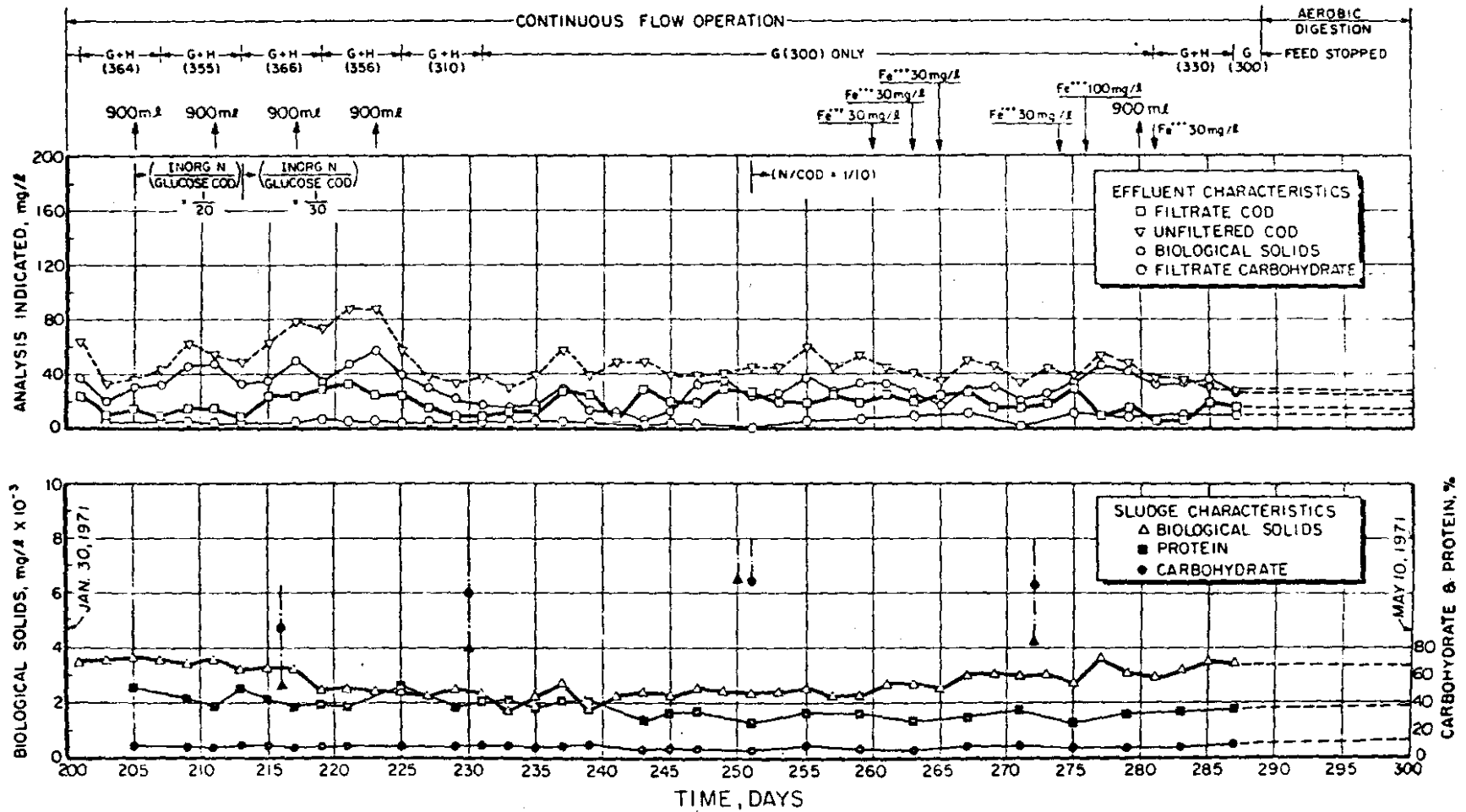


Figure 4. Performance characteristics of a hydrolytically-assisted extended aeration process pilot plant from day 200 through day 300 of operation.

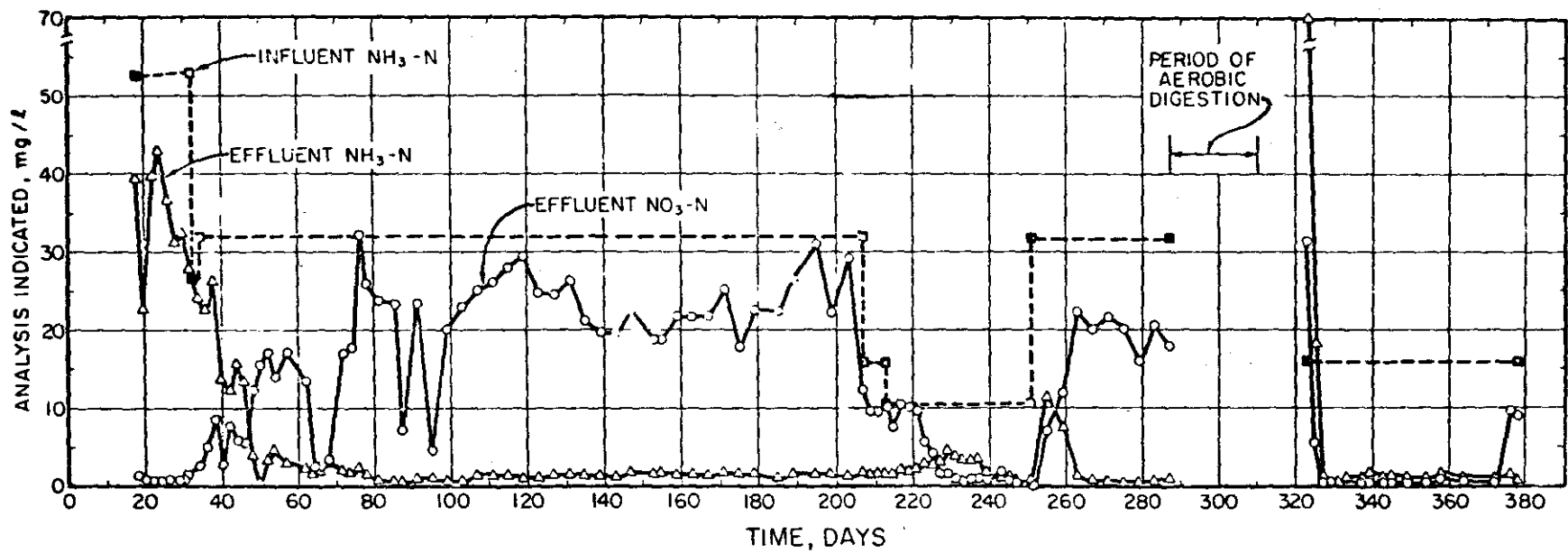


Figure 5. Influent and effluent nitrogen during operation of hydrolytically-assisted extended aeration pilot plant (Phase B).

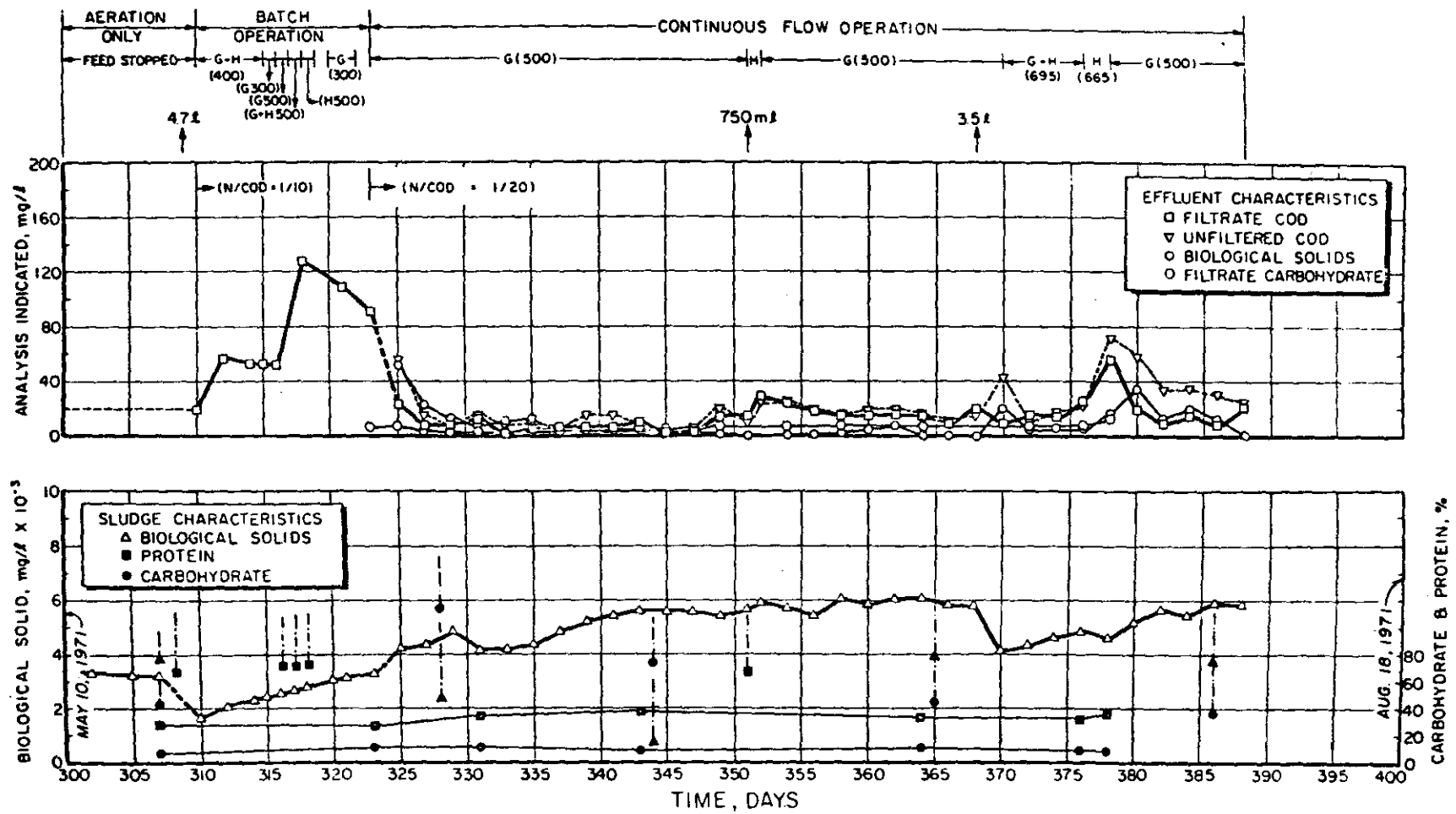


Figure 6. Performance characteristics of a hydrolytically-assisted extended aeration process pilot plant from day 300 through day 388 of operation.