STUDIES ON EXTENDED AERATION ACTIVATED SLUDGE AND A MODIFICATION OF THE PROCESS EMPLOYING CHEMICAL HYDROLYSIS OF PORTIONS OF THE RETURN SLUDGE

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STUDIES ON EXTENDED AERATION ACTIVATED SLUDGE AND A MODIFICATION OF THE PROCESS EMPLOYING CHEMICAL HYDROLYSIS OF PORTIONS OF THE RETURN SLUDGE

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CHAPTER I

INTRODUCTION

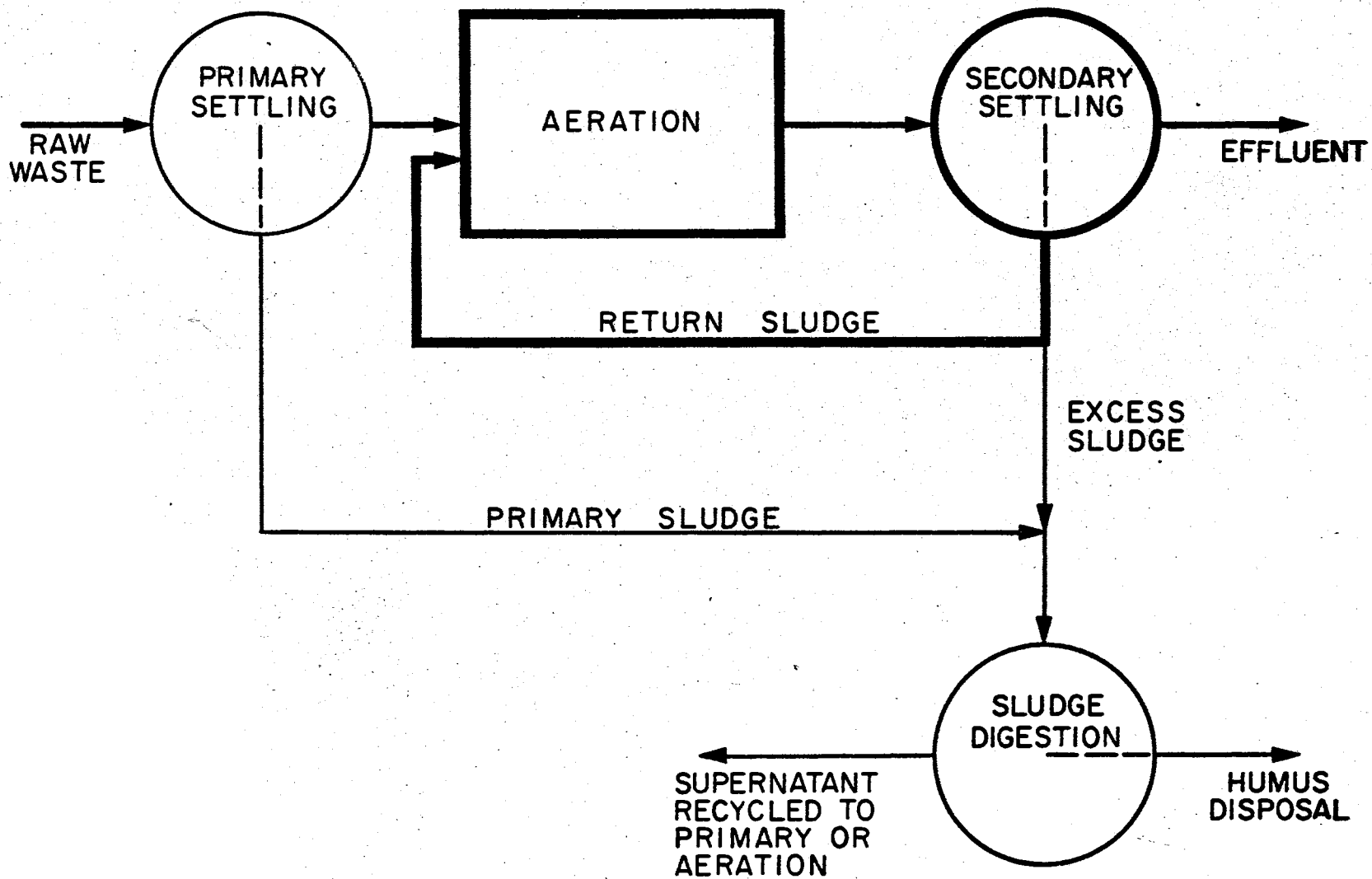
An evergrowing population and increasing industrial production have emphasized the need for secondary treatment of organic-containing wastewaters. Secondary treatment involves the removal of organic matter which could be used as substrate by microorganisms in the receiving water, thus drawing down the oxygen supply in the water resource. Secondary treatment of the organic matter in the wastewater is used for the controlled growth of microorganisms prior to releasing the aqueous effluent to the receiving stream, and the water resource is thus protected. Such biological treatment has come into prominent use, and today the terms "secondary treatment" and "biological treatment" are used synonymously, although there are other possible means of removing organic matter.

The activated sludge process has become one of the most frequently used secondary treatments for waste effluents. The early stages of development of the activated sludge process can be traced to approximately the year 1913 in England, when experiments on the oxidation of sewage employing supplemental aeration were conducted. During the succeeding 30 years, many experiments were conducted aimed at understanding and improving the process, adapting it to special situations, and reducing costs. Since World War II, basic research on the process

has been accelerated. In over half a century of investigation, many modifications of the process have been proposed and installed in the field. One of the most interesting and potentially useful modifications is that one which has become known as the "extended aeration" or "total oxidation" process. The experimentation to be reported in the present thesis is directly applicable to this process.

The extended aeration process which was proposed in the early 1950s differs from other modifications of the activated sludge process in that it combines sludge disposal and wastewater purification. The major elements of the process are shown in heavy lines in Figure 1. It can be seen that all sludge is returned to the aerobic reactor; there is in theory no excess sludge; therefore, sludge handling treatment and ultimate disposal facilities are not required. When the process was proposed, it was thought that endogenous respiration of the organic matter produced during the removal or metabolism of the organic substrates in the waste would balance new growth of biological cells, and that an equilibrium solids concentration would eventually be established. If such a situation did occur, it could truly be said that the incoming organic matter in the waste would be totally oxidized to CO_2 and water. The proposed process engendered a considerable research interest on the part of investigators in the pollution control field, and the conclusion of most of the early researchers was that the process was biologically unsound, i.e., it was theoretically impossible to oxidize totally the organic matter to CO_2 and water. It was generally felt that there would be some net accumulation of organic material which was inert, i.e., it could neither be used as a substrate nor was it biologically active in removal of substrate. Thus, it could be

Figure 1. Comparison of extended aeration and conventional activated sludge process


PRIMARY
SETTLING
AERATION
SECONDARY
SETTLING
RAW
WASTE
EFFLUENT
RETURN SLUDGE
EXCESS
SLUDGE
PRIMARY SLUDGE
SLUDGE
DIGESTION
SUPERNATANT
RECYCLED TO
PRIMARY OR
AERATION
HUMUS
DISPOSAL

envisioned that the biologically inert fraction would continually build up, unless some excess sludge was wasted, either purposely or inadvertently over the weir of the clarifier.

Regardless of these general conclusions warning against the use of the process, it has been used in the field to an increasing extent. For example, beginning with three installations in 1950, there were, by 1962, over 2600 extended aeration plants in use. Many small communities and commercial establishments have employed the process because of the high cost of separate sludge treatment and disposal facilities. There have been some conflicting field reports, but many have claimed that the process has worked exceptionally well, yielding a high degree of purification with no sludge wasting or inadvertent loss of biological solids from the system. One gains the impression from reviewing the technological literature and the present status of the process that it would enjoy more extensive use were it not for the onus of theoretical unsoundness which has been assigned to the process.

Within the past five years, an extensive investigative effort regarding this process has been mounted in the bioengineering laboratories of the Oklahoma State University. In initiating these studies, it was felt that there were not sufficient data to warrant the general conclusions which had been made regarding the theoretical unsoundness of the process. Also, from a practical standpoint, there was some question regarding how long it might take before an inactive organic fraction (if, indeed, one did exist), would build up to such an extent as to cause biochemical failure of the process. Thus, from the standpoint of theoretical concepts of biological engineering, and from the standpoint of practical engineering utility of the extended aeration,

or total oxidation, process, further work on the concept seemed warranted.

In general, four main lines of investigation have been pursued. The first two channels for investigation were envisioned at the outset of the work, whereas the latter two developed as the investigations proceeded.

The major experimental study envisioned was the operation of an extended aeration laboratory pilot plant over a long period of time with positive retention of biological solids, in order to determine when and if such a system would lose its substrate removal capability because of the buildup of so-called inert materials. Concurrently with the operation of this system under relatively steady conditions, another system was developed to which were applied various shock loadings. It was essential to investigate the ability of the extended aeration process to accommodate shock loadings. The shock loading studies have been completed, and some of these results have recently been reported. Those which have been reported embody, in the main, the work conducted by Wisut Ragthaidee during the course of his Master's thesis research. Also, many of the results obtained during long-term operation of the extended aeration process have already been reported by the author. These include much of the work conducted during research pertinent to the Master of Science thesis. Also already reported is some of the research on the process which followed the MS thesis research. The research already reported provided definite indication that a so-called inert biological fraction did not continually build up in the system, and that the system could not be expected to undergo metabolic failure. This research led to the third and fourth major avenues of

investigation. Basic metabolic studies on the metabolism of various fractions of microbial cells has recently been completed by Alan Obayashi, and will be reported in his doctoral thesis. The fourth line of investigation involved various ways and means to enhance the initiation of the autodigestive cycle and provide some engineering control over the process. It was reasoned that control of the biological solids concentration could be enhanced by providing a "chemical assist" in which some sludge could be withdrawn from the bottom of the clarifier, solubilized by chemical hydrolysis, then neutralized and returned to the aeration tank as soluble sludge, i.e., as new substrate for the intact sludge. It is these studies, along with continued investigation of the first major line of study, which are the subject of the present report.

CHAPTER II

LITERATURE REVIEW

The concept of the extended aeration process (or the total oxidation process) was first presented by Hoover, et al. (1) and Porges, et al. (2) as a result of their studies on the aerobic treatment of dairy wastes. In these studies they employed skimmed milk (1000 mg/l) as the substrate. They found that the endogenous respiration rate of the sludge grown on skimmed milk solids was approximately 3 to 4 mg O_2/l/hr, whereas during the active growth or substrate removal phase, the oxygen uptake amounted to 40 to 50 mg/l/hr. Thus the endogenous oxygen uptake was approximately one-tenth the respiration rate during the substrate removal period. In previous studies (3)(4), they found that about 32 to 40 percent of the oxygen demand of the skimmed milk solids was expressed as O_2 uptake during the substrate removal phase, and that approximately 60 percent of the oxygen demand of the substrate was incorporated as cell components in the sludge. It was reasoned that by employing a somewhat lengthy detention time in the aerator, the 60 percent of the oxygen demand of the initial substrate which had been incorporated into the cells could be expressed, or dissipated, as endogenous respiration. It was reasoned that an equilibrium between net autoxidation and net synthesis of new biological solids could be established, and the solids concentration could be expected to reach some equilibrium

value. It can be seen that in such a system there would theoretically be no sludge to dispose of, and it is readily understood that the suggestion of such a process modification stimulated a considerable amount of research on the process by workers in the water pollution control field.

Based upon the findings of Porges and his co-workers, Thayer (5)(6) developed a waste disposal plan applicable to small dairies. Work was accomplished at the George Shell Creamery Plant, near Germantown, Pa., the Blossom Hill Dairy, in Dayton Ohio, and the Cherry Grove Dairy Plant, near Toledo, Ohio. These three plants represented the first actual application of the laboratory studies on the extended aeration process. The plants were operated with detention times varying from 36 to 41 hours in the aeration tank and two to four hours in the settling tank. The influent BODs varied from 567 to 926 mg/l, and the final effluent BODs obtained varied from 19 to 31 mg/l. For these systems, in which all sludge from the settling tank was returned to the head end of the aeration tank, the BOD reduction was approximately 96 percent. The process was put into field application by the Chicago Pump Company, of Chicago, Illinois.

In 1956, Eckenfelder (7) reported on the results of his studies on the oxidation kinetics of biological sludges. He found that sludge accumulated during the bioxidation of organic wastes will undergo endogenous oxidation at varying rates, depending upon such factors as temperature, waste characteristics, microbial content of the sludge, and age of the sludge. He also concluded that the endogenous degradation of the sludge proceeds (approximately) in accordance with first-order decreasing rate kinetics. The rate progressively decreases and

approaches a limit of about 40 to 60 percent volatile solids reduction. He concluded that the remaining constituents were resistant to further oxidation, and would provide a residue for sludge disposal. He suggested that the nitrogen-rich liquor remaining after oxidation had proceeded might be usable in systems requiring supplemental nitrogen.

In 1958, Tapleshay (8) reported on the results of pilot plant studies at a B. F. Goodrich Company plant. Primary settling and sludge digestion were eliminated. An aeration period of 24 hours was provided, and it was recommended that the BOD loading not exceed 30 lbs/day/1000 cu ft aeration capacity. A 4-hour detention time in the settling tank was provided. The sludge was returned from the settling tank to the aeration tank at a high rate in order to assure completely aerobic conditions in the sludge at all times. Also, a sludge holding tank was provided in order to avoid high levels of solids concentration in the system. It was generally found that the solids concentration in the aeration tank was maintained at relatively fixed levels without the necessity of wasting any sludge. The process accomplished BOD reductions of 75 to 90 percent, and an effluent BOD of less than 20 mg/l was generally obtained using the process. Tapleshay also found that the process could handle fairly wide variations in influent organic loading with little effect on the treatment efficiency. He concluded that the process was relatively inexpensive, due to the elimination of costly substructures and extensive piping, and he felt that operational control primarily involved mechanical maintenance and housekeeping, thus enhancing the low cost of the treatment.

Not all reports regarding or bearing upon the extended aeration process were favorable. In 1958, Symons and McKinney (9) in their

study on the biochemistry of nitrogen in the synthesis of activated sludge concluded that biological solids (measurable as MLVSS, mixed liquor volatile suspended solids) will accumulate in the form of extracellular polysaccharides. In their studies, the feed consisted of 1000 mg/l of acetic acid; the studies were conducted under batch operation conditions for a duration of 35 days, and the concentration of nitrogen source in the synthetic waste was varied. They concluded that the extracellular polysaccharide was resistant to biological degradation even under prolonged periods of aeration. Their general conclusion was that batch fed or conventionally fed activated sludge systems cannot be operated without wasting sludge, and that in such a system in which no sludge was wasted, there would be a gradual buildup of biological solids unless some sludge escaped with the effluent. In 1956, Porges, et al. (10) had reported that the stored material in the sludge they examined was an internal insoluble glycogen-like substance which was metabolized when the concentration of substrate in solution was lowered.

In 1958, Jasewicz and Porges (11) reported on their studies of the biological treatment of whey wastes. They varied the nitrogen content (COD:N = 30:1 and 50:1). The higher nitrogen concentration enhanced the rate of sludge oxidation, but in operation of the laboratory pilot plant, they found that feeding whey and employing both levels of nitrogen supplementation, the settling characteristics of the sludge gradually deteriorated and serious bulking problems were encountered within three months. They pointed out that the cheese-making process probably removed various growth factors, and made the whey a relatively unbalanced food material for bacterial growth. Therefore they suggested that periodic additions of milk were necessary to avoid the bulking

problem. They concluded that sludge accumulation might occur as a result of impaired oxidation of intracellular material.

In later work (1959), Jasewicz and Porges (12) studied the COD and solids balance during aerobic biological treatment of whey wastes, and noted that, on the average, 75 percent of the influent whey COD was oxidized. Although a sludge COD oxidation rate of 5.2 percent/day was obtained, sludge accumulation occurred. The period of bench-scale operation was 61 days, during which time the concentration of whey fed was 1000 mg/l, and the biological solids concentration in the aeration chamber was 2000 mg/l. The biochemical efficiency of the process was 97 percent. They made calculations which indicated that a dynamic equilibrium would be possible when 100 units of sludge were employed to metabolize or treat 10 units of whey, if the sludge autoxidation rate were 6.3 percent/day. They felt that it would be theoretically possible to operate a total oxidation plant without any excess sludge production, provided no insoluble and non-fermentable solids were introduced with the waste. However, they were forced to point out that under actual operational conditions, such an ideal environment for total sludge oxidation is rarely attained, and small quantities of substances that cannot be oxidized in the aeration tank are formed. Thus, after almost 10 years of research on the aerobic treatment of dairy wastes, Porges and his co-workers concluded that occasional wasting of sludge is necessary.

In 1959, Kountz and Forney (13) reported on their studies of the operation of a continuous flow pilot plant employing a waste of known chemical composition. They concluded that total endogenous oxidation of the sludge was not possible with reasonable times and sizes of

treatment units. There was a residual material equivalent to 20 to 25 percent by weight of the new activated sludge produced. They estimated the actual endogenous loss of solids per day to be two percent of the total weight of activated sludge, and the accumulation of non-oxidizable sludge per day was estimated at 0.6 percent of the total weight of activated sludge. Even though they considered total oxidation to be impossible, they suggested that the system could be operated at relatively low organic loadings with a loss of approximately 20 mg/l of activated sludge in the effluent, and an effluent biochemical oxygen demand of less than 10 mg/l.

Busch and Myrick (14) investigated the total oxidation process in both batch and continuous flow laboratory bench-scale operation, using a completely defined soluble organic waste. Glucose was used as substrate. No wasting of biological solids was practiced, although biological solids carried over in the clarifier effluent were not recycled. They found that soluble BOD in the supernatant and plant effluent were consistently below 10 mg/l at BOD loadings ranging from 0.05 to 1.7 lbs BOD/lb volatile suspended solids. They also observed that no equilibrium solids level was attained even after 103 days of operation at BOD loadings of 0.05 lbs/lb volatile suspended solids. They concluded that total oxidation is neither theoretically nor practically attainable, since the buildup of biological solids is inevitable unless the amount of biological solids carried over in the effluent is sufficient to balance that which is accumulating in the system.

By the early 1960s a considerable amount of field experience had been amassed regarding the extended aeration process, and reports by Porges, et al. (15) and by Baker (16) cited the rapid growth in the use

of the process for both permanent and temporary installations. It was generally concluded that these plants could be designed and operated to produce rather high removal efficiencies without noticeable odors, but that there were wide differences of opinion concerning the need for sludge wasting facilities and attendant sludge handling and disposal problems. There was the general recognition that wasting of some activated sludge would enhance effluent quality, and there was general acceptance regarding the simplicity, flexibility, small space requirement, and ease of architectural treatment of the process, all factors which enhance its acceptability and use.

In 1962, the theory of operation of extended aeration processes and the parameters affecting BOD removal efficiencies were evaluated by McCarty and Broderson (17). They felt that if no facilities for disposal of excess sludge were provided, the system would accumulate solids and discharge the excess suspended solids in the effluent. Thus the purification efficiency of the extended aeration system could be expected to decrease as a result of the continual increase of biological solids concentration. They felt that the sludge which accumulated in the unit would include the synthesized biological solids and the biologically nondegradable suspended solids which were originally present in the influent waste, for example, small inorganic grit particles and certain biologically resistant organics such as lignins and cellulose. They therefore suggested that soluble industrial waste and municipal waste must receive separate consideration when designing extended aeration processes. They also pointed out that the efficiency of operation of the extended aeration process was closely related to the effectiveness of the settling tank in retaining the suspended solids.

They suggested that in order to maintain 85 percent BOD_5 removal efficiency, the organic loading should be less than 40 lbs BOD_5/day/1000 cu ft. They found that although fairly high average efficiencies could be maintained at higher loadings, fluctuation of effluent quality would be greater. They also cautioned that nitrification in the aeration tank could cause false values for BOD removal efficiency, as well as enhance possibilities for a rising sludge in the settling tank.

During the late 1950s and prior to 1962, most of the laboratory research studies on the extended aeration process yielded results indicating that a total cell recycle system would indeed show some net accumulation of volatile (organic) sludge. There was some doubt concerning the possibilities of total oxidation with various substrates and loading levels, and Washington and Symons (18) decided to make a study pertaining to volatile solids accumulation on various substrates and at various feeding rates under controlled experimental conditions over an extended period of time. They concluded that relatively inert excess volatile solids did accumulate in activated sludges developed on several types of soluble feeds. They noted that polysaccharides were major constituents; also fatty acids and organic nitrogen compounds comprised a significant portion of the stabilized solids. The relatively inert materials amounted to 10 to 15 percent of the ultimate BOD for carbohydrate or fatty acid feeds. Further, when glycine was fed, a smaller amount of residual solids accumulated. They felt that the inert materials would be cell capsules and external slime, since these were thought to be the least degradable by the organisms. Thus was added one more study which concluded that there were constituents of microbial cells which were non-biodegradable.

Since it was suspected that biological solids would be carried over in the effluent, and since the number of extended aeration plants being installed was ever increasing, it was inevitable that investigators would study possible ways and means to polish the effluent. Culp, et al. (19) studied effluent polishing by mixed-media filtration. A high rate mixed-media filtration unit was recommended for polishing the effluent from an extended aeration plant. It was concluded that such a unit in series with the extended aeration plant would increase BOD removal to more than 98 percent, even when the average detention time in the aeration chamber was decreased from 24 to 12 hours, and when there were severe fluctuations in flow.

In 1964, Simpson (20) described the use of extended aeration systems for treatment of sewage in England. He found that approximately 20 percent of the applied BOD was channelled into accumulated solids. He observed that a high concentration of aeration solids interfered with settling, and he suggested that if the solids concentration reached about 8500 mg/l, partial de-sludging, or sludge wasting, should be accomplished. He recommended retention time in the settling tank of six hours, and a surface overflow rate of 150 gpd/ft^2 at maximum design flow rates.

In 1965, Sawyer discussed some of the milestones in the development of activated sludge processes (21) and presented some guidelines for the operation of one of the most recent modifications of the process, i.e., the extended aeration process. These general guidelines consisted of an aeration time of 24 hours, a BOD loading of approximately 15 lbs BOD/day/1000 cu ft, and 5000 to 8000 mg/l biological solids concentration. It could be expected that at temperatures of 15.5^{o}C and above,

nitrification would be essentially complete, and new sludge growth would be approximately balanced by losses of relatively inert suspended solids which were carried out of the system in the effluent. When the temperature dropped below 15.5°C, it was to be expected that some sludge wasting must be practiced in order to prevent unreasonable losses of solids in the effluent. It was also stated that sludges from extended aeration processes were usually found to be so well oxidized that they could be dried on sand beds without development of nuisance odors.

Over the past five or six years there have been continued studies in both the laboratory and the field regarding the extended aeration process. Ludzack (22) has studied the extended aeration process in bench-scale operation in the laboratory, and on the basis of laboratory experimentation, has made suggestions as to field operation. He warned against the carrying of too high a solids concentration in the aeration chamber, since this would hamper settling in the settling compartment, thereby enhancing solids carryover. He suggested that sludge wasting could not be expected to correct for floating solids problems, but might be expected to limit them somewhat. Prompt return of settled solids to the aerator was recommended as probably the best control for floating solids.

Westrick, et al. (23), and Eye, et al. (24) reported on field evaluations of the performance of extended aeration plants, and have indicated that performance of such plants could be enhanced by enlargement of clarifier capacity or by providing a positive means for controlled sludge wastage.

Middlebrooks and Garland (25) have performed laboratory-scale pilot plant studies on the treatment of comminuted, degritted, raw

waste collected at the local sewage treatment plant. They observed that some solids were lost in the effluent as suspended solids, and they found that the sludge decay rate increased as the influent substrate concentration increased.

The design and operation of extended aeration processes are of continuing interest, and in 1971, Pillai, et al. (26) reviewed various aspects pertinent to the process. They concluded that the unusual amounts of solids which are sometimes discharged are caused in general by higher hydraulic loadings, and by rising sludge in the clarifier due to denitrification. They also suggested that aeration systems in many cases are inefficient, and the return sludge lines often clog and sludge escapes in the effluent. They made recommendations and suggestions for improving design and operation which included: (a) use of "sudden expansion-type" aerators, (b) use of dual aeration tanks equipped with one-way valves permitting back mixing and the recirculation of sludge from both aerator and clarifier, (c) installation of a denitrification tank between the aeration tank and the clarifier, and (d) introduction of a final "polishing" clarifier.

From the above brief review, it can be seen that since its inception approximately 20 years ago, the extended aeration, or total oxidation, process has come into rather wide but not extensive use, and that the premise upon which it is based--that is, total oxidation of biological sludge--is a controversial one. The general belief of many researchers in the field is that biologically inert organic solids will gradually accumulate in the system unless these biological solids inadvertently escape in the effluent from the settling chamber or are purposely withdrawn from the system. It can be seen that continued

buildup of inert material in the sludge would eventually cause the system to undergo biochemical failure. Some of the previous results obtained in the bioengineering laboratories of Oklahoma State University provided some evidence that the total oxidation of biological sludges was not theoretically impossible. Also the very complicated nature of the ecosystem which exists in an activated sludge and possibilities for predatory activity made it rather difficult to accept the concept of continual buildup of inert organic matter, and the studies to be described herein were therefore initiated. Recently, studies on the ability of the process to accommodate quantitative shock loadings have been reported from these laboratories (27). Also some of the research included in this thesis has been recently reported (28)(29), and these works will be cited in more detail as the report progresses.

It can be seen from the literature cited thus far that questions concerning the theoretical validity of the total oxidation concept as well as the practical application of the process revolve in considerable measure around whether microbial cells can serve as a source of nutrition and food supply. During the investigations reported in this thesis, a method for preparing cells for use as biological foodstuffs was conceived and tested. It is appropriate, therefore, to review pertinent literature which had some bearing on the results, which will be presented later.

Various methods of disintegrating microbial cells were categorized by Edebo (30). In general, physical, chemical, and mechanical methods are available. Among the chemical methods are included alkaline, or detergent breakdown, solvent extraction, and enzymatic digestion. Physical methods include freeze-thawing, heat-treatment rupture,

osmotic disruption, decompression, and sonic vibration. Mechanical methods include agitation with abrasive particles, grinding, and pressure extrusion. Most of these methods are of somewhat limited use in most biological studies, since many of the methods can bring about considerable changes in the nature of the biological material, and some of the methods are of low breakage, or disintegration, efficiency. In general, the methods which are usually employed for biological studies involve agitation with abrasive particles and pressure extrusion. More severe treatment of microbial cells usually involves hydrolysis under acid conditions. Such methods are usually employed to solubilize or liquify biological cells. Howe, et al. (31) in their studies of the amino acid composition of certain bacterial proteins, hydrolyzed 100 mg of cells with 5 ml of 6 M HCl in sealed tubes at 121°C for 16 hours. Also, in the preparation of protein hydrolysate for the determination of amino acid composition, it has been reported that a protein sample can be hydrolyzed by 4 N to 6 N HCl or H_2SO_4 and autoclaving for five hours (32). Again, in the study of the extraction of protein from bacteria, Samejima, et al. (33) found that extraction with aqueous urea was facilitated by briefly contacting the cells with heated dilute acid. It was pointed out that this treatment was especially effective for Gram-positive bacteria. In their studies, 100 gm of dried cells of Corynebacterium glutamicum were heated for 30 minutes at 95°C in 500 ml 1 N HCl prior to neutralization and extraction.

In the water pollution control field there have been some recent studies on the determination of available amino acids in activated sludge, and on optimum conditions for acid hydrolysis of the sludge (34)(35). Preparations of dried activated sludge obtained from the

treatment of food industry wastes, as well as other industrial wastes and domestic wastes, were studied to evaluate kinds and amounts of amino acid present. Adequate recoveries of amino acids were found for the following conditions of hydrolysis: 1.05 kg/cm^2 autoclaving pressure for four hours with 4-6 N hydrochloric acid (270 mg/gm dried sludge) or sulfuric acid (290 mg/gm dried sludge).

Hoshino, et al. (34) found that the content of the sulfur-containing amino acids was low, as was the content of tryptophan. In samples obtained from an activated sludge waste treatment plant in the field, the crude protein and ash ranged from 10-50 percent, and 20-55 percent, respectively, and the crude protein and ash in samples from a laboratory activated sludge plant were 50-55 percent, and 10 percent, respectively.

It is interesting to note that in his studies of bacteria, molds, and yeast, as sources of vitamins and protein and related factors pertinent to the use of these products in animal rations, Hall (36) indicated that the average nitrogen content of bacteria, yeast, and molds was 11.1, 8.2, and 5.7 percent of the dry weight, respectively. The bacteria and yeasts were good sources of the ten essential amino acids, whereas the essential amino acid content of the filamentous fungi was more variable and generally lower than that of either the bacteria or the yeast. Thus it would appear that from the standpoint of possible nutritional value of the sludge, as well as creation of adverse settling conditions, the filamentous forms are to be avoided.

In addition to the employment of external forces to disintegrate and hydrolyze cells, cell disintegration is also brought about through biological means. For example, in 1968, Kawata, et al. (37) reported

that cells of Clostridium botulinum, type A strain 190, harvested during the logarithmic growth phase, rapidly autolysed in phosphate buffer, and most of the cells were converted autolytically into spheroplasts in 0.5 M sucrose-phosphate buffer within 2-3 hours at 37°C. A crude cell wall fraction isolated from log phase cultures by sonication and fractionation rapidly autolysed in phosphate buffer. The production of lytic enzymes by various microbial species is also fairly well documented. For example, Carenberg and Heden (38) found that soil bacteria and actinomycetes formed lytic enzymes which could be used to lyse living cells of yeast-like organisms, such as Eremothecium ashbyii.

It is seen from the brief review above that there are various ways in which the integrity of microbial cells can be destroyed, and the breakup, disintegration, or hydrolysis by whatever means should enhance or increase the availability of the cellular carbon as food material. This cellular carbon may at some time in the future serve as animal food, but the primary interest in the present investigation is the determination of whether it serves as bacterial food. Some studies on the metabolic availability of hydrolyzed microbial cells have already been reported from these laboratories (29). Rather extensive studies on the long-term operation of an extended aeration activated sludge pilot plant incorporating periodic hydrolysis of a portion of the sludge and its return to the aeration tank are reported in this thesis.

CHAPTER III

MATERIALS AND METHODS

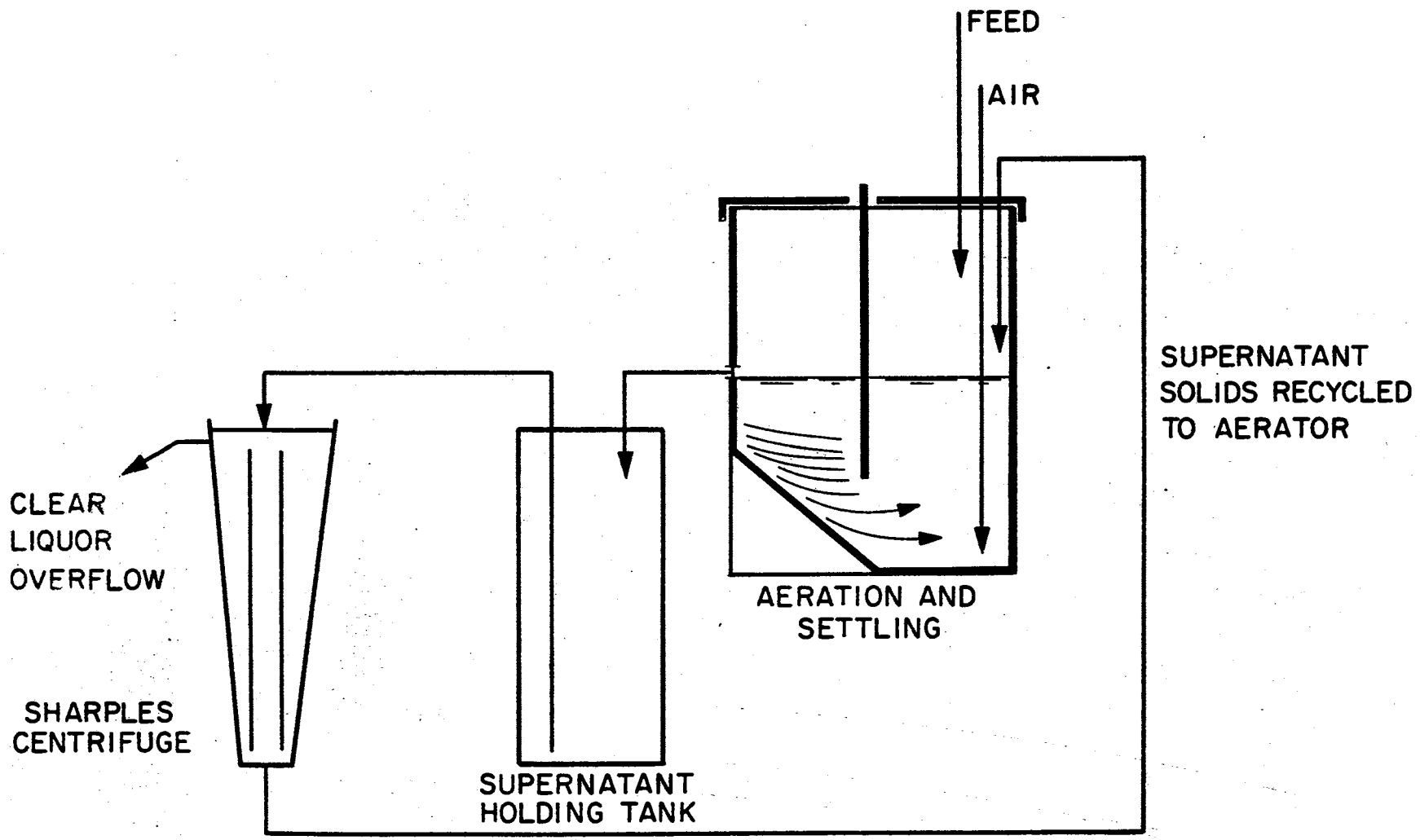
A. Experimental Design

Throughout these investigations, heterogeneous microbial populations were employed. These activated sludges were developed from initial sewage seeds obtained from the effluent of the primary clarifier at the municipal sewage treatment plant at Stillwater, Oklahoma.

1. Studies on the Operational Stability of the Extended Aeration Process Without Hydrolytic Pretreatment

The bench-scale extended aeration pilot plant employed in this study was essentially the same as the one used in the shock loading studies reported previously (27). A flow diagram showing the aeration and settling chambers, the supernatant holding tank, and the Sharples centrifuge through which the holding tank liquor was processed is shown in Figure 2. A photograph showing the plexiglass aeration and settling chamber is shown in Figure 3. The total volume of the system is 9.4 liters (6.2 liters, aeration chamber, and 3.2 liters, settling chamber). A movable baffle was used to separate the aeration and settling chambers. Compressed air provided not only mixing and oxygen supply to the biological solids, but also provided "suction" to recycle settled solids from the settling chamber. Airflow rate was maintained at 2000 cc/min/l. Temperature was maintained at $23 \pm 2^{o}C$.

Figure 2. Continuous flow extended aeration pilot plant


FEED
AIR
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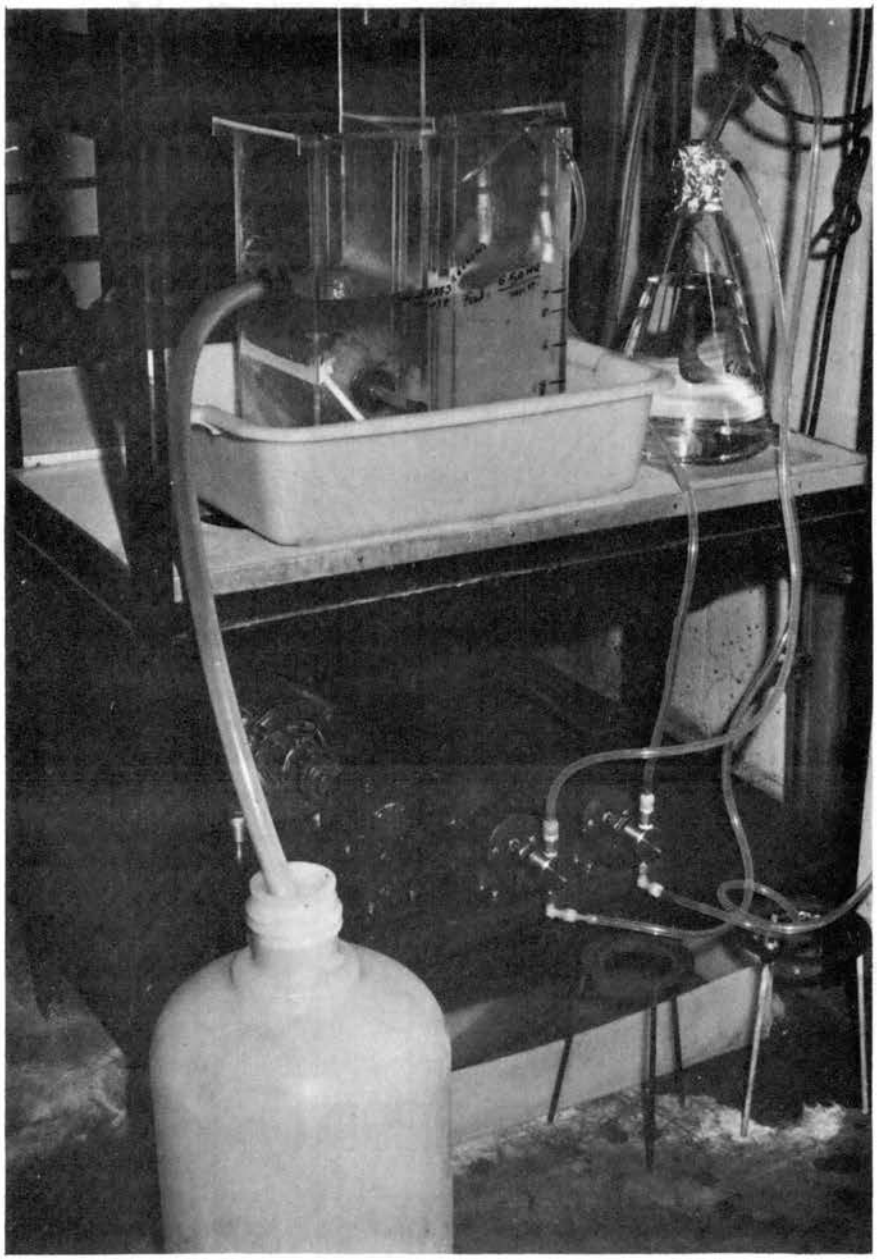
SOLIDS RECYCLED
TO AERATOR
CLEAR
LIQUOR
OVERFLOW
AERATION AND
SETTLING
SHARPLES
CENTRIFUGE
SUPERNATANT
HOLDING TANK

During operation under continuous flow conditions, the feed rate was set to provide an overall detention time of 24 hours (approximately 16 hours aeration and 8 hours settling). During periods of batch operation, the baffle separating the two chambers was removed and the unit was fed once daily. During these periods, a 23-hour aeration period was employed. The sludge was permitted to settle for one hour after the daily sample (15 ml) was taken. After the one-hour settling period, one liter of supernatant was removed and centrifuged, and the biological solids were returned with one liter of feed solution to the reactor; thus, the unit was again brought back to the operating level (9.4 liters). During continuous flow operation, the feed solution was channelled to the aeration chamber through a dual positive displacement pump arrangement (minipump, Milton Roy, Model MM2-B-96R) shown in Figure 3. Alternately, each of the feed lines was cleaned by pumping Clorox solution (1%) and distilled water. Thus one of the lines was being disinfected while the other was in use. The effluent collected in the holding tank (plastic bottle, bottom Figure 3) was periodically (once daily or once every two days when the effluent was not so turbid) passed through the centrifuge (Sharples Superspeed), and any solids which had been carried over from the settling chamber were scraped from the bowl and returned to the aeration chamber. It is emphasized that no biological solids were either inadvertently or intentionally lost from the system throughout this study. The only solids "lost" from the system were those taken for samples to assess operational behavior.

Daily (or nearly so), 15 ml-samples of mixed liquor were removed for analysis. At less frequent intervals (approximately twice per

Figure 3. Laboratory-scale pilot plant.

The plastic aeration and settling chamber, pumps, and effluent holding tank are shown. The pump on the right-hand side is delivering the synthetic waste for the feedholding tank (not shown), and the pump on the left is circulating mild chlorine solution, sterilizing the alternate set of feed lines.



month) 20 ml of mixed liquor were removed for measurement of endogenous uptake, sludge composition (carbohydrate and protein), and periodic examination of substrate removal rate. The daily samples for the measurement of biological solids concentration were taken in such a way that the data represent the sludge concentration in the total system, not in the aeration chamber alone. Just prior to removing a sample, the settling chamber outlet was closed, the feed flow was stopped, the movable chamber baffle was lifted and the contents of the two chambers were allowed to mix. The sample of mixed liquor was then removed and the baffle was immediately replaced, the settling tank outlet opened, and feeding resumed.

The composition of synthetic waste during the continuous feeding operations is shown in Table I. During periods of batch operation, the concentration of each constituent was increased 9.4-fold. The daily organic loading was approximately 50 lb COD/1000 cu ft aeration capacity (32 lb BOD_5/1000 cu ft) during continuous flow operations, and 32 lb COD/1000 cu ft (22 lb BOD_5/1000 cu ft) on the basis of total volume of the system (or aeration volume) during batch operation.

2. Studies on the Operational Stability of the Extended Aeration Process With Hydrolytic Pretreatment of Some Recycled Sludge

The general operational procedure for this process was the same as for the operation without hydrolytic pretreatment of portions of the sludge, except that the composition of the feed solution was different, and there was no centrifugation of biological solids suspended in the effluent. The flow scheme for the process is shown in Figure 4. The feed solution used is shown in Table II.

The sludge hydrolysate was prepared by partial hydrolysis of

TABLE I

COMPOSITION OF FEED FOR 500 mg/l GLUCOSE AS SUBSTRATE

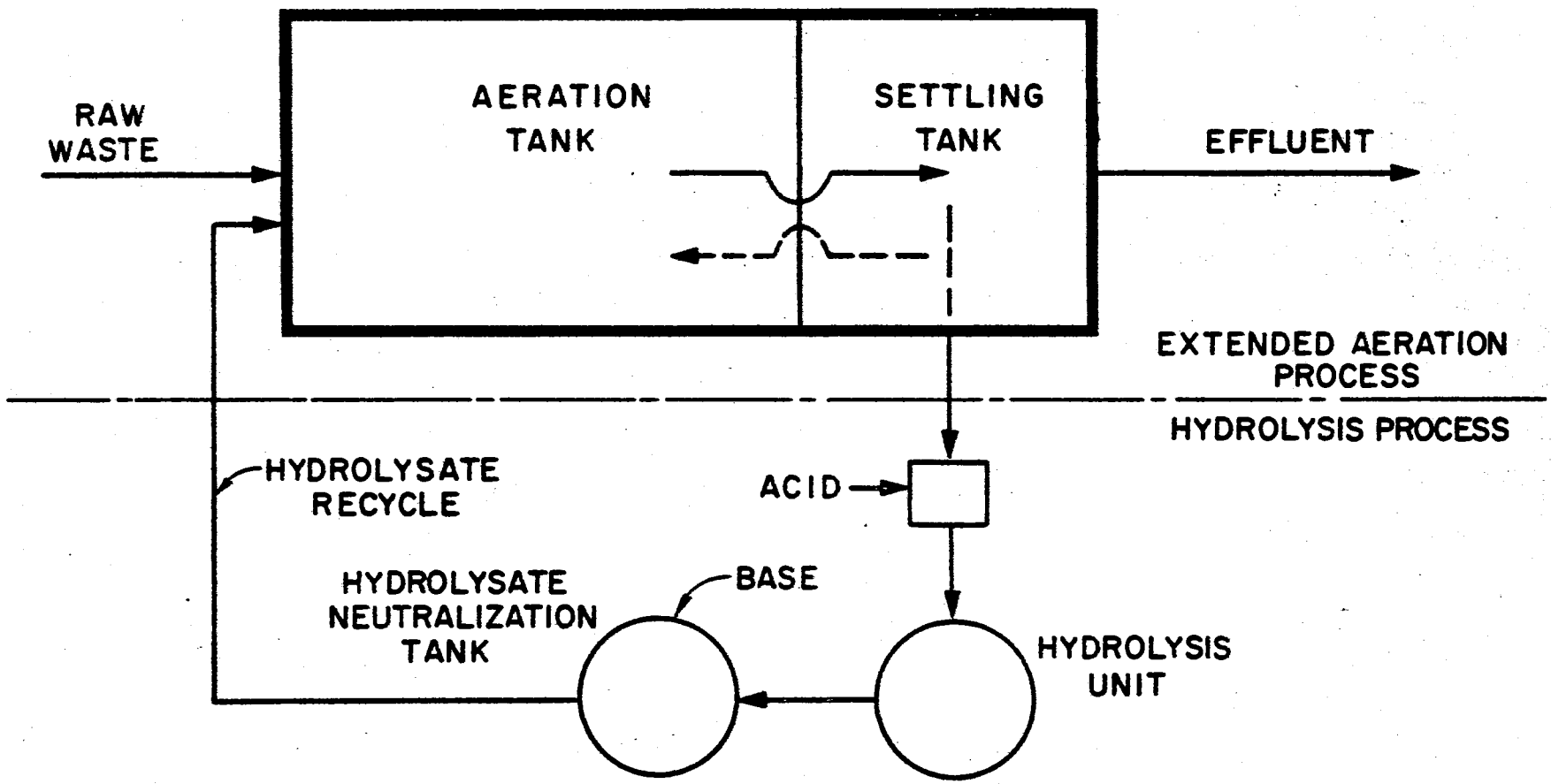
Glucose	500	mg/l
$(NH_4)_2SO_4$	250	mg/l
$MgSO_4 \cdot 7\ H_2O$	50	mg/l
$FeCl_3 \cdot 6\ H_2O$	0.25	mg/l
$CaCl_2$	3.75	mg/l
$MnSO_4 \cdot H_2O$	4	mg/l
Phosphate Buffer, 1.0 M, pH 7.0 (KH_2PO_4 + K_2HPO_4)	5 ml/l to 30	ml/l
Tap Water	50	ml/l

TABLE II

COMPOSITION OF FEED FOR 300 mg/l GLUCOSE AND 45-100 mg/l SLUDGE HYDROLYSATE COD

Glucose	300	mg/l
Hydrolysate COD	45-100	mg/l
$(NH_4)_2 \cdot SO_4$	150	mg/l
$MgSO_4 \cdot 7\ H_2O$	30	mg/l
$FeCl_3 \cdot 6\ H_2O$	0.15	mg/l
$CaCl_2$	2.25	mg/l
$MnSO_4 \cdot H_2O$	3	mg/l
Phosphate Buffer, 1.0 M, OH 7.0 (KH_2PO_4 + K_2HPO_4)	3 ml/l to 12	ml/l
Tap Water	30	ml/l

Figure 4. Proposed extended aeration activated sludge process incorporating chemical hydrolysis for control of sludge concentration.


RAW
WASTE
AERATION
TANK
SETTLING
TANK
EFFLUENT
EXTENDED AERATION
PROCESS
HYDROLYSIS PROCESS
HYDROLYSATE
RECYCLE
ACID
HYDROLYSATE
NEUTRALIZATION
TANK
BASE
HYDROLYSIS
UNIT

portions of the sludge (taken from the settling chamber) under acid conditions, pH = 1, in a laboratory autoclave (five hours at 15 psi, 121°C). This hydrolyzed sludge was neutralized to pH 7 with NaOH, and fed with glucose minimal medium to the system.

3. Studies on the Growth and Substrate Removal Characteristics of Cells Taken From the Extended Aeration Unit With and Without Hydrolytic Pretreatment

The apparatus used in these experiments is shown in Figure 5. For these studies, a small inoculum of cells from the extended aeration unit was placed in the growth apparatus along with fresh glucose minimal medium (Table I). The growth and substrate removal characteristics of cells were investigated.

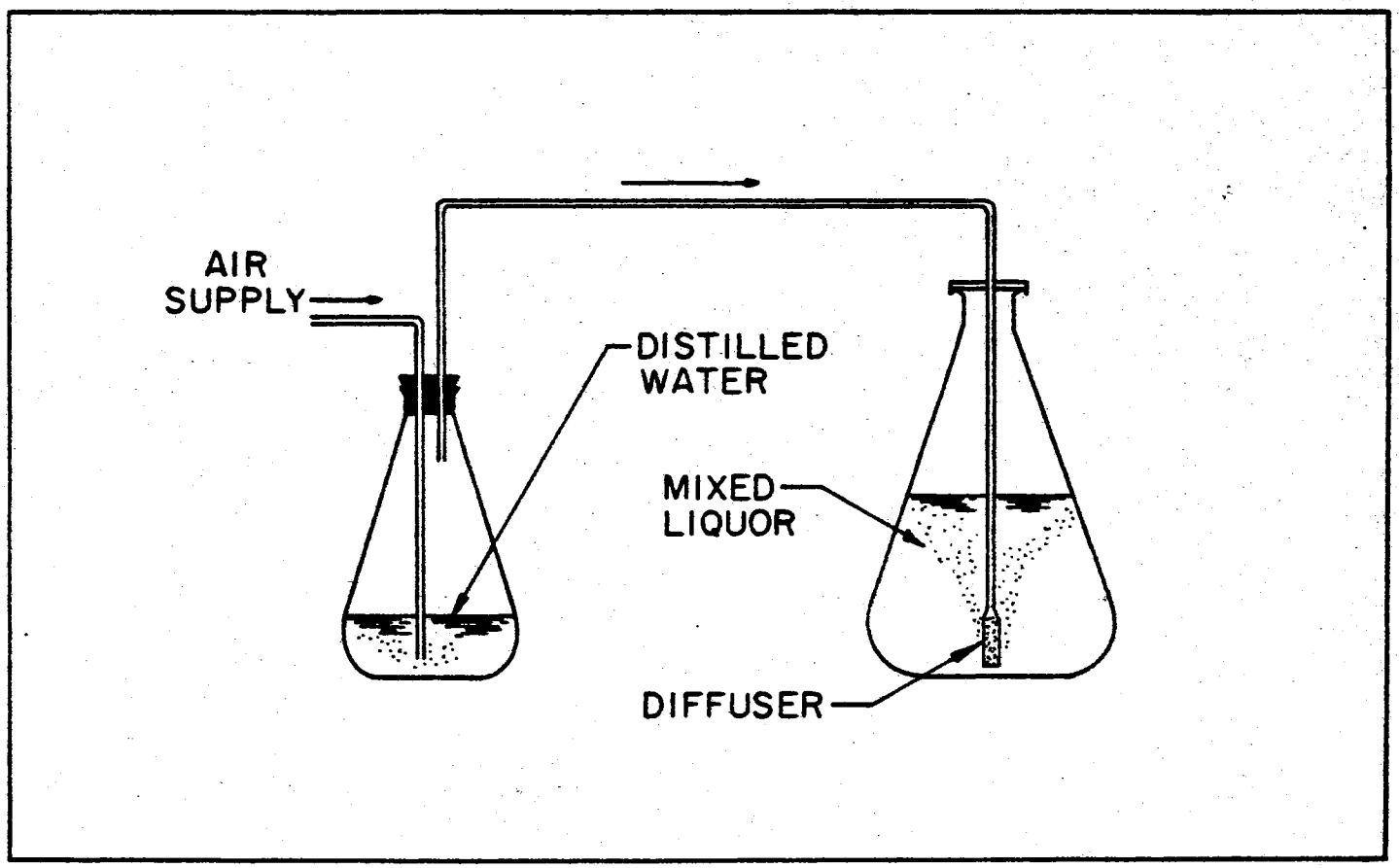
4. Studies on the Endogenous Oxygen Uptake of Cells Taken From the Extended Aeration Unit With and Without Hydrolytic Pretreatment

About twice per month, cells were harvested by centrifugation of a 10-20 ml sample of reactor mixed liquor, washed twice in 0.1 M phosphate buffer solution and resuspended in buffer for measurement of the rate of endogenous O_2 uptake. A reaction volume of 40 ml was employed in Warburg respirometer studies, using 1.5 ml of 20 percent KOH in the center well. The apparatus was operated at 25°C and 90 osc/min.

5. Studies on the Response of the Extended Aeration Activated Sludge to Slug Dosage of Glucose at Various Cell Ages

About twice per month, the rate of substrate removal was measured in the extended aeration unit. The procedure for this measurement was as follows: Just prior to adding the slug dose of glucose minimal medium, the settling chamber outlet was stoppered, and the feed was

Figure 5. Experimental setup for substrate utilization rate with low initial solids concentration.


AIR
SUPPLY
DISTILLED
WATER
MIXED
LIQUOR
DIFFUSER

shut off. The dividing baffle was then lifted, and the contents of the settling and aeration chambers were allowed to mix completely. Also, while the solids were mixing, the solids in the effluent which had been passed through the centrifuge were returned to the system. Then concentrated (9.4 x) glucose minimal medium was added as a slug dosage to the reactor. The COD removal and biological solids concentration were determined every fifteen minutes until no further COD removal was observed.

6. Studies on the Metabolism of Cell Hydrolysate in the Extended Aeration Unit and in Separate Batch Experiments

The apparatus used for this study was shown in Figures 3 and 5. The experiments were accomplished using the cell hydrolysate (instead of glucose) as the substrate. The procedure was the same as described above (items 3 and 5). Cell growth and COD removal characteristics were measured.

7. Studies on Chemical Flocculation of Biological Solids in the Effluent of the Extended Aeration Unit

Cell suspensions taken from the effluent of the extended aeration unit were subjected to jar tests with different chemical coagulants and dosages at various pH values. The treatment efficiency was assessed by measuring the optical density (turbidity) of the supernatant at a wave length of 540 mμ. The tests were run by adding the coagulants, flash mixing (100 rpm, one min), flocculating (50 rpm, 10 min), and settling (20 min). The supernatant was withdrawn and the optical density was measured using the Spectronic 20 (Bausch & Lomb, Inc.). The jar test apparatus was manufactured by Phipps & Bird, Inc., Richmond, Va.

8. Studies on the Effects of Different Nitrogen Levels in the Synthetic Waste During the Period of Operation With Hydrolytic Pretreatment

The operational procedure and apparatus employed were the same as those described above (item 2). The composition of the feed was that shown in Table II, except that varied nitrogen levels were employed. The purification efficiency, biological solids concentration, sludge composition (protein and carbohydrate), and nitrogen concentration (NH_3-N, NO_3-N, NO_2-NO) in the effluent were measured.

B. Analytical Methods

Biological solids concentration was determined by the membrane filter technique (Millipore Filter Co., Bedford, Mass., HA 0.45 μ) as outlined in Standard Methods (39). Chemical oxygen demand (COD) was also run on the filtrate (39). At periodical intervals, CODs were also run on the effluent from the settling chamber (unfiltered COD). Protein and carbohydrate contents of the sludge were determined by, respectively, the biuret and anthrone tests (40). At various times, the concentrations of phosphate (41), NH_3-N (42), NO_3-N (39), and NO_2-N (39) in the effluent were determined. The anthrone test (in addition to CODs) was run on the membrane filtrate. Periodically throughout the experimentation, the dissolved oxygen in the aeration chamber mixed liquor and in the settling chamber effluent was measured by a galvanic cell oxygen analyzer (Precision Scientific), in accordance with the procedures recommended by the manufacturer (43). The pH was checked periodically with a pH meter (Beckman Zerometric).

CHAPTER IV

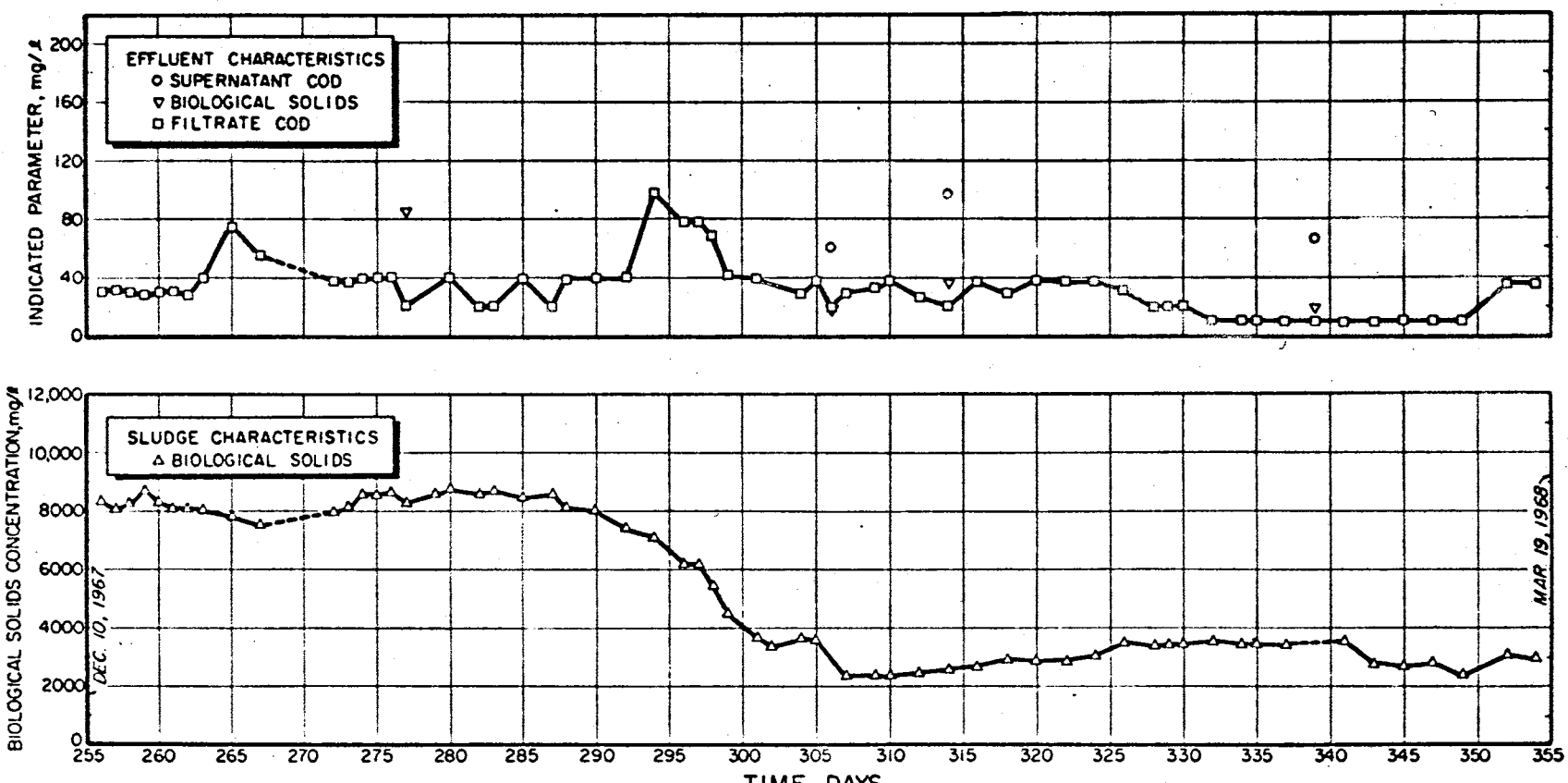
RESULTS

The extended aeration activated sludge pilot plant was put into operation on March 30, 1967. The history of development of this activated sludge has been reported in detail (28)(44). The ability of the system to accommodate shock loads (quantitative and qualitative) has also been described (27)(45). The work herein reported pertains to the long-term behavior of the extended aeration process, and the performance of an extended aeration process incorporating chemical hydrolysis for control of sludge concentration. Although portions of this work have been previously reported (28)(29)(44), certain aspects deserve to be re-emphasized and are herein reviewed and expanded. The data on operational stability for the first 1000 days of experimental pilot plant operation in phase A are also given in the author's MS thesis (44). It was deemed essential to the continuity of presentation of the overall study and to interpretation of results leading to phase B, which comprises the major contribution of this PhD thesis, that the previous results of the author be included in this chapter.

A. Studies on the Operational Stability of the Extended Aeration Activated Sludge Process Without Chemical Hydrolysis of Portions of the Sludge for Control of Sludge Concentration

It can be seen in Figure 6 that the biological solids concentration

Figure 6. Performance data for continuous flow extended aeration pilot plant, December 10, 1967, to March 19, 1968.


EFFLUENT CHARACTERISTICS
○ SUPERNATANT COD
▽ BIOLOGICAL SOLIDS
□ FILTRATE COD
INDICATED PARAMETER, mg/ℓ
0
40
80
120
160
200
SLUDGE CHARACTERISTICS
△ BIOLOGICAL SOLIDS
BIOLOGICAL SOLIDS CONCENTRATION, mg/ℓ
0
2000
4000
6000
8000
10,000
12,000
DEC. 10, 1967
MAR 19, 1968
255
260
265
270
275
280
285
290
295
300
305
310
315
320
325
330
335
340
345
350
355
TIME, DAYS

in the system after 285 days of operation was slightly above 8000 mg/l. The solids concentration then decreased, and by day 310 was between 2000 and 3000 mg/l. It is emphasized that solids were not "wasted" purposely or inadvertently. After completion of this decreasing cycle, the sludge concentration in the system followed an irregular but generally increasing trend (see Figures 6 through 9). The biochemical purification efficiency remained at approximately 90 percent or above throughout the decreasing and increasing cycle, and sludge settleability was very good (these results were the subject of one of the previous reports)(28). Between day 600 and day 1000 (see Figures 10 through 13) there were periods during which biological solids in the settling chamber were carried over the effluent weir (see effluent characteristics, top portion of figures). The solids carryover was not a result of dispersed growth, but of floc carryover. The biochemical purification efficiency remained above 90 percent. Again, it is emphasized that the solids were not lost to the system, but were collected, centrifuged, and returned to the aeration chamber. Unfortunately, the raw data sheets for the pilot plant for days 655 through 679 were lost before they had been transferred to the summary log sheet. However, behavior of the system during this period was very similar to that shown for days 637 through 654.

Although the previous reports (28)(29) covering 1000 days of operation provided sound data regarding the operational stability of the extended aeration process without sludge wasting, it was considered desirable to continue operation and study further the unique, mature ecosystem which had been developed.

Performance data on the system from day 1000 to day 1200 are shown in Figures 14 and 15.

Figure 7. Performance data for continuous flow extended aeration pilot plant, March 19, 1968, to June 27, 1968.

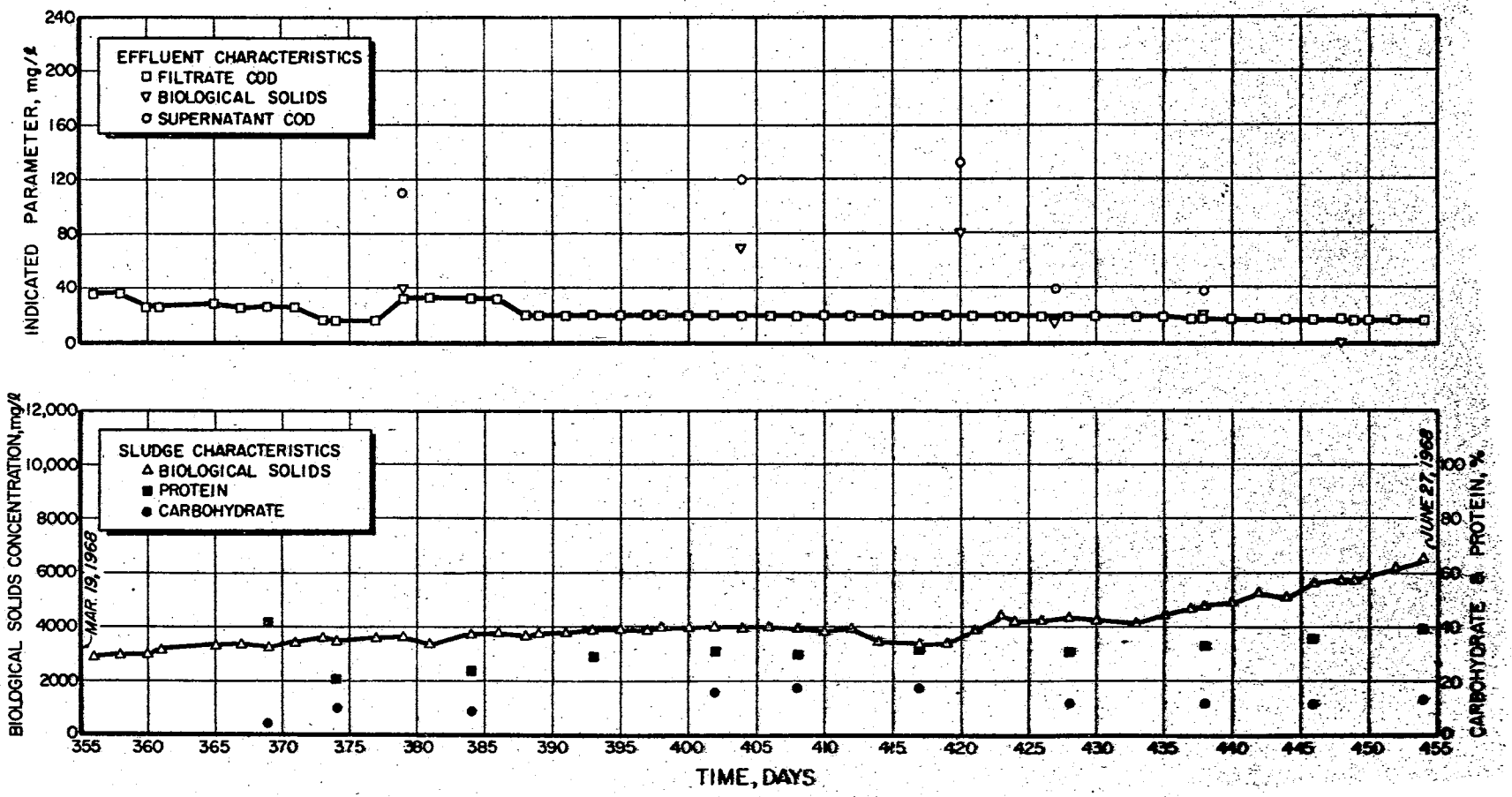

EFFLUENT CHARACTERISTICS
FILTRATE COD
BIOLOGICAL SOLIDS
SUPERNATANT COD
INDICATED PARAMETER, mg/ℓ
240
200
160
120
80
40
0
SLUDGE CHARACTERISTICS
BIOLOGICAL SOLIDS
PROTEIN
CARBOHYDRATE
BIOLOGICAL SOLIDS CONCENTRATION, mg/ℓ
12,000
10,000
8000
6000
4000
2000
0
CARBOHYDRATE & PROTEIN, %
100
80
60
40
20
0
MAR. 19, 1968
JUNE 27, 1968
355
360
365
370
375
380
385
390
395
400
405
410
415
420
425
430
435
440
445
450
455
TIME, DAYS

Figure 8. Performance data for continuous flow extended aeration pilot plant, June 27, 1968, to October 5, 1968.

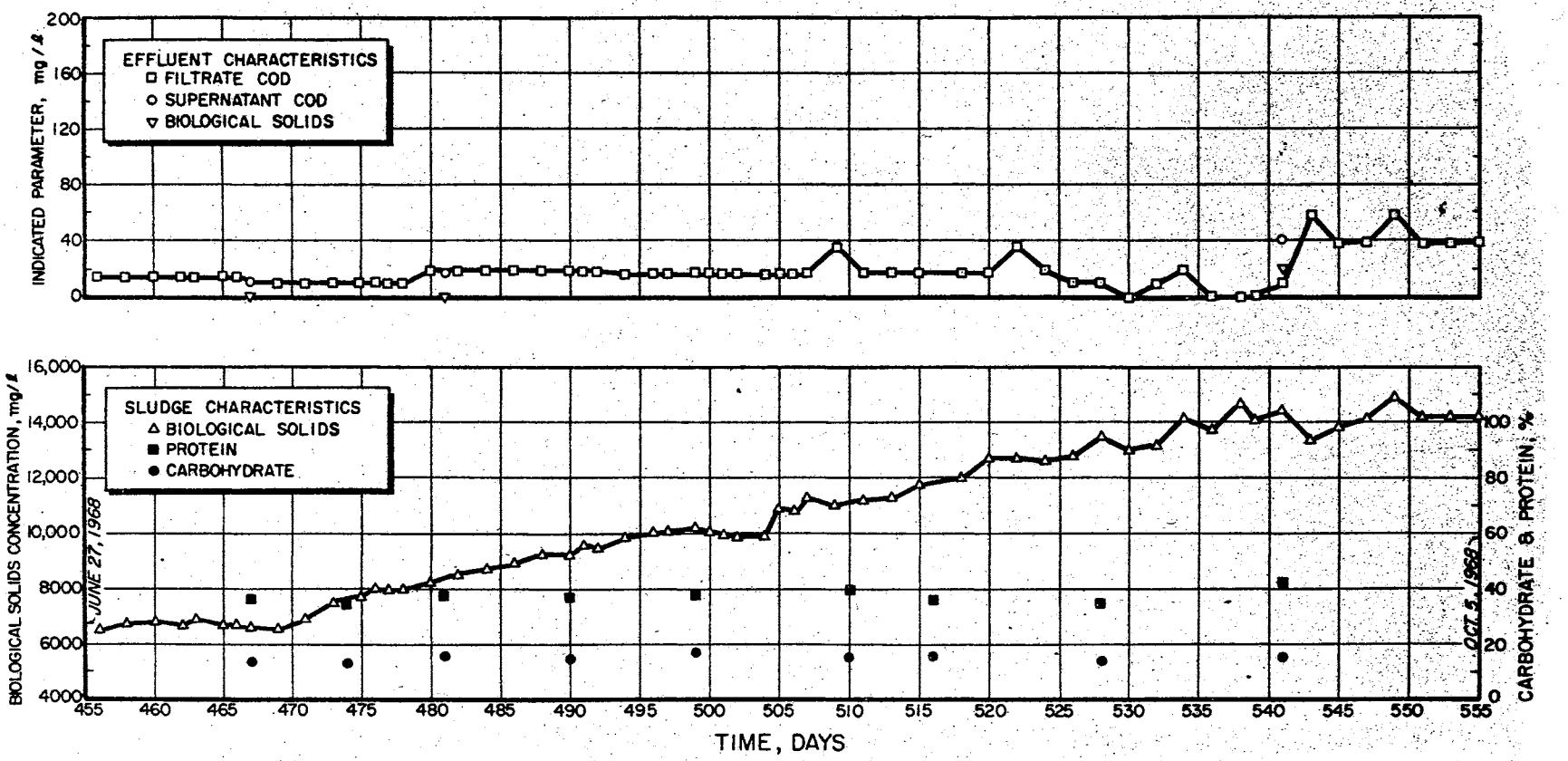

EFFLUENT CHARACTERISTICS
FILTRATE COD
SUPERNATANT COD
BIOLOGICAL SOLIDS
INDICATED PARAMETER, mg/ℓ
200
160
120
80
40
0
SLUDGE CHARACTERISTICS
BIOLOGICAL SOLIDS
PROTEIN
CARBOHYDRATE
BIOLOGICAL SOLIDS CONCENTRATION, mg/ℓ
16,000
14,000
12,000
10,000
8000
6000
4000
JUNE 27, 1968
OCT. 5, 1968
CARBOHYDRATE & PROTEIN, %
100
80
60
40
20
0
455
460
465
470
475
480
485
490
495
500
505
510
515
520
525
530
535
540
545
550
555
TIME, DAYS

Figure 9. Performance data for continuous flow extended aeration pilot plant, October 5, 1968, to January 13, 1969.

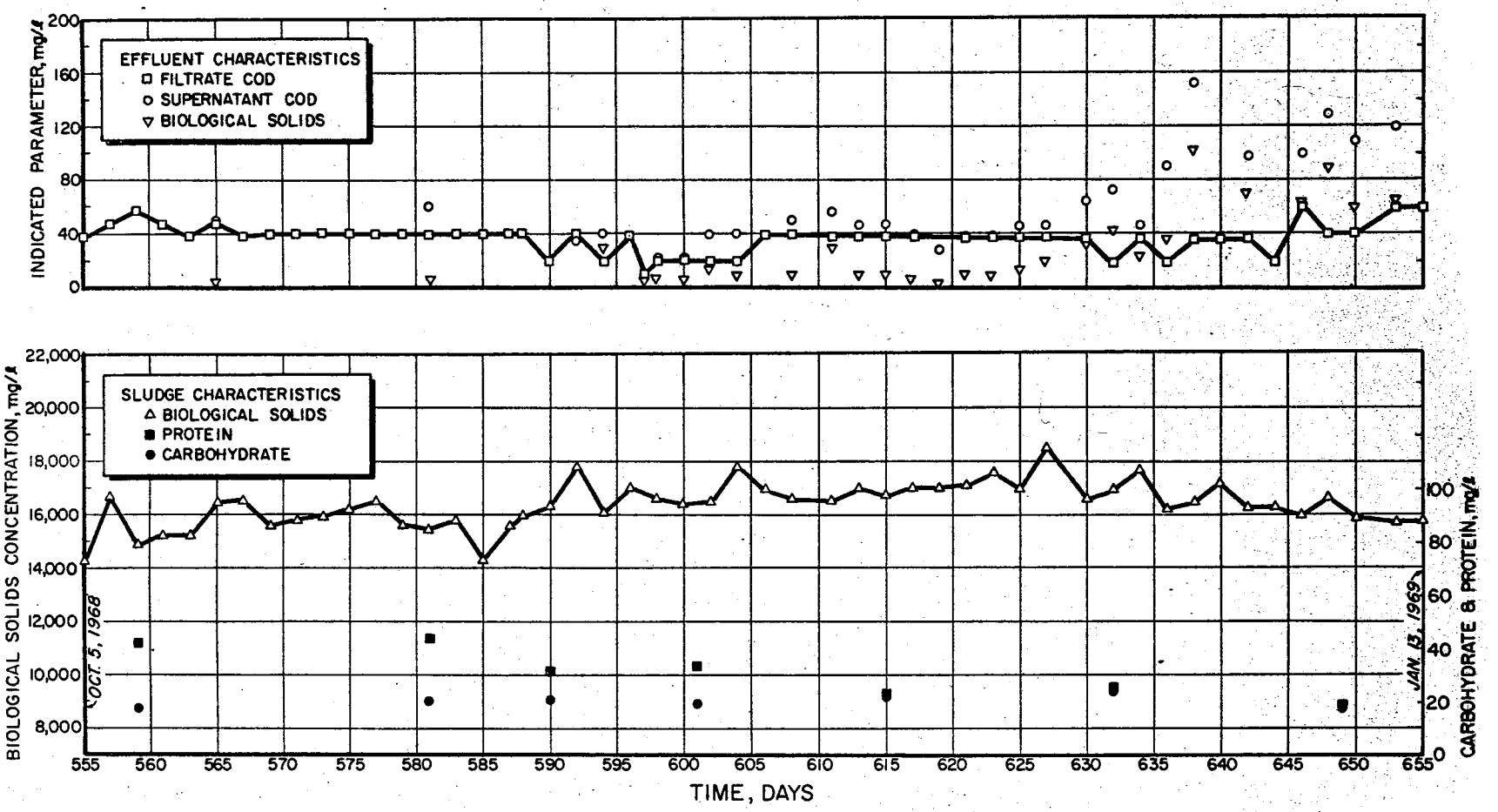

EFFLUENT CHARACTERISTICS
FILTRATE COD
SUPERNATANT COD
BIOLOGICAL SOLIDS
INDICATED PARAMETER, mg/ℓ
SLUDGE CHARACTERISTICS
BIOLOGICAL SOLIDS
PROTEIN
CARBOHYDRATE
BIOLOGICAL SOLIDS CONCENTRATION, mg/ℓ
CARBOHYDRATE & PROTEIN, mg/ℓ
OCT. 5, 1968
JAN. 13, 1969
TIME, DAYS

Figure 10. Performance data for continuous flow extended aeration pilot plant, November 19, 1968, to February 27, 1969.

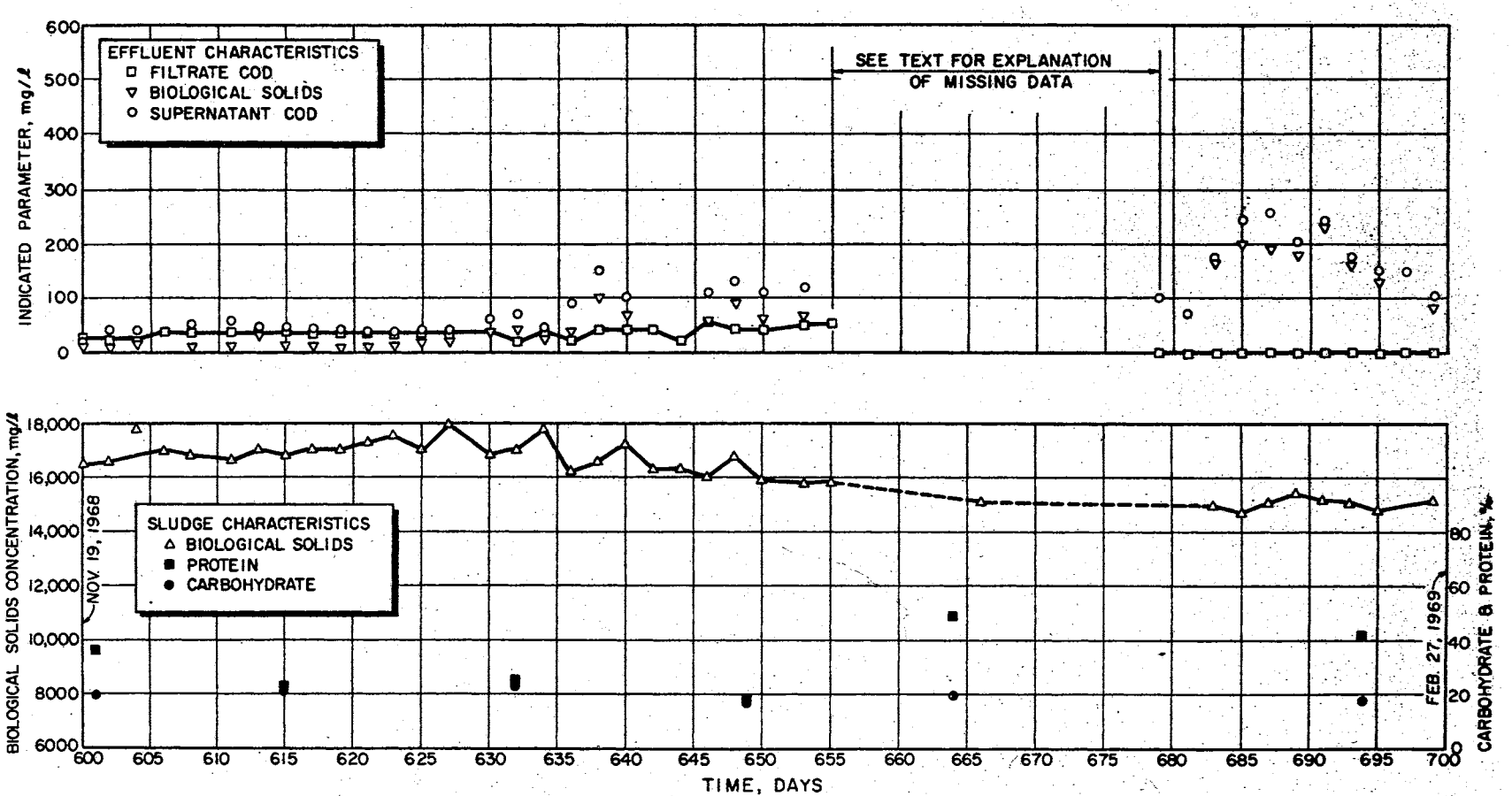

EFFLUENT CHARACTERISTICS
FILTRATE COD
BIOLOGICAL SOLIDS
SUPERNATANT COD
SEE TEXT FOR EXPLANATION OF MISSING DATA
INDICATED PARAMETER, mg/ℓ
SLUDGE CHARACTERISTICS
BIOLOGICAL SOLIDS
PROTEIN
CARBOHYDRATE
NOV. 19, 1968
FEB. 27, 1969
BIOLOGICAL SOLIDS CONCENTRATION, mg/ℓ
CARBOHYDRATE & PROTEIN, %
TIME, DAYS

Figure 11. Performance data for continuous flow extended aeration pilot plant, February 27, 1969, to June 7, 1969.

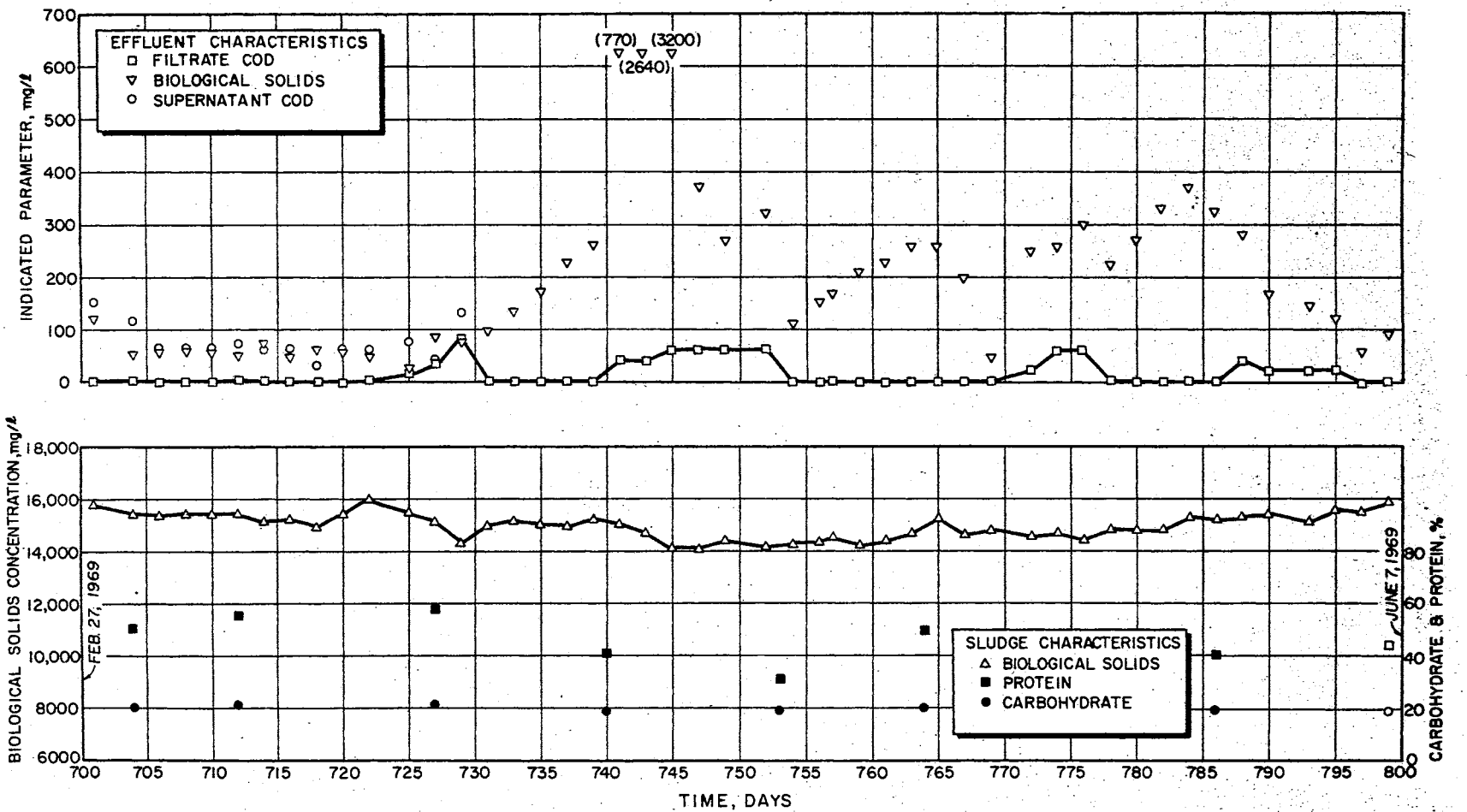

EFFLUENT CHARACTERISTICS
FILTRATE COD
BIOLOGICAL SOLIDS
SUPERNATANT COD
(770) (3200)
(2640)
INDICATED PARAMETER, mg/ℓ
700
600
500
400
300
200
100
0
BIOLOGICAL SOLIDS CONCENTRATION, mg/ℓ
18,000
16,000
14,000
12,000
10,000
8000
6000
FEB. 27, 1969
JUNE 7, 1969
SLUDGE CHARACTERISTICS
BIOLOGICAL SOLIDS
PROTEIN
CARBOHYDRATE
CARBOHYDRATE & PROTEIN, %
80
60
40
20
0
700 705 710 715 720 725 730 735 740 745 750 755 760 765 770 775 780 785 790 795 800
TIME, DAYS

Figure 12. Performance data for continuous flow extended aeration pilot plant, June 7, 1969, to September 15, 1969.

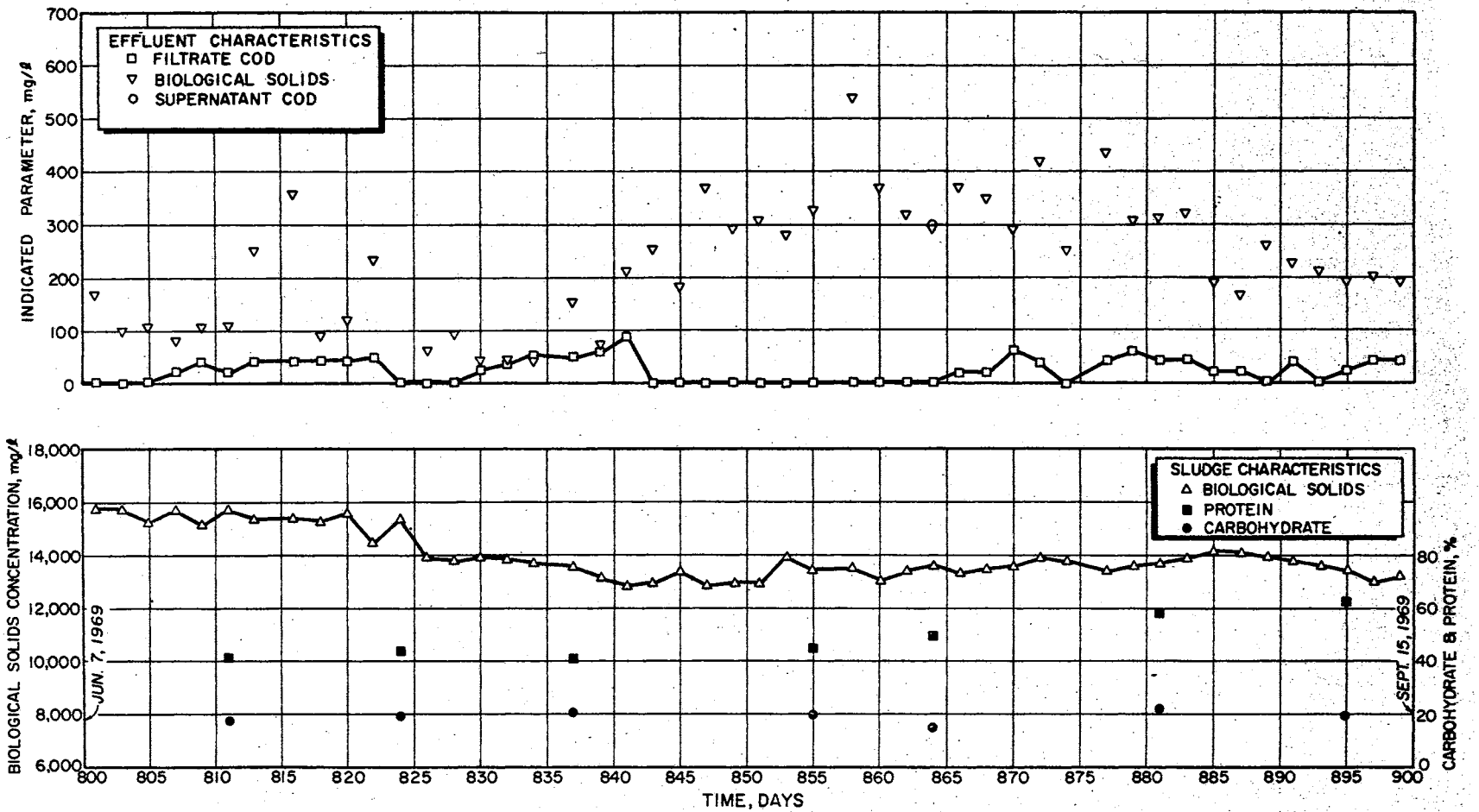

EFFLUENT CHARACTERISTICS
FILTRATE COD
BIOLOGICAL SOLIDS
SUPERNATANT COD
INDICATED PARAMETER, mg/ℓ
700
600
500
400
300
200
100
0
SLUDGE CHARACTERISTICS
BIOLOGICAL SOLIDS
PROTEIN
CARBOHYDRATE
BIOLOGICAL SOLIDS CONCENTRATION, mg/ℓ
18,000
16,000
14,000
12,000
10,000
8,000
6,000
CARBOHYDRATE & PROTEIN, %
80
60
40
20
0
JUN. 7, 1969
SEPT. 15, 1969
800
805
810
815
820
825
830
835
840
845
850
855
860
865
870
875
880
885
890
895
900
TIME, DAYS

Figure 13. Performance data for continuous flow extended aeration pilot plant, September 15, 1969, to December 24, 1969.

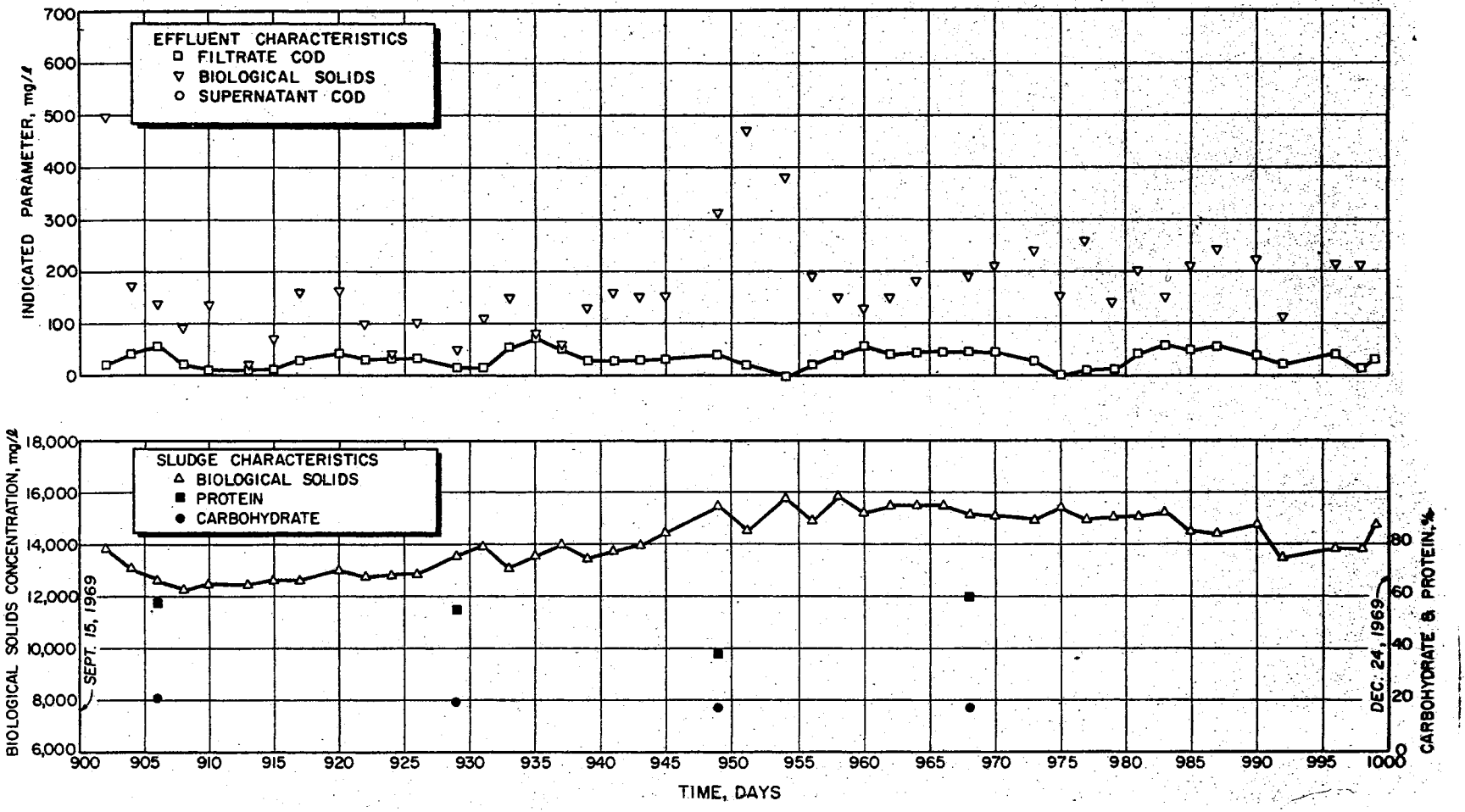

EFFLUENT CHARACTERISTICS
FILTRATE COD
BIOLOGICAL SOLIDS
SUPERNATANT COD
INDICATED PARAMETER, mg/ℓ
700
600
500
400
300
200
100
0
SLUDGE CHARACTERISTICS
BIOLOGICAL SOLIDS
PROTEIN
CARBOHYDRATE
BIOLOGICAL SOLIDS CONCENTRATION, mg/ℓ
18,000
16,000
14,000
12,000
10,000
8,000
6,000
SEPT. 15, 1969
DEC. 24, 1969
CARBOHYDRATE & PROTEIN, %
80
60
40
20
0
900
905
910
915
920
925
930
935
940
945
950
955
960
965
970
975
980
985
990
995
1000
TIME, DAYS

Figure 14. Performance data for continuous flow extended aeration pilot plant, December 24, 1969, to April 3, 1970.

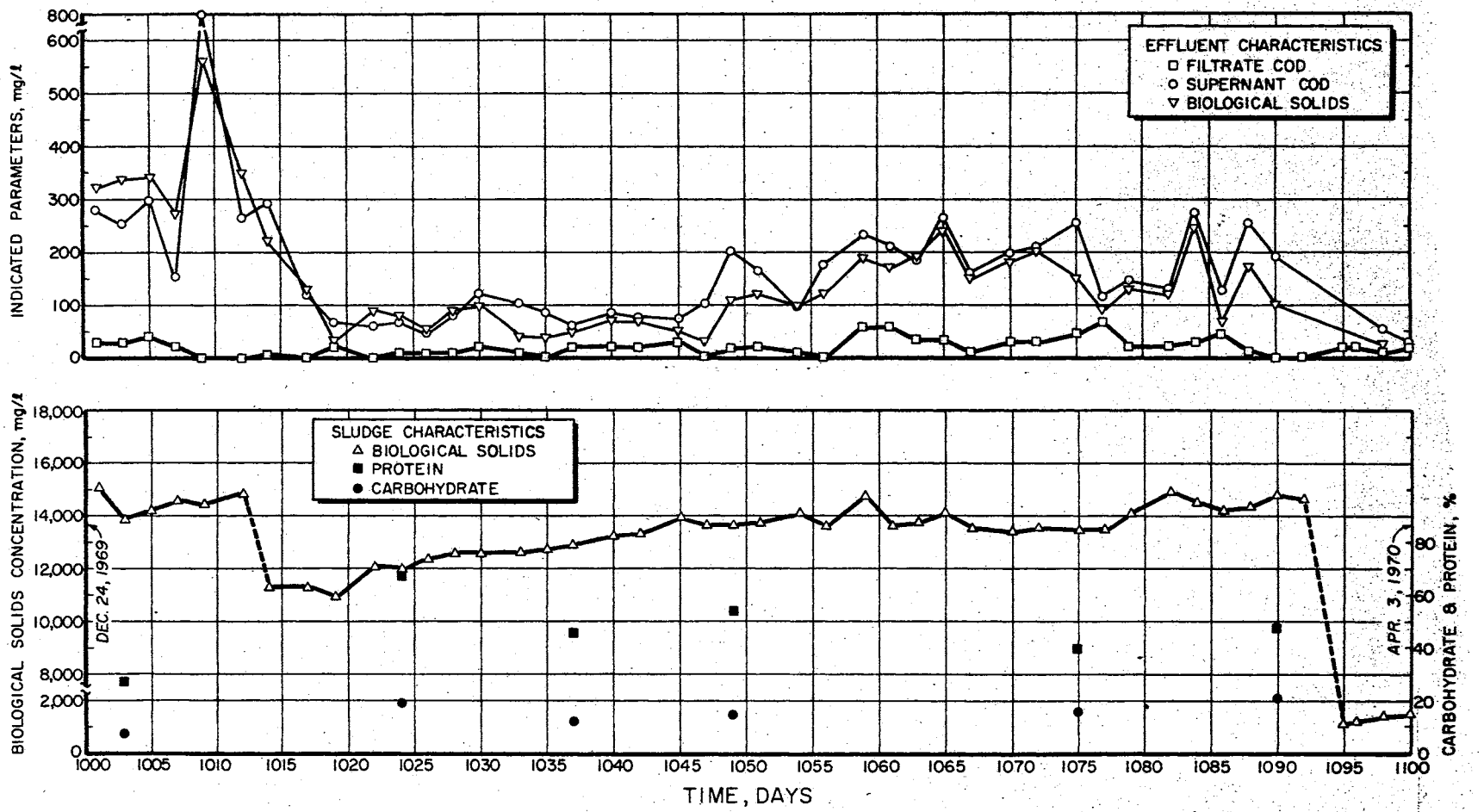
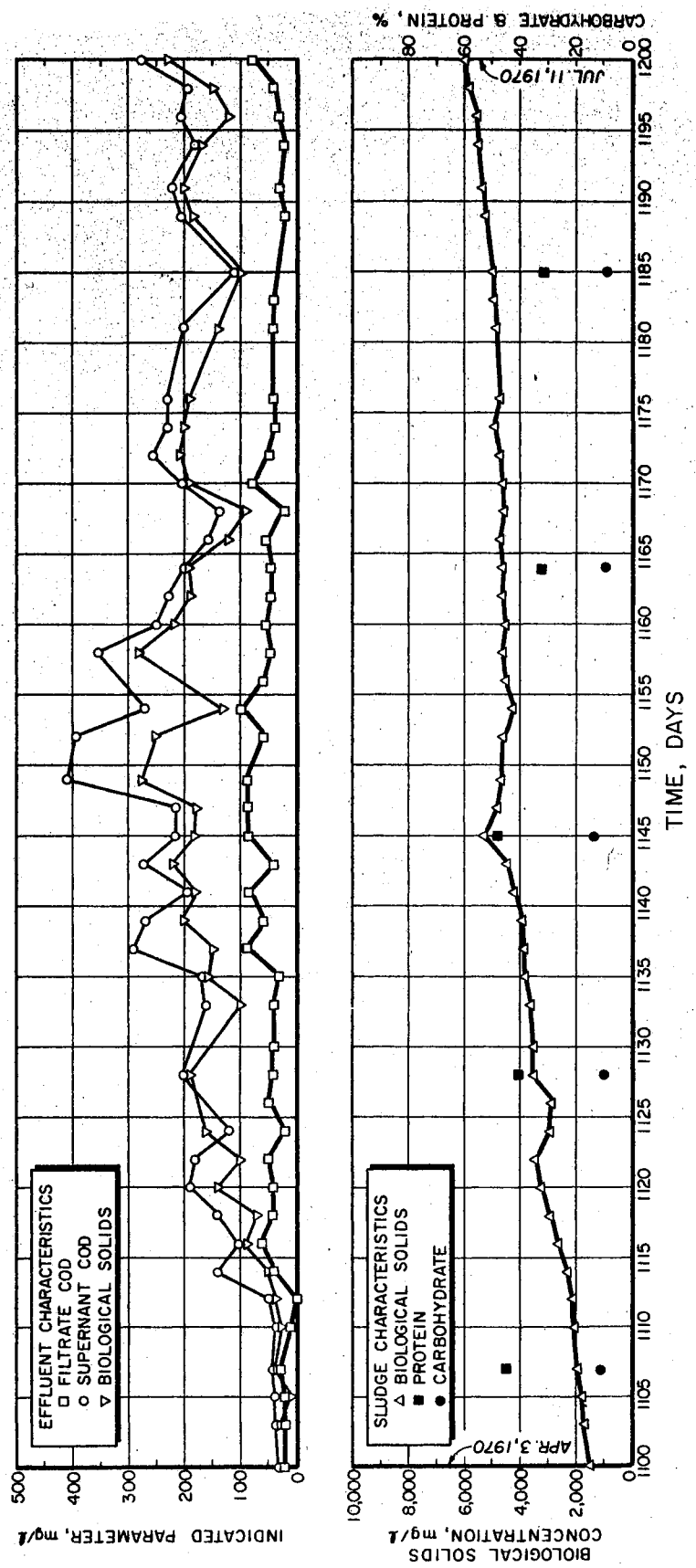

EFFLUENT CHARACTERISTICS
FILTRATE COD
SUPERNANT COD
BIOLOGICAL SOLIDS
INDICATED PARAMETERS, mg/ℓ
SLUDGE CHARACTERISTICS
BIOLOGICAL SOLIDS
PROTEIN
CARBOHYDRATE
BIOLOGICAL SOLIDS CONCENTRATION, mg/ℓ
CARBOHYDRATE & PROTEIN, %
DEC. 24, 1969
APR. 3, 1970
TIME, DAYS

Figure 15. Performance data for continuous flow extended aeration pilot plant, April 3, 1970, to July 11, 1970.


EFFLUENT CHARACTERISTICS
□ FILTRATE COD
○ SUPERNANT COD
▽ BIOLOGICAL SOLIDS
INDICATED PARAMETER, mg/ℓ
SLUDGE CHARACTERISTICS
△ BIOLOGICAL SOLIDS
■ PROTEIN
● CARBOHYDRATE
APR. 3, 1970
JUL. 11, 1970
BIOLOGICAL SOLIDS CONCENTRATION, mg/ℓ
CARBOHYDRATE & PROTEIN, %
TIME, DAYS

1. Sludge Characteristics (Biological Solids Concentration, Carbohydrate, and Protein Content) and Effluent Characteristics

In Figure 14, it is seen that from day 1000 to day 1012, the biochemical efficiency was high (92 percent), but there was a rather large carryover of biological solids in the effluent. As always, these solids were collected in the effluent, centrifuged, and returned to the system. No sludge was wasted until January 7, 1970 (day 1014), when a side joint of the plexiglass reactor cracked, and this structural failure of the activated sludge tank caused a loss of approximately 24 percent of the solids (i.e., solids concentration decreased from 14,800 mg/l to 11,250 mg/l). From day 1014 to day 1092 (Figure 14), the solids concentration in the system fluctuated, but in general experienced a rising trend from 11,000 mg/l to 15,000 mg/l. The biochemical removal efficiency remained above 90 percent. The biological solids concentration in the effluent fluctuated from 20 mg/l to 250 mg/l. In general, if the solids concentration remained between 12,000 and 16,000 mg/l, the sludge level in the settling compartment was usually less than 1/4th inch below the outlet line; thus the supernatant layer was extremely thin, leading to poor mixed liquor separation.

At some time after midnight on the morning of March 26, 1970, the unit was vandalized and the system destroyed by addition of acid. The pH of the material in the unit had been reduced to pH 0.6. Since the synthetic waste contained a considerable amount of phosphate buffer, concentrated acid must have been added. The acid was not HCl, since it was possible to run a COD determination on the tank contents. Violent reaction must have ensued, judging by the amount of cell substance spilled on the floor. Judging by the amount of synthetic waste

remaining in the feed container, the feed pump was shut off at approximately 1:00 A.M. Since the pump was in all probability turned off by the vandal immediately after (or before) the acid was admitted, the approximate time of the incident is fixed. This unnatural, irresponsible act is rather distasteful to report in a scientific research document such as this; however, it is a part of the operational history of this activated sludge and is the reason for the drastic decrease in solids concentration shown in Figure 14. Fortunately, the large amount of experimental data and the previous uninterrupted period of operation were more than ample to permit conclusions relative to the operational stability of the extended aeration process. The only unaffected biological solids were those in the holding tank. These were recovered and used to re-start the unit. Between day 1094 and day 1114 (Figures 14 and 15), the feed concentration of glucose was reduced to 250 mg/l. During this period, the biochemical efficiency remained above 90 percent. The solids concentration in the effluent was below 100 mg/l. On day 1114 the glucose concentration was increased to 500 mg/l. Between day 1114 and day 1200 (Figure 15), the solids concentration gradually increased from 2500 mg/l to 6000 mg/l. The biochemical removal efficiency fluctuated between 80 percent and 90 percent. The biological solids concentration in the effluent fluctuated from 60 mg/l to 280 mg/l. The protein content of the sludge ranged from 30 percent to 45 percent (less than before), and carbohydrate content remained approximately 10 percent.

The unit had been re-started primarily to observe its capacity for recovery and to determine if a decreasing cycle of biological solids concentration would emerge in a relatively short time. There was a

slight dropoff between day 1122 and day 1126. It may have been of interest to operate the unit until a more pronounced downward cycle had re-occurred; however, it was important to pursue experimentation on another mode of operation.

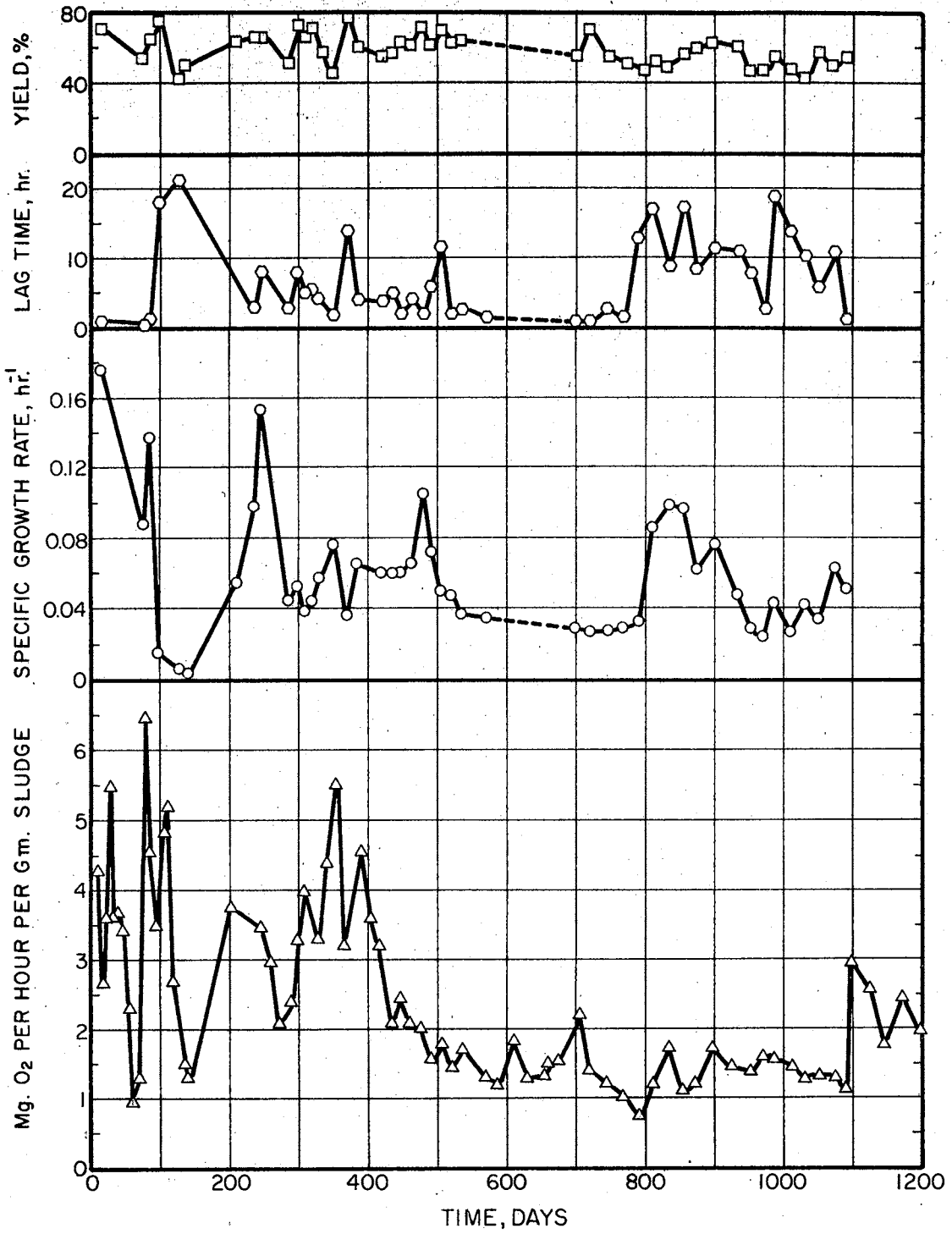
On July 14, 1970 (day 1203), the unit was shut off and another unit was started for the study of the operational feasibility of the extended aeration activated sludge process with incorporation of chemical hydrolysis for control of sludge concentration. Thus ended what is believed to be the longest term experimentation on the extended aeration process ever attempted under controlled laboratory conditions.

2. Characteristics of Extended Aeration Activated Sludge With Respect to Endogenous O_2 Uptake, Substrate Removal Rate (High and Low Initial Sludge Concentration), Specific Growth Rate, Yield, and Lag Time (Low Initial Solids)

In this section, various separate experiments made in order to gain further insight into the metabolic capability of the extended aeration sludge are reported.

At various periods throughout the experiments a small sample of sludge was taken for measurement of its endogenous respiration rate (expressed as mg O_2/hr/gm sludge). The unit O_2 uptake rate of the sludge for approximately 1000 days of operation of extended aeration activated sludge have been previously reported (28)(29). The lower graph in Figure 16 shows the values during the entire 1200 days of operation. After approximately 600 days of operation, it appeared that the endogenous O_2 uptake rate was attaining a lower limit of about 1.0-1.5 mg O_2/hr/gm sludge. This lower value was found to be approximately 5 to 10 percent that of young cells grown from a small inoculum of cells

Figure 16. Effect of aging on cell yield, lag time, specific growth rate, and endogenous O_2 uptake of extended aeration activated sludge.


YIELD, %
80
40
0
LAG TIME, hr.
20
10
0
SPECIFIC GROWTH RATE, hr.$^{-1}$
0.16
0.12
0.08
0.04
0
Mg. O_2 PER HOUR PER Gm. SLUDGE
6
5
4
3
2
1
0
0
200
400
600
800
1000
1200
TIME, DAYS

taken from the aeration chamber. It is noted that, after 1100 days of operation, the unit O_2 uptake showed a sharp increase to 3 mg O_2/hr/gm sludge. The rise corresponds to the time the unit was re-started (see Figures 14 and 15).

At various times during the study, the specific growth rate (hr^{-1}), lag time (hr), and yield (percent) were calculated from the results of separate batch experiments using seed from the unit, and these are plotted in Figure 16 for comparison with the unit O_2 uptake rate. In general, it is noted that there is a correlation between O_2 uptake rate and specific growth rate. The average value of cell yield calculated from the 46 "low initial solids" batch experiments is 57.4 percent. The lowest yield value was 42 percent; the highest, 78.5 percent. There were marked differences in the macroscopic and microscopic appearances of the microbial population when these extreme values were obtained, indicating rather drastic changes in predominance. The standard deviation(s), the coefficient of variance (CV), and the 95 percent confidence limits of the mean (CL)(46) were: s = 8.8, CV = 15.3, CL = 57.4 ± 2.6.

Direct assessment of the metabolic capability (i.e., substrate removal) of the extended aeration sludge, as its age increased, was obtained by conducting batch experiments with both low and high initial solids concentrations. In these experiments, the course of biological solids accumulation and COD removal were assessed. In some of these experiments, removal of glucose was also measured by using the anthrone test. In calculating the specific substrate utilization rate (SSUR) for the low initial solids experiments, the lag time was subtracted. The SSUR of both low and high initial solids batch experiments was calculated as follows:

$$SSUR = \frac{COD_i - COD_f}{(T - T_l) \cdot \frac{(X_i + X_f)}{2}}$$

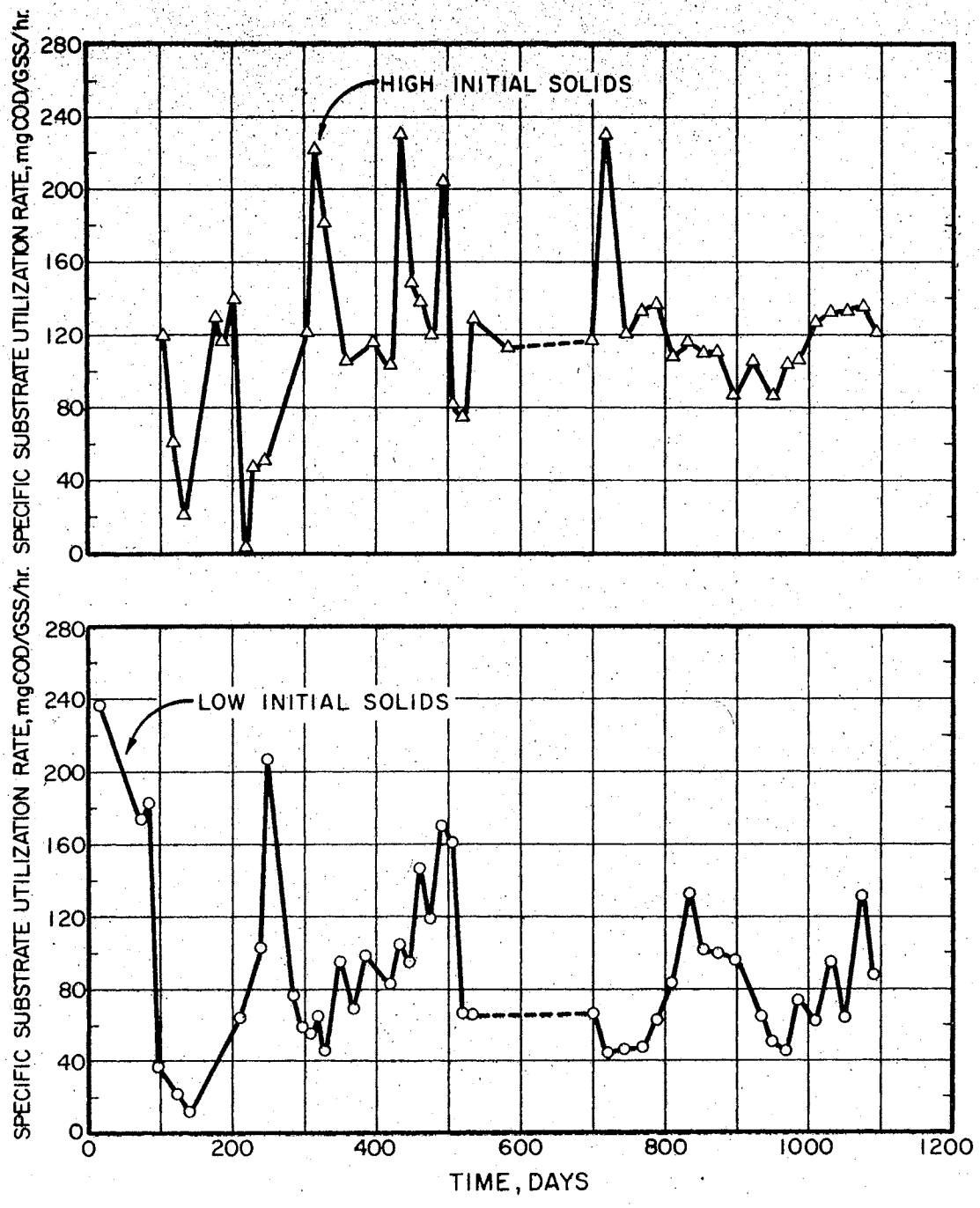
where COD_i = initial COD, mg/l; COD_f = final COD, mg/l; T = time for COD removal, hr; T_l = lag time, hr; X_i = initial solids concentration, g/l; and X_f = final solids concentration, g/l.

In Figure 17, the specific substrate utilization rates (expressed as mg COD removed/hr/gm sludge) for both the low and the high initial solids batch experiments are compared. After 720 days of operation, the values for substrate utilization rate at high initial solids concentration levelled off in the range of 88-136 mg COD/gm sludge/hr, whereas for the low initial solids concentrations, a range of 45-135 mg COD/gm sludge/hr was obtained.

In the interest of brevity, the primary data from which the SSUR, cell yield, lag time, and specific growth rates were calculated, are not shown in this chapter; however, the course of substrate removal and biological solids accumulation for the batch experiments at both high and low initial biological solids concentration after day 700 are presented in Appendix A. Results for such batch experiments before day 700 were previously reported (44). Since the significance of the numerical calculation of the specific substrate utilization rate may be subject to debate for the lower solids system, the primary data are included in the appendix in order to permit the reader to examine the actual experimental results rather than to assess only the calculated parameter. The figures in Appendix A can be used in conjunction with Figures 11 through 15, as well as with Figures 16 and 17.

It can be seen in all of the high initial solids batch experiments (the results of eight representative experiments are shown in Figures

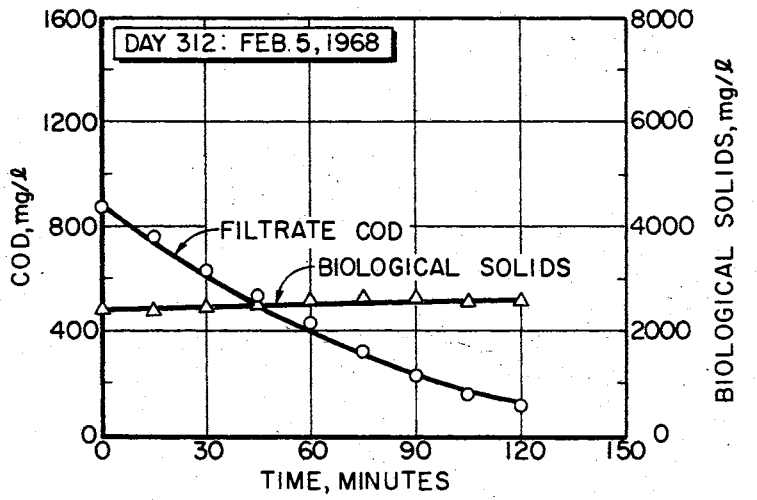
Figure 17. Effect of aging on specific substrate utilization rate of extended aeration activated sludge.

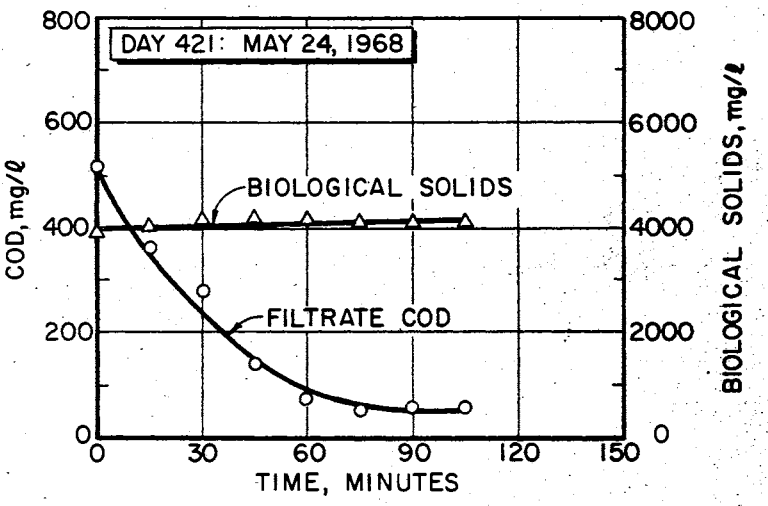

HIGH INITIAL SOLIDS
LOW INITIAL SOLIDS
SPECIFIC SUBSTRATE UTILIZATION RATE, mgCOD/GSS/hr.
SPECIFIC SUBSTRATE UTILIZATION RATE, mgCOD/GSS/hr.
TIME, DAYS
0
40
80
120
160
200
240
280
0
200
400
600
800
1000
1200

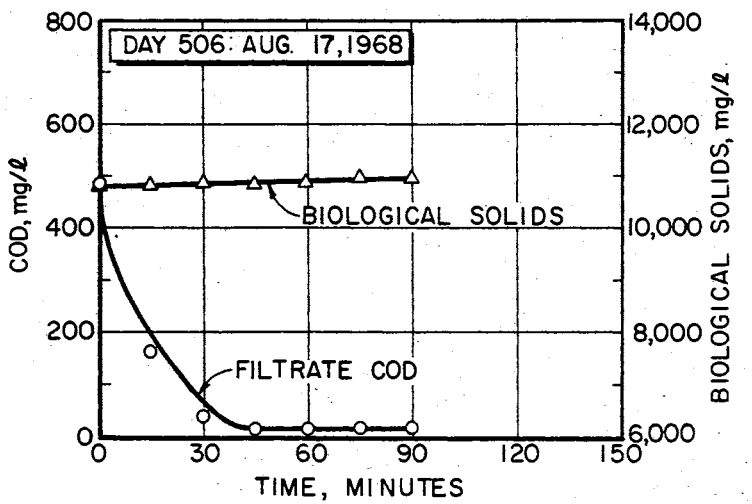
18 and 19), that the feed COD was removed rather rapidly--within approximately 30 minutes--except for the experiments of days 312 and 421. Thus, the detention time in the unit was much in excess of that absolutely required; during continuous flow operation, the detention time in the aeration chamber was 16 hours. It is noted that with few exceptions (day 312, Figure 18 was one), the amount of glucose added was calculated to give an initial filtrate COD of 530 mg/l at zero time. The difference between this amount and the measured initial filtrate COD (sample taken immediately after mixing and plotted in the graphs at zero time) represents the amount of substrate removed by the cells in the time required to mix the substrate and obtain the filtered sample, i.e., about three minutes. Since the overall substrate removal rate was so rapid, the actual initial filtrate COD could not be measured. The COD removal rate per unit weight of sludge ranged from 88 to 230 mg COD/hr/gm solids. Rates ranging from approximately 300 to 700 mg COD/hr/gm solids can be calculated from data for more "normal" activated sludge systems, i.e., ones in which sludge wasting is practiced (47)(48)(49). Thus the substrate removal rate as well as the endogenous O_2 uptake rate is lower for sludge developed under conditions of total solids retention (extended aeration) than for systems in which sludge is routinely wasted.

The low initial solids batch experiments indicate that the cells do not go into a log phase rapidly, and that the population is a rather slow-growing one. From Figure 16 it is seen that the specific growth rate ranged from 0.029 to 0.092 hr^{-1}. A small amount of sludge was taken from the extended aeration unit and cultivated in nutrient broth for about 24 hours. A small portion of this young population was taken

Figure 18. COD removal capability of extended aeration activated sludge on the day indicated.


DAY 312: FEB. 5, 1968
FILTRATE COD
BIOLOGICAL SOLIDS
COD, mg/ℓ
BIOLOGICAL SOLIDS, mg/ℓ
TIME, MINUTES
0
400
800
1200
1600
2000
4000
6000
8000
30
60
90
120
150


DAY 421: MAY 24, 1968
BIOLOGICAL SOLIDS
FILTRATE COD
COD, mg/ℓ
BIOLOGICAL SOLIDS, mg/ℓ
TIME, MINUTES
0
200
400
600
800
2000
4000
6000
8000
30
60
90
120
150


DAY 506: AUG. 17, 1968
BIOLOGICAL SOLIDS
FILTRATE COD
COD, mg/ℓ
BIOLOGICAL SOLIDS, mg/ℓ
TIME, MINUTES
0
200
400
600
800
6,000
8,000
10,000
12,000
14,000
30
60
90
120
150

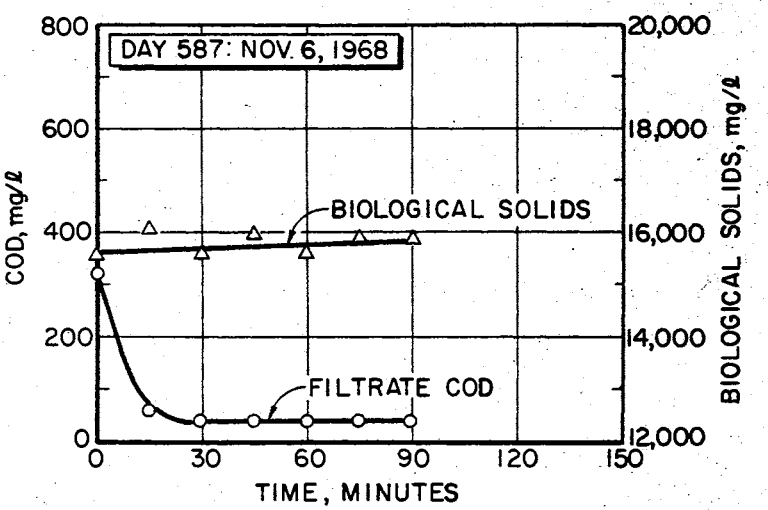
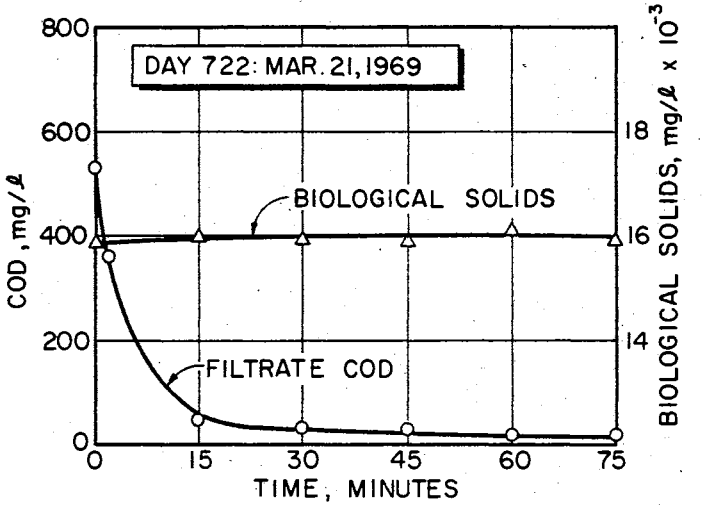
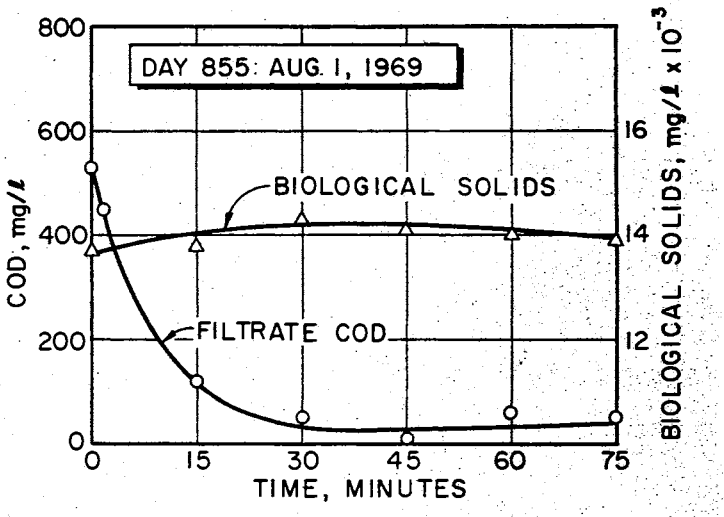
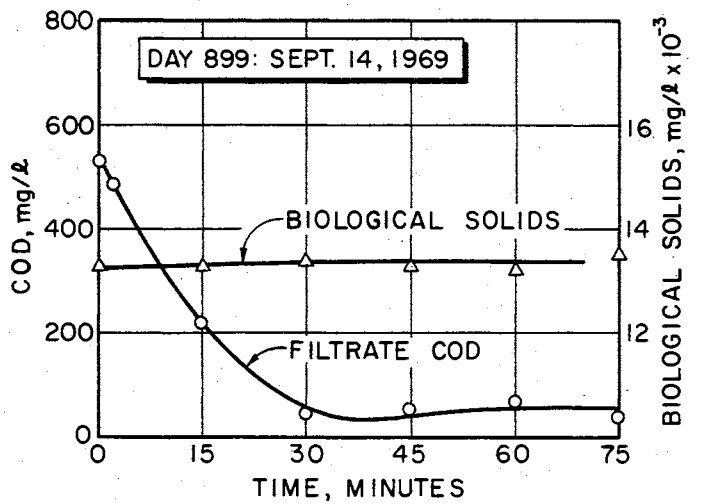
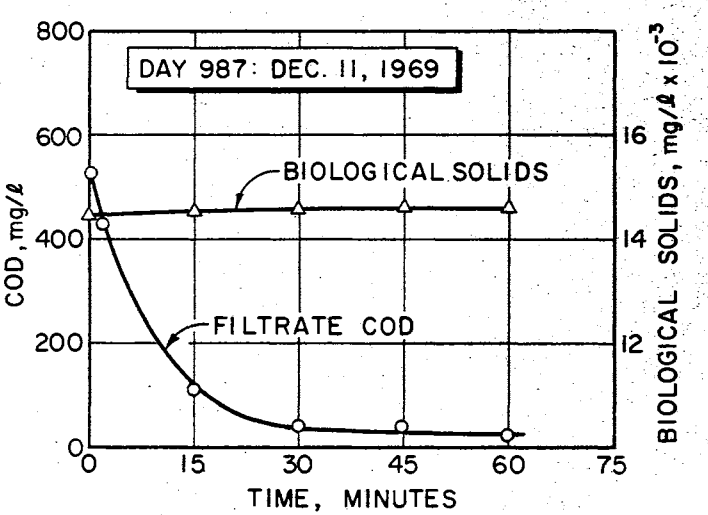

DAY 587: NOV. 6, 1968
BIOLOGICAL SOLIDS
FILTRATE COD
COD, mg/ℓ
BIOLOGICAL SOLIDS, mg/ℓ
TIME, MINUTES
0
200
400
600
800
12,000
14,000
16,000
18,000
20,000
30
60
90
120
150

Figure 19. COD removal capability of extended aeration activated sludge on the day indicated.


DAY 722: MAR. 21, 1969
BIOLOGICAL SOLIDS
FILTRATE COD
COD, mg/ℓ
BIOLOGICAL SOLIDS, mg/ℓ x 10⁻³
TIME, MINUTES
0
200
400
600
800
0
15
30
45
60
75
14
16
18


DAY 855: AUG. 1, 1969
BIOLOGICAL SOLIDS
FILTRATE COD
COD, mg/ℓ
BIOLOGICAL SOLIDS, mg/ℓ x 10⁻³
TIME, MINUTES
0
200
400
600
800
0
15
30
45
60
75
12
14
16


DAY 899: SEPT. 14, 1969
BIOLOGICAL SOLIDS
FILTRATE COD
COD, mg/ℓ
BIOLOGICAL SOLIDS, mg/ℓ x 10⁻³
TIME, MINUTES
0
200
400
600
800
0
15
30
45
60
75
12
14
16


DAY 987: DEC. 11, 1969
BIOLOGICAL SOLIDS
FILTRATE COD
COD, mg/ℓ
BIOLOGICAL SOLIDS, mg/ℓ x 10⁻³
TIME, MINUTES
0
200
400
600
800
0
15
30
45
60
75
12
14
16

from the nutrient broth, grown in the usual glucose minimal medium (1000 mg/l of glucose) through four transfers, and finally was inoculated into 500 mg/l of glucose minimal medium to measure the specific growth rate. The specific growth rate of this young acclimated sludge was 0.33 hr^{-1}. Thus, the specific growth rate of the extended aeration sludge was approximately 10 to 30 percent of that of young cells grown from a small inoculum of cells taken from the aeration chamber. For many of the experiments at low initial solids concentration, the course of substrate removal was determined by the anthrone test as well as COD analyses (see Appendix A). In general, both analyses give similar values; thus, for these slower growing cells there was no evidence for the accumulation of metabolic intermediates or end products.

3. Characteristics of the Effluent (Filtrate) From the Extended Aeration Activated Sludge Process With Regard to PO_4-P, NH_3-N, NO_3-N, and NO_2-N

The increase in algal activity in receiving water caused by inorganic nutrients in waste water effluents (phosphorus and/or nitrogen) has led to consideration of the need for treatment processes to remove these constituents. The removal of phosphate and nitrogen by extended aeration plants or, on the other hand, the discharge of these materials by such plants, is of increasing interest. It was therefore appropriate to gain some insight into this aspect. Such analyses were not run routinely, but between days 351 and 1202 (see Table III) 64 samples were taken for these determinations. The amount of phosphate in the influent after 740 days was increased in order to counteract the tendency for the pH to drop.

TABLE III

COD, INORGANIC PHOSPHORUS, AND NITROGEN CONTENT IN THE INFLUENT AND FILTERED (Phase A) EFFLUENT, mg/l

		Influent, (mg/l)			Effluent (Filtrate), (mg/l)						COD, P & N removed by the sludge, (mg/l)			% P & N in the Effluent		COD/P & COD/N removed by the sludge, (mg/l)	
Date	Age of Sludge (Days)	COD	PO_4-P	NH_3-N	COD	PO_4-P	NH_3-N	NO_3-N	NO_2-N	Approx. Total Inorg. N.	COD	PO_4-P	Inorg. N.	PO_4-P	Inorg. N.	$\frac{COD}{P}$	$\frac{COD}{N}$
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1968																	
3-15	351	530	155.2	53	20	125	25	10	<0.1	35.0	510	30.2	18.0	80.5	66.0	16.8	28.3
3-22	358	"	"	"	38	130	31.5	7.5	"	39.0	492	25.2	14.0	83.7	73.5	19.5	35.1
4- 4	371	"	"	"	25	129	26	9.5	"	35.5	505	26.2	17.5	83.1	66.9	19.2	28.8
4-10	377	"	"	"	20	138	25.5	6.3	"	31.8	510	17.2	21.2	88.9	60.0	29.6	24.0
4-20	387	"	"	"	20	127	23	6.3	"	29.3	510	28.2	23.7	81.8	55.2	18.0	21.5
4-29	396	"	"	"	20	184	26	4.3	"	30.3	510	-28.8	22.7	118.5	57.1	-	22.4
5- 8	405	"	"	"	20	180	26	2.8	"	28.8	510	-24.8	24.2	115.9	54.3	-	21.0
5-14	411	"	"	"	20	145	22	3.2	"	25.2	510	10.2	27.8	93.4	47.5	50	18.3
5-23	420	"	"	"	20	145	26	3.2	"	29.2	510	10.2	23.8	93.4	55.0	50	21.4
6- 3	431	"	"	"	20	145	30.5	3.2	"	33.7	510	10.2	19.3	93.4	63.5	50	26.4
6-13	441	"	"	"	20	153	27.5	3.2	"	30.7	510	2.2	22.3	98.5	57.9	231.8	22.8
6-21	449	"	"	"	20	-	25	3.2	"	28.2	510	-	24.8	-	53.2	-	20.5
6-29	457	"	"	"	18	124	25	3.2	"	28.2	512	31.2	24.8	79.8	53.2	16.4	20.6
7-12	470	"	"	"	10	176	25	3.2	"	28.2	520	-20.8	24.8	113.4	53.2	-	20.9
7-19	477	"	"	"	10	166	49	2.1	"	51.1	520	10.8	1.9	106.9	96.4	48.1	273.6
7-26	484	"	"	"	20	137	25.5	3.2	"	28.7	510	18.2	24.3	88.2	54.1	28	20.9
8- 4	493	"	"	"	20	129	25.5	3.1	"	28.6	510	26.2	24.4	83.1	53.9	19.4	20.9
8-13	502	"	"	"	20	142	25.5	3.2	"	28.7	510	13.2	24.3	91.4	54.1	24.3	20.9
8-24	513	"	"	"	20	150	25.6	2.2	"	27.8	510	5.2	25.2	96.6	52.4	98.0	20.2
9- 1	521	"	"	"	20	153	25	3.2	"	28.2	510	2.2	24.8	98.5	53.2	231.8	20.5
9-11	531	"	"	"	10	153	26.2	3.7	"	29.9	520	2.2	23.1	98.5	56.4	236.3	22.5
9-24	544	"	"	"	40	260	37	8	"	45.0	490	-104.8	8.0	167.5	84.9		61.2
10- 9	559	"	"	"	60	130	28.8	1.9	"	51.2	470	25.2	1.8	83.7	96.6	18.6	261.1
10-12	562	"	"	"	40	153	28.2	23	"	51.2	490	2.2	1.8	98.5	96.6	222.7	272.2
11- 3	584	"	"	"	40	260	33	3.4	"	36.4	490	-104.8	16.6	167.5	68.6	-	29.5
11-12	593	"	"	"	40	222	41	1.4	"	42.4	490	-66.8	10.6	143.0	80.0	-	46.2
11-23	604	"	"	"	20	195	40	2.5	0	42.5	510	-39.8	10.5	125.6	80.1	-	48.5
12- 7	618	"	"	"	40	202	36	3.4	0.12	39.4	490	-46.8	13.6	130.1	74.3	-	36.0
12-24	635	"	"	"	30	170	42.8	8.3	0	51.1	500	-14.8	1.9	109.5	96.4	-	263.1

TABLE III (Continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1969																	
1-10	652	"	"	"	45	214	40	7.2	0.04	47.2	485	-58.8	5.8	137.8	89.0	-	83.6
1-22	664	"	"	"	-	90	16.5	4	0.04	20.5	-	65.2	32.5	57.9	38.6	-	-
2-21	694	"	"	"	0	189	35	10.9	0.08	45.98	530	-33.8	7.1	121.7	86.7	-	74.6
3- 3	704	"	"	"	0	206	25.4	19.8	0.04	45.24	530	-50.8	7.8	132.7	85.3	-	67.9
3-11	712	"	"	"	0	176	26.2	19	0.10	45.3	530	-20.8	7.7	113.4	85.4	-	68.8
3-26	727	"	"	"	35	195	38	7.8	0.05	45.85	495	-39.8	7.2	125.6	86.5	-	68.7
4- 8	740	"	186.24	"	40	195	23.6	18.2	0.16	41.96	490	-8.7	11.1	104.7	79.1	-	44.1
4-11	753	"	310.4	"	20	595	2.3	23.3	0.15	25.75	510	-284.5	27.3	191.6	48.5	-	18.6
5- 2	764	"	620.8	"	0	545	3.1	27.3	0.39	30.79	530	75.8	22.3	87.7	58	6.9	23.7
5-24	786	530	931.2	53	0	990	2.3	24	0.36	26.66	530	-58.8	26.4	106.3	50.3	-	20.0
6- 6	799	"	"	"	0	930	1.97	20.6	0.88	23.45	530	1.2	29.6	99.8	44.2	441.6	17.9
6-18	811	"	"	"	20	870	3.95	29.3	0.32	33.57	510	61.2	19.5	93.4	63.3	8.3	26.1
7- 1	824	"	"	"	0	685	1.97	28.6	0.32	30.89	530	246.2	22.2	73.5	58.2	2.1	23.8
7-14	837	"	"	"	50	805	1.32	28.6	0.12	30.04	480	126.2	23.0	86.4	56.6	3.8	20.8
8- 1	855	"	"	"	0	990	0.66	25.0	0.12	25.78	530	-58.8	27.3	106.3	48.6	-	19.4
8-10	864	"	"	"	0	805	0.66	24.6	0.10	25.36	530	126.2	27.7	86.4	47.8	4.1	19.1
8-27	881	"	"	"	45	805	1.65	37	0.06	38.71	485	126.2	14.3	86.4	73.0	3.8	33.9
9-10	895	"	"	"	25	728	1.65	37.5	0.05	39.2	505	203.2	13.8	78.1	73.9	2.4	36.5
9-21	906	"	"	"	50	555	1.65	47.5	0.06	49.21	480	376.2	3.8	59.6	92.8	1.2	126.3
10-14	929	"	"	"	15	965	2.96	34.5	3.96	41.42	515	-33.8	11.6	103.6	78.1	-	44.3
11- 3	949	"	"	"	40	805	1.81	28	1.1	30.91	490	126.2	22.1	86.4	58.3	3.8	22.1
11-22	968	"	"	"	50	823	1.32	43.5	3.96	48.78	480	108.2	4.3	88.3	92.0	4.4	111.6
12- 5	981	"	"	"	40	475	1.65	41	0.51	43.16	490	456.2	9.9	51.0	81.4	1.0	49.4
12-27	1003	"	"	"	30	465	1.65	32.5	0.01	34.16	500	466.2	18.9	49.9	64.4	1.0	26.4
1970																	
1-17	1024	"	"	"	10	435	0.495	36	1.72	38.215	520	496.2	14.8	46.7	72.1	1.0	35.1
1-30	1037	"	"	"	20	780	1.65	27.5	0.77	29.92	510	151.2	23.1	83.7	56.4	3.3	22.0
2-11	1049	"	"	"	20	496	0.41	34.5	1.57	36.48	510	435.2	16.6	53.2	68.8	1.1	30.7
3- 9	1075	"	"	"	48	403	0.495	31	2.11	33.605	482	528.2	19.4	43.2	63.4	0.9	24.8
3-24	1090	"	"	"	0	560	0.495	22.5	2.4	25.395	530	371.2	27.7	60.1	47.9	1.4	19.1
4-10	1107	265	465.6	"	28	270	0.0825	21	0.48	21.562	237	195.6	31.5	57.9	40.6	1.2	7.5
5- 1	1128	530	931.2	"	40	570	0.0825	42.5	0.35	42.932	490	361.2	10.1	61.2	81.0	1.3	48.5
5-18	1145	"	"	"	87	465	0.41	34.5	0.07	34.98	443	466.2	18.1	49.9	66.0	0.9	24.4
6- 6	1164	"	"	"	44	728	0.495	39.5	0.09	40.085	486	203.2	13.0	78.1	75.6	2.3	37.3
6-26	1185	"	"	"	40	775	0.0825	41	2.4	43.482	490	156.2	9.6	83.2	82.0	3.1	51.0
7-13	1202	"	"	"	39	465	0.33	28.5	1.1	29.93	491	466.2	23.7	49.9	56.4	1.0	20.7

In Table III, the influent and effluent concentrations of COD, inorganic phosphorus, and inorganic nitrogen are compared. Inorganic nitrogen in the effluent is recorded as the total of the NH_3-N, NO_3-N, and NO_2-N. The table also shows the concentrations of COD, phosphorus, and nitrogen which were retained in the sludge or in the system at the particular sampling date (columns 12, 13, and 14). Also shown are the percentages of phosphorus and nitrogen which passed out in the effluent. The last two columns of the table show the ratio of the amount of COD removed to phosphorus taken up by the system, and the ratio of COD removed to the amount of nitrogen retained in the system, respectively. It can be seen in column 9 that, as might be expected by the rather lengthy aeration period employed, the system produced a nitrified effluent. It should be recalled that the amount of phosphorus in the system was rather high because phosphorus was used as the buffering system. It is seen that at times a considerable amount of phosphorus was retained in the sludge, and also at times there was considerably more phosphorus in the effluent than in the influent. Thus, the retention and the release of phosphorus appeared to follow a somewhat cyclic process. Taking the average of values in column 15, it can be calculated that 93.8 percent of the influent phosphorus appeared in the effluent. Thus, the amount of phosphorus retained in the system on the average was 6.2 percent. Sekikawa, et al. (50) presented data from which it may be calculated that 10 percent of the phosphorus they added was retained in the sludge. The BOD:phosphorus ratio employed in their studies was approximately 8:1. They also observed that phosphorus uptake or retention of phosphorus in the system appeared to go through several cycles. Throughout the present study, the ratio of COD in the

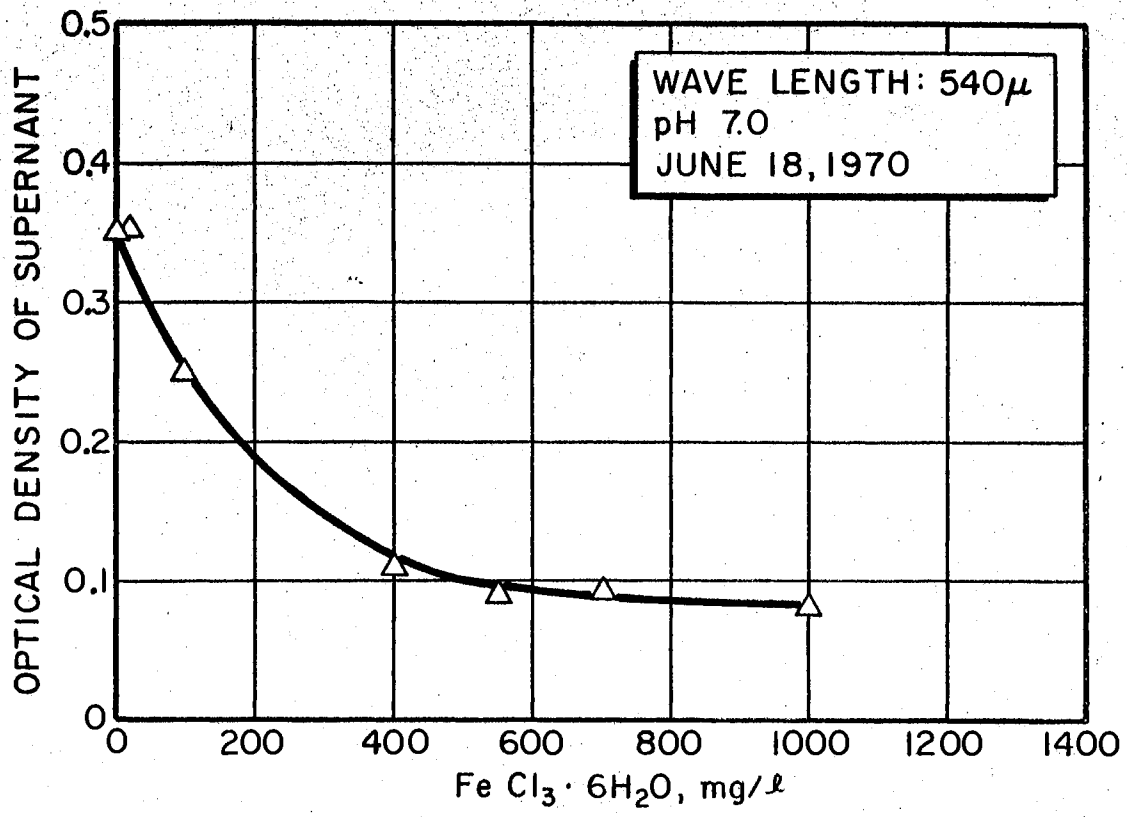
feed was 10. Comparison of influent and effluent concentrations of inorganic nitrogen indicates that at times the nitrogen concentration in the effluent approached that in the feed, indicating that much of the nitrogen contained in the sludge was being recycled or re-used by the biological system. The ratio of COD removed to nitrogen removed is shown in the last column (column 18), and it is seen that this value varied over a considerable range. The average ratio of COD removed to nitrogen utilized was calculated by dividing the sums of columns 12 and 14. This calculation yielded an average ratio of 27.8. This value compares favorably with values reported by Gaudy, et al. for systems not limited by nitrogen concentration (51). It is apparent that the COD: nitrogen concentration in the feed could have been reduced considerably from the 10:1 ratio which was used, since there was a rather high concentration of nitrogen in the effluent. It has been shown by Gaudy, et al. (51) that excellent COD removals can be obtained with COD:N ratios as high as 70:1. Furthermore, Simpson indicated that the nitrogen supplementation for extended aeration activated sludge processes could be reduced to 1/5th of the BOD:nitrogen ratios normally employed in the field (20). Measurement of the total inorganic nitrogen in the effluent (column 11) showed that on the average, 66.6 percent of the nitrogen fed as NH_3-N was discharged in the effluent. It is interesting to note that the NH_3-N concentration in the effluent decreased after day 753, while the NO_3-N concentration increased, indicating the presence of nitrifying bacteria.

4. Chemical Flocculation of the Suspended Biological Solids in the Effluent of the Extended Aeration Activated Sludge Process

The extended aeration activated sludge process could be considered

one that should enhance development of flocculated microbial populations, since it is operated under severe starvation conditions. However, from time to time, due perhaps to changes in predominance of species, to small changes in pH, and to high solids concentrations (about 12,000 -16,000 mg/l) which sometimes accumulated in the system, it was observed that some biological solids were carried over in the effluent. While in the present study these were removed and returned to the aerator, their presence in the effluent would cause a lowering of treatment efficiency in a field installation. The biological flocculation phenomenon in waste treatment processes is one that is difficult to control, since the biological system is a non-homogeneous and mixed population, and at times some type of operational remedial measure may be needed. One possible way to produce a good (low biological solids content) effluent may be by the employment of chemical flocculation, or of flocculation aids (e.g., polyelectrolytes). Bacteria and other microorganisms such as viruses and protozoa can be considered to be hydrophilic biocolloids. They carry a net negative charge within the pH range of interest and exhibit variability regarding surface composition (i.e., proteins, lipids, and polysaccharides, combined in various portions)(52). Experiments were designed to gain some insight into the optimum concentration and kinds of flocculating agents (i.e., multivalent metal ions). Some of the results of these studies are shown in Figures 20 through 22. The chemical coagulating agents examined included $FeCl_3 \cdot 6\ H_2O$, $Fe_2SO_4 \cdot 7\ H_2O$, $Fe_2(SO_4)_3$, $Al_2(SO_4)_3 \cdot 18\ H_2O$, $Ca(OH)_2$, and Purifloc 401 (Dow Chemical Co.). It was noted that with the exception of Purifloc 401 and $Ca(OH)_2$ (data not shown) the other flocculating agents provided greater than 65 percent removal of biological solids. From these

Figure 20. Effect of concentration of indicated coagulant on the flocculation of biological solids retained in the effluent of the extended aeration pilot plant.


WAVE LENGTH: 540μ
pH 7.0
JUNE 18, 1970
OPTICAL DENSITY OF SUPERNANT
0.5
0.4
0.3
0.2
0.1
0
0
200
400
600
800
1000
1200
1400
Fe Cl3 · 6H2O, mg/ℓ

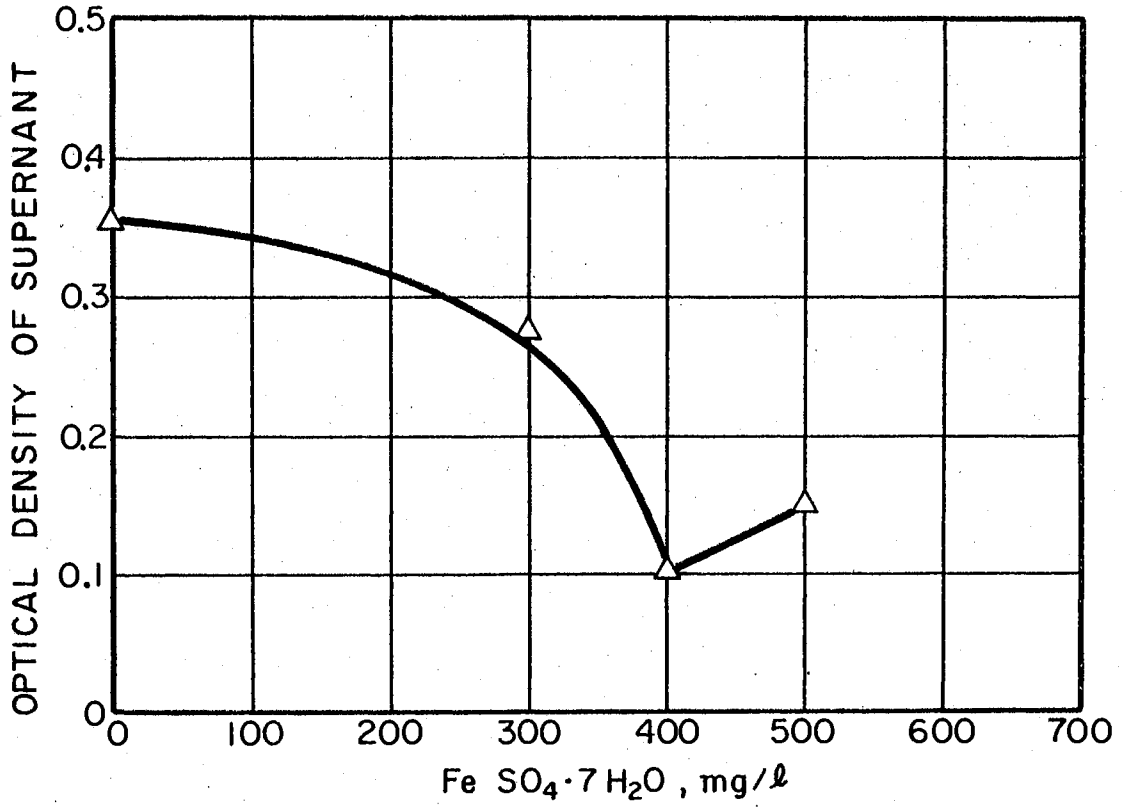
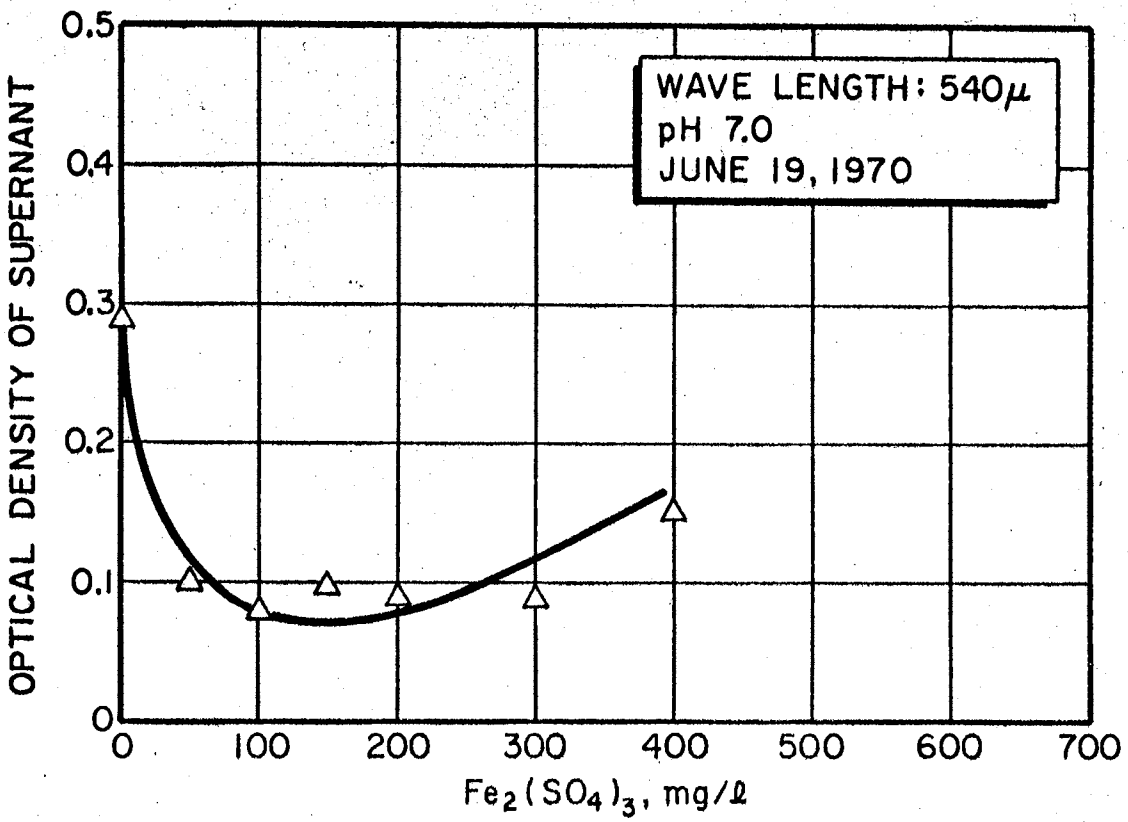

OPTICAL DENSITY OF SUPERNANT
0.5
0.4
0.3
0.2
0.1
0
0
100
200
300
400
500
600
700
Fe SO4 · 7 H2O , mg/ℓ

Figure 21. Effect of concentration of indicated coagulant on the flocculation of biological solids retained in the effluent of the extended aeration pilot plant.


WAVE LENGTH: 540μ
pH 7.0
JUNE 19, 1970
OPTICAL DENSITY OF SUPERNANT
0.5
0.4
0.3
0.2
0.1
0
0
100
200
300
400
500
600
700
Fe2(SO4)3, mg/ℓ

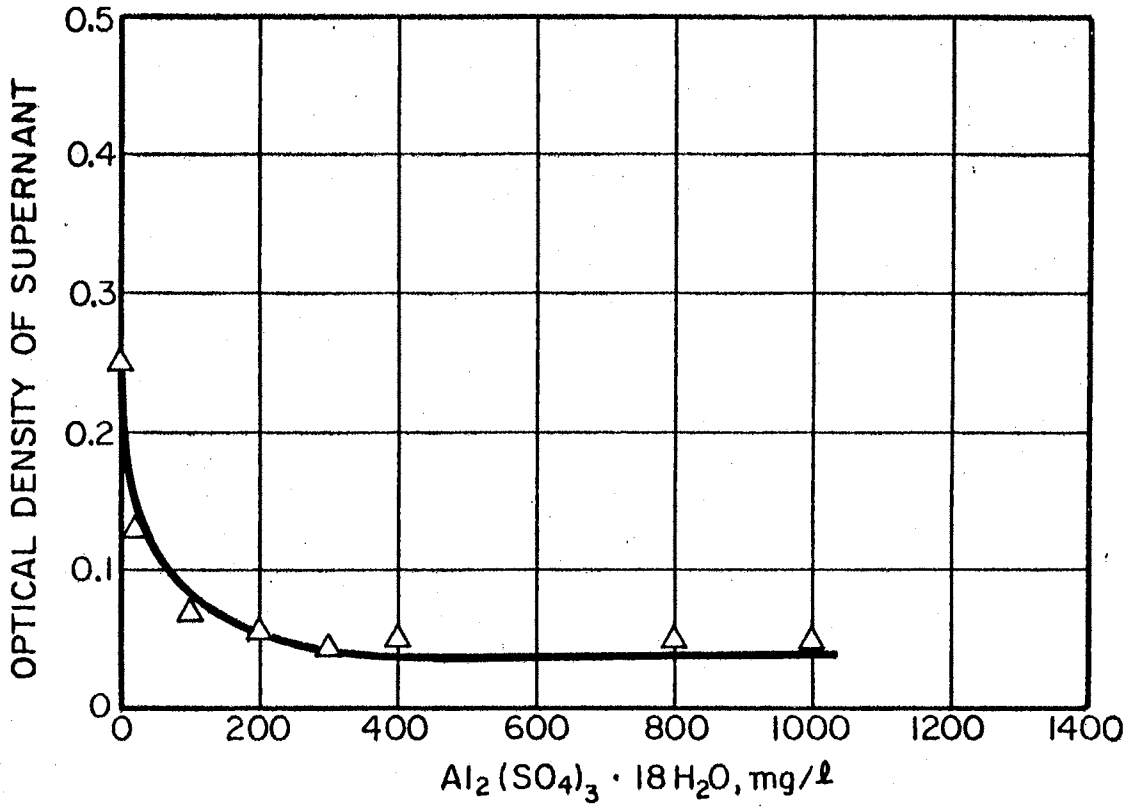
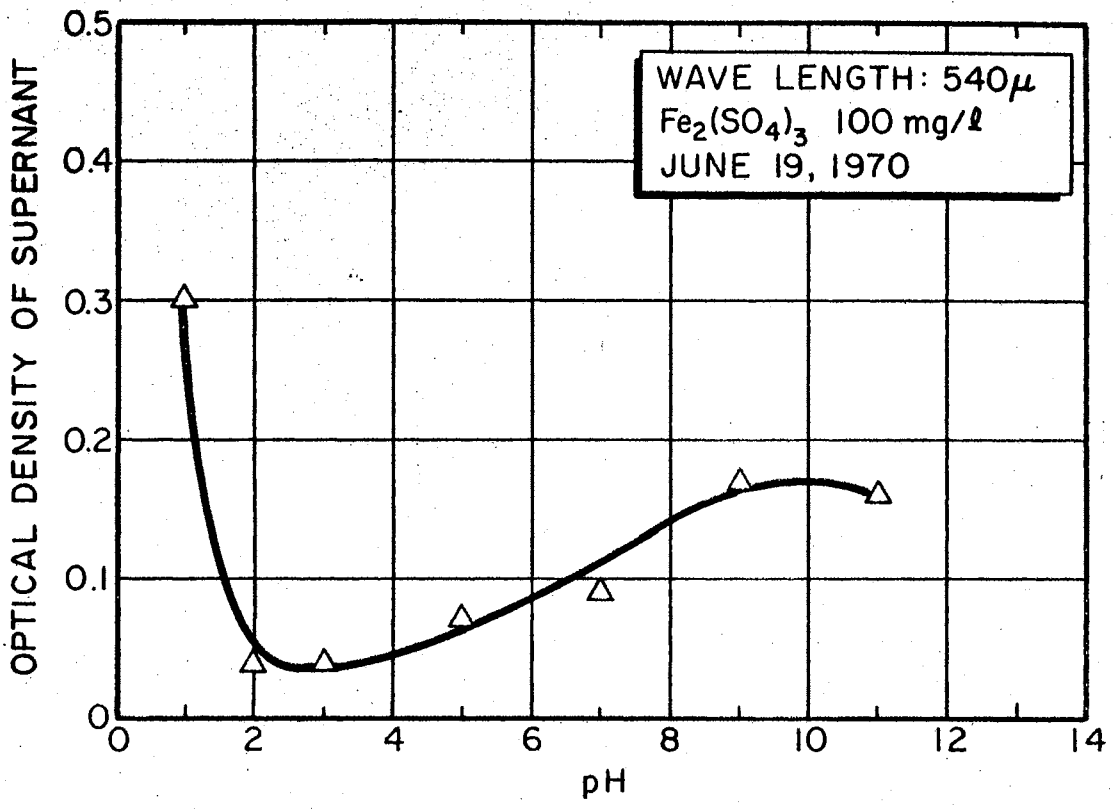
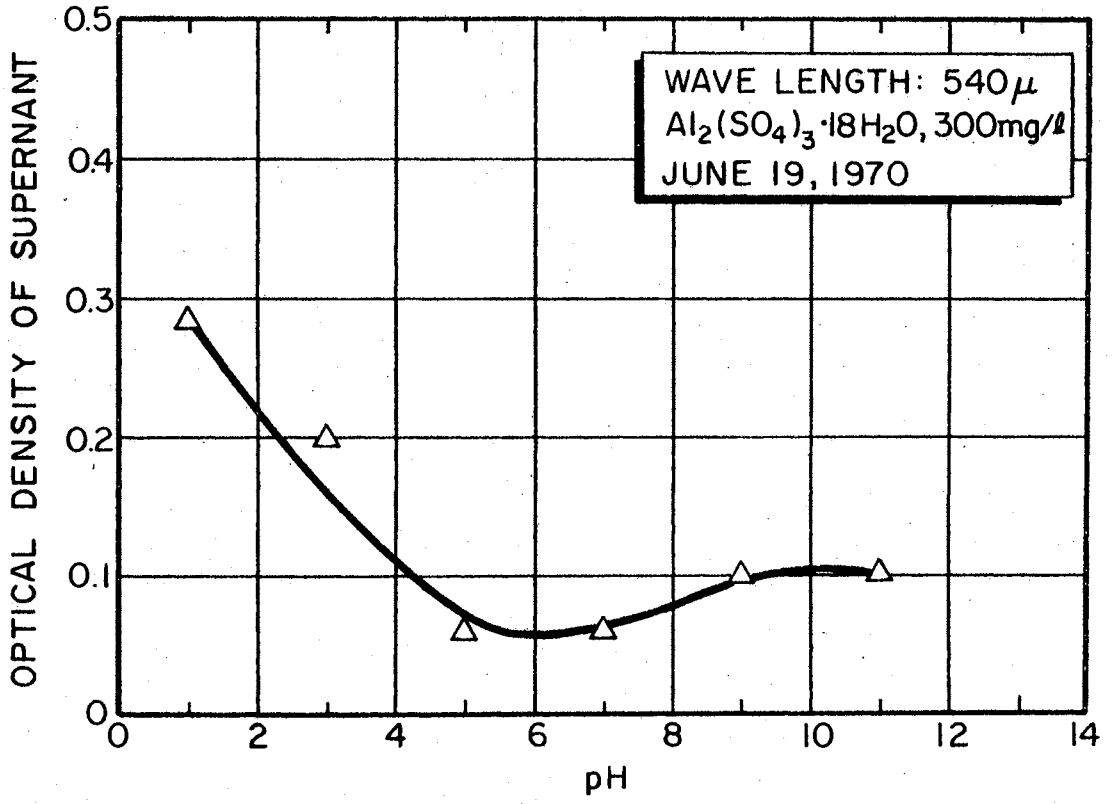

OPTICAL DENSITY OF SUPERNANT
0.5
0.4
0.3
0.2
0.1
0
0
200
400
600
800
1000
1200
1400
Al2(SO4)3 · 18H2O, mg/ℓ

Figure 22. Effect of pH on the flocculation of biological solids retained in the effluent of the extended aeration pilot plant by the application of ferric sulfate and aluminum sulfate at the concentrations indicated.


WAVE LENGTH: 540μ
$Fe_2(SO_4)_3$ 100 mg/ℓ
JUNE 19, 1970
OPTICAL DENSITY OF SUPERNANT
0.5
0.4
0.3
0.2
0.1
0
0
2
4
6
8
10
12
14
pH


WAVE LENGTH: 540μ
$Al_2(SO_4)_3 \cdot 18H_2O$, 300mg/ℓ
JUNE 19, 1970
OPTICAL DENSITY OF SUPERNANT
0.5
0.4
0.3
0.2
0.1
0
0
2
4
6
8
10
12
14
pH

results, it appeared that $Al_2(SO_4)_3 \cdot 18\ H_2O$ and $Fe_2(SO_4)_3$ would be the best reagents to flocculate the biological solids in the effluent of the extended aeration activated sludge process. Therefore, these two agents were used in experiments to determine the effect of pH upon the efficiency of flocculation in this system. Results are shown in Figure 22.

B. Performances and Operational Stability of Extended Aeration Activated Sludge Process With Incorporation of Chemical Hydrolysis for Control of Sludge Concentration

After three years' operation of the extended aeration activated sludge process without wasting biological solids, it was found that natural periodic phases of solids accumulation and subsequent de-accumulation or accelerated autodigestion were possible (28)(29). During periodic decreases in biological solids concentration, purification efficiency remained high (28). Although natural periods of decreasing biological solids concentration without deterioration of biochemical purification efficiency would be ideal for the operation of extended aeration activated sludge processes, the duration of such a cycle (increasing or decreasing) may at times be rather long, and the concentration of biological solids may increase to such an extent as to exceed the capacity of the settling chamber for handling the solids. Since the results of experimentations on the extended aeration process have indicated that the concept of so-called "total oxidation" is not theoretically unsound or impossible from a microbiological standpoint (28)(29), the major problem is one of controlling the autodigestive process or finding a way to retain the sludge until a natural period of accelerated autodigestion, which will reduce the sludge concentration, occurs.

The natural period of accelerated autodigestion may require a long time period to develop, since the major organic loading in an extended aeration system is indeed the sludge itself (29). The organic materials contained in the sludge represent a very complex carbon source, and a considerable amount of time may be needed for various autodigestive processes (i.e., establishment of predominance of predatory cells, induction of enzymes by one cell which could lyse other cells, and the development of the necessary enzyme systems to hydrolyze the slime or capsule layers and cell wall of some cells). The first step in the autodigestion of complex cell material is the degradation of the macromolecules to less complex organic molecules. It was reasoned that such an hydrolysis was in all probability one of the most difficult to perform biologically but might be more easily performed chemically, thus effecting some degree of engineering control over the initiation and extent of solids degradation. It was suggested that a portion of the biological solids might be hydrolyzed chemically, and the sludge hydrolysate recycled to the oxidation tank (29). In this section, experimental results on the metabolism of the hydrolysate and on the operational performance of the extended aeration process incorporating chemical hydrolysis (the "chemical assist") will be presented in Figures 25 through 33, and Figures 34 through 37, respectively. Concurrently with the operation of this pilot plant, various experiments were conducted to determine the optimum conditions for hydrolysis of the sludge and to characterize the hydrolysate. Results of these experiments will be presented before presenting data concerning operation of the pilot plant.

1. Conditions for Acid Hydrolysis of Activated Sludge and Characterization of the Hydrolysate

Usually, the protein concentration in activated sludge consisting of microbial cells is about 40 to 60 percent of the total biological solids concentration. This considerable amount of material is thus the major constituent subjected to acid hydrolysis, and methods considered for hydrolysis of sludge were chosen primarily for their efficiency in hydrolysis of protein. Both hydrochloric and sulfuric acids have been employed in the hydrolysis of proteins (53). When hydrochloric acid is the hydrolytic agent, the protein is usually treated with five to ten times its weight of strong acid (6 to 12 N) at 100 to 110°C for six to 20 hours. By hydrolysis with either HCl or H_2SO_4, the protein of activated sludge can be converted to amino acids, to a certain degree, by using a pressure of 15 lb/in^2 and temperature of 121°C for four to six hours (35). However, the presence of large amounts of Cl^- ion in the hydrolysate would interfere with the COD analysis (by the dichromate method). Therefore, sulfuric acid was employed in place of hydrochloric acid for the acid hydrolysis of activated sludge in the present study.

The two disadvantages of complete acid hydrolysis are the destruction of certain amino acids (especially true of the amino acid tryptophan) and the carbohydrate in the sample is degraded to a variety of compounds. However, in the present study there was no need of complete hydrolysis of protein to amino acids, since the shorter soluble molecules (like some short-chain peptides) could be expected to be metabolized by the organisms in the activated sludge process. Therefore, hydrolysis was conducted using relative low amounts of acid, and autoclaving at 15 psi.

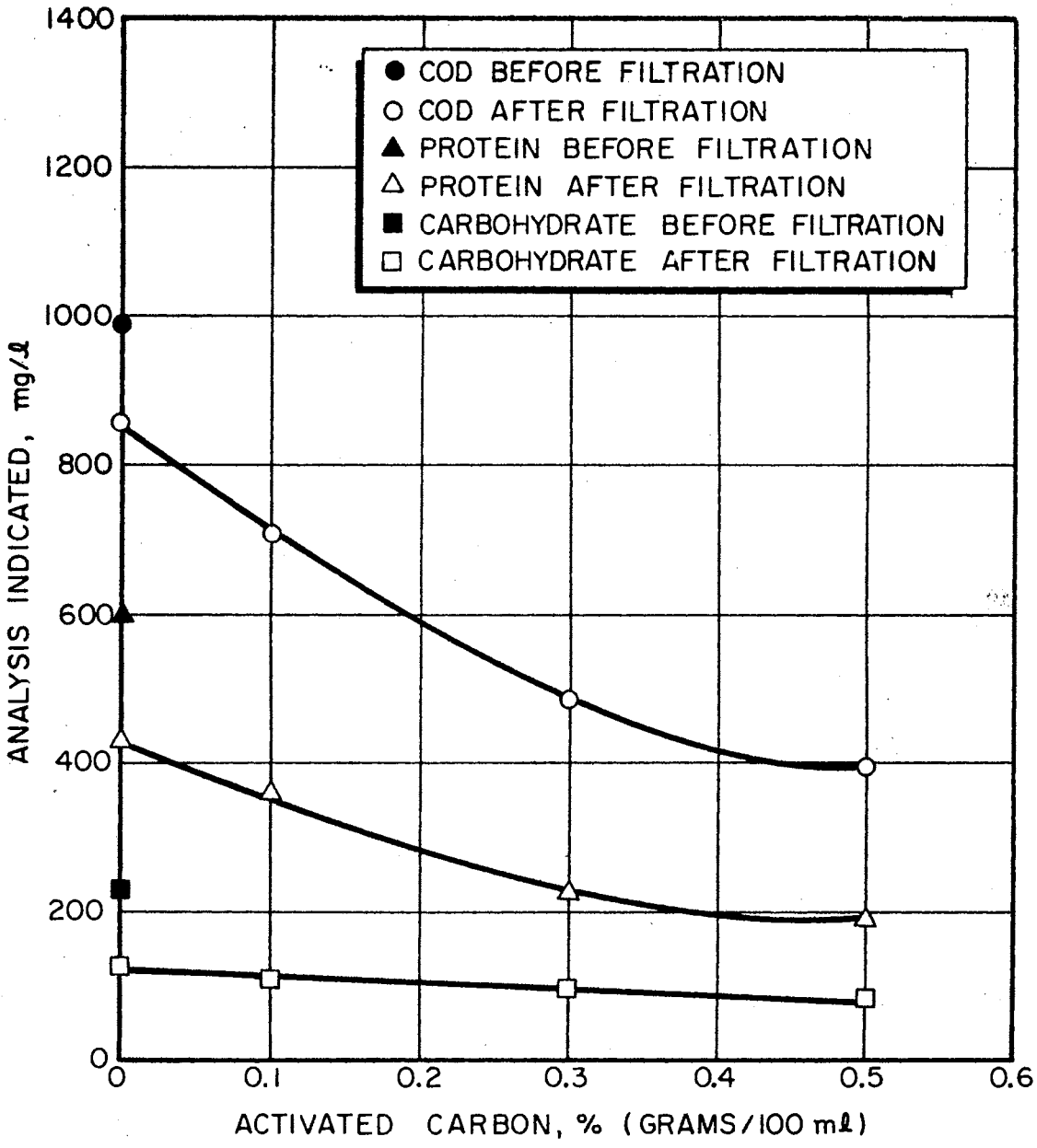
In preliminary experiments using cells grown up for this purpose in glucose minimal medium, suspensions containing 5000 mg/l biological solids were adjusted to various levels of pH (1.0 to 4.0) by adding sulfuric acid. Each suspension was then autoclaved at 15 psi, 121°C, for five hours. Results of these experiments indicated that pH 1.0 provided conditions which yielded the highest apparent solubility of the cell components. Therefore, standard procedure adopted for experiments to characterize the hydrolysate employed, about 0.35 N acid for the hydrolysis of about 5000 mg/l of biological solids concentration.

It was important to determine by performing materials balances, the fraction of COD lost during hydrolysis and the fraction which would be retained by the Millipore filter used to measure biological solids during growth studies. Before materials balances could be made, it was necessary to adjust the pH of the hydrolysate to 7.0 by adding sodium hydroxide solution, since the low pH of the hydrolysate had an adverse effect on the Millipore filters. The cells used for this experiment were harvested from a separate batch culture. A small inoculum of cells taken from the extended aeration unit was transferred to 1000 ml of minimal medium containing 1.0 gm of glucose and aerated for 36 hours; then this one-liter culture was used to inoculate ten liters of glucose minimal medium. After growth of this large-scale culture, the cells were centrifuged and diluted with distilled water to a concentration of about 5000 mg/l, and used for more detailed studies on acid hydrolysis. Four such batches of relatively young cells were grown up for hydrolytic studies. For two of them, the COD of the cells before hydrolysis was the same as the COD of the hydrolyzed sludge. In one, 23 percent of the COD was removed by the hydrolytic procedure, and in another, 16 percent

was removed. It is interesting to note that under the hydrolytic conditions employed using these freshly grown cells, all cell components were generally solubilized. After hydrolysis, there was usually a small amount of fluffy precipitate which was apparently inorganic in nature, since the COD of the neutralized hydrolysate was the same before and after passage through a Millipore filter (0.45 μ), which retained the small amount of precipitate formed. It was interesting to note that the conditions of hydrolysis employed did not break down the cell protein completely to amino acids. Approximately 50 to 60 percent of the hydrolysate COD reacted in the biuret test, which requires the presence of two or more peptide bonds. Approximately eight to 15 percent registered as carbohydrate, as determined by the anthrone test.

The experiments described above showed that the conditions of hydrolysis did not completely hydrolyze many of the complex macromolecules, e.g., protein. These molecules, as well as free amino acids, can be expected to carry an electrical charge, and in order to gain further insight into the characteristics of the acid hydrolysate of activated sludge, the adsorption characteristics of the material in the hydrolysate were examined. For the experiments described below, the activated sludge which was hydrolyzed was that existing in the system at the end of the pilot plant operation of phase B. The COD of the acid hydrolysate was approximately 7600 mg/l; it was neutralized to pH 7.0 and diluted to about 1000 mg COD per liter. This diluted hydrolysate was treated with various concentrations of activated carbon (Nuchar-C 190-N, Fisher Scientific Co.). The hydrolysate and activated carbon were mixed at 40-50^{o}C for two minutes, and filtered through Whatman No. 1 filter paper. The results are shown in Figure 23. The figure

Figure 23. Adsorption of sludge hydrolysate (388-day old sludge) by activated carbon.

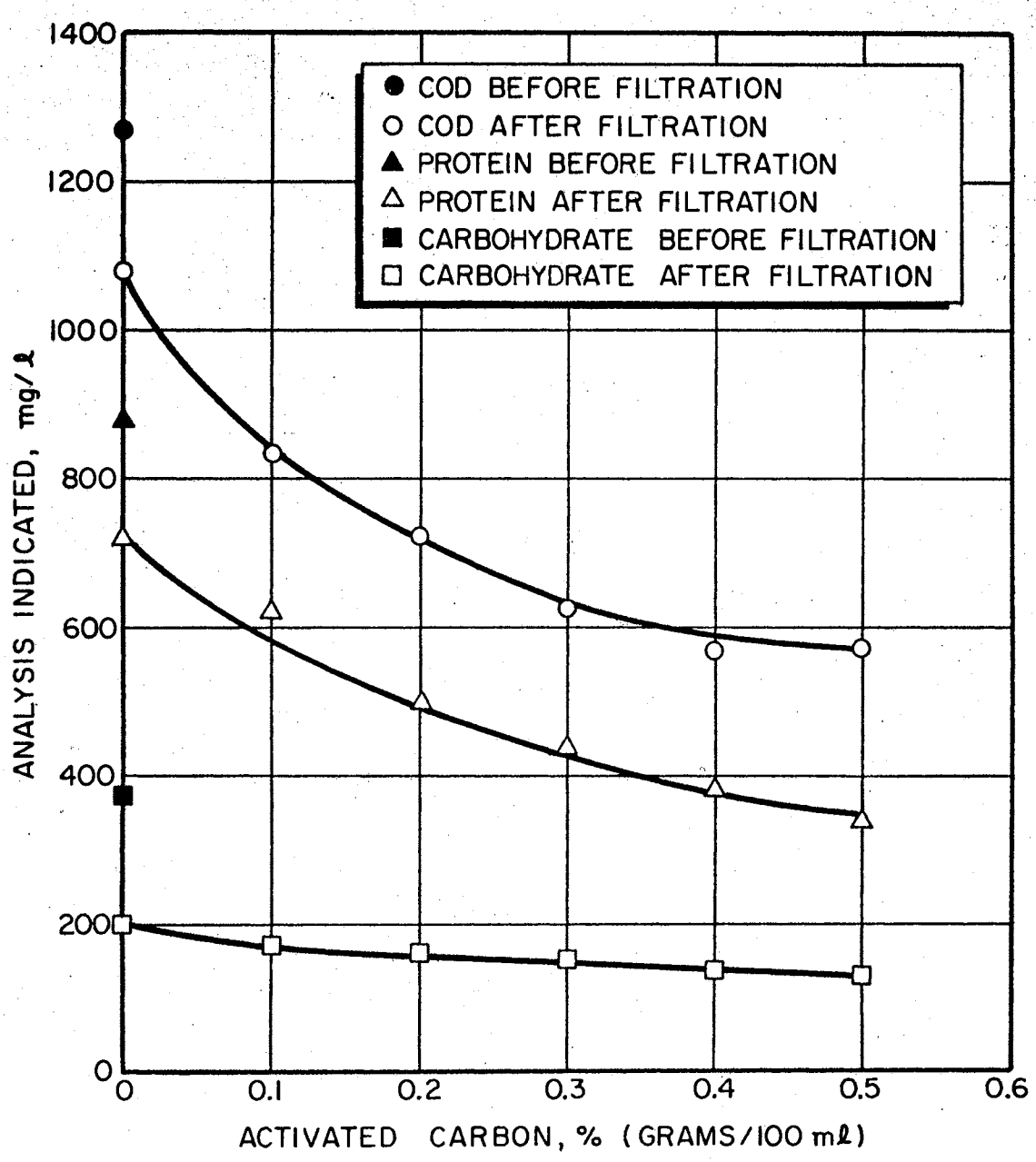

COD BEFORE FILTRATION
COD AFTER FILTRATION
PROTEIN BEFORE FILTRATION
PROTEIN AFTER FILTRATION
CARBOHYDRATE BEFORE FILTRATION
CARBOHYDRATE AFTER FILTRATION
ANALYSIS INDICATED, mg/ℓ
1400
1200
1000
800
600
400
200
0
0
0.1
0.2
0.3
0.4
0.5
0.6
ACTIVATED CARBON, % (GRAMS/100 mℓ)

shows that approximately 60 percent of the COD, 66 percent of the protein, and 64 percent of the carbohydrate were removed by activated carbon at a concentration of 0.5 percent. Filtration of the hydrolysate without adding activated carbon removed 15, 28.3, and 46.5 percent of the COD, protein, and carbohydrate concentrations, respectively. Approximately 60 percent of the hydrolysate COD was due to protein, and approximately 20 percent was due to carbohydrate. From this experimentation it must be concluded that the conditions of acid hydrolysis (pH 1.0, 121°C, and 15 psi) in the autoclaving which solubilized young cells used in previous experiments were not quite severe enough to solubilize all components of the cells taken from the extended aeration activated sludge unit after 388 days of operation of phase B.

Another experiment was run on hydrolysate prepared using 0.5 N H_2SO_4 and the sludge sample prepared after 1202 days in operation of phase A and stored for later use. As before, the sludge hydrolysate was treated with different concentrations of activated carbon, i.e., contacting at 40 to 50°C for two minutes followed by filtration through Whatman No. 1 filter paper. The results are shown in Figure 24. In these experiments, approximately 55 percent of the COD, 53 percent of the protein, 66 percent of the carbohydrate of the hydrolysate were removed by activated carbon at a concentration of 0.5 percent. Comparing these results with those of the previous experiments (388-day old sludge), it will be noted that the hydrolysate from the older sludge (1202 days) was adsorbed to a lesser extent. Again, filtration alone removed appreciable amounts of COD, protein, and carbohydrate.

Experiments were also conducted to determine the effect of increasing the concentration of acid on the hydrolysis of aged activated

Figure 24. Adsorption of sludge hydrolysate (1202-day old sludge) by activated carbon.


COD BEFORE FILTRATION
COD AFTER FILTRATION
PROTEIN BEFORE FILTRATION
PROTEIN AFTER FILTRATION
CARBOHYDRATE BEFORE FILTRATION
CARBOHYDRATE AFTER FILTRATION
ANALYSIS INDICATED, mg/ℓ
1400
1200
1000
800
600
400
200
0
0
0.1
0.2
0.3
0.4
0.5
0.6
ACTIVATED CARBON, % (GRAMS/100 mℓ)

sludge. The 1202-day sludge taken from the extended aeration unit at the end of phase A was employed with various concentrations of sulfuric acid (i.e., 0.5 N, 1.0 N, 2.0 N, 3.0 N, 4.0 N, and 5.0 N). The results are shown in Table IV. It can be seen that increasing the acid concentration did not change the COD concentration in the sludge hydrolysate, nor did it appreciably increase the solubility of the hydrolysate. Solubility was assessed by passing a neutralized sample through a membrane filter (pore size 0.45 μ). With this older activated sludge, 19.3 to 25.3 percent of insoluble COD remained in the acid hydrolysate. From analysis of the carbohydrate content in these acid hydrolysates it can be seen that the more acid used in the hydrolysis, the higher was the insoluble carbohydrate remaining in the hydrolysate. From the analysis of protein content of the filtrate, it can be seen that the higher acid concentration permitted more extensive hydrolysis of the protein. Unfortunately, data on protein content before filtration could not be obtained because of interference by some component which was apparently retained on the filter. However, the COD data indicate that the decrease in filtrate protein was not due to increased loss of protein during filtration. It is readily seen that the protein contained in the sludge was not completely hydrolyzed to amino acids even by the application of 5 N acid.

2. Studies on the Metabolism of Neutralized Cell Hydrolysate by an Extended Aeration Activated Sludge

Separate batches of cells were grown up, harvested, and subjected to acid hydrolysis, as previously described, then adjusted to pH 7.0, added to the mineral salts and phosphate buffer and employed as the growth medium. For all experiments, the initial cell inoculum consisted

TABLE IV

EFFECT ON CHARACTERISTICS OF THE SLUDGE HYDROLYSATE OF TREATMENT AT DIFFERENT ACID CONCENTRATIONS

Normality of Acid Used	COD Before Filtration (mg/l)	COD After Filtration (mg/l)	COD Lost During Filtration (mg/l)	Carbohydrate Before Filtration (mg/l)	Carbohydrate Removed After Filtration (mg/l)	Carbohydrate Removed During Filtration (%)	Protein After Filtration (mg/l)
0.5	14180	10580	25.3	2096	1961	6.4	6211
1.0	13720	11070	19.3	2042	1763	13.6	5306
2.0	14120	11220	20.5	1706	1388	18.6	4246
3.0	14320	10710	25.2	1531	1120	26.8	4545
4.0	14100	10820	23.2	1161	795	31.5	3541
5.0	15100	11450	24.1	1159	794	31.4	3866

of cells from the extended aeration plant. The results are presented in Figures 25 through 28. Observation of the course of COD removal and biological solids accumulation clearly indicates that the hydrolysate served as a carbon source on the extended aeration activated sludge. The residual COD for the three experiments shown in Figures 25, 26, and 27 was approximately 50 mg/l. Figure 28 shows the course of biological solids accumulation and COD removal when cell hydrolysate was fed directly to the pilot plant as a slug dose in a batch feeding experiment (upper portion of figure). The experiment was conducted after 998 days of continuous operation without wasting of solids, and it is seen that the cell hydrolysate was rapidly removed. The bottom portion of the figure shows the course of growth and substrate removal when a small inoculum of the extended aeration sludge was fed with the hydrolysate. This experiment was similar to those shown in Figures 25, 26, and 27, and gave similar results. The results shown in Figures 25 through 28 indicated that cell hydrolysates prepared from cells grown in batch cultures could be readily metabolized by cells in the extended aeration pilot plant. However, it was also desirable to determine whether cell hydrolysate prepared from the mature activated sludge in the extended aeration pilot plant was readily metabolizable. After March 27, 1970, i.e., the day the pilot plant was vandalized, it was no longer of any particular advantage to save all sludge for return to the aerator, and at various times, significant portions were taken for experimentation. Figures 29 and 30 show the results of experiments using hydrolysate prepared from sludge taken from this extended aeration pilot plant and fed to a low initial inoculum of cells taken from the extended aeration pilot plant on days 1097 and 1169, respectively. Approximately

Figure 25. Metabolism of hydrolyzed biological sludge using a low initial inoculum of cells taken from the extended aeration activated sludge pilot plant on day 907 (September 22, 1969).

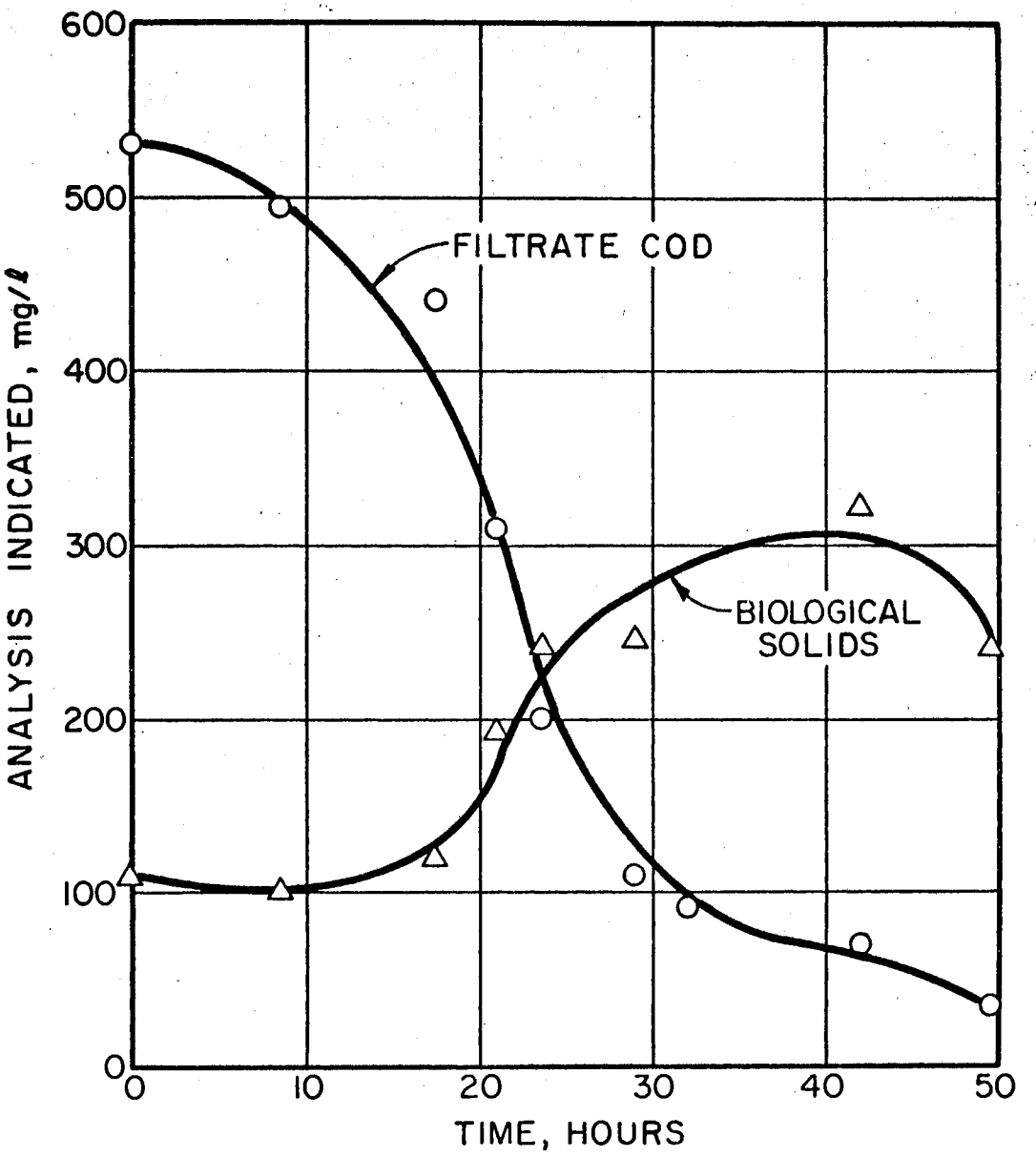

600
500
400
300
200
100
0
ANALYSIS INDICATED, mg/ℓ
FILTRATE COD
BIOLOGICAL SOLIDS
0
10
20
30
40
50
TIME, HOURS

Figure 26. Metabolism of hydrolyzed biological sludge using a low inoculum of cells taken from the extended aeration activated sludge pilot plant on day 917 (October 2, 1969).

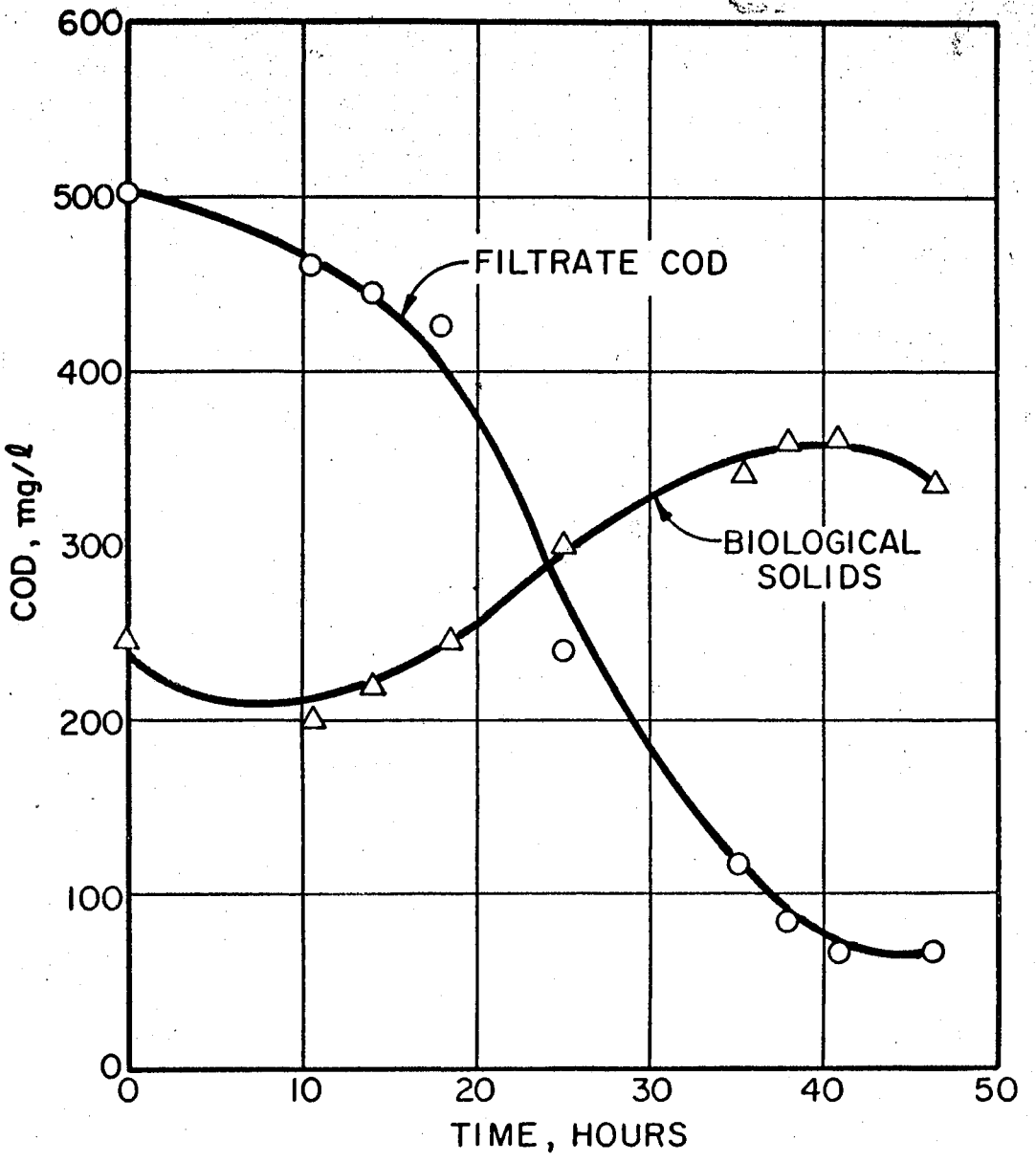

600
500
400
300
200
100
0
COD, mg/l
FILTRATE COD
BIOLOGICAL SOLIDS
0
10
20
30
40
50
TIME, HOURS

Figure 27. Metabolism of hydrolyzed biological sludge using a low initial inoculum of cells taken from the extended aeration activated sludge pilot plant on day 955 (November 9, 1969).

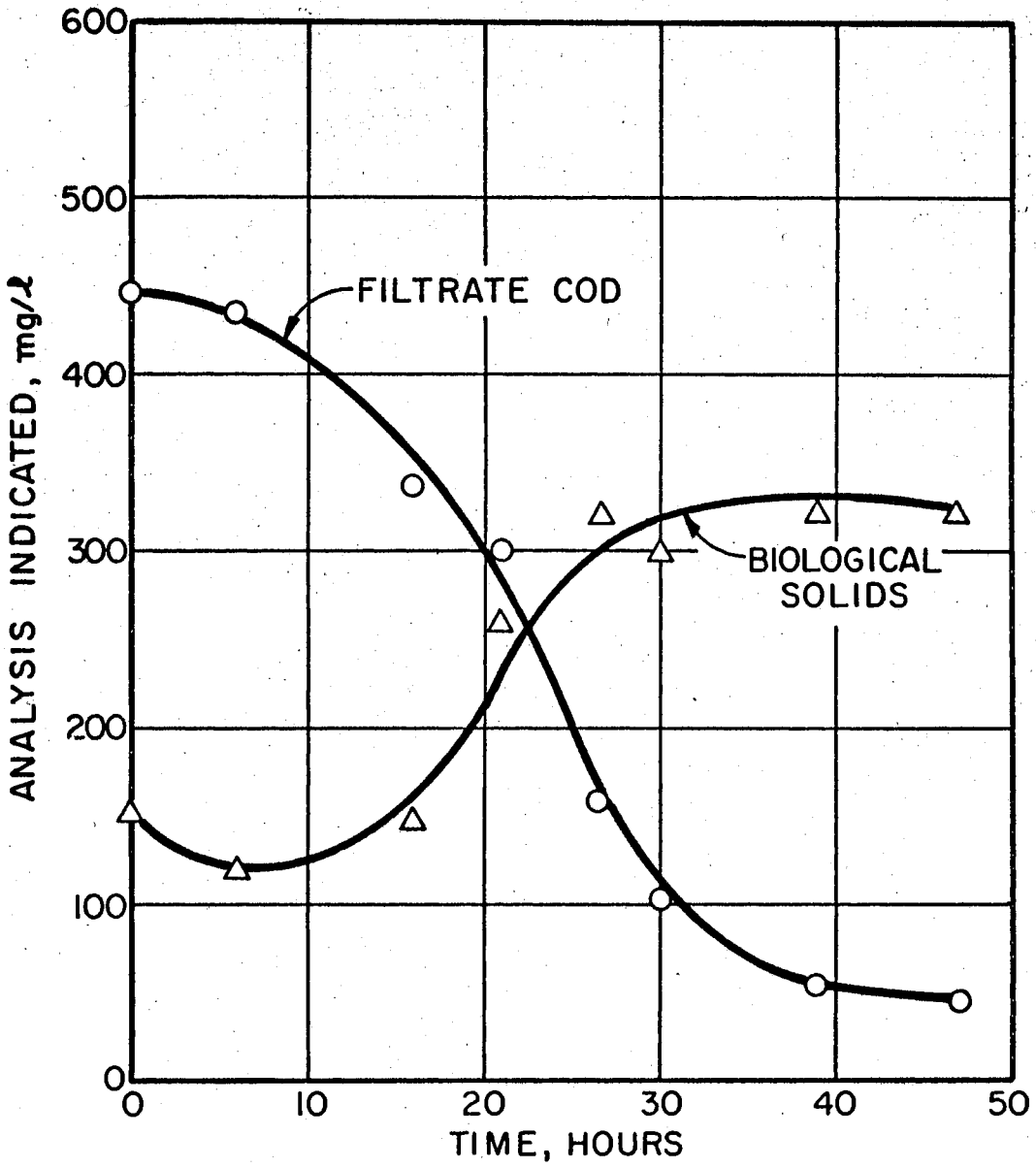
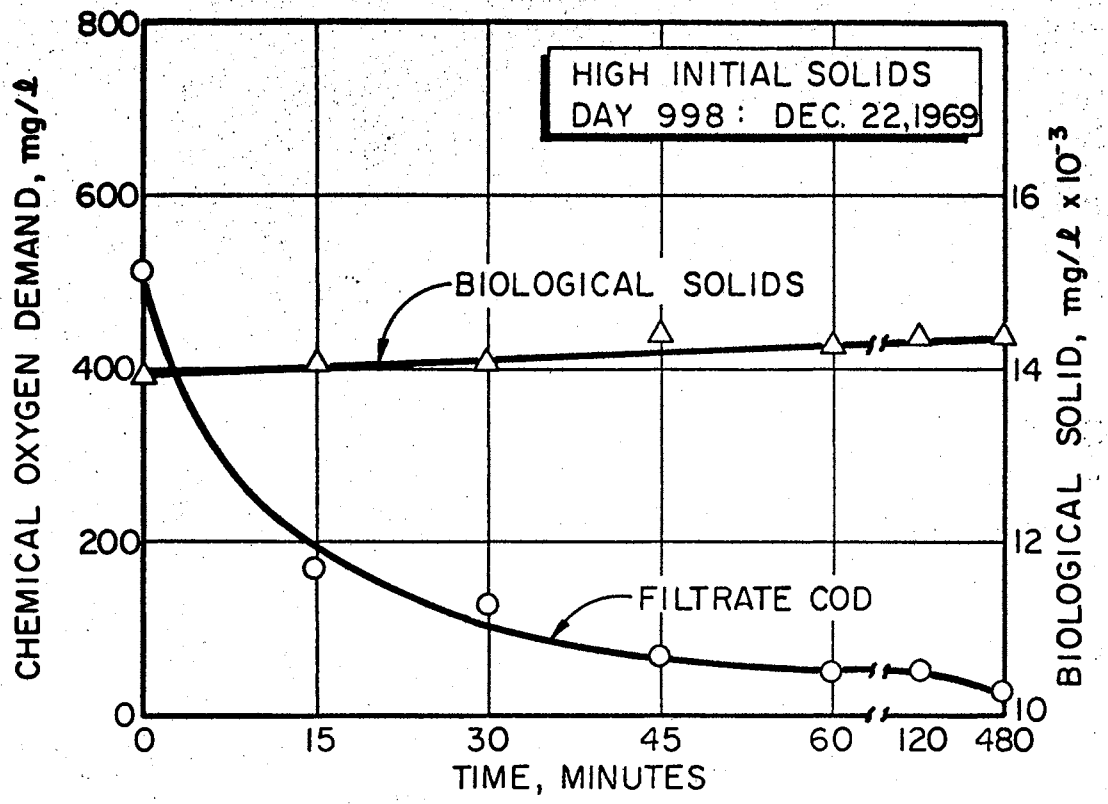

600
500
400
300
200
100
0
ANALYSIS INDICATED, mg/ℓ
FILTRATE COD
BIOLOGICAL SOLIDS
0
10
20
30
40
50
TIME, HOURS

Figure 28. Metabolism of hydrolyzed biological sludge by extended aeration activated sludge at both low and high initial solids levels on day 998 (December 22, 1969).


HIGH INITIAL SOLIDS
DAY 998 : DEC. 22,1969
BIOLOGICAL SOLIDS
FILTRATE COD
CHEMICAL OXYGEN DEMAND, mg/ℓ
BIOLOGICAL SOLID, mg/ℓ x 10⁻³
TIME, MINUTES
800
600
400
200
0
16
14
12
10
0
15
30
45
60
120
480

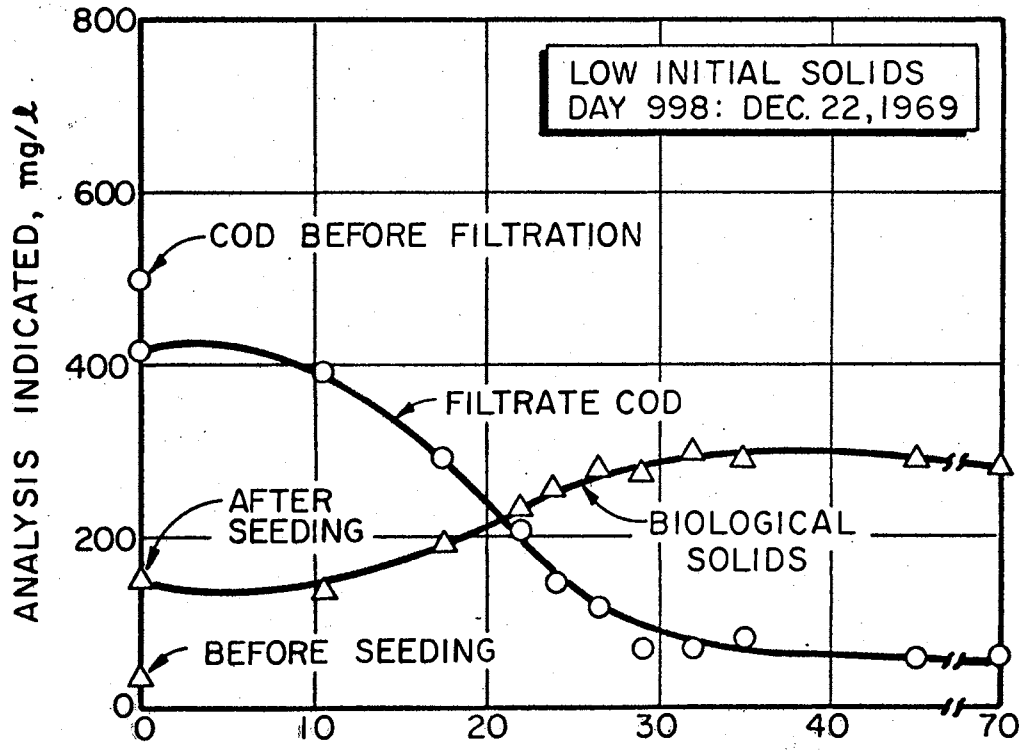

LOW INITIAL SOLIDS
DAY 998: DEC. 22,1969
COD BEFORE FILTRATION
FILTRATE COD
AFTER SEEDING
BIOLOGICAL SOLIDS
BEFORE SEEDING
ANALYSIS INDICATED, mg/ℓ
800
600
400
200
0
0
10
20
30
40
70

Figure 29. Metabolism of hydrolyzed extended aeration sludge using a low initial inoculum of cells taken from the extended aeration activated sludge pilot plant on day 1097 (March 31, 1970).

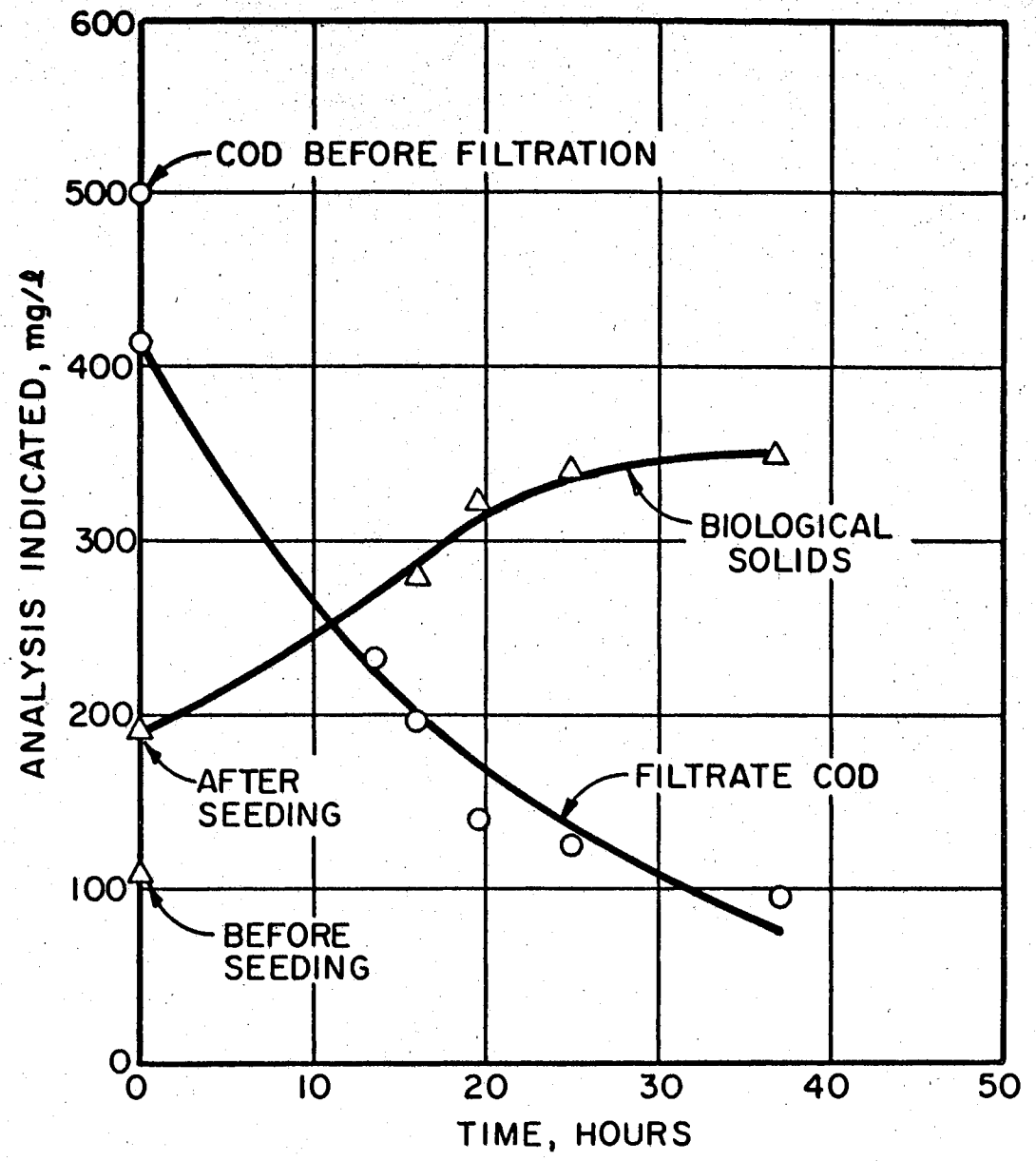
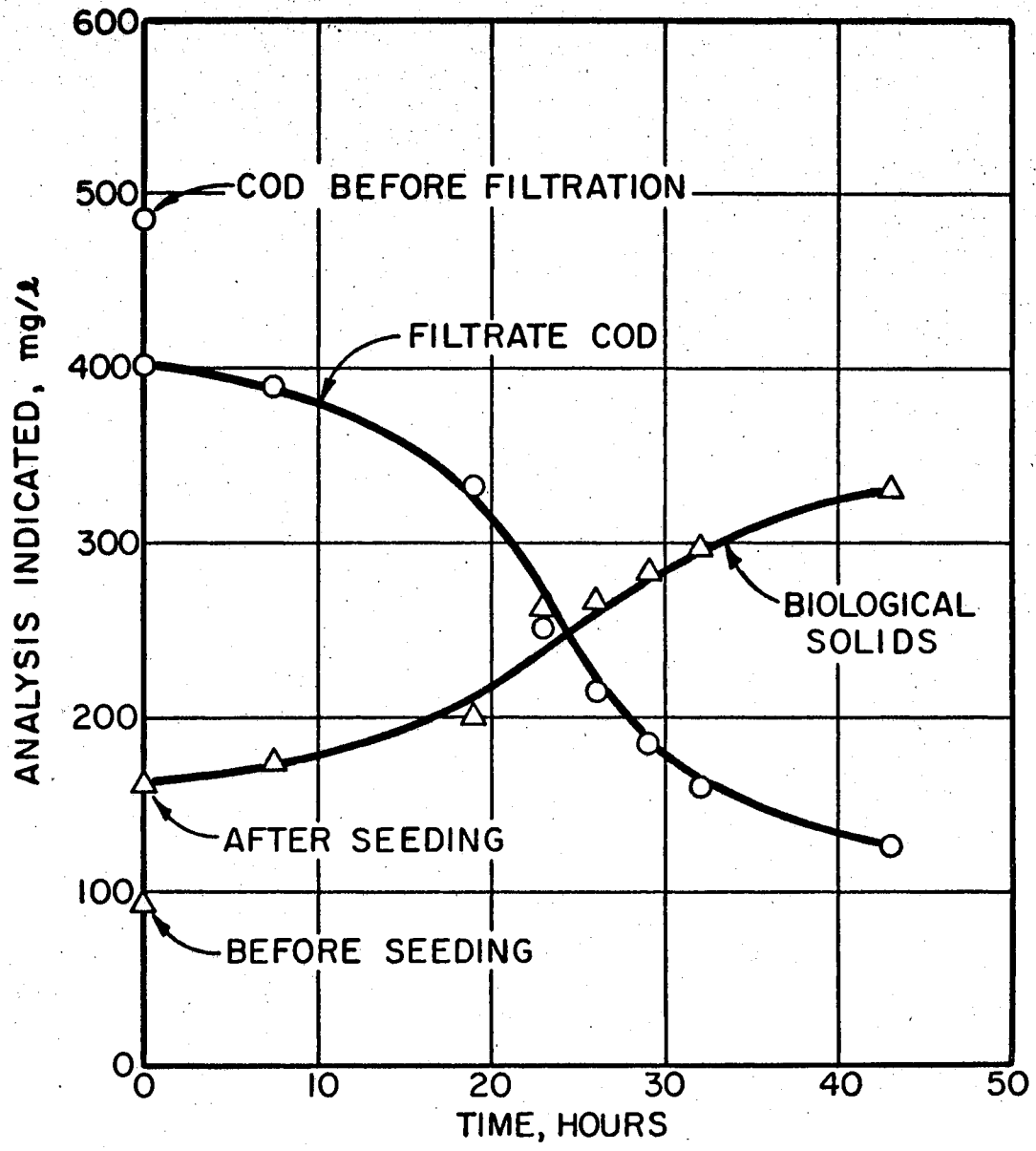

600
500
400
300
200
100
0
ANALYSIS INDICATED, mg/ℓ
COD BEFORE FILTRATION
BIOLOGICAL SOLIDS
FILTRATE COD
AFTER SEEDING
BEFORE SEEDING
0
10
20
30
40
50
TIME, HOURS

Figure 30. Metabolism of hydrolyzed extended aeration sludge using a low initial inoculum of cells taken from the extended aeration activated sludge pilot plant on day 1169 (June 11, 1970).


600
500
400
300
200
100
0
0
10
20
30
40
50
ANALYSIS INDICATED, mg/ℓ
TIME, HOURS
COD BEFORE FILTRATION
FILTRATE COD
BIOLOGICAL SOLIDS
AFTER SEEDING
BEFORE SEEDING

80 percent of the cell hydrolysate was soluble (i.e., passed through a 0.45 μ membrane filter). The hydrolysate was utilizable by a mature (extended aeration) activated sludge microbial population. The experiments were terminated after about 40 hours, and the residual COD was somewhat higher than when hydrolysate from younger cells was employed (compare residual COD in Figures 29 and 30 with those in Figures 25, 26, 27, and 28).

It was seen previously that addition of chemical flocculating agents could be used to enhance settleability and retention of biological solids in the system. If such an expedient were employed in a field installation and the precipitated solids were then subjected to hydrolysis, there would be some question as to the metabolizability of the hydrolysate, since it would contain a considerable amount of the flocculating agent (for example, aluminum) which could conceivably exhibit an inhibitory effect. Therefore it was of interest to determine the metabolic availability of hydrolysate from sludge which had been subjected to chemical precipitation. Suspended solids in the effluent of the settling chamber of the pilot plant on day 1182 were treated with $Fe_2(SO_4)_3$ at 100 mg/l (pH 7.0), and the effluent on days 1188 and 1198 were treated with $Al_2(SO_4)_3 \cdot 18\ H_2)$ at 200 mg/l (pH 7.0). These flocculating chemicals were effective in precipitating the suspended solids (as previously shown, see Figures 21 and 22). With $Fe_2(SO_4)_3$, 80 percent of the effluent COD was removed by flocculation and settling, and in the experiments with $Al_2(SO_4)_3 \cdot 18\ H_2O$, removals of 73 and 88 percent were obtained. The settled solids were harvested, acidified to pH 1.0 and autoclaved (15 psi, 5 hours). The hydrolysate was neutralized, and the COD was determined. Portions of the neutralized hydrolysates were

filtered (membrane filter, 0.45 μ), and the COD of the filtrate was measured. Volatile solids determinations were run on the material remaining on the membrane filter used in filtering the hydrolysate from the Fe^{+++} floc.

It was found that from the hydrolyzed Fe^{+++} floc, 21.3 percent of the COD of the hydrolysate was filtered out (i.e., 78.7 percent was soluble), and from the Al^{+++} hydrolysates, 26.6 and 36 percent of the hydrolysate COD was retained on the filter. Volatile solids determination on the material retained on the filter indicated that only 28.6 percent of this material was organic matter, i.e., the major portion of it was inorganic chemical floc. However, it is apparent that the conditions of hydrolysis employed were not severe enough to solubilize all of the organic matter. It is noted that the same conditions of hydrolysis were sufficient to hydrolyze freshly grown populations of cells. Additional experiments were run in which samples of chemically precipitated extended aeration sludge were acidified to pH 1.0 as before and to pH 0.4 (required addition of sulfuric acid to approximately 0.5 N), and subjected to the hydrolytic period as before. The sample which was dosed with the higher amount of acid exhibited the highest percent soluble COD (as measured by the amount passing a 0.45 μ membrane filter), 87.6 percent as compared to 73.4 percent. However, in previous experiments (see Table IV), use of acid (H_2SO_4) in greater concentrations than 0.5 N did not appear to increase the solubilized fraction.

In Figures 31, 32, and 33, COD removed and cell growth on the hydrolysate from chemically precipitated suspended solids from the extended aeration unit are shown. Figure 31 shows results for the cells flocculated with $Fe_2(SO_4)_3$, and Figure 32 for cells flocculated with

Figure 31. Metabolism of hydrolyzed biological sludge which had been previously flocculated using $Fe_2(SO_4)_3$, 100 mg/l.

The sludge was obtained by treating the effluent from the extended aeration pilot plant during the period when microbial flocculation was poor. The experiment was using a low initial solids inoculum of cells taken from the extended aeration unit on day 1182 (June 23, 1970).

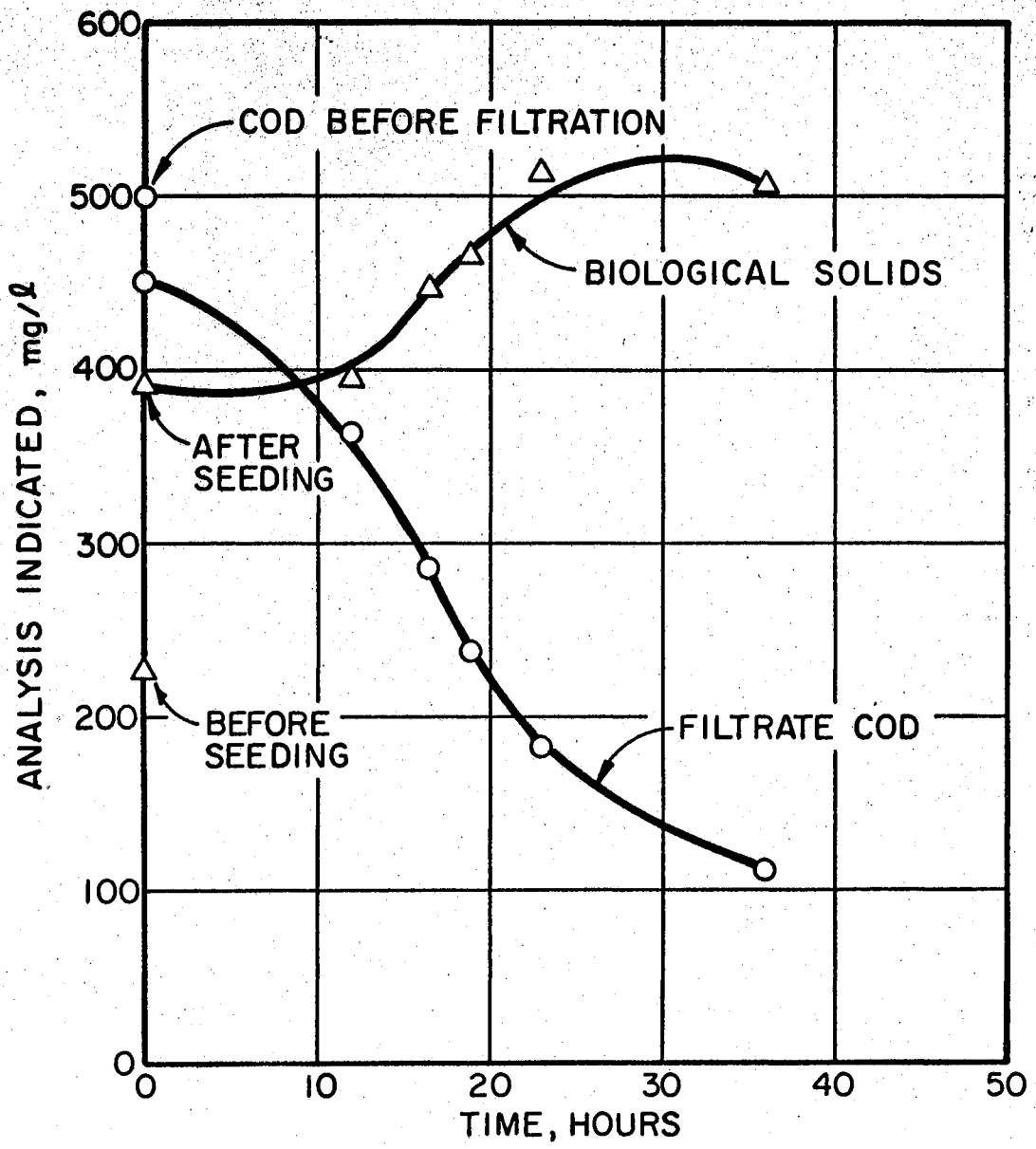

600
500
400
300
200
100
0
0
10
20
30
40
50
ANALYSIS INDICATED, mg/ℓ
TIME, HOURS
COD BEFORE FILTRATION
BIOLOGICAL SOLIDS
AFTER SEEDING
BEFORE SEEDING
FILTRATE COD

Figure 32. Metabolism of hydrolyzed biological sludge which had been previously flocculated using $Al_2(SO_4)_3 \cdot 18\ H_2O$, 200 mg/l.

The sludge was obtained by treating the effluent from the extended aeration pilot plant during the period when microbial flocculation was poor. The experiment was using a low initial solids inoculum of cells taken from the extended aeration unit on day 1188 (June 29, 1970).

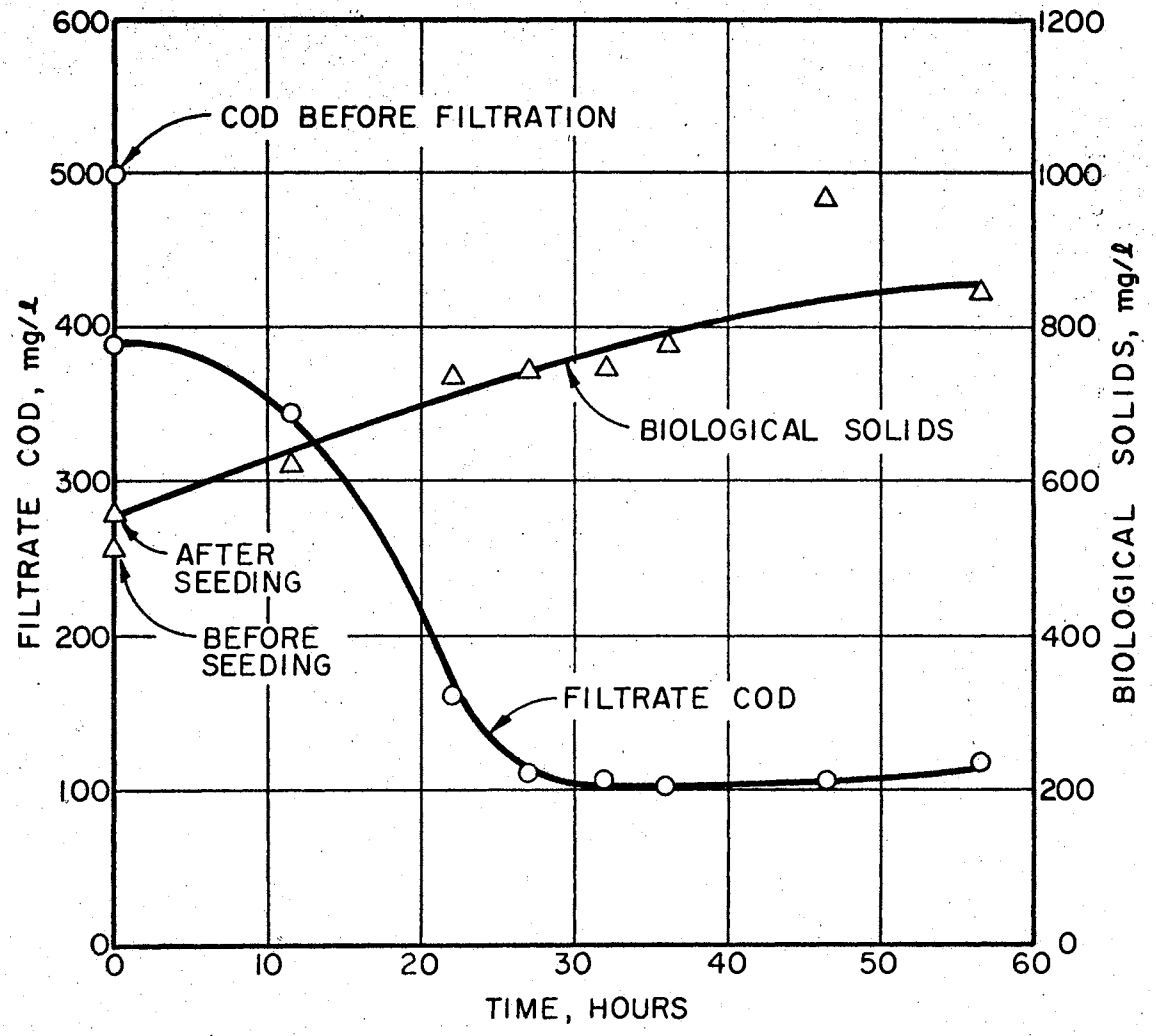
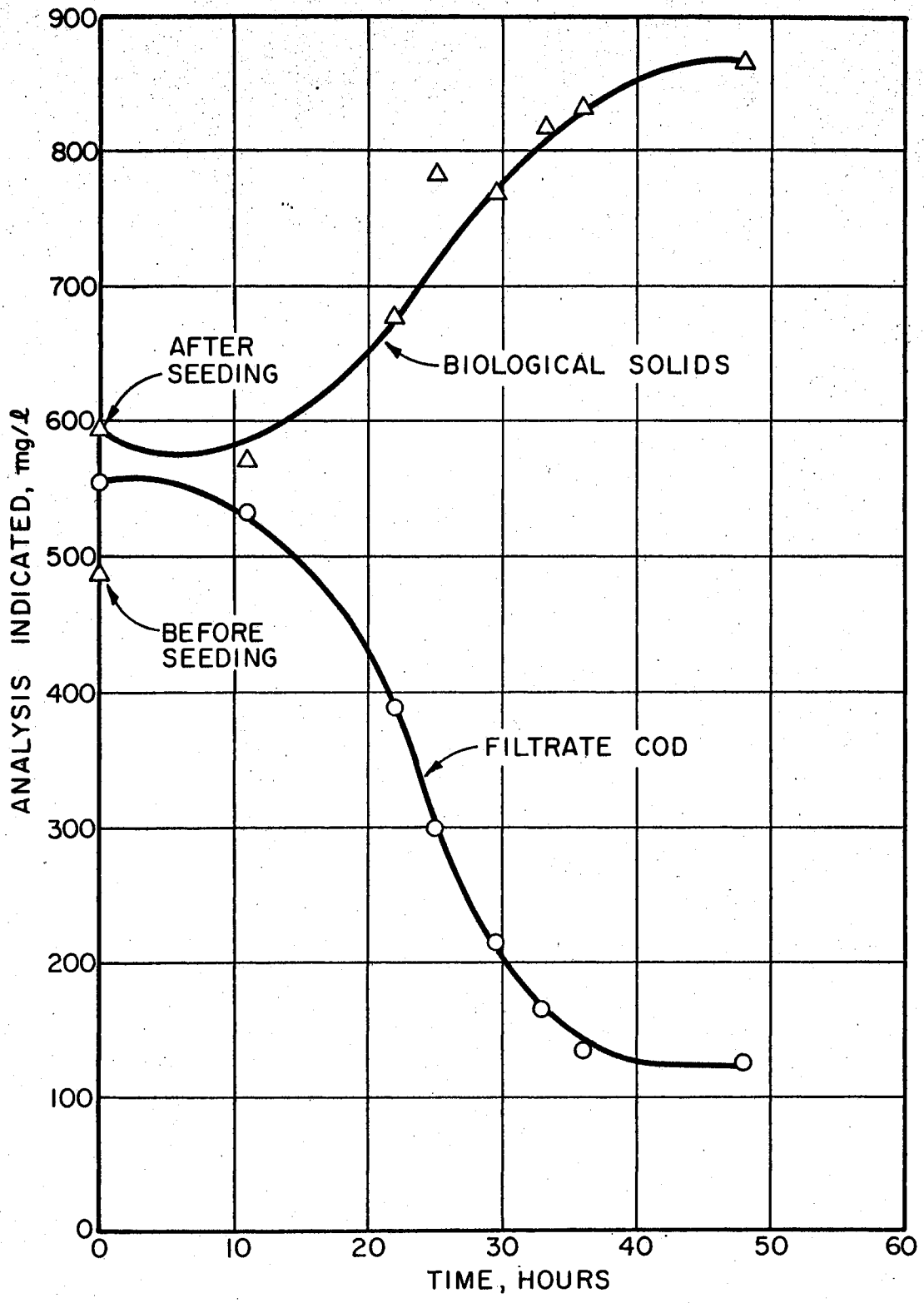

COD BEFORE FILTRATION
BIOLOGICAL SOLIDS
AFTER SEEDING
BEFORE SEEDING
FILTRATE COD
FILTRATE COD, mg/ℓ
BIOLOGICAL SOLIDS, mg/ℓ
TIME, HOURS
0
100
200
300
400
500
600
0
200
400
600
800
1000
1200
0
10
20
30
40
50
60

Figure 33. Metabolism of hydrolyzed biological sludge which had been previously flocculated using $Al_2(SO_4)_3 \cdot 18\ H_2O$, 200 mg/l.

The sludge was obtained by treating the effluent from the extended aeration pilot plant during the period when microbial flocculation was poor. The experiment was using a low initial solids inoculum of cells taken from the extended aeration unit on day 1198 (July 9, 1970). (This experiment was run under nonproliferating conditions, i.e., no exogenous nitrogen was added.)

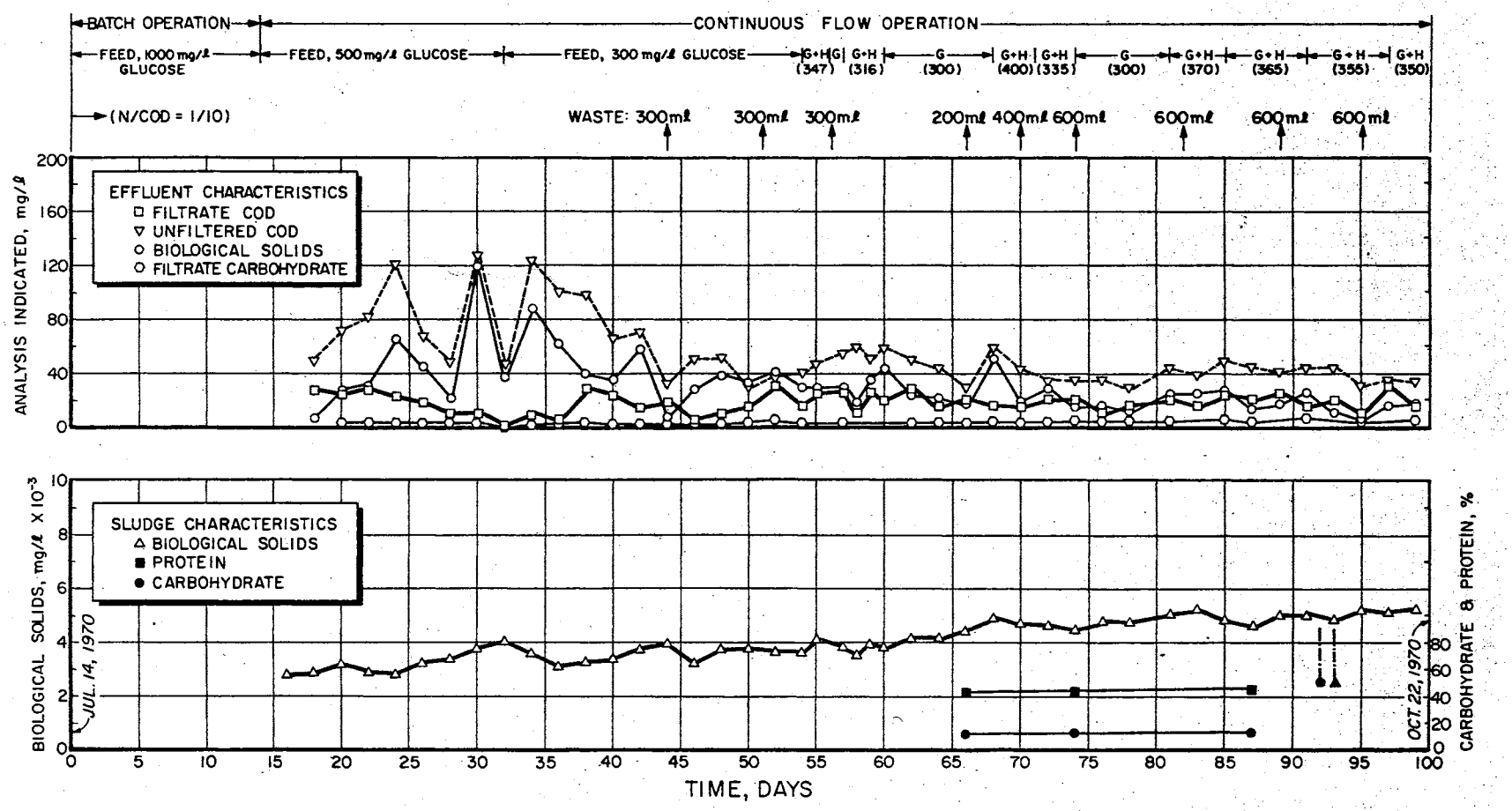

900
800
700
600
500
400
300
200
100
0
0
10
20
30
40
50
60
ANALYSIS INDICATED, mg/ℓ
TIME, HOURS
AFTER SEEDING
BIOLOGICAL SOLIDS
BEFORE SEEDING
FILTRATE COD

$Al_2(SO_4)_3 \cdot 18\ H_2O$. In both cases, the hydrolysate was added as the carbon source to standard minimal medium containing ammonium ion. For the experimental results shown in Figure 33, in which hydrolysate from Al^{+++} flocculated cells was employed, the ammonium ion was withheld from the standard mineral salts in the medium, and the only source of nitrogen for growth was that contained in the hydrolysate. In all of these cases, the hydrolysates were metabolized, and it would appear that chemical dosage of the cell suspension (at the chemical dosages herein employed) when needed to help clear up the effluent would not seriously hamper subsequent metabolism of hydrolysate prepared from the chemically coagulated sludge.

3. Studies on the Operation of an Extended Aeration Process Employing Periodic Chemical Hydrolysis of Portions of the Sludge

Before operating the pilot plant in accordance with the new process modification, an activated sludge was developed under batch feeding conditions, without wasting sludge, using minimal medium with glucose fed at 1000 mg/l per day. An initial seed of microorganisms was taken from the supernatant of the primary clarifier of the municipal treatment plant at Stillwater, Oklahoma. After two weeks' development of the extended aeration activated sludge, the mode of operation was switched to continuous flow, with a feed containing 500 mg/l glucose (see Figure 34). This mode of operation was similar to the previous one, except that the clarifier effluent which was collected in the holding reservoir was not centrifuged. The biological solids concentration in the aeration chamber accumulated to 4000 mg/l after 32 days of operation. The biochemical removal efficiency achieved (based on filtrate COD) was 94 to 98 percent. During this period, the

Figure 34. Operational characteristics of the extended aeration pilot plant with periodic withdrawal, hydrolysis and refeeding of portions of the activated sludge; days 0-100 (July 14, 1970, to October 22, 1970).


BATCH OPERATION
CONTINUOUS FLOW OPERATION
FEED, 1000 mg/ℓ GLUCOSE
FEED, 500 mg/ℓ GLUCOSE
FEED, 300 mg/ℓ GLUCOSE
G+H (347)
G+H (316)
G (300)
G+H (400)
G+H (335)
G (300)
G+H (370)
G+H (365)
G+H (355)
G+H (350)
(N/COD = 1/10)
WASTE: 300mℓ
300mℓ
300mℓ
200mℓ
400mℓ
600mℓ
600mℓ
600mℓ
600mℓ
EFFLUENT CHARACTERISTICS
FILTRATE COD
UNFILTERED COD
BIOLOGICAL SOLIDS
FILTRATE CARBOHYDRATE
ANALYSIS INDICATED, mg/ℓ
SLUDGE CHARACTERISTICS
BIOLOGICAL SOLIDS
PROTEIN
CARBOHYDRATE
BIOLOGICAL SOLIDS, mg/ℓ X 10⁻³
CARBOHYDRATE & PROTEIN, %
JUL. 14, 1970
OCT. 22, 1970
TIME, DAYS

biological solids concentration in the effluent fluctuated between 50 and 200 mg/l. In order to bring the organic loading closer to those commonly employed in extended aeration plants in the field, and to determine whether a lower loading would enhance flocculation of the effluent, the glucose concentration in the feed was changed from 500 mg/l to 300 mg/l on day 32. This procedure did appear to have a steadying influence upon the system; on day 44, the very low level of 10 mg/l suspended solids was recorded. The first of a series of sludge withdrawals was made on day 44. Sludge was hydrolyzed in accordance with procedures previously described; it was then neutralized and fed at desired intervals to the aerator along with incoming synthetic waste. A tentative operational decision was made to hold the biological solids concentration in the aeration chamber to a level between 3000 and 5000 mg/l. 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. Letter G is used to designate glucose in the feed, and H is used to designate hydrolysate in the feed. Unless otherwise noted, the glucose concentration in the feed was 300 mg/l. Figures 34 through 37 show the operational characteristics of the system during a 388-day period of operation. Throughout the operation of the pilot plant, the effluent was characterized by determinations of filtrate COD and effluent (unfiltered) COD; also the carbohydrate content of the filtrate was determined, and the biological solids concentration in the effluent was assessed.

Sludge characterization (bottom portions of figures) included biological solids concentration and periodic determination of protein and

Figure 35. Operational characteristics of the extended aeration pilot plant with periodic withdrawal, hydrolysis and re-feeding of portions of the activated sludge; days 100-200 (October 22, 1970, to January 30, 1971).

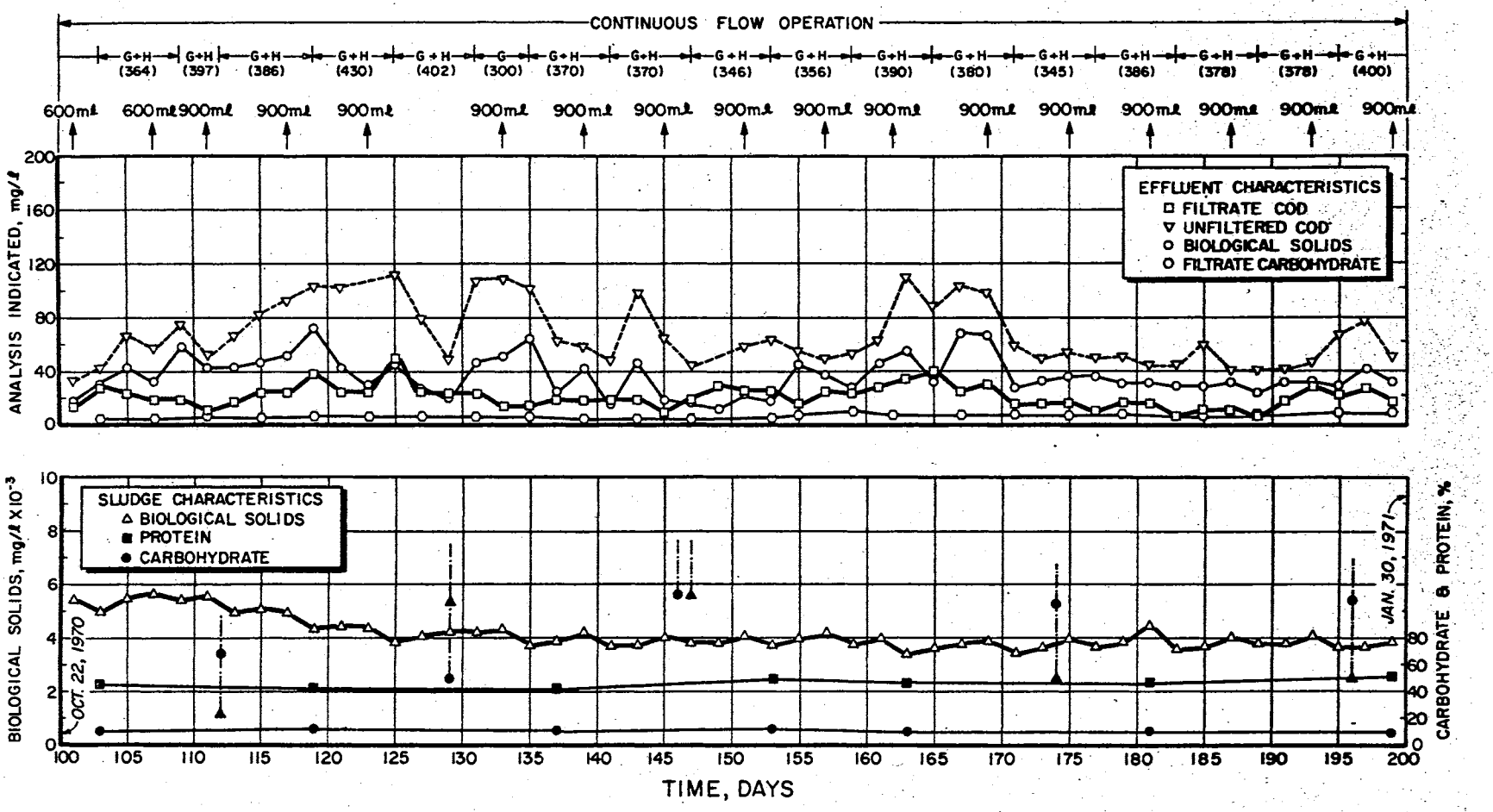

CONTINUOUS FLOW OPERATION
G+H (364)
G+H (397)
G+H (386)
G+H (430)
G+H (402)
G (300)
G+H (370)
G+H (370)
G+H (346)
G+H (356)
G+H (390)
G+H (380)
G+H (345)
G+H (386)
G+H (378)
G+H (378)
G+H (400)
600ml
900ml
ANALYSIS INDICATED, mg/ℓ
EFFLUENT CHARACTERISTICS
FILTRATE COD
UNFILTERED COD
BIOLOGICAL SOLIDS
FILTRATE CARBOHYDRATE
SLUDGE CHARACTERISTICS
BIOLOGICAL SOLIDS
PROTEIN
CARBOHYDRATE
BIOLOGICAL SOLIDS, mg/ℓ X 10⁻³
CARBOHYDRATE & PROTEIN, %
OCT. 22, 1970
JAN. 30, 1971
TIME, DAYS

Figure 36. Operational characteristics of the extended aeration pilot plant with periodic withdrawal, hydrolysis and re-feeding of portions of the activated sludge; days 200-300 (January 30, 1971, to May 10, 1971).

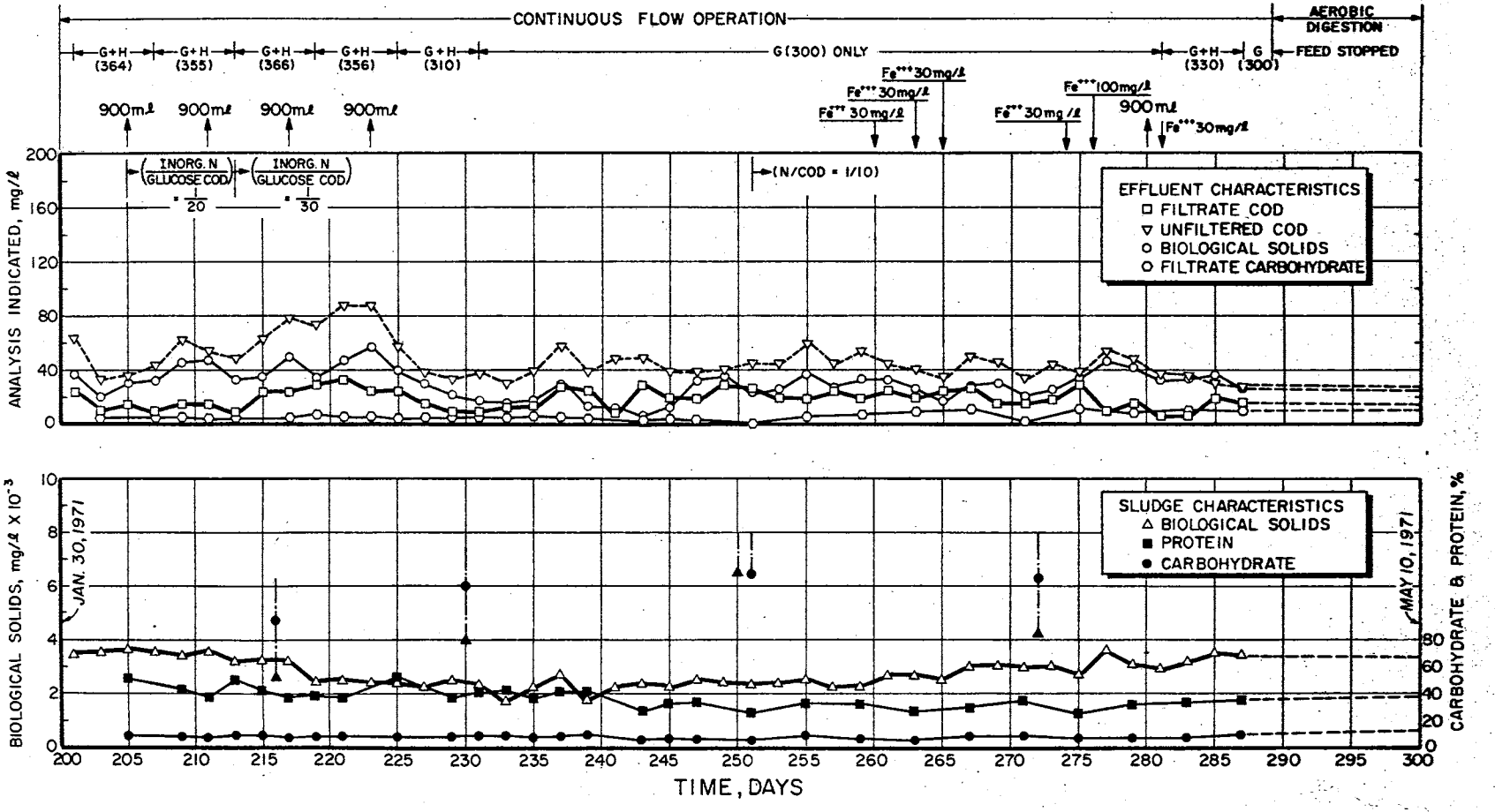
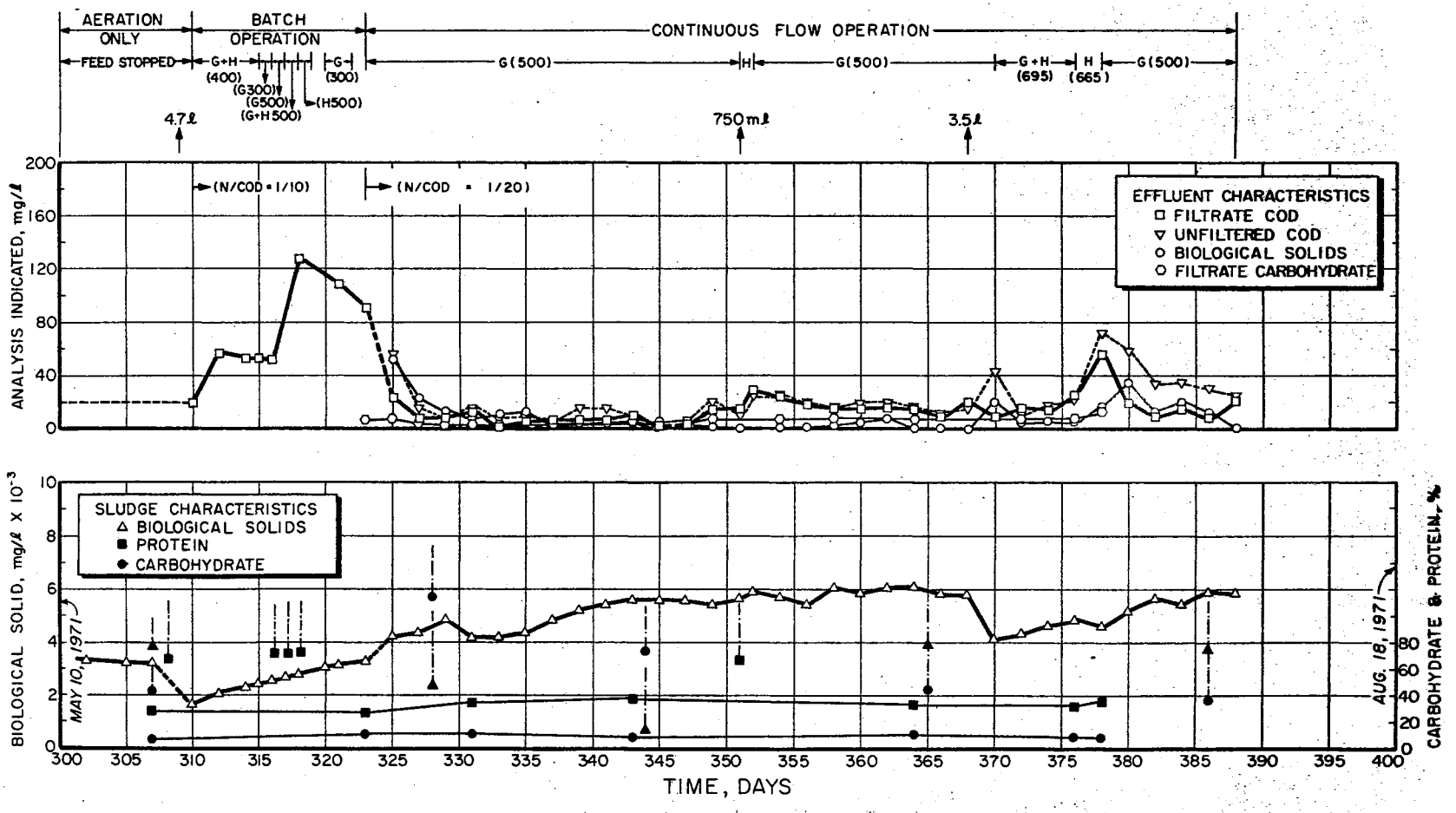

CONTINUOUS FLOW OPERATION
AEROBIC DIGESTION
G+H (364)
G+H (355)
G+H (366)
G+H (356)
G+H (310)
G(300) ONLY
G+H (330)
G (300)
FEED STOPPED
900mℓ
900mℓ
900mℓ
900mℓ
Fe+++ 30mg/ℓ
Fe+++ 30mg/ℓ
Fe+++ 30mg/ℓ
Fe+++ 30mg/ℓ
Fe+++ 100mg/ℓ
900mℓ
Fe+++ 30mg/ℓ
INORG. N / GLUCOSE COD * 1/20
INORG. N / GLUCOSE COD * 1/30
(N/COD = 1/10)
EFFLUENT CHARACTERISTICS
FILTRATE COD
UNFILTERED COD
BIOLOGICAL SOLIDS
FILTRATE CARBOHYDRATE
ANALYSIS INDICATED, mg/ℓ
SLUDGE CHARACTERISTICS
BIOLOGICAL SOLIDS
PROTEIN
CARBOHYDRATE
JAN. 30, 1971
MAY 10, 1971
BIOLOGICAL SOLIDS, mg/ℓ X 10⁻³
CARBOHYDRATE & PROTEIN, %
TIME, DAYS

Figure 37. Operational characteristics of the extended aeration pilot plant with periodic withdrawal, hydrolysis and refeeding of portions of the activated sludge; days 300-388 (May 10, 1971, to August 6, 1971).


AERATION ONLY
FEED STOPPED
BATCH OPERATION
G+H (400)
(G300)
(G500)
(G+H500)
(H500)
G (300)
CONTINUOUS FLOW OPERATION
G(500)
H
G(500)
G+H (695)
H (665)
G(500)
4.7ℓ
750mℓ
3.5ℓ
(N/COD = 1/10)
(N/COD = 1/20)
ANALYSIS INDICATED, mg/ℓ
EFFLUENT CHARACTERISTICS
FILTRATE COD
UNFILTERED COD
BIOLOGICAL SOLIDS
FILTRATE CARBOHYDRATE
SLUDGE CHARACTERISTICS
BIOLOGICAL SOLIDS
PROTEIN
CARBOHYDRATE
BIOLOGICAL SOLID, mg/ℓ X 10⁻³
CARBOHYDRATE & PROTEIN, %
MAY 10, 1971
AUG. 18, 1971
TIME, DAYS

carbohydrate content of the biological solids. Also indicated in the lower portion of each figure are the times when samples of biological solids were taken from the aeration chamber for auxiliary experiments. For example, in Figure 34, the broken vertical line terminating in a triangle indicates the time when a sample was extracted to use as seed in a low biological solids batch growth experiment. Also on the preceding day (see day 92), the broken vertical line terminating in a circle indicates that a sample was withdrawn for measurement of endogenous O_2 uptake in the Warburg apparatus.

It is seen in Figure 34 that the operational procedure did provide for rather good and stable operation from day 50 onward. Biological solids concentration in general ranged between 4000 and 5000 mg/l. 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 was approximately 20 mg/l. Carbohydrate content in the residual COD was approximately 5 mg/l.

Between days 100 and 111 (Figure 35), 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 ml to 900 ml. The increased withdrawal 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 the

incoming feed, glucose. The biochemical removal efficiency fluctuated between 88 and 98 percent. Biological solids concentration in the effluent fluctuated from 12 mg/l 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 Figure 35 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 wastes, 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 extended aeration for high strength industrial wastes.

It will be recalled that the operational results shown in Figures 34 and 35 were obtained with the pilot plant running at a COD:nitrogen ratio of 10:1. Analyses for inorganic nitrogen and phosphorus were made throughout the entire operational period using the hydrolytic assist process, and these results are shown in graphical form in Figures 38 and 39. The effluent inorganic nitrogen concentrations plotted in Figures 38 and 39 represent the sum of NH_3-N, NO_2-N, and NO_3-N. As was noted in the unit used in the previous phase, after approximately 50 days of operation, the predominant form of effluent nitrogen changed from NH_3-N to NO_3-N, indicating the development of a nitrifying population. This is shown graphically in Figure 40.

It is seen from Figure 38 that during the first 200 days of operation, wherein the NH_3-N to glucose COD ratio in the feed was 1:10,

Figure 38. Comparison of phosphorus and nitrogen in the feed and in the effluent of the extended aeration pilot plant (July 14, 1970, to January 30, 1971).

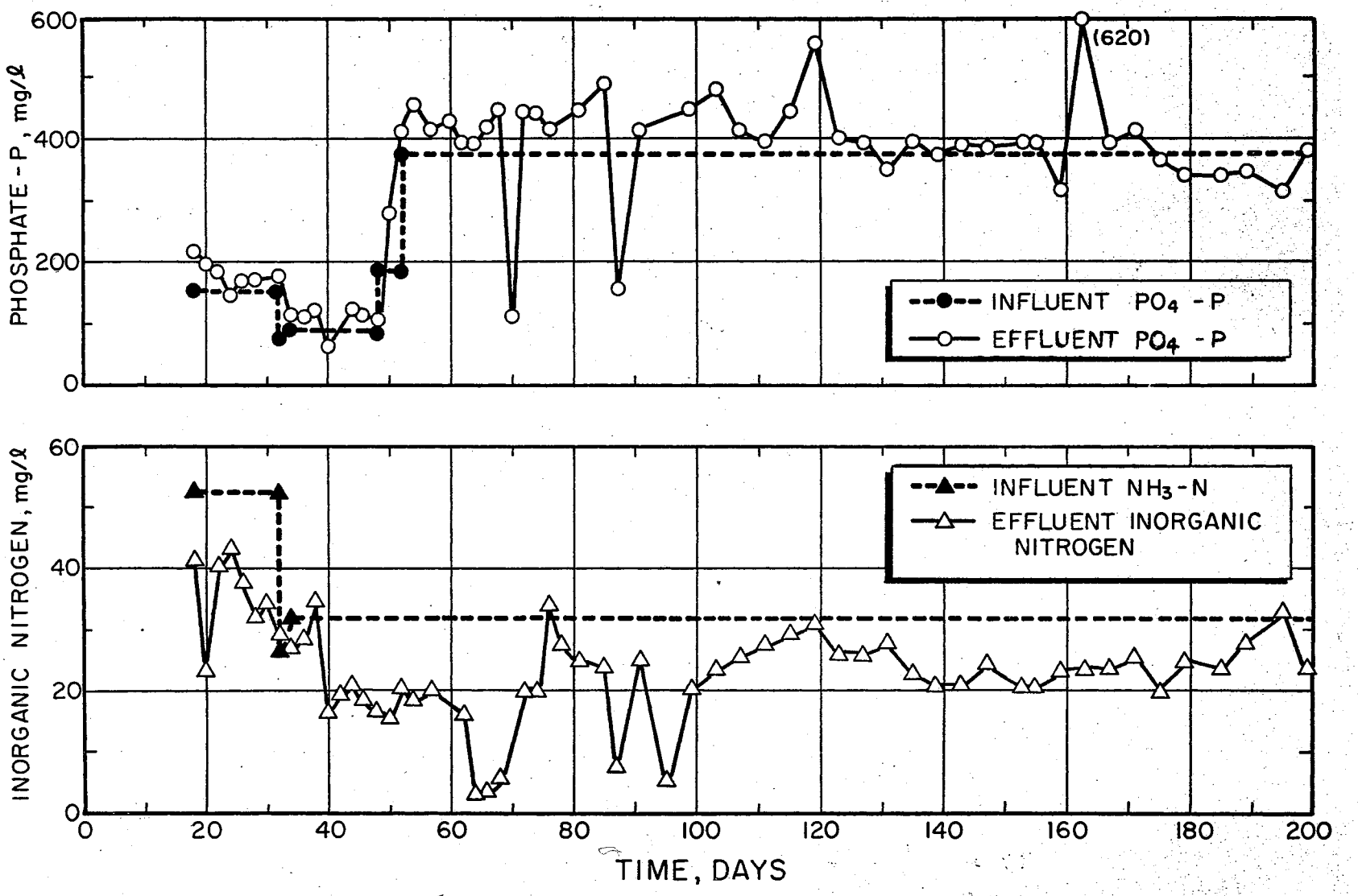

PHOSPHATE - P, mg/ℓ
600
400
200
0
(620)
INFLUENT PO4 - P
EFFLUENT PO4 - P
INORGANIC NITROGEN, mg/ℓ
60
40
20
0
INFLUENT NH3 - N
EFFLUENT INORGANIC NITROGEN
0
20
40
60
80
100
120
140
160
180
200
TIME, DAYS

Figure 39. Comparison of phosphorus and nitrogen in the feed and in the effluent of the extended aeration pilot plant (January 30, 1971, to July 27, 1971).

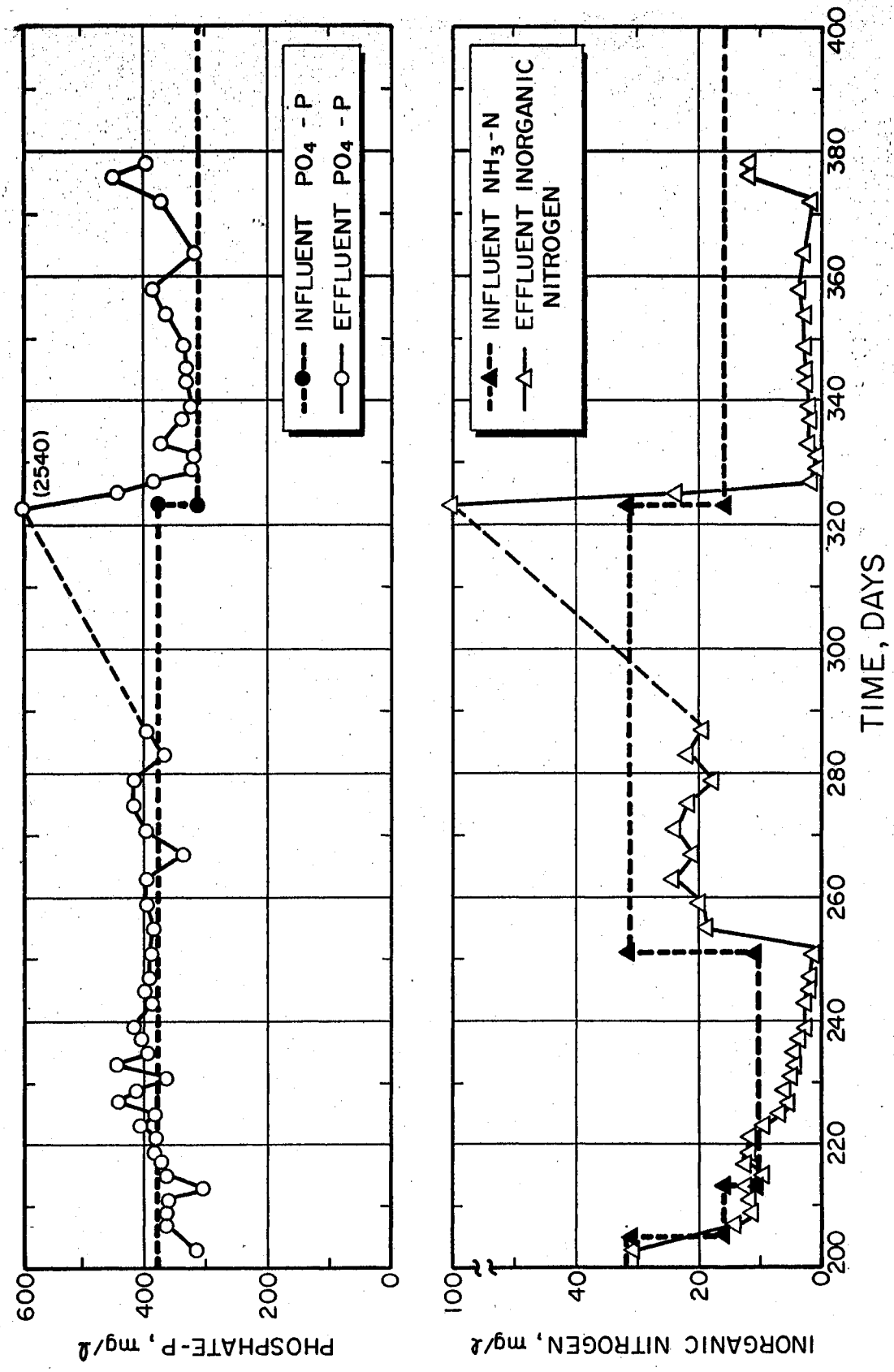
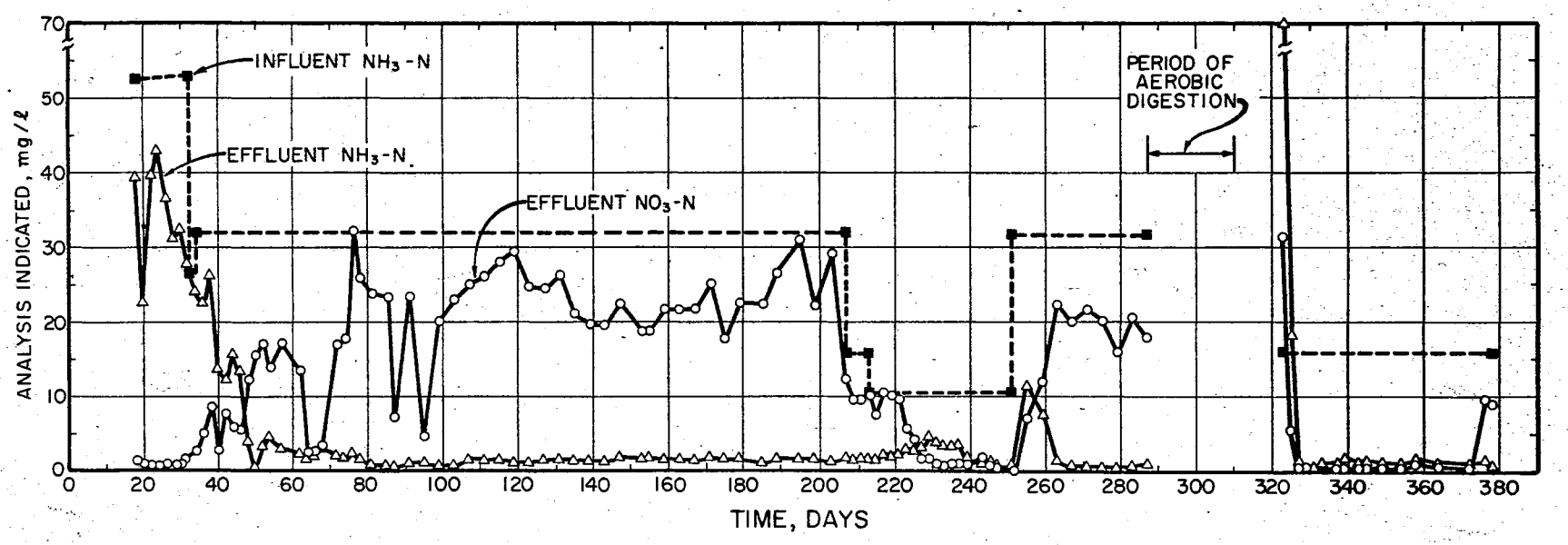

INFLUENT PO4 - P
EFFLUENT PO4 - P
(2540)
PHOSPHATE-P, mg/ℓ
600
400
200
0
INFLUENT NH3-N
EFFLUENT INORGANIC NITROGEN
INORGANIC NITROGEN, mg/ℓ
100
40
20
0
200
220
240
260
280
300
320
340
360
380
400
TIME, DAYS

Figure 40. Relating between NH_3-N in the feed, and NH_3-N and NO_3-N in the effluent of the extended pilot plant (August 1, 1970, to July 27, 1971).


INFLUENT NH_3-N
EFFLUENT NH_3-N
EFFLUENT NO_3-N
PERIOD OF AEROBIC DIGESTION
ANALYSIS INDICATED, mg/ℓ
TIME, DAYS
0
10
20
30
40
50
70
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
320
340
360
380

approximately two-thirds of the influent nitrogen appeared in the effluent stream from the system. It was, therefore, of interest to investigate the effect on the operational behavior of the system as well as on the concentration of inorganic nitrogen in the effluent of reducing the amount of ammonia nitrogen in the synthetic waste. On day 205 (see Figure 36), the ratio of ammonia nitrogen in the feed to glucose COD in the feed was decreased from 1/10 to 1/20. One week of operation at this nitrogen level did not appear to cause any drastic changes in the system, and on day 213 the N:glucose COD ratio was changed to 1:30. During the next 35 days of operation at this nitrogen level, the biological solids concentration remained relatively constant at approximately 2500 mg/l. Biochemical removal efficiency of the system fluctuated between 39 and 93 percent. Protein concentration of the sludge remained at approximately 40 percent. The biological solids concentration in the effluent ranged from 8 to 58 mg/l. On day 225, it was noted that the sludge in the settling chamber of the pilot plant began to exhibit characteristics of a bulking sludge. Microscopic examination indicated that filamentous organisms were increasing in the sludge. It was decided to increase the nitrogen:COD ratio to determine if such an increase would cause a shift in predominant species toward non-filamentous organisms. From day 251 to day 287, the nitrogen concentration was increased to the 1/10 level. It can be seen that during the ensuing 10-day period there were no drastic changes in system behavior due to the addition of the greater amount of ammonia nitrogen in the feed. Beginning on day 260, ferric sulfate was added periodically to the mixed liquor. These additions appeared to have an immediately beneficial effect on sludge settling, but the effect was not

long-lived. The system continued to provide good substrate removal efficiency; however, the supernatant layer in the settling chamber was very thin and the abundance of filamentous organisms remained. The relationship between influent and effluent concentrations of inorganic phosphorus and nitrogen throughout the period of operation will be discussed in a subsequent section.

From day 289 to day 310 (see Figures 36 and 37), the feeding pump was turned off, and the system was subjected to aerobic digestion for a three-week period to observe the effect of aerobic digestion on the predominance of filamentous organisms in the system. During this time, samples were not taken but frequent checks were made to assure that pH remained at the neutral level. Very little autodigestion occurred during the three-week autodigestive period. Biological solids concentration decreased by only approximately 6 percent. Filamentous organisms still existed quite abundantly in the system, and the light fluffy floc, characteristic of the sludge, was still in evidence. It was decided to determine whether withdrawal and hydrolysis of a massive amount of biological solids would be effective in providing some means of bioengineering control over biological predominance in the system. Accordingly, 4.7 liters of mixed liquor were withdrawn and subjected to acid hydrolysis. From day 310 through day 322 (see Figure 37), the system was operated as a batch process, and the neutralized hydrolysate was fed back to the aeration chamber along with glucose (details of the batch feeding procedure are shown at the top of the graph). During this period, residual glucose COD in the system accumulated to 125 mg/l. The accumulation of residual COD under batch conditions has been discussed previously (27)(44). At the end of this two-week period of batch

operation there was a decrease in the amount of filamentous organisms present in the system, and the filaments which were present seemed to be considerably shorter than those which existed in the system prior to the withdrawal and hydrolysis of this large portion of biological solids.

On day 323, continuous flow operation was initiated using a feed of 500 mg/l glucose and a ratio of nitrogen:COD = 1:20. The biological solids gradually rose to 5600 mg/l by day 345. The biochemical purification efficiency during this period was above 90 percent, and the biological solids concentration in the effluent was below 15 mg/l. During this period, some filamentous organisms still existed in the system, but the sludge settled excellently. Carbohydrate content of the sludge remained at approximately 10 percent, but there was a slight increase in the protein content. Microscopic examination of the sludge indicated that short filaments were present, but unlike the previous observations, there were now many individual bacterial cells which appeared to be adsorbed on the surface of the filamentous forms.

On day 351, 750 ml of sludge from the settling chamber was removed, hydrolyzed, and fed to the system as a slug dose in a batch experiment. The system responded rather successfully to this shock loading, although it is seen that the residual COD on the following day (352) did exhibit a slightly higher value than before the shock loading experiment. Multiple samples were taken during the batch feeding experiment to assess the immediate response to the shock load, and the detailed results are presented along with those of other batch experiments in the series of Figures 42 through 53, which are presented later in this section (see Figure 52 for results of the batch shock loading experiment on day 351). After resumption of the normal feedings schedule, the system performed

rather well; the biochemical purification efficiency was above 95 percent, and it can be seen from Figure 37 that only small amounts of biological solids appeared in the effluent.

A final experiment on the proposed process modification was initiated on day 368 with the removal of a rather large volume of sludge (3.5 liters). It was decided to feed this material back to the system over a rather short period of time to gain an insight into the ability of the system to accommodate hydrolysate feedback. From day 370 to day 376, hydrolysate was fed back to the system 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, the biological solids concentration in the effluent and the unfiltered COD, i.e., supernatant COD or effluent COD, remained rather 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 ten days (from day 378 to day 388), synthetic waste was fed and the biological solids accumulated to approximately 6000 mg/l. Chemical removal efficiency of 97 percent efficiency was again attained. At this time, pilot plant operation was terminated, and the extended aeration sludge was hydrolyzed and stored for possible future experimentations.

4. Effluent Characteristics With Regard to PO_4-P, NH_3-N, NO_3-N, NO_2-N, and Total Inorganic Nitrogen in the Pilot Plant

Data for phosphorus and inorganic nitrogen throughout the period of pilot plant operation were shown in Figures 38, 39, and 40. Since phosphate buffer was used to maintain the pH during the pilot plant study, it was expected that the phosphorus in the effluent would be approximately equal to the phosphorus in the influent, unless there was a significant "luxury uptake" of phosphorus in this type of activated sludge system. The results indicate that there was essentially no luxury uptake of phosphorus throughout the period of study.

During the first 205 days of operation, the system was operated at an NH_3-N:glucose COD ratio of 1:10, and it is seen that approximately two-thirds of the ammonia fed to the system appeared as total inorganic nitrogen in the effluent. The nitrogen:COD ratio was changed on day 205 to 1:20, and on day 213, to 1:30, and was maintained at this ratio until day 251. After one week of operation at the nitrogen:COD ratio of 1:30, the effluent contained approximately the same concentration of inorganic nitrogen as was being fed in the form of ammonia nitrogen in the influent. During this week of operation, the biological solids concentration in the aerator was decreasing from 3200 mg/l to 2500 mg/l (see days 213 to 221, Figure 36). It would thus appear that the inorganic nitrogen released due to the decrease in solids was sufficient to provide for the nitrogen requirement of the system, and there was no net utilization of nitrogen. In succeeding days of operation at this nitrogen:COD level, the total inorganic nitrogen concentration in the effluent decreased until by day 251 it amounted to only two percent of the influent inorganic nitrogen. On day 251, the nitrogen concentration

was increased to yield the initial nitrogen:COD ratio of 1:10, and again approximately two-thirds of the influent concentration was registered as total inorganic nitrogen in the effluent. From day 287 to day 310, the system was not fed, and was subjected to continuous aeration and pH adjustment. Following this, it was operated on a batch basis and was fed a combination of glucose and cell hydrolysate. The rise in phosphorus and nitrogen content was due largely to the batch operation.

From day 323 to day 370, the N:COD ratio was fixed at 1:20, and the inorganic nitrogen appearing in the effluent amounted to approximately 20 percent of the inorganic nitrogen in the feed. Following this, the unit was fed a mixture of glucose and hydrolysate, and the ratio of nitrogen:COD in the feed was maintained at 1:20, and it can be seen that the concentration of inorganic nitrogen in the effluent increased.

5. Sludge Characterization Including Endogenous O_2 Uptake Rate, Substrate Removal Rate, Specific Growth Rate, Yield, and Lag Time

Pertinent results from the auxiliary experiments which were conducted on the extended aeration activated sludge during the period of operation using the hydrolytic assist modification are summarized in Figure 41, and the individual experiments from which the summary graphs were plotted are shown in Figures 42 through 53. The experiments used in the summary graphs of Figure 41 were obtained only from the experiments labeled "low initial solids" in Figures 42 through 53; all experiments labeled "high initial solids" were conducted under batch feeding conditions in the pilot plant on the day indicated in Figure 37.

From Figure 41, it is seen that most of the cell yield values fall between 40 and 60 percent. The mean cell yield, $\bar{Y}$, the standard deviation, s, the coefficient of variance, CV, and the 95 percent

Figure 41. Effect of aging on cell yield, specific substrate utilization rate (SSUR), specific growth rate, endogenous O_2 uptake, and lag time of extended aeration activated sludge.

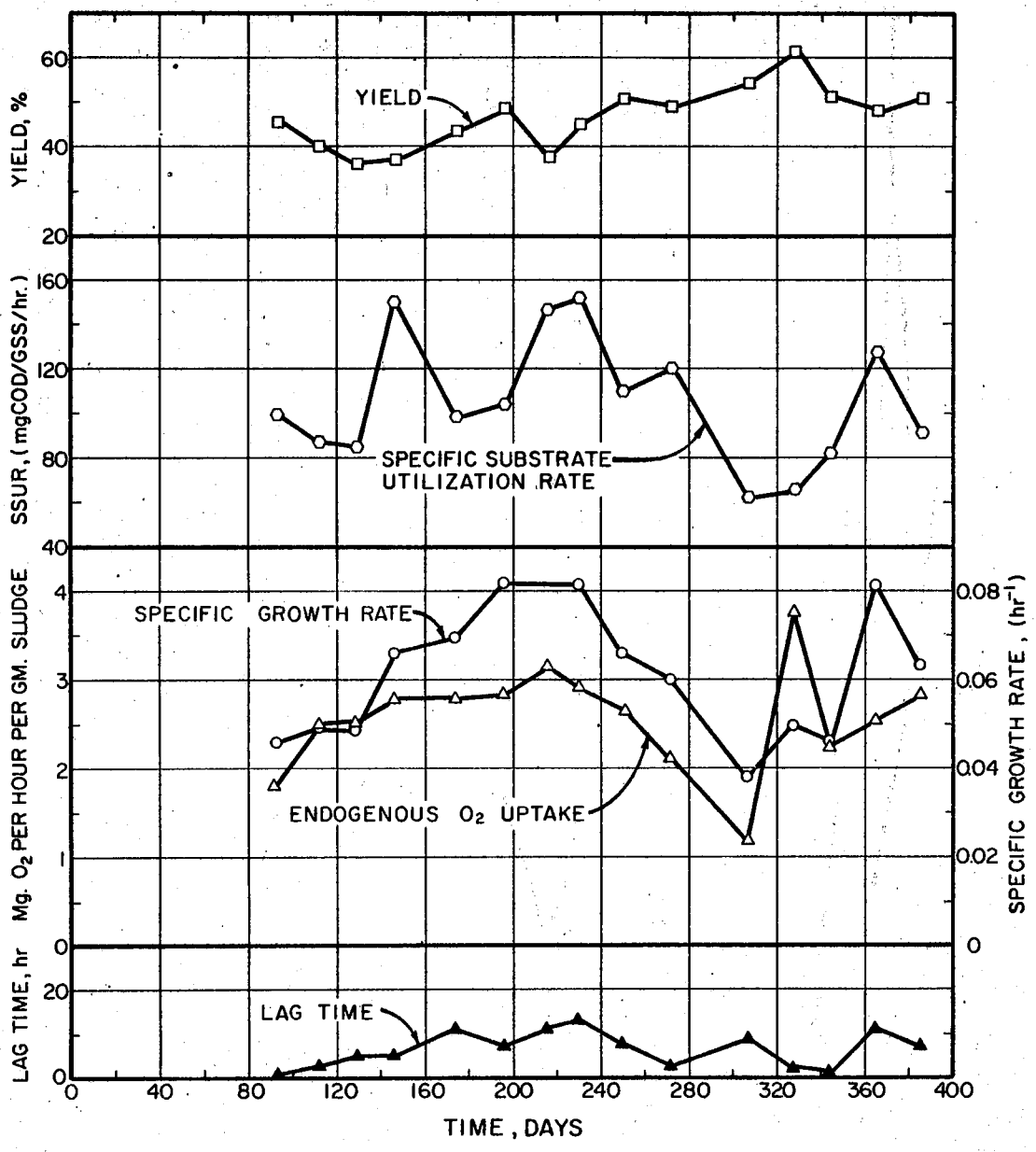
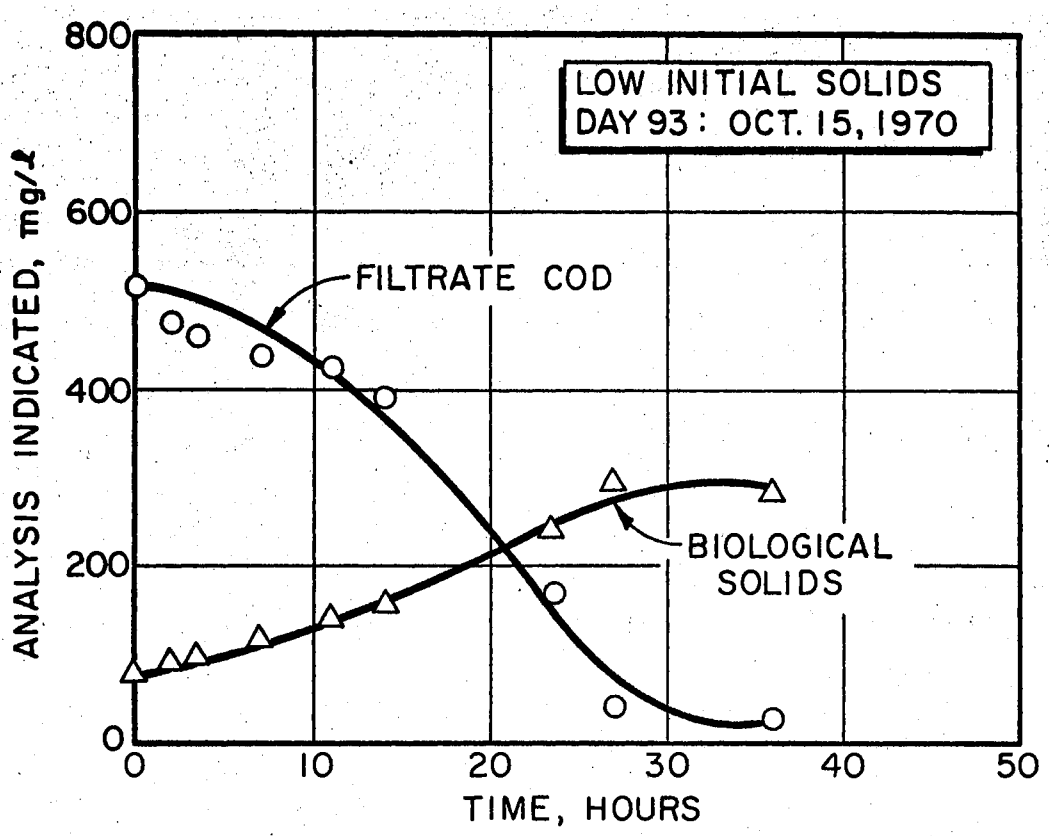

YIELD, %
YIELD
SSUR, (mgCOD/GSS/hr.)
SPECIFIC SUBSTRATE UTILIZATION RATE
Mg. O2 PER HOUR PER GM. SLUDGE
SPECIFIC GROWTH RATE
ENDOGENOUS O2 UPTAKE
SPECIFIC GROWTH RATE, (hr-1)
LAG TIME, hr
LAG TIME
TIME, DAYS

Figure 42. Response of the extended aeration activated sludge to a slug dose of glucose after 93 days of operation and after 112 days of operation.


LOW INITIAL SOLIDS
DAY 93 : OCT. 15, 1970
FILTRATE COD
BIOLOGICAL SOLIDS
ANALYSIS INDICATED, mg/ℓ
TIME, HOURS
0
200
400
600
800
0
10
20
30
40
50

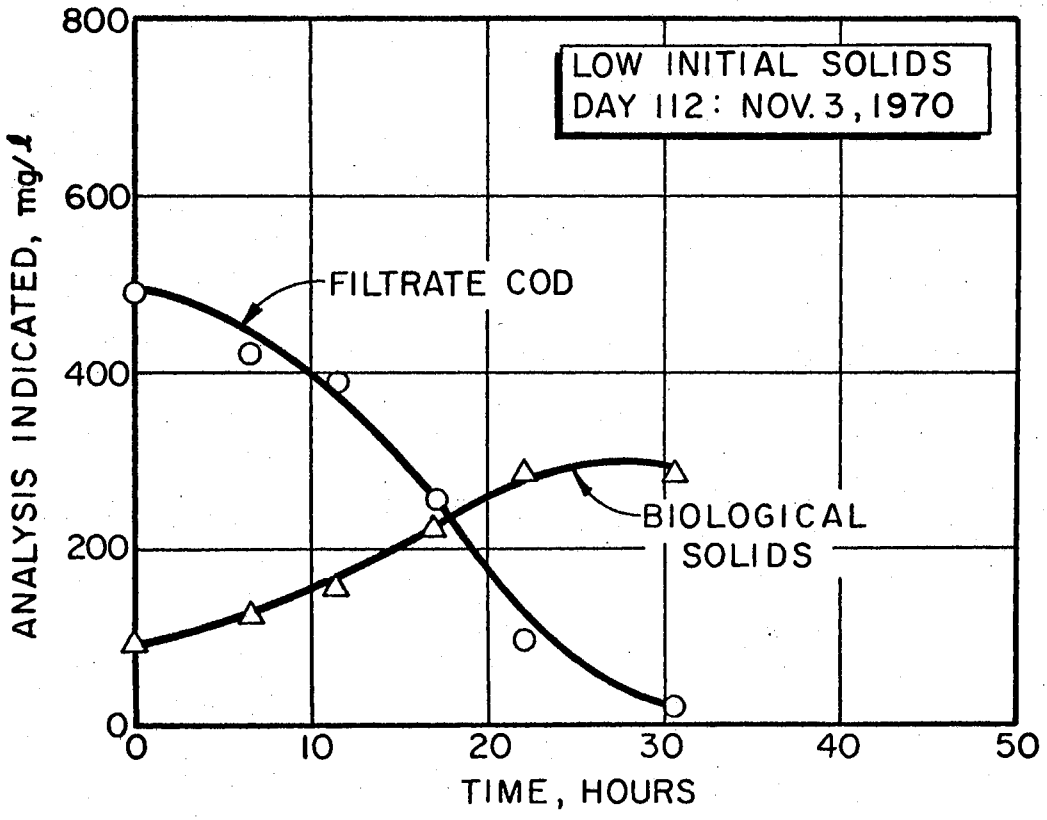
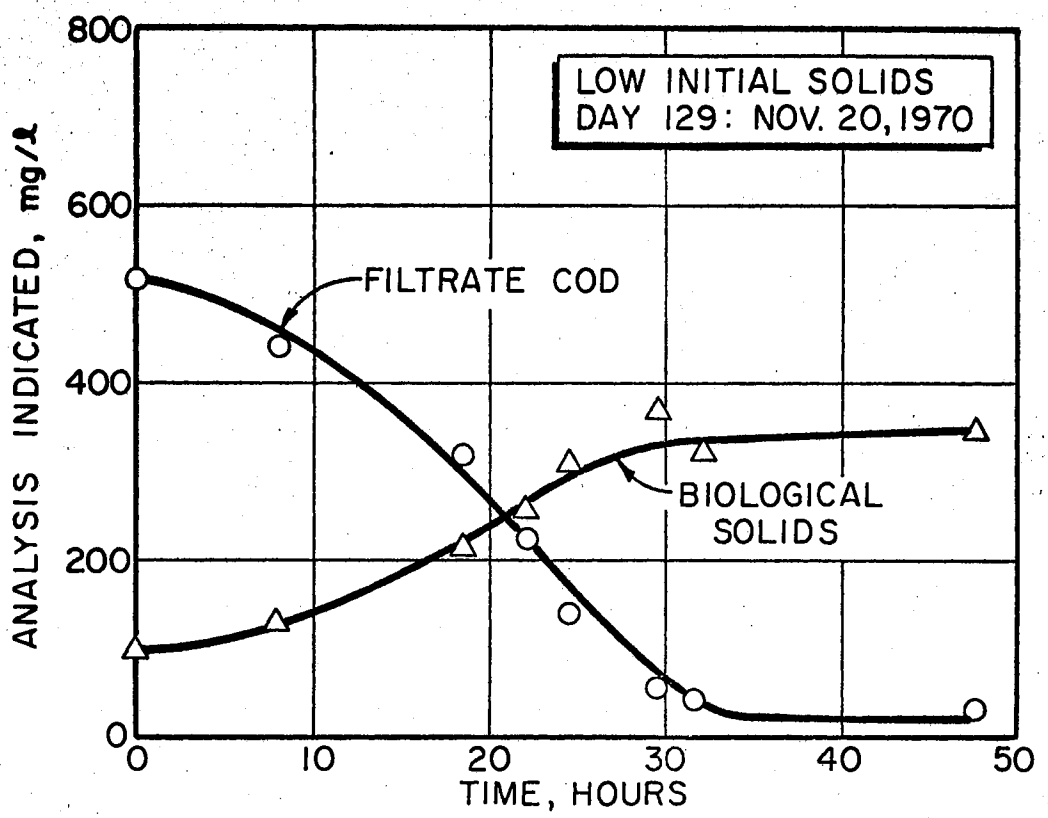

LOW INITIAL SOLIDS
DAY 112 : NOV. 3, 1970
FILTRATE COD
BIOLOGICAL SOLIDS
ANALYSIS INDICATED, mg/ℓ
TIME, HOURS
0
200
400
600
800
0
10
20
30
40
50

Figure 43. Response of the extended aeration activated sludge to a slug dose of glucose after 129 days of operation and after 146 days of operation.


LOW INITIAL SOLIDS
DAY 129: NOV. 20, 1970
FILTRATE COD
BIOLOGICAL SOLIDS
ANALYSIS INDICATED, mg/ℓ
TIME, HOURS
0
200
400
600
800
0
10
20
30
40
50

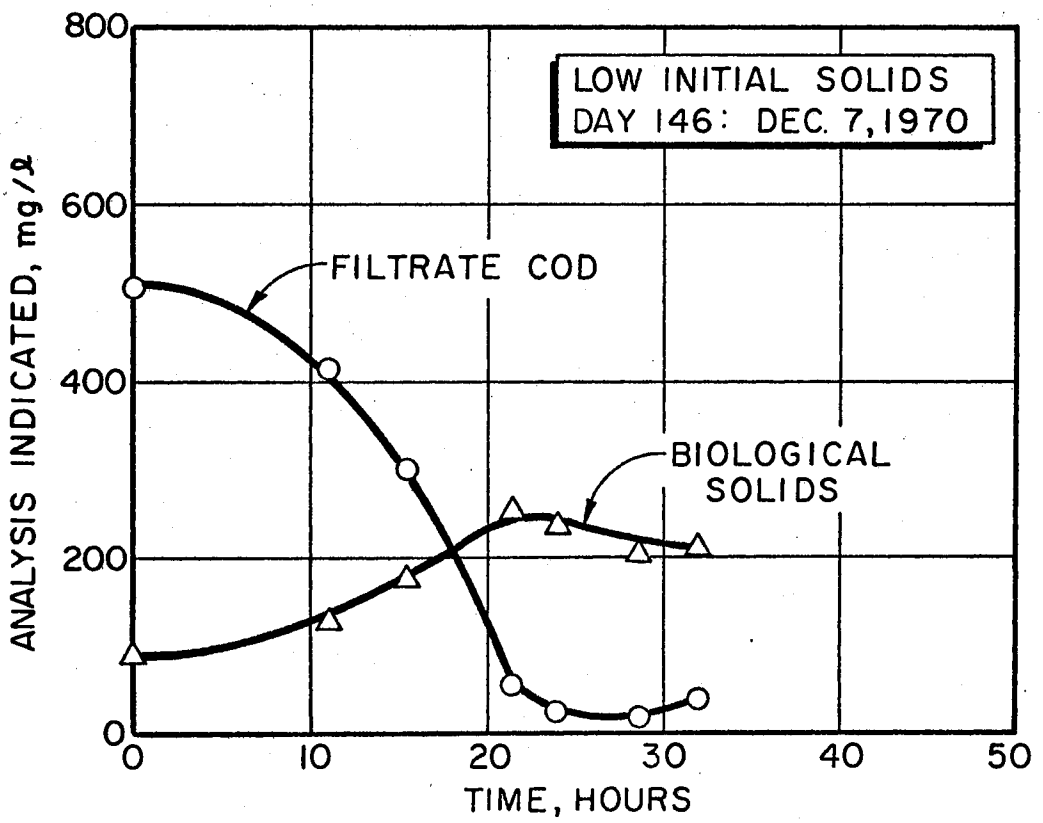
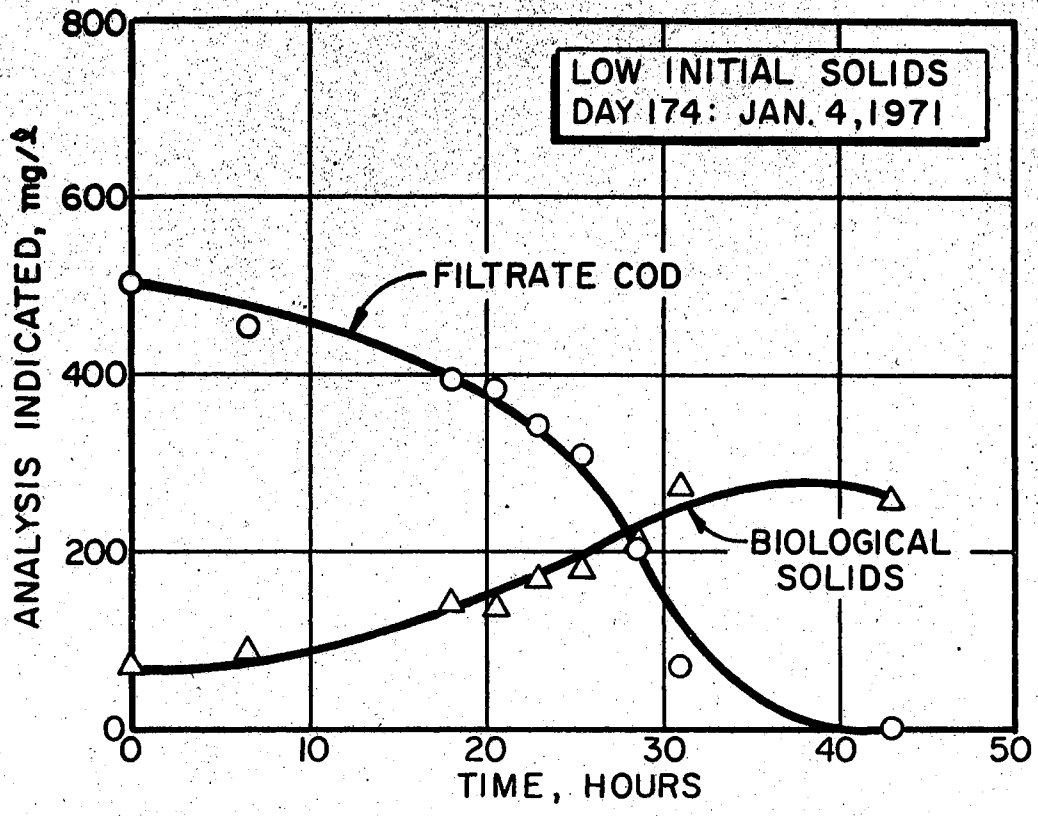

LOW INITIAL SOLIDS
DAY 146: DEC. 7, 1970
FILTRATE COD
BIOLOGICAL SOLIDS
ANALYSIS INDICATED, mg/ℓ
TIME, HOURS
0
200
400
600
800
0
10
20
30
40
50

Figure 44. Response of the extended aeration activated sludge to a slug dose of glucose after 174 days of operation and after 196 days of operation.


LOW INITIAL SOLIDS
DAY 174: JAN. 4, 1971
FILTRATE COD
BIOLOGICAL SOLIDS
ANALYSIS INDICATED, mg/ℓ
TIME, HOURS
0
200
400
600
800
0
10
20
30
40
50

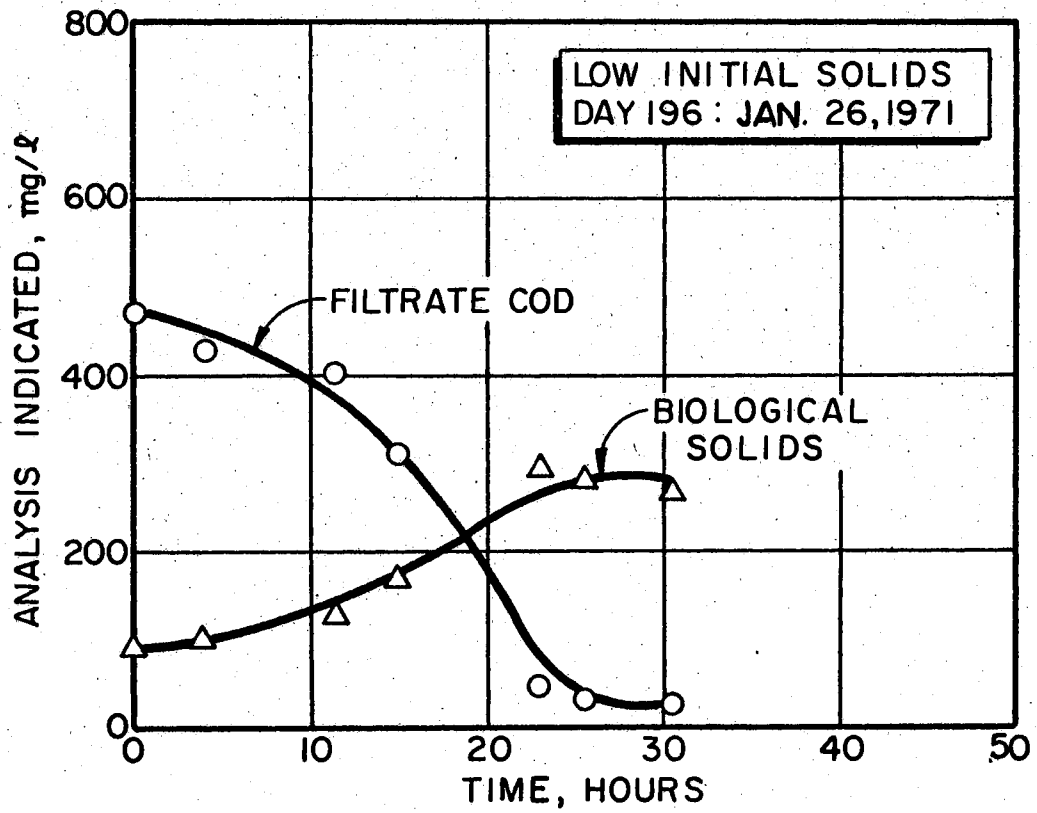
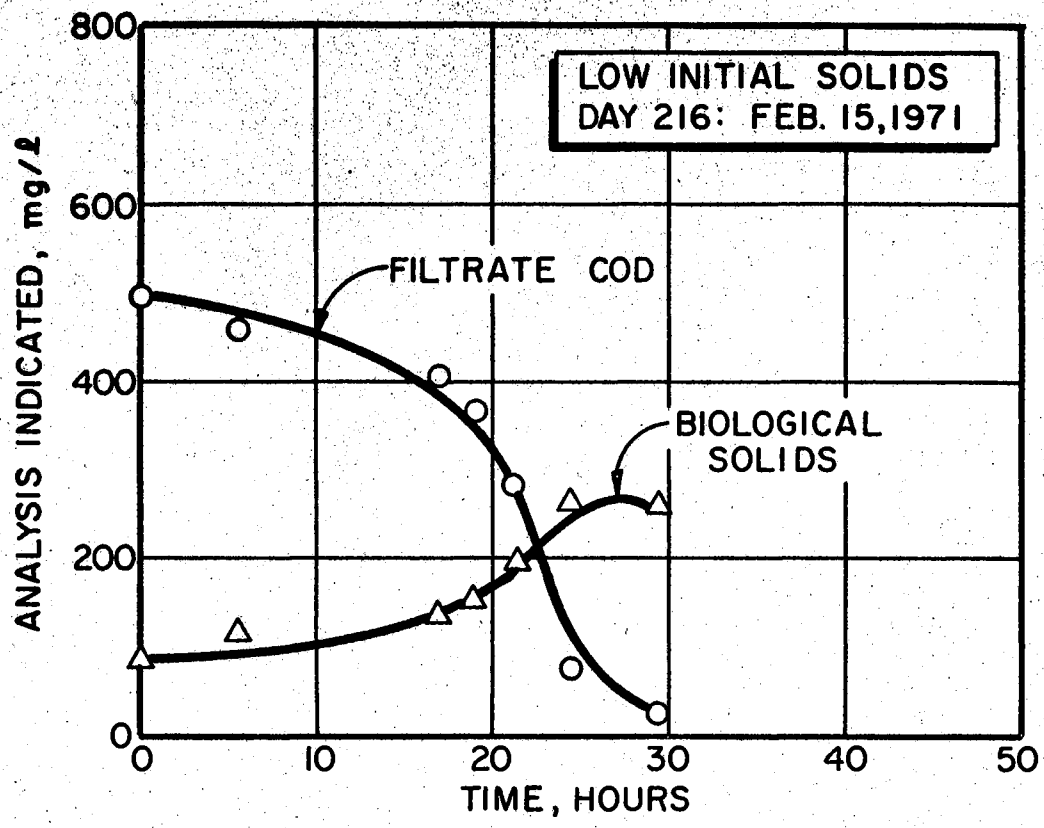

LOW INITIAL SOLIDS
DAY 196 : JAN. 26, 1971
FILTRATE COD
BIOLOGICAL SOLIDS
ANALYSIS INDICATED, mg/ℓ
TIME, HOURS
0
200
400
600
800
0
10
20
30
40
50

Figure 45. Response of the extended aeration activated sludge to a slug dose of glucose after 216 days of operation and after 230 days of operation.


LOW INITIAL SOLIDS
DAY 216: FEB. 15,1971
FILTRATE COD
BIOLOGICAL SOLIDS
ANALYSIS INDICATED, mg/ℓ
TIME, HOURS
0
200
400
600
800
0
10
20
30
40
50

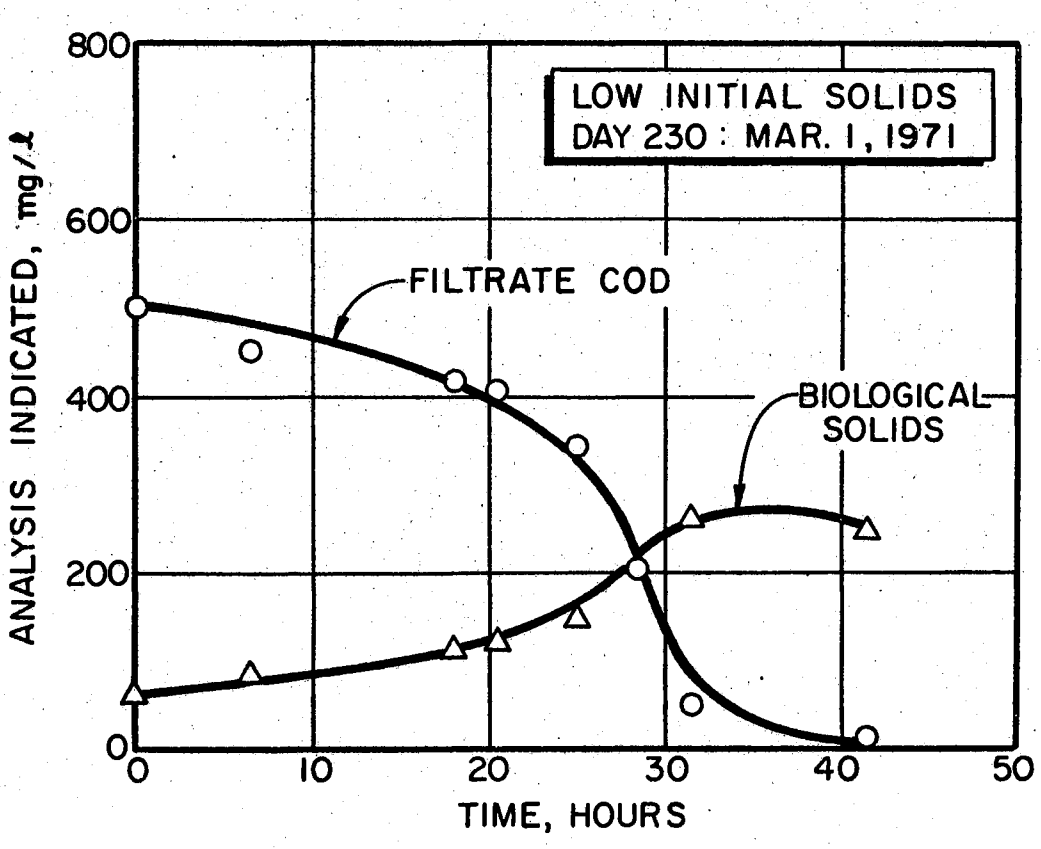
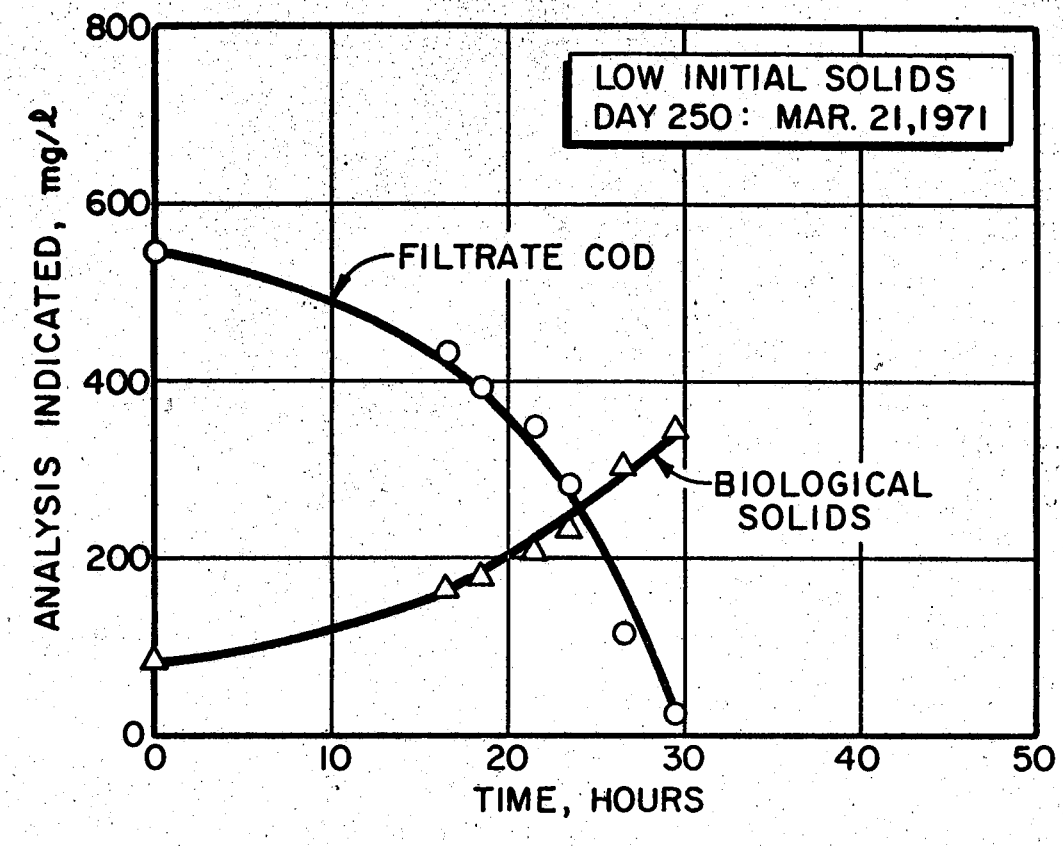

LOW INITIAL SOLIDS
DAY 230 : MAR. 1, 1971
FILTRATE COD
BIOLOGICAL SOLIDS
ANALYSIS INDICATED, mg/ℓ
TIME, HOURS
0
200
400
600
800
0
10
20
30
40
50

Figure 46. Response of the extended aeration activated sludge to a slug dose of glucose after 250 days of operation and after 272 days of operation.


LOW INITIAL SOLIDS
DAY 250 : MAR. 21,1971
FILTRATE COD
BIOLOGICAL SOLIDS
ANALYSIS INDICATED, mg/l
TIME, HOURS
0
200
400
600
800
0
10
20
30
40
50

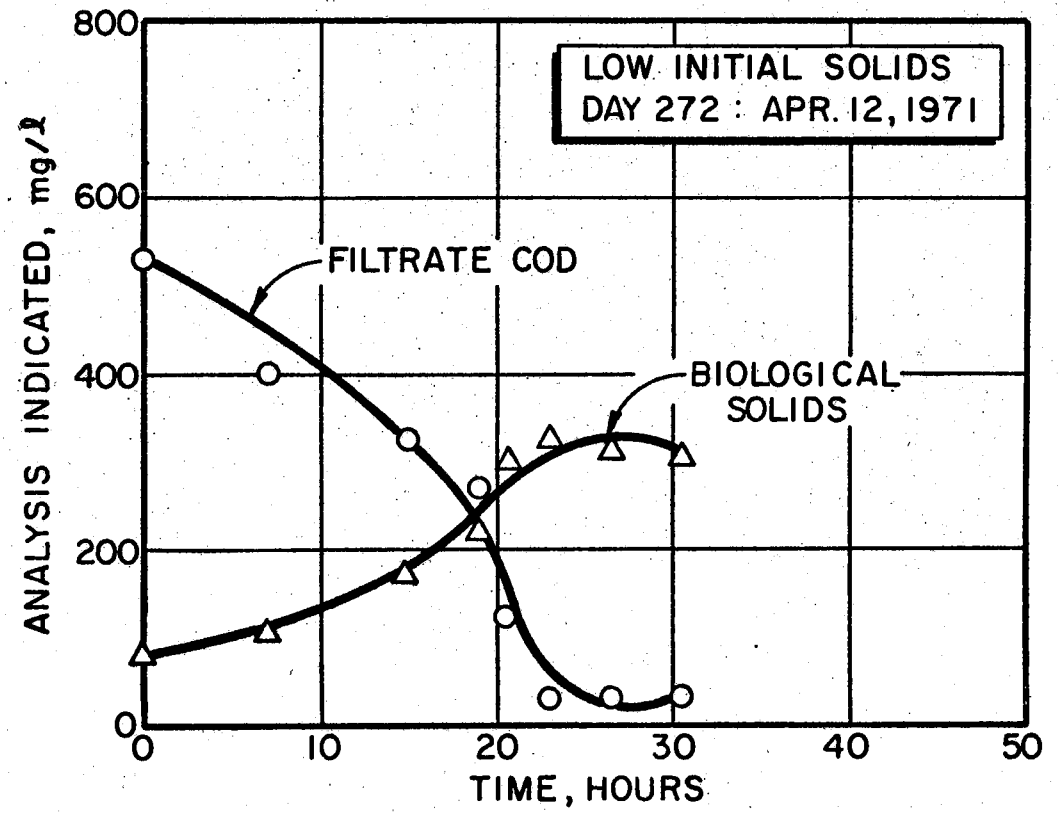
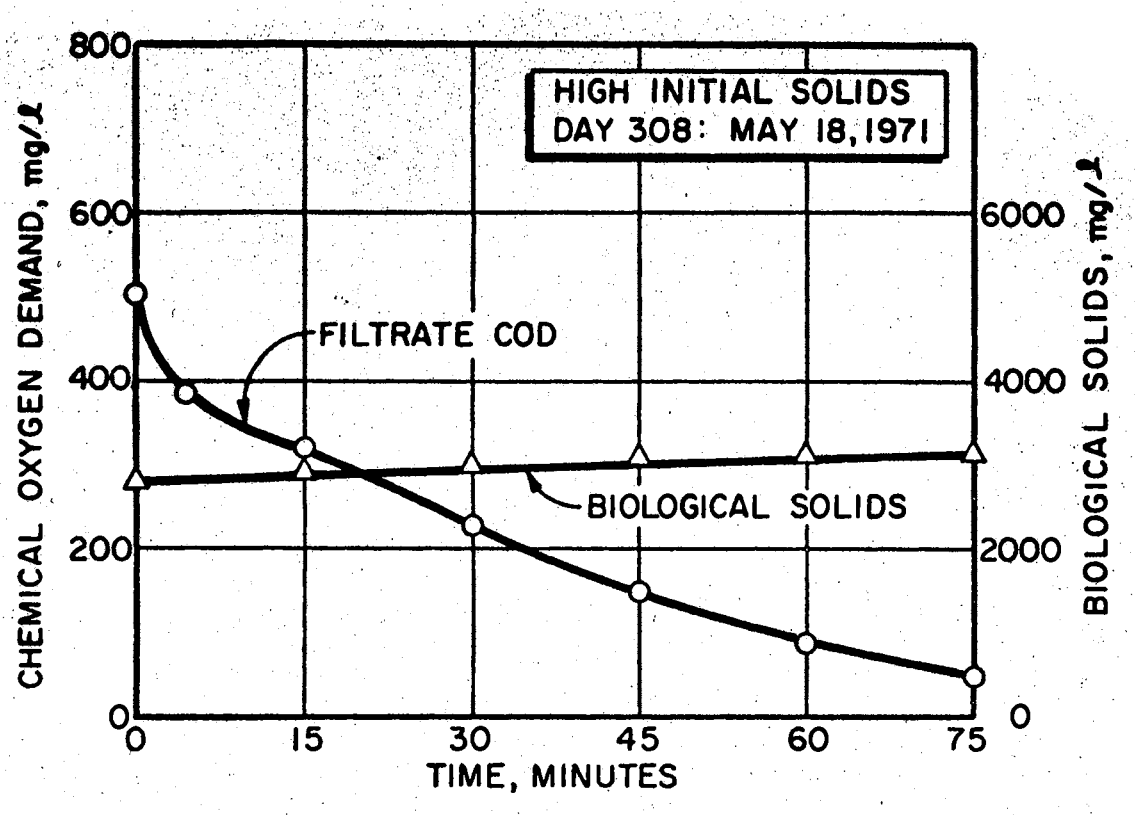

LOW INITIAL SOLIDS
DAY 272 : APR. 12, 1971
FILTRATE COD
BIOLOGICAL SOLIDS
ANALYSIS INDICATED, mg/l
TIME, HOURS
0
200
400
600
800
0
10
20
30
40
50

Figure 47. Response of the extended aeration activated sludge to a slug dose of glucose after 307 days of operation and after 308 days of operation.

(High initial batch experiment was conducted in the extended aeration pilot plant.)


HIGH INITIAL SOLIDS
DAY 308: MAY 18, 1971
FILTRATE COD
BIOLOGICAL SOLIDS
CHEMICAL OXYGEN DEMAND, mg/l
BIOLOGICAL SOLIDS, mg/l
TIME, MINUTES
0
200
400
600
800
2000
4000
6000
15
30
45
60
75

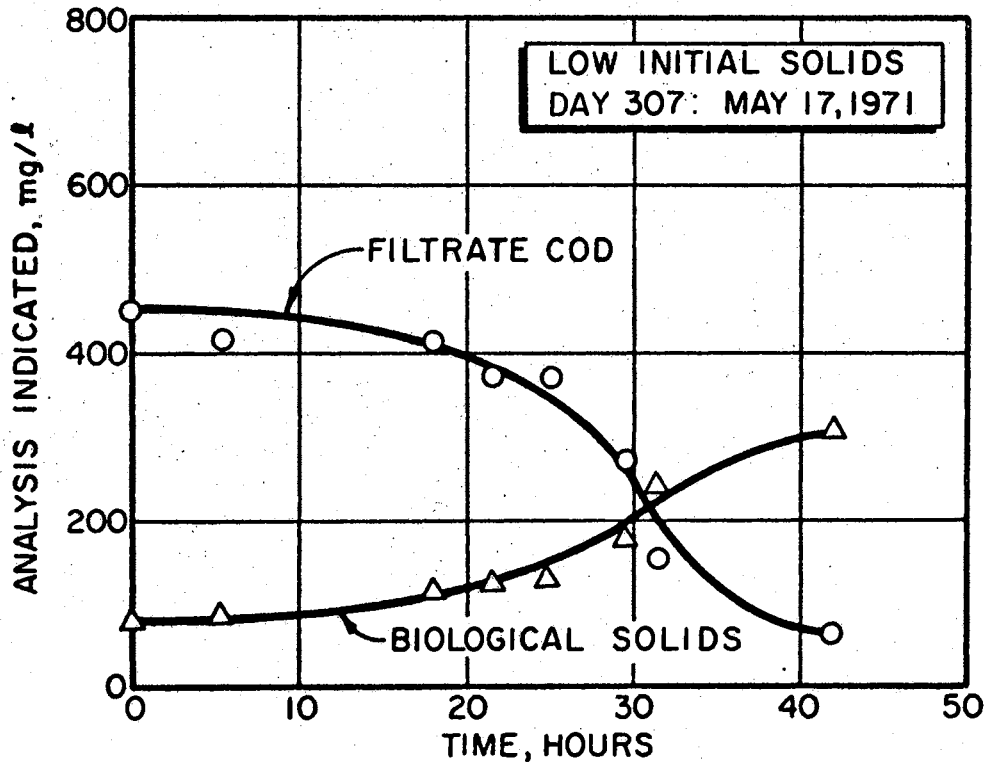

LOW INITIAL SOLIDS
DAY 307: MAY 17, 1971
FILTRATE COD
BIOLOGICAL SOLIDS
ANALYSIS INDICATED, mg/l
TIME, HOURS
0
200
400
600
800
10
20
30
40
50

Figure 48. Response of the extended aeration activated sludge to a slug dose of glucose after 316 days of operation.

(Experiment was conducted in the extended aeration pilot plant.)

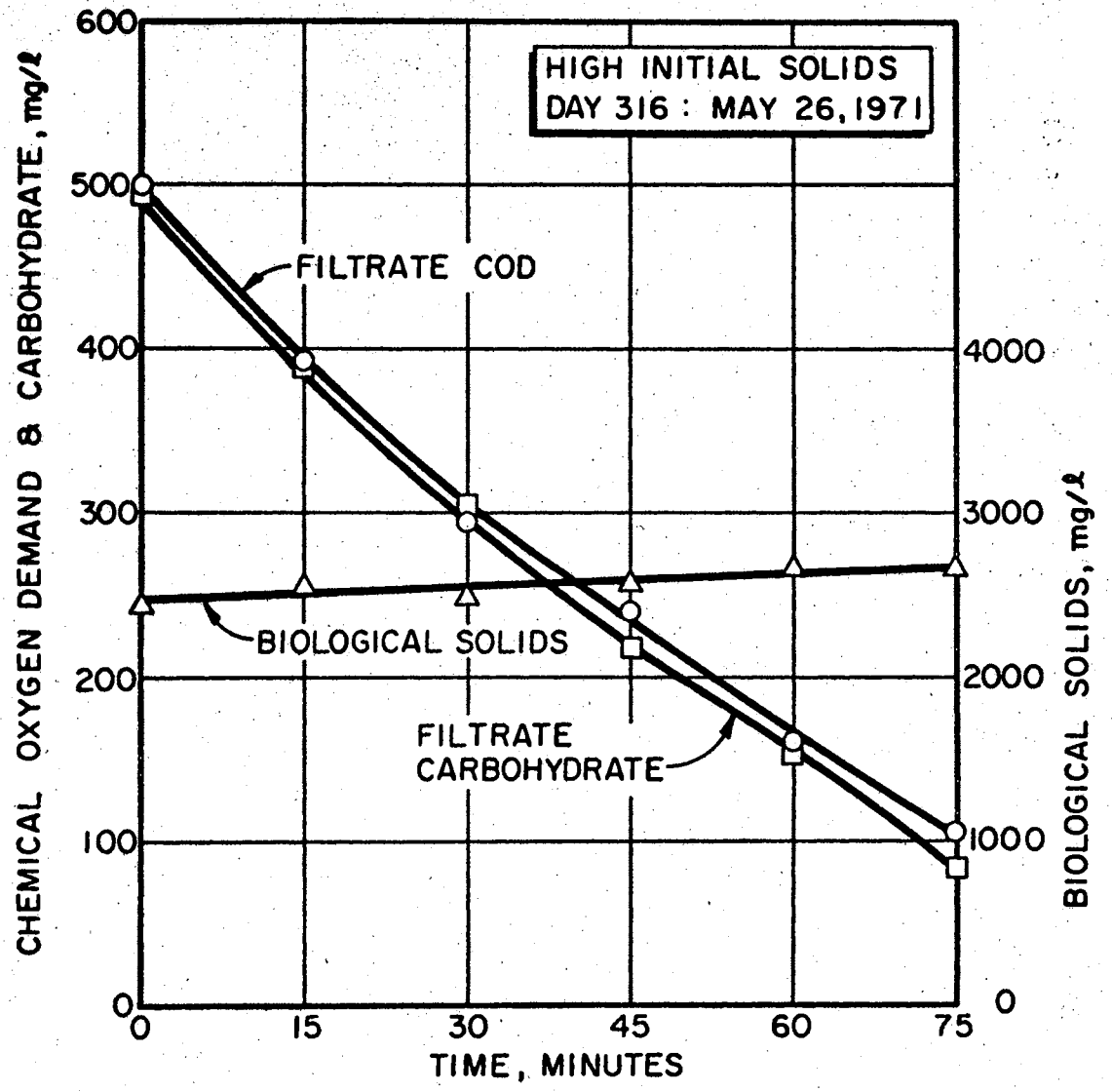

HIGH INITIAL SOLIDS
DAY 316 : MAY 26, 1971
FILTRATE COD
BIOLOGICAL SOLIDS
FILTRATE
CARBOHYDRATE
CHEMICAL OXYGEN DEMAND & CARBOHYDRATE, mg/ℓ
BIOLOGICAL SOLIDS, mg/ℓ
TIME, MINUTES
600
500
400
300
200
100
0
4000
3000
2000
1000
0
0
15
30
45
60
75

Figure 49. Response of the extended aeration activated sludge to a slug dose consisting of glucose (COD = 250 mg/l) and sludge hydrolysate (COD = 250 mg/l), after 317 days of operation.

(Experiment was conducted in the extended aeration pilot plant.)

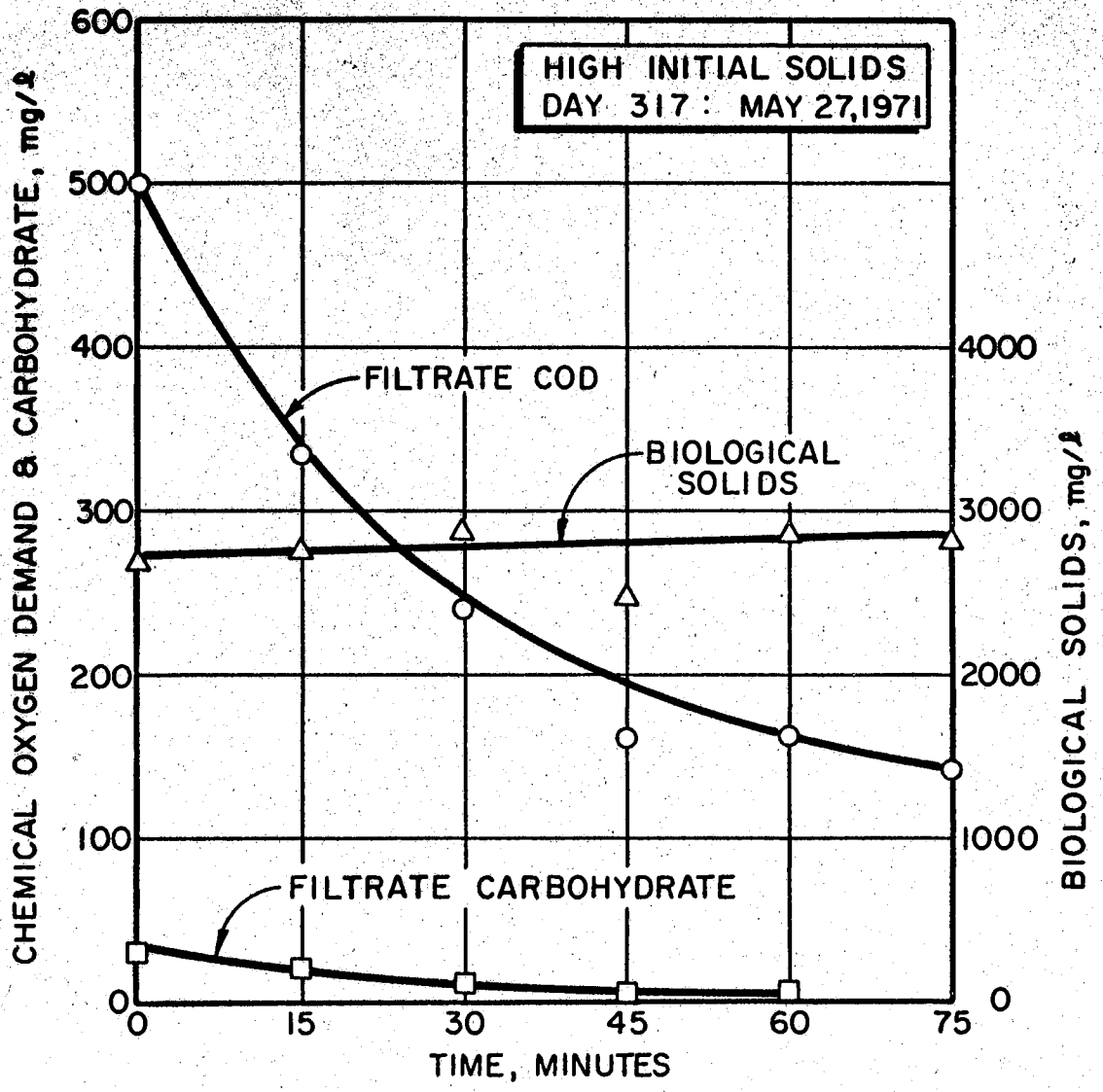

HIGH INITIAL SOLIDS
DAY 317 : MAY 27,1971
FILTRATE COD
BIOLOGICAL SOLIDS
FILTRATE CARBOHYDRATE
CHEMICAL OXYGEN DEMAND & CARBOHYDRATE, mg/ℓ
BIOLOGICAL SOLIDS, mg/ℓ
TIME, MINUTES
600
500
400
300
200
100
0
4000
3000
2000
1000
0
0
15
30
45
60
75

Figure 50. Response of the extended aeration activated sludge to a slug dose of sludge hydrolysate after 318 days of operation.

(Experiment was conducted in the extended aeration pilot plant.)

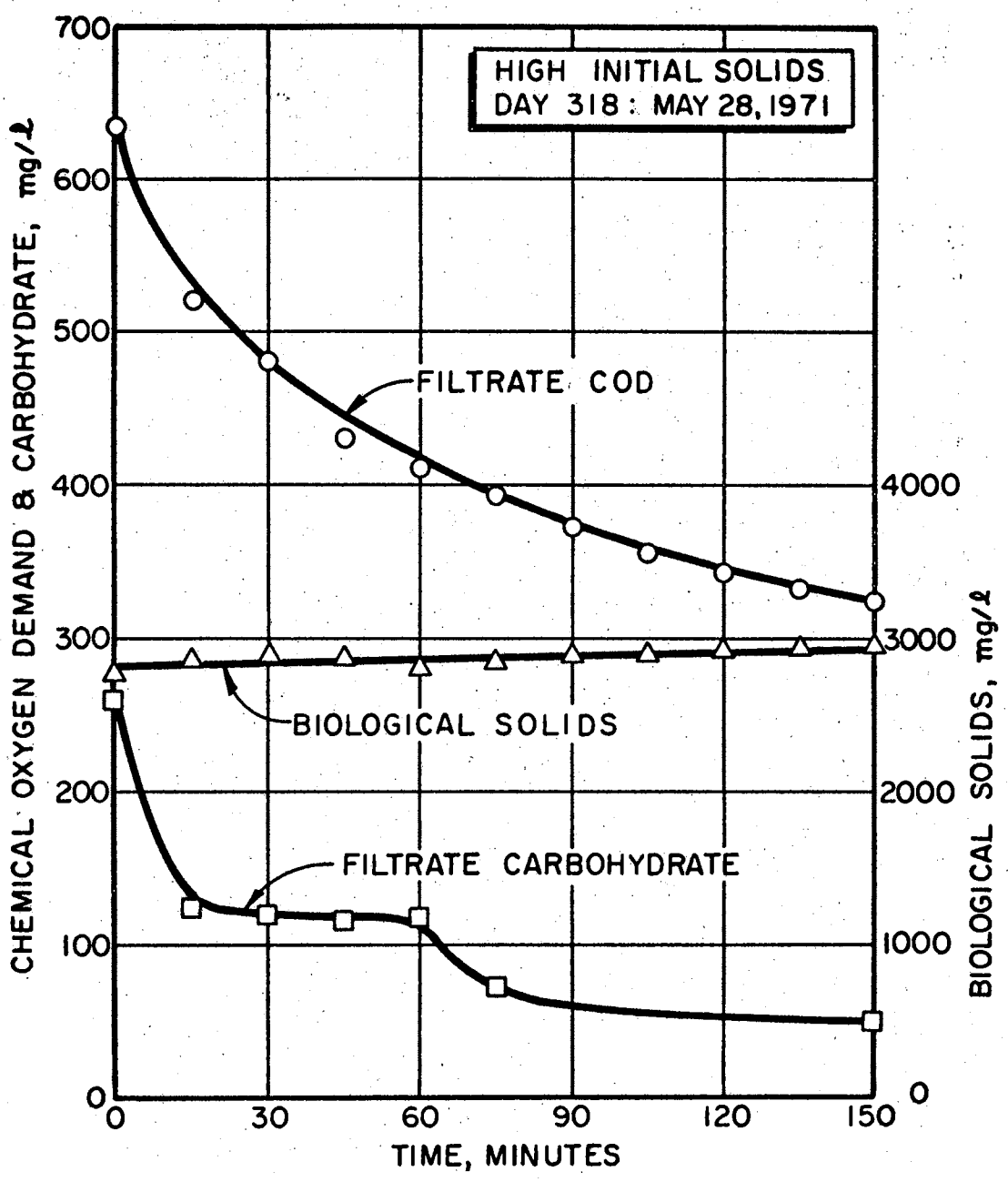
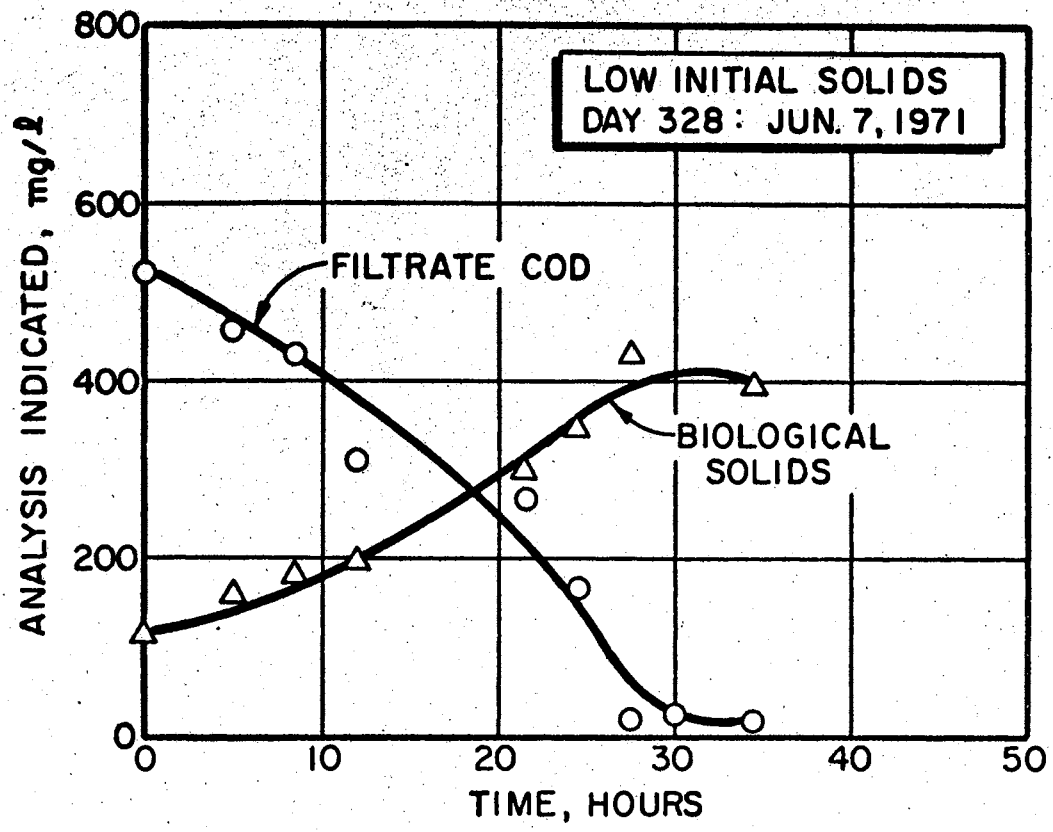

HIGH INITIAL SOLIDS
DAY 318 : MAY 28, 1971
FILTRATE COD
BIOLOGICAL SOLIDS
FILTRATE CARBOHYDRATE
CHEMICAL OXYGEN DEMAND & CARBOHYDRATE, mg/ℓ
BIOLOGICAL SOLIDS, mg/ℓ
TIME, MINUTES
0
100
200
300
400
500
600
700
1000
2000
3000
4000
0
30
60
90
120
150

Figure 51. Response of the extended aeration activated sludge to a slug dose of glucose after 328 days of operation and after 344 days of operation.


LOW INITIAL SOLIDS
DAY 328 : JUN. 7, 1971
FILTRATE COD
BIOLOGICAL SOLIDS
ANALYSIS INDICATED, mg/ℓ
TIME, HOURS
0
200
400
600
800
0
10
20
30
40
50

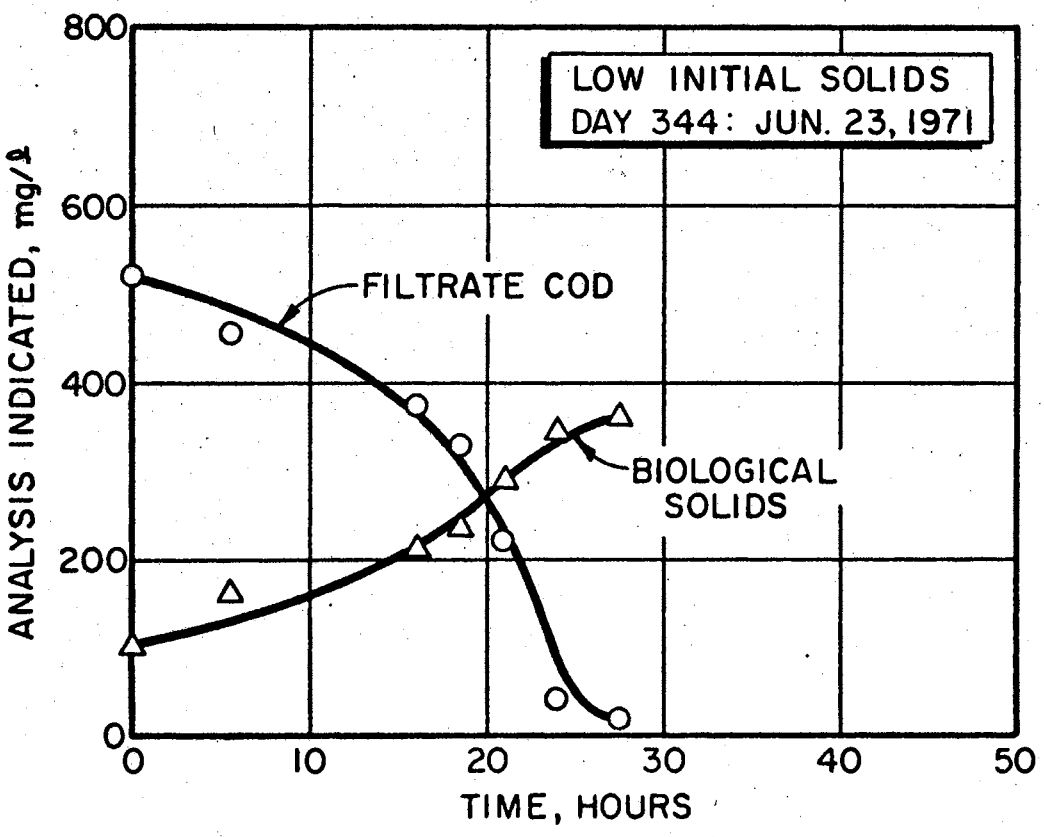

LOW INITIAL SOLIDS
DAY 344 : JUN. 23, 1971
FILTRATE COD
BIOLOGICAL SOLIDS
ANALYSIS INDICATED, mg/ℓ
TIME, HOURS
0
200
400
600
800
0
10
20
30
40
50

Figure 52. Response of the extended aeration activated sludge to a slug dose of sludge hydrolysate after 351 days of operation.

(Experiment was conducted in the extended aeration pilot plant.)

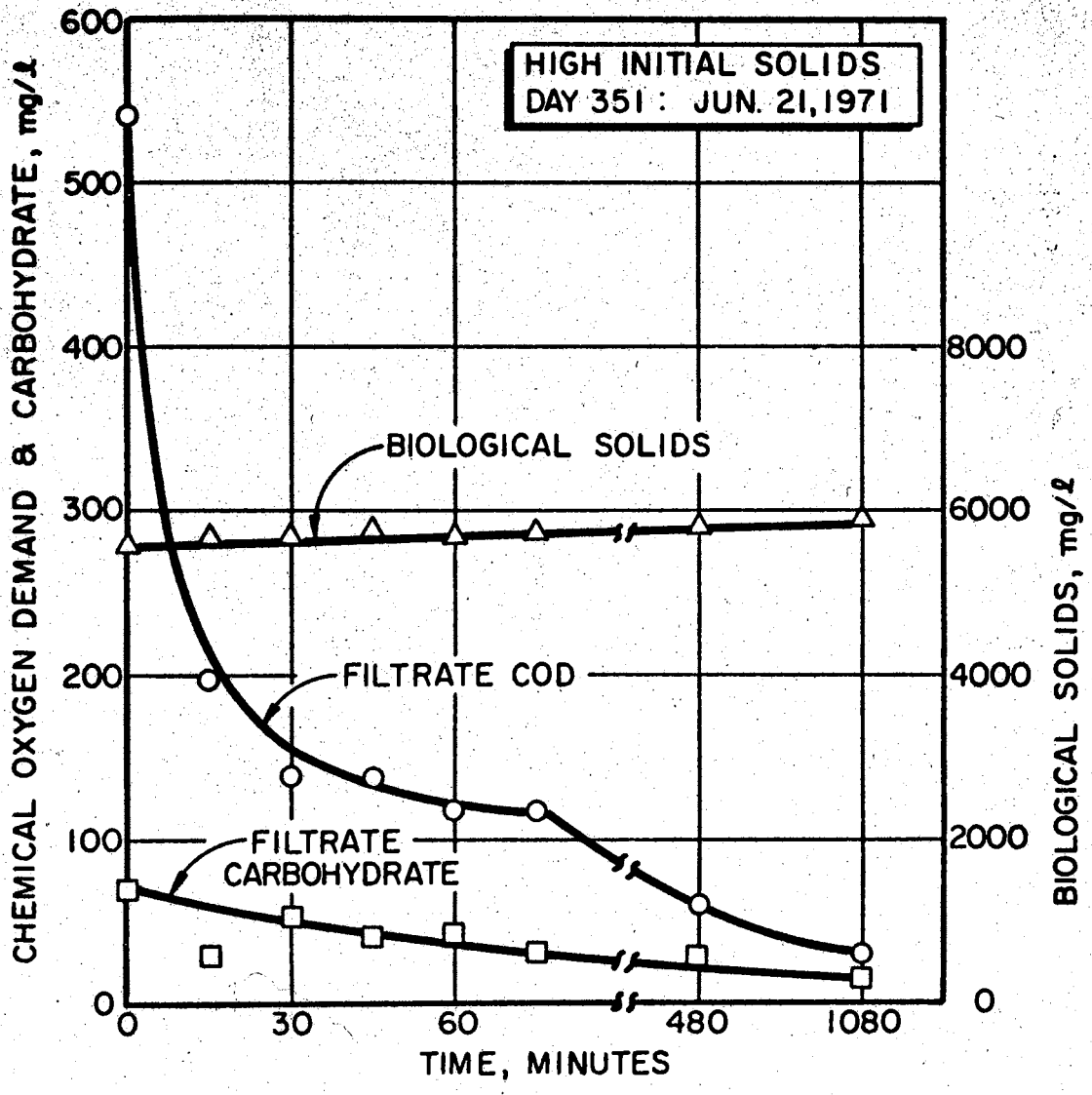
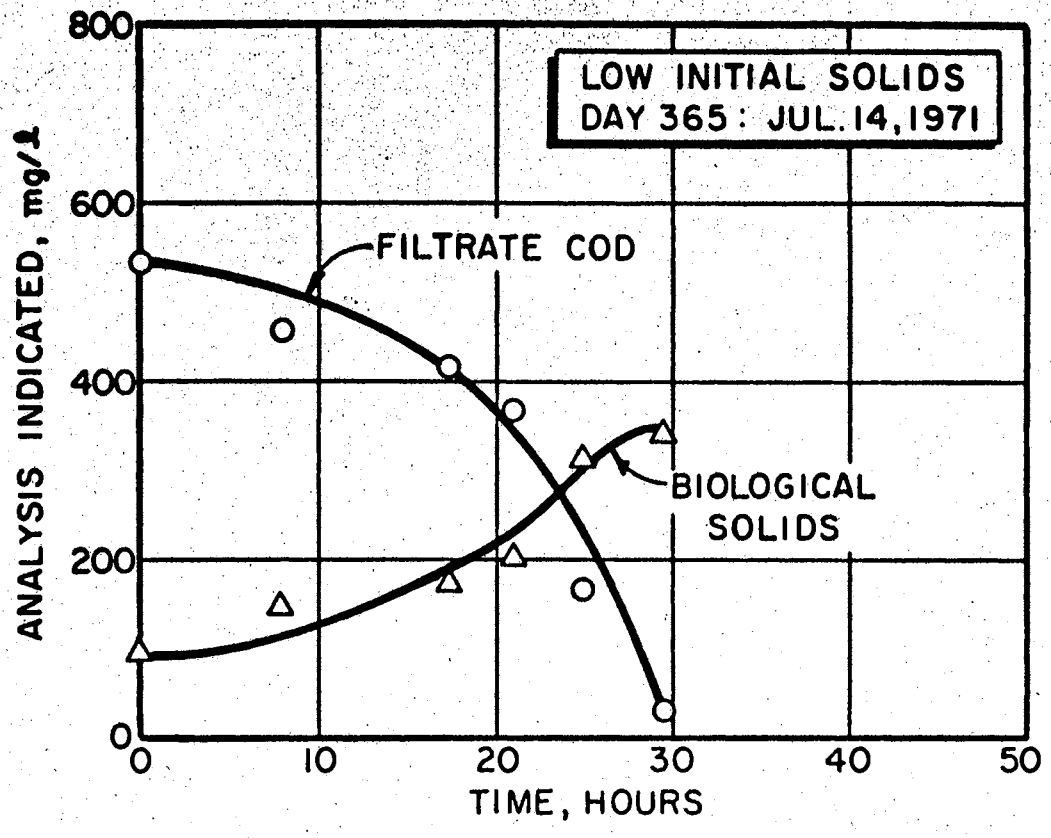
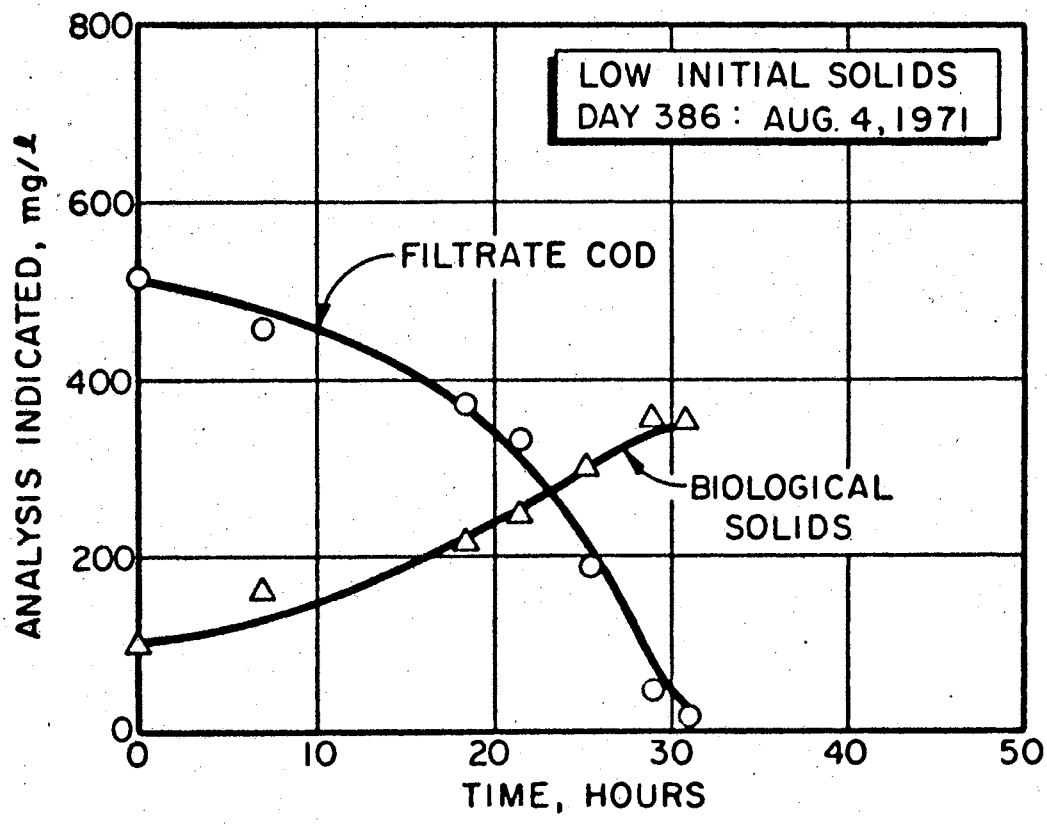

HIGH INITIAL SOLIDS
DAY 351 : JUN. 21, 1971
BIOLOGICAL SOLIDS
FILTRATE COD
FILTRATE
CARBOHYDRATE
CHEMICAL OXYGEN DEMAND & CARBOHYDRATE, mg/ℓ
BIOLOGICAL SOLIDS, mg/ℓ
TIME, MINUTES
600
500
400
300
200
100
0
8000
6000
4000
2000
0
0
30
60
480
1080

Figure 53. Response of the extended aeration activated sludge to a slug dose of glucose after 365 days of operation and after 386 days of operation.


LOW INITIAL SOLIDS
DAY 365 : JUL. 14, 1971
FILTRATE COD
BIOLOGICAL SOLIDS
ANALYSIS INDICATED, mg/ℓ
TIME, HOURS
0
200
400
600
800
0
10
20
30
40
50


LOW INITIAL SOLIDS
DAY 386 : AUG. 4, 1971
FILTRATE COD
BIOLOGICAL SOLIDS
ANALYSIS INDICATED, mg/ℓ
TIME, HOURS
0
200
400
600
800
0
10
20
30
40
50

confidence limit of the mean, CL, were calculated (46), and the following values were obtained: $\bar{Y}$ = 46.9 percent, s = 7.0, CV = 14.9, and CL = 46.9 $\pm$ 3.8.

The specific substrate utilization rate (corrected for lag time) was also plotted, and it is observed that there was no particular pattern for the value. The lowest value was obtained on day 307, during the time when the unit was nearing the end of the period of aerobic digestion (see Figure 37). The endogenous uptake rate and the specific growth rate appear to follow somewhat similar patterns. In general, the values of these parameters run from 40 to 50 percent higher than the same parameters assessed in phase A on the extended aeration system operated with total cell retention, i.e., without hydrolysis of portions of the sludge. In the lower graph of Figure 41, the lag time or time to initiate the exponential phase of growth is plotted, and it is seen that except for a generally rising trend during the first 230 days of operation, there was no general pattern; lag time fluctuated over a rather wide range.

Direct assessment of the metabolic capability of the extended aeration sludge used in this phase of the study (hydrolytic assist) by conducting high initial solids batch experiments in the unit, as was done under the previous mode of operation of the pilot plant, was not considered necessary since removal of sludge and refeeding of the solubilized sludge was expected to maintain the substrate removal capability. However, assessment of the metabolic capability of the sludge, after the long period (about 19 days) of aerobic digestion, was of considerable interest. Three such experiments were run during the period of batch operation following the aerobic digestion period. The first of

these was conducted on day 308 (see Figure 47), and it is noted that the substrate (glucose) removal rate was rather high. On days 316, 317, and 318, the specific substrate utilization rate was assessed, using glucose (see Figure 48), glucose plus hydrolysate (see Figure 49), and acid hydrolysate (see Figure 50). The values obtained were 126.4, 103.6, and 68.1 mg COD/gm/hr, respectively. All three SSUR values were calculated over a time period of 75 minutes for the purpose of making comparisons.

On day 351, the ability of the sludge to metabolize acid hydrolysate was again assessed. The results are shown in Figure 52. The substrate utilization rate was 60 mg COD/gm/hr (calculated at 75 minutes). This experiment was continued for 18 hours, and it is seen that residual COD was very low.

CHAPTER V

DISCUSSION

The use of the extended aeration process has been increasing since the early 1960s. Although it has been claimed by some to be simple in operation, low in cost, and rather stable to such environmental changes as shock loads, there have been warnings by researchers against the use of the process on the basis of its theoretical unsoundness, and these warnings may have led in some degree to a restricted use of the process. Most of the researchers who have investigated the process have concluded that biological solids concentration will continue to build up in the system, that the buildup is caused by inert biological material, and that systems which do not provide for wasting of sludge will eventually undergo biochemical failure. A search of the literature reveals that no one seems to have run an experimental extended aeration system long enough to have observed a biochemical failure of the system, nor has anyone really provided for total retention of biological cells in the system. Thus, no studies other than the present one have attempted to investigate the soundness of the process by attaining positive control of the retention of sludge in the aeration system, and no other experimentation has provided for long-term observation under the closely controlled experimental conditions which are required in order to form sound conclusions from the experimental observations.

At the start of these experiments, it was thought that the biological solids concentration might in all probability steadily increase, and that the system would eventually undergo biochemical failure. One of the main purposes in initiating the experiments was to determine how long the system could continue to function effectively in removing the incoming soluble organic carbon source in the feed. Indeed, had this not been the aim of the study, and had the duration of the experiment been shorter or had some sludge wasting been practiced, the findings, and the conclusions based upon them, would in all probability be very similar to those of other workers who have studied the process. However, it was seen in the previous chapter that after nearly three years of operation of an extended aeration system with no sludge wasting, there was no loss in biochemical removal efficiency. COD removal efficiency was, in general, above 90 percent throughout the three-year operational period, for a feed COD of approximately 530 mg/l and an aeration chamber detention time of 16 hours. The sludge did not steadily accumulate, but there were periodic cycles of decreasing biological solids concentration followed by succeeding periods of solids accumulation. During the periods of decreasing biological solids concentration, no gross leakage of COD in the effluent was observed. Downward cycles in biological solids concentration can in no way be attributed to escape of biological solids in the effluent, and the causation for the initiation of the decreasing solids concentration cycle cannot be attributed to external causes (e.g., changes in temperature, pH, organic loading, etc.).

There are several natural explanations or theoretical possibilities for periodic acceleration of the autodigestion of a bio-mass. The fact

that cells undergo a decrease in mass during endogenous respiration is well known, but it is obvious that the endogenous respiration of any particular microbial cell cannot be expected to lead to total oxidation of its bio-mass. However, it is known that some species undergo complete or nearly complete autolysis after attaining the maximum growth level (54)(55). The released material can then become food or carbon sources for intact cells in the population. Considerations of the concept of endogenous respiration are indeed very complex. It has been pointed out by Gaudy and Engelbrecht (47) that the specific endogenous respiration rate in heterogeneous populations appears to vary not only with the type of organisms and substrate, but also with the substrate concentration and time. Dietrich and Burris (56) have observed that the effect of exogenous substrates upon endogenous respiration differs among organisms from inhibition to no effect to enhancement. In the present experimentation using a heterogeneous bio-mass, the endogenous O_2 uptake rate of the extended aeration activated sludge varied with the age and the predominance of species in the system when substrate concentration of the influent was not changed. In general, the endogenous respiration rate decreased with increasing age of the system, and it appeared to become asymptotic to a lower limit. In any case, the endogenous respiration of microorganisms cannot be expected to provide a complete explanation for the decreasing cycle in sludge concentration in extended aeration processes. In cases where autolysis of cells leads to their dissolution (i.e., solubilization of structural components), or to simple disruption or tearing of the wall and subsequent release of internal components, the entire bio-mass of the cell would become food for another cell of the same species, but more importantly,

in a heterogeneous population it could become carbon source for another species of microorganisms. Thus, through a series of passages through the ecological food chain, the carbon of one cell could, indeed, be totally oxidized.

Considering again the complicated ecology of a heterogeneous bio-mass, it is known that various microorganisms produce enzymes which cause lysis or complete dissolution of other cells (57)(58). Thus, autolysis may play only a minor role in making the carbon of one cell available to other cells in a mixed population. Bacteriophage may also play an important role in induced cell lysis. Either enzymic or bacteriophage attack would be expected to be rather specific for certain species within the bio-mass; thus these mechanisms could relieve the sludge accumulation but not cause total kill of the population. In the water pollution control field, Simpson has demonstrated the presence of lytic agents in the cell-free filtrate obtained from natural microbial populations undergoing endogenous respiration (59).

In addition to autolysis and lysis induced by other bacterial species, there is also in the heterogeneous population the decided probability of predatory attack by higher organisms, such as protozoa and slime molds, which ingest intact bacterial cells as sources of carbon and other nutrients. Thus, the possibilities for autolysis, lysis by enzymes produced by other bacteria, cell disruption by bacteriophage, and ingestion by higher organisms, all of which can and undoubtedly do go on in the very complicated ecosystem represented by an activated sludge, contribute explanations for the continued successful functioning of an activated sludge process in which all biological solids are returned to the aerator.

All of the above mechanisms can convert cell material into exogenous substrate. In order for the substrate to be used, it is necessary that species which possess the metabolic capability to utilize it as a substrate, be present. If such were not the case, the quality of the effluent would deteriorate, i.e., the efficiency of substrate removal or the purification efficiency (COD removal) would decrease as the biological solids concentration underwent a decreasing cycle. In the present study, such feeding species were apparently present, since the effluent COD did not rise appreciably during cyclic decreasing biological solids concentration.

In the early 1950s the design of extended aeration processes was based generally on the expectation of establishment of an equilibrium between the rate of cell synthesis and cell oxidation. From the results of the present experimentation, it is obvious that cell disruption or dissolution does not stay in balance with cell synthesis, i.e., the operation of this process at some equilibrium solids concentration cannot be expected. Attainment of constant biological solids concentration would require a very precise balance between the specific natural lytic agents and species which could metabolize the various cell components which are made available as substrate. Such a situation can exist at times, but it cannot be expected to be the normal state in the system. Throughout the experimentation, regular observation of the biological solids (both microscopic and macroscopic) as well as the various values recorded for endogenous O_2 uptake, specific growth rate, sludge yield, and lag time indicated that an ever-changing population exists in an activated sludge process. It has been shown by Gaudy, et al. (60), and Ramanathan and Gaudy (61) that for heterogeneous

populations, a pseudo-steady state with respect to biological solids concentration and substrate concentrations in the reactor can be approached, but even in continuously fed, once-through, completely mixed reactors, some variation, particularly in the concentration of biological solids, is always observed. Also, it must be emphasized that of all activated sludge processes, an extended aeration system is the one which exists under most severe starvation conditions. Cells which predominate in such a system are those which possess an ability to compete for a very limited supply of substrate which is comprised of many types of complicated molecules. Demand for carbon source greatly exceeds the supply; at times the greatest supply of food for one species is comprised of other members of the population, and predatory and parasitic relationships can be expected to be operative in such natural ecosystems; all of these factors militate against development of a stable or equilibrium condition with respect to biological solids concentration.

The time required to complete a cycle of net accumulation and net decrease of solids concentration cannot be predicted, nor can the periodicity of such cycles be predicted, but the results of this study surely attest to the fact that such cycles do exist. One other report has suggested that cyclic reduction in biological solids accumulation can occur in extended aeration processes. Washington, et al. (62) employed batch systems and provided no positive control of solids loss in the effluent. However, during the period of operation of approximately one year and four months (July, 1962-November, 1963), they observed a gradual rise in solids concentration followed by a gradual decline and subsequent rise. According to their data, the decline could not be attributed to loss of solids in the effluent. They felt

that the decline in solids was not the result of lysis of a portion of the population, but that a portion of the biologically inert volatile solids fraction was metabolized by an organism (or organisms) which adapted to this material. Their basis for ruling out lysis was that the decrease in solids concentration was not accompanied by an increase in effluent COD. However, it is entirely expectable that the lysed material would be metabolized by the remaining intact cells. Regardless of the various interpretations to be placed upon their results, the fact remains that the results do indicate that there was a period of accelerated autodigestion which relieved solids accumulation. They concluded that it was uncertain that there was any long-term cyclic reduction in the accumulated volatile solids. It is somewhat unfortunate that they terminated the experiment when they did; their data indicate that the system may have been poised for a second cycle of decreasing solids concentration.

Based upon the results of the present study, it is apparent that it is not axiomatic that an inactive fraction which cannot be metabolized and/or which cannot metabolize the incoming organic waste and/or the lysate of sludge itself will continue to build up under conditions of total cell recycle. It seems obvious that a given microbial cell cannot totally oxidize itself, but it is not impossible or improbable that all of the organic matter from any particular microbe can serve as food for other species in the complex mixed population system which comprises an extended aeration system or, for that matter, any activated sludge system. Concerning capsular material (largely polysaccharide in nature), it is well known that some organisms utilize the capsular material which they have produced as a source of energy, and some do

not (63). However, even in cases where capsular material is not utilized by the particular species which produced it, it is certainly possible that this material or any other cellular material can be used as a source of carbon by another species. If an organism can produce the hydrolytic enzymes to split the polymers, thus solubilizing the material, it can be expected that various species will induce enzymes for the metabolism of these carbon sources. It is known, from the results of recent experiments with heterogeneous populations, that the soluble portion of the cells released after breakage of the cell walls and the slime or capsular layer surrounding the cell wall provide excellent substrates for microbial growth (29)(64). In these recent experiments, cell components released by sonication were used as feed or source of carbon in systems in which the seed population consisted of a small inoculum of cells grown from sewage or cells from the extended aeration unit. In some cases, there was a rather long lag period, followed by very rapid growth, whereas in others, the sonicate COD was removed with no acclimation period, but at a rather slow rate. Also, cell sonicate has been fed to the extended aeration pilot plant directly. Results indicated that the sonicate COD could be removed or metabolized by the extended aeration activated sludge. In other recent work (65) it was shown that during prolonged endogenous metabolism, total oxidation of an amount of sludge equal to that synthesized in the previous growth phase was possible. Also, more recently in the Oklahoma State University bioengineering laboratories, 20 batch experiments were conducted under prolonged endogenous aeration conditions using a heterogeneous biological mass which had been grown previously on acetic acid as carbon source, and the results of these studies indicated that total oxidation of the synthesized sludge was possible (66).

The results of the present study as well as those cited above indicate that a heterogeneous bio-mass existing under starvation conditions such as those obtaining in an extended aeration activated sludge process is characterized more by biological activity than by biological inertness. Thus some of the previous conclusions by other workers indicating concern over the continual buildup of biologically inert materials in an extended aeration system must be concluded to be somewhat premature and unsound in view of the recent results. The theoretical or mechanistic "soundness" of the operation of an activated sludge process with return of all biological solids to the aerator is confirmed by these studies, and it is felt that the findings should cause water pollution control engineers to re-evaluate the useful possibilities for total cell recycle in the treatment of soluble organic wastes, particularly industrial wastes.

Experimental demonstration of the biological soundness of total oxidation systems does much to remove the onus of unsoundness which has been attached to the system for so many years; it does not automatically confer endorsement of the use of the process from a practical standpoint, since there is, during a period of solids accumulation, the problem of retaining the biological solids in the system. For example, it can be seen in Figures 10, 11, 12, and 13, that a large amount of biological solids would have exited the system had settling been the only means employed for separating the cells. When biological solids concentration is high, there is a very real and practical engineering problem with regard to separation of the mixed liquor in the clarifier. However, in vew of the problems which a total oxidation process can solve (for example, it can provide for oxidative disposal of the

sludge), it does seem a wise expenditure of effort to seek some engineering solutions to the problem of sludge retention, rather than to overlook the advantages of the total oxidation process because there are settling problems from time to time.

If one could retain all of the solids, the time required to initiate a decreasing cycle in sludge concentration would not be a particularly important factor; however, engineering control over retention of biological solids in any activated sludge system has generally proven to be rather difficult, and in the present study, another means of controlling biological solids was envisioned. If one could control the onset of the decreasing cycle in solids concentration, engineering expedients for physically controlling the retention of high solids concentrations might be unnecessary. In the present investigations, it was reasoned that perhaps the most difficult metabolic action for one cell to perform upon another was solubilization of the insoluble fractions of the cell; e.g., cell walls and cell capsules or slime material. Therefore, whole cells or portions of an activated sludge were hydrolyzed chemically under conditions which solubilized most or all of the cellular material. It was found that these solubilized cell components did provide a usable substrate for a heterogeneous population, and the engineering process modification to the extended aeration process which was shown in Figure 4 was envisioned as a means of exerting engineering control over the operation of a total oxidation process. Under this mode of operation, biological solids could, at times of accumulating concentration, be removed from the underflow of the settling tank and hydrolyzed, and the neutralized hydrolysate, i.e., chemically prepared cell material, could then be recycled or bled back to the aeration

chamber, where it would be used as carbon source by the intact cells in the sludge. Thus, a bioengineering control over the operation of a total oxidation process could be achieved. It was felt that such a process, which embodies a "chemical assist" to the biological process, accomplishes chemically a function which is biologically difficult and accomplishes biologically a function which is difficult and costly to accomplish chemically.

The new process was proposed based upon the findings of batch experimentation regarding the metabolism of sludge hydrolysates, and various modifications and possible advantages for the system, in particular, waste water situations, have been discussed (29). The results of the present study show that this modification of the process does possess considerable engineering potential. Such a system has been successfully operated in the laboratory pilot plant stage for over a year, and when one compares the results of such operation (Phase B) with those of Phase A in regard to system efficiency, endogenous O_2 uptake of the sludge, specific growth rate, and substrate removal rate, it is apparent that the "chemically assisted" process provides a somewhat more active sludge.

The fact that results reported in Phase A of this thesis remove the onus of theoretical unsoundness from the extended aeration process, and the fact that results reported in Phase B show that the process employing the hydrolytic assist provides a means of considerable engineering control over the operation of the process, do much to enhance and extend the scope and future use of biological treatment for the removal and ultimate disposal of organic carbon in waste water streams. However, it is recognized that in this process, as well as any other

fluidized biological process, the successful operation of the system using normal clarification procedures is dependent upon the flocculation and settling characteristics of the biological pppulations which are developed in the process. The results of the studies in Phase B provide some insight into solution of some of these important considerations. On day 205, the ratio of COD:nitrogen was shifted from 10:1 to 20:1, and then on day 213, the ratio was changed from 20:1 to 30:1. After this reduction of nitrogen source in the influent, there was first observed the onset of "floating sludge" in the settling chamber, and this was followed by the development of a "bulking sludge." During this period, the dissolved oxygen concentrations in the aeration tank and settling chamber were 6.5 and 3.0 mg/l, respectively. Therefore, there was no oxygen deficiency. The bulking sludge was characterized by copious growth of filamentous organisms. Many possible causes for the predominance of filamentous organisms in an activated sludge have been cited (67)(68)(69)(70)(71)(72). After surveying all of these reports, possibilities for the onset of the growth of filamentous organisms in the present study could include the reduction of nitrogen concentration in the influent feed (69), and the incorporation of neutralized acid hydrolysate as feed to the system or as liquified return sludge, since Harrison and Heukelekian (72) have concluded that (in the case of Sphaerotilus) luxurious growth is dependent upon a source of organic nitrogen. However, since the onset of predominance of filamentous organisms can be observed in systems from time to time without the exertion of any such external pressure, it is difficult to say whether the growth of filamentous organisms was incidental to these periodic changes in environmental conditions, or was caused by them.

Although, as suggested by Adamse (70), there are many possible ways we might attempt to prevent or eliminate the growth of filamentous organisms, a truly effective way is still unknown. In the present study, the addition of Fe^{+++} ion temporarily increased the settleability of the sludge; however, unless the dosage was maintained, the beneficial effects soon disappeared. In any event, the addition of ferric ion had no effect on predominating species, and it is quite obvious that the most effective way to attain good settling flocculent sludge would be to foster the predominance of organisms which exhibited these characteristics. Insights into this latter aspect would surely be as desirable as definitive insights into the mechanism of biological flocculation, and are in all probability just as elusive.

In view of the lack of knowledge in this regard, it was interesting to determine whether the hydrolytic assist process could be employed to help the system rid itself, so to speak, of the undesirable predominance of filamentous organisms. In the present study, during the period when most of the sludge consisted of filamentous organisms, half of the sludge was removed, hydrolyzed, and fed back to the system (see day 309). On days following this operation, it was observed that filamentous organisms were still present in the system; however, there was some improvement in settling characteristics and clarity of the effluent.

Although the present studies have shown that the organic content of bacterial cells is an available substrate for microbial populations, the studies have also shown that some of the cellular materials cannot be expected to be degraded at a very rapid rate. Also, it has been observed recently in studies in the Oklahoma State University

bioengineering laboratories that a residual COD (approximately 100-200 mg/l) which was removed only very slowly, remained in the system in experiments in which cell hydrolysate was used as substrate by a low initial inoculum of cells. This "residual" COD could be removed (reduced by approximately 90 percent) by application of 0.5 percent (W/V) activated carbon in a contact time of two minutes (73). It is apparent that the application of activated carbon is not only useful for the partial removal of the original materials in the hydrolysate, but also for the nearly complete removal of the "residual" COD remaining in the system when hydrolysate is used as carbon source for the rapid development of a new population. The residual COD which persisted in the system in which hydrolysate was used as carbon source for the growth of an initially small population of cells can be considered to be material which is not readily metabolized by the new population which has been recently grown in an environment consisting of readily available sources of carbon. Apparently these are materials which can be metabolized only by a complex mature ecosystem of an extended aeration activated sludge, since these same materials could be removed nearly completely by feeding them directly to the extended aeration process which consisted of a rather high concentration of mature biological solids. The possibility also exists that some of the COD removal effected by the rather high concentration of mature solids in the extended aeration unit was due to adsorption and slower later metabolism of some of the organic matter which could be expected to carry a considerable electrical charge, e.g., proteins and some of the polysaccharide material.

In summation, all studies to date indicate that the hydrolytic

assist process is feasible, since the biochemical purification efficiency of the process was found to be almost the same as that which was observed in Phase A, in which acid hydrolysate was not incorporated. It has been found that biological solids can be positively controlled in the system without increasing the filtrate COD of the effluent. Statistical comparison of the values of sludge yield for the studies in Phase A and Phase B indicates that the average cell yield was somewhat lower (approximately 10.5 percent) during the studies in which sludge hydrolysate was recycled to the aeration chamber. Thus, the somewhat lower cell yield coupled with the continued high substrate removal efficiency augurs well for the process. Before the "chemical hydrolysis" assist process was conceived and developed as a modification to the extended aeration process, various expedients to retain biological solids in the system and prevent their carryover to the receiving stream had been proposed. Such suggestions included lengthening of the detention time in the aeration tank and/or the settling tank, the application of mixed media filters to remove suspended solids in the effluent, the installation of a denitrification system between the aerator and the settling chamber to avoid flotation of the sludge, all expedients aimed at prevention of solids carryover to the receiving stream. In this regard, these suggestions are useful, but they do not necessarily solve the problem without creating new problems. For example, larger settling tanks could provide for the development of anaerobic conditions, thereby enhancing rising sludge. The installation of mixed media filters requires a backwashing operation, and denitrification systems can be expected to change the characteristics of the biological solids which must be returned to the aerator. Most

importantly, none of these expedients is addressed directly to the problem or the question of wet biological oxidation of the organic sludges. The process herein examined is addressed directly to enhancement of total oxidation of organic carbon originating in waste waters. It provides a way of attaining engineering control over this very important portion of the natural carbon cycle, in which concurrent secondary treatment and sludge disposal are achieved. Its continued study and further development and use in the field should help foster pollution control for a wide variety of municipal, commercial, and industrial waste waters.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The first phase of this study consisted of a three-year investigation on the operational stability of an extended aeration activated sludge process with positive control of the retention of biological solids in the system, and the results warrant the conclusion that the basic premise of total oxidation of biological solids is not inconsistent with sound microbiological and ecological theory. This conclusion is in contrast with opinions held by a rather wide number of researchers in the water pollution control field. Throughout the three years' investigation, the biological solids concentration underwent cyclic periods of accumulation and de-accumulation, i.e., the system did not attain a balanced or equilibrium solids concentration. During periods of de-accumulation, the biochemical efficiency of the system remained high. The periodicity of the cyclic accumulation and de-accumulation could not be predicted, but the existence of such cycles is attested to by the present results and such behavior is in accord with expected behavior of microbial ecological systems, which involves various shifts in predominating species, and the periodic occurrence of cannibalizing cells in the system. There was no buildup of biologically inert material, and the system never approached a condition of imminent biochemical failure. The studies of Phase A offer considerable promise

for the extension of the scope of use of extended aeration processes.

In addition to providing sound evidence for the theoretical validity of the total oxidation process, the three-year investigation of Phase A also pointed up one of the most important engineering problems associated with the extended aeration process. There were times when biological solids concentration accumulated to such an extent as to cause serious settling problems in the clarification compartment. As a means of initiating a downward cycle in biological solids concentration and of controlling biological solids concentration in the system, the concept of the "chemical assist" was envisioned, and testing of this concept comprised investigations conducted under Phase B of the present report.

Phase B was concerned primarily with the operation of a new process modification (see Figure 4). A pilot plant was operated for over one year under the new mode of operation, and the results of this study indicated the operational feasibility of periodic withdrawals of sludge, hydrolysis of the cells, and recirculation of hydrolysate to the aeration chamber. The new process offers a means of attaining engineering control over the biological solids concentration in an extended aeration or total oxidation process, and should permit wider use of this system for concurrent secondary treatment and disposal of secondary sludge.

CHAPTER VII

SUGGESTIONS FOR FUTURE WORK

The results of the present investigation have provided a more sound theoretical basis for the concept of total oxidation, and the new mode of operation incorporating periodic chemical hydrolysis of portions of the sludge has been shown to offer considerable promise in attaining engineering control over the process. There are various aspects of the work which warrant future investigation. Some of these are given below:

Laboratory pilot plant investigations using specific industrial wastes rather than synthetic wastes would be of use in order to demonstrate the feasibility of the chemical hydrolysis process for specific "real" wastes.

Laboratory pilot plant studies using the highly controlled specific synthetic waste would also be useful in determining various operational criteria for withdrawal, hydrolysis, and recycle of liquified sludge.

Further work on the utilization of activated sludge hydrolysate as sole substrate for the growth of heterogeneous microbial populations would be useful for design purposes. Also, further work on the physical removal of complex macromolecules (e.g., by adsorption on activated carbon) would be useful.

Investigations on the possibility of employing alkaline hydrolysis as an alternative to acid hydrolysis may be useful, especially for specific field situations wherein sodium or potassium hydroxide may be more readily available than is sulfuric acid.

Lastly, it would be of considerable basic interest to investigate further possibilities of enhancing changes in predominant species by removal and hydrolysis of large portions of the extended aeration activated sludge when operational conditions indicated that a change in predominance was advisable; e.g., at times when filamentous organisms were beginning to predominate in the system, thus indicating the onset of bulking sludge problems. This aspect was investigated to some extent in the present study, and continued investigations along these lines might provide a useful avenue of approach to solution of this rather important operational problem concerning activated sludge processes.

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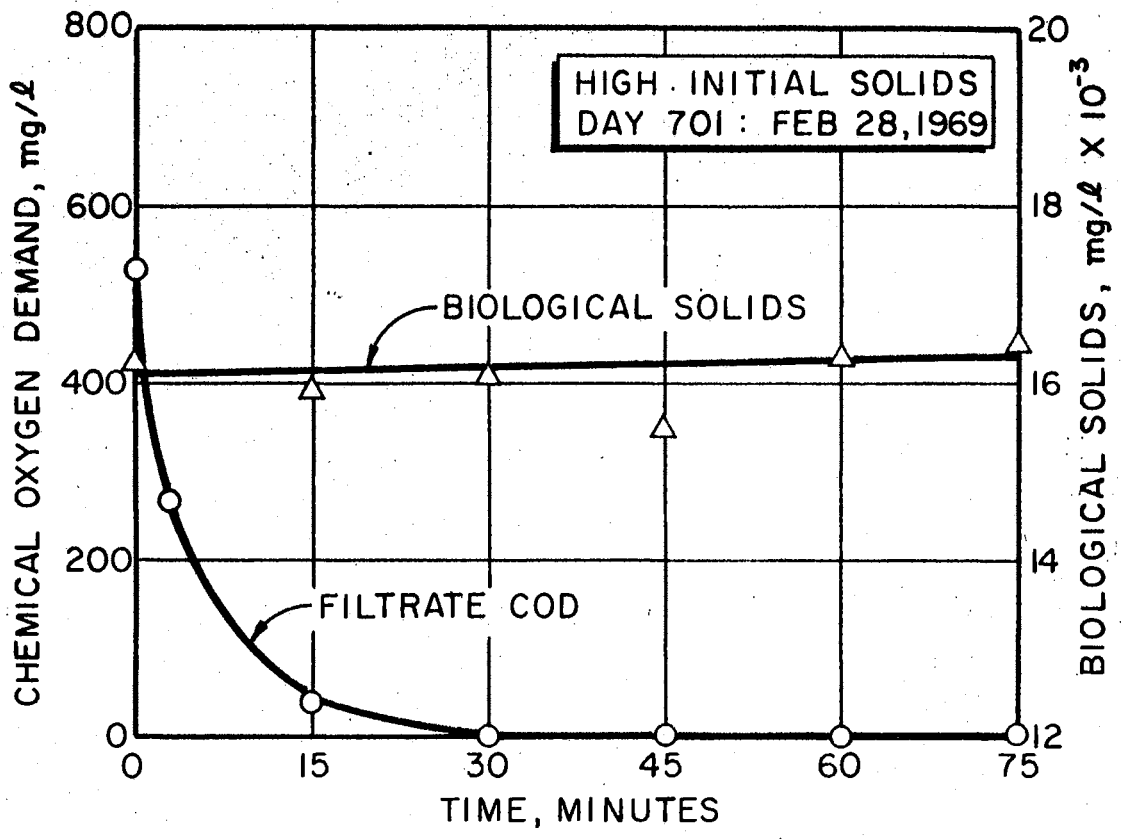
APPENDIX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DAY 701 : FEB 28,1969
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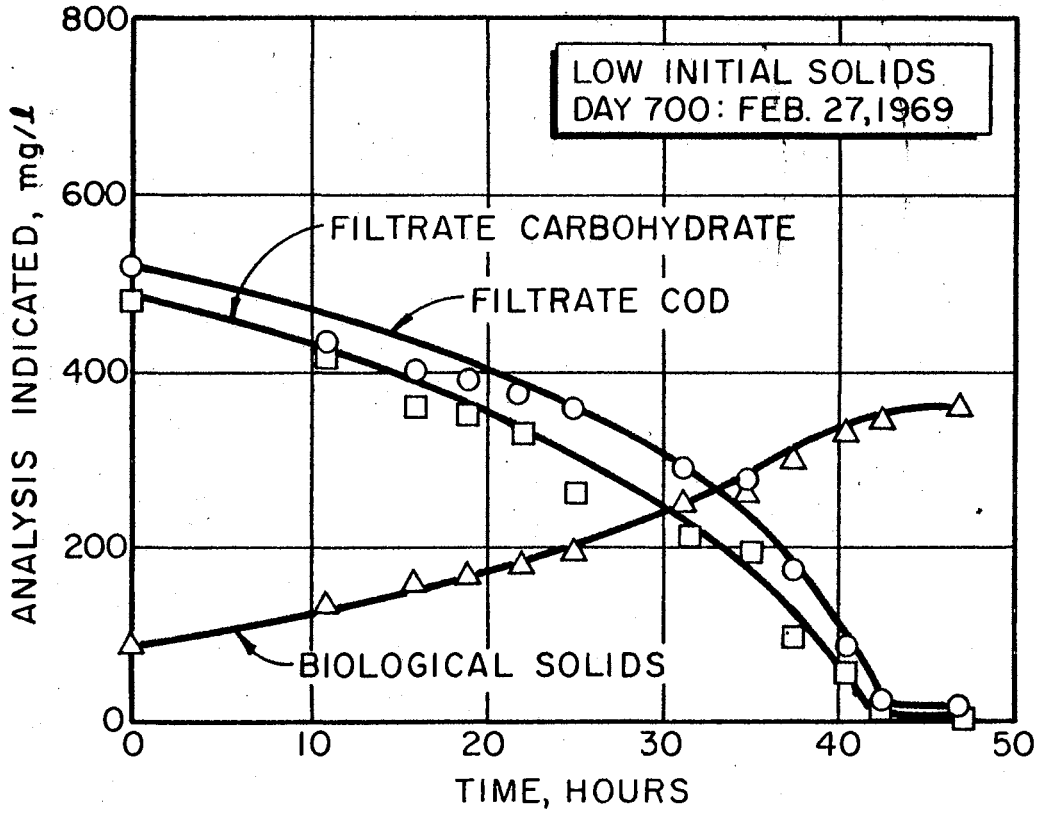
FILTRATE COD
CHEMICAL OXYGEN DEMAND, mg/ℓ
BIOLOGICAL SOLIDS, mg/ℓ X 10⁻³
TIME, MINUTES
0
200
400
600
800
12
14
16
18
20
0
15
30
45
60
75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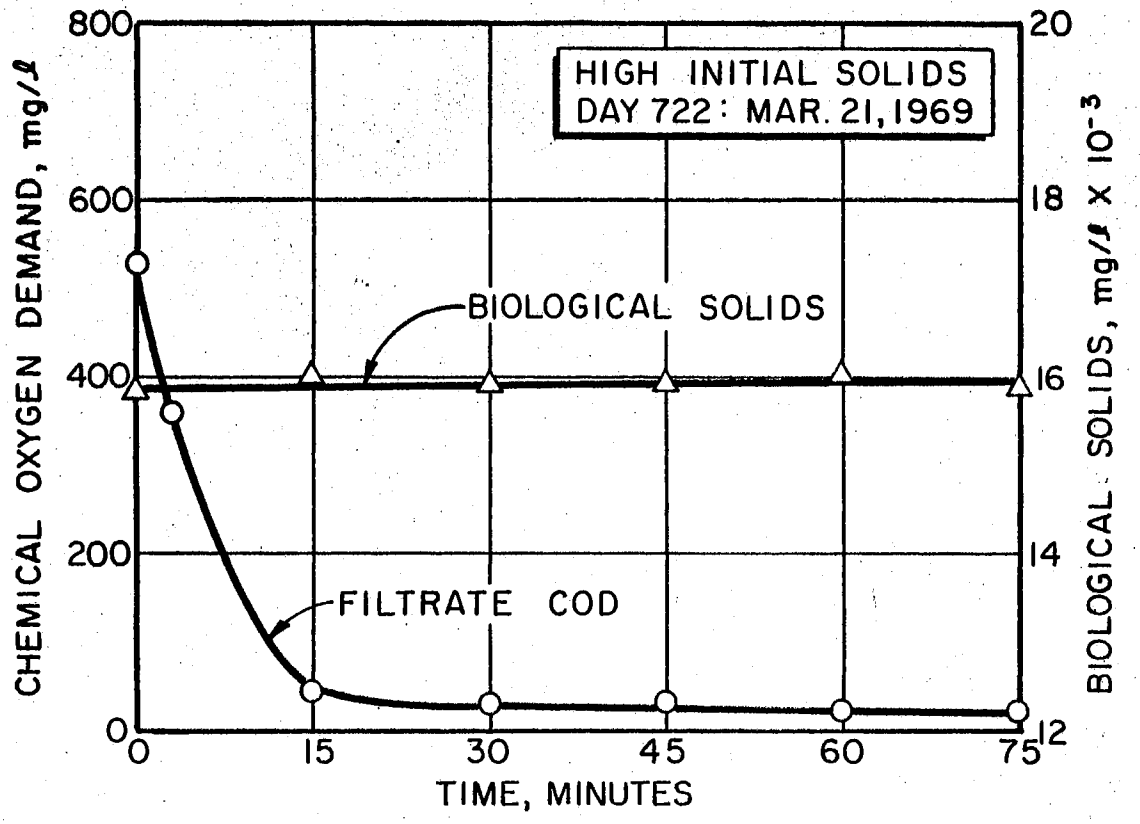
DAY 700: FEB. 27,1969
FILTRATE CARBOHYDRATE
FILTRATE COD
BIOLOGICAL SOLIDS
ANALYSIS INDICATED, mg/ℓ
TIME, HOURS
0
200
400
600
800
0
10
20
30
40
50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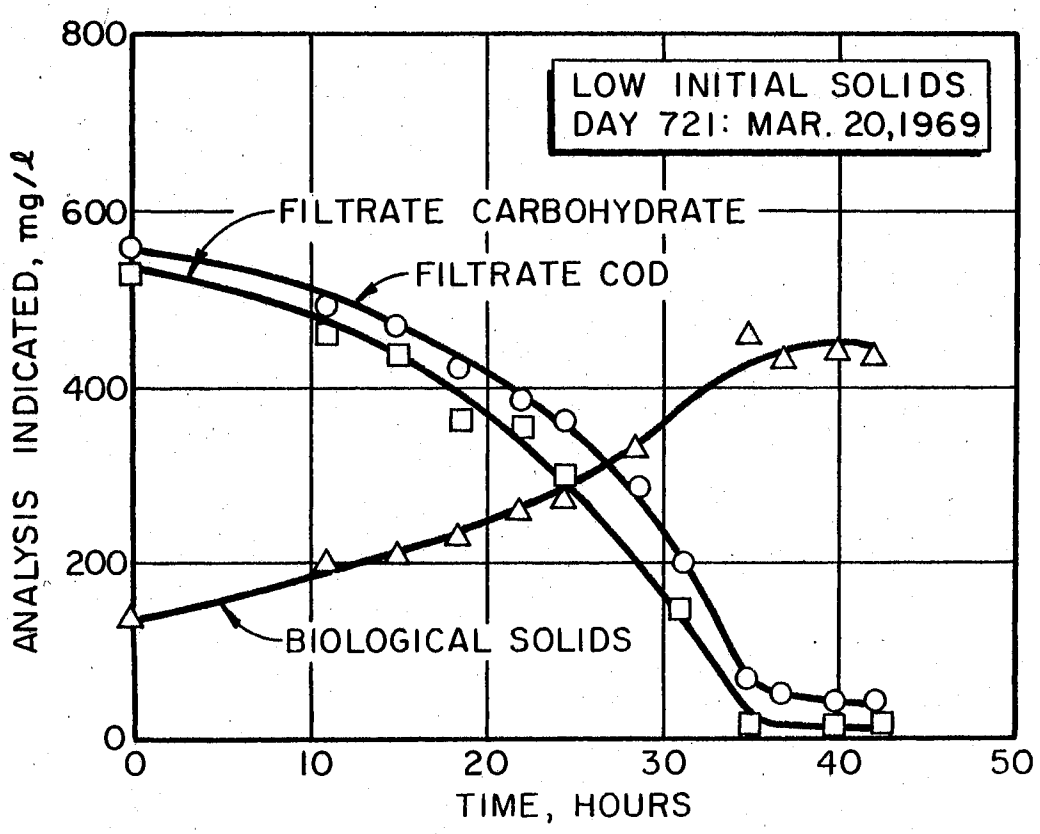
DAY 722: MAR. 21, 1969
BIOLOGICAL SOLIDS
FILTRATE COD
CHEMICAL OXYGEN DEMAND, mg/ℓ
BIOLOGICAL SOLIDS, mg/ℓ X 10⁻³
TIME, MINUTES
0
200
400
600
800
12
14
16
18
20
0
15
30
45
60
75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FILTRATE COD
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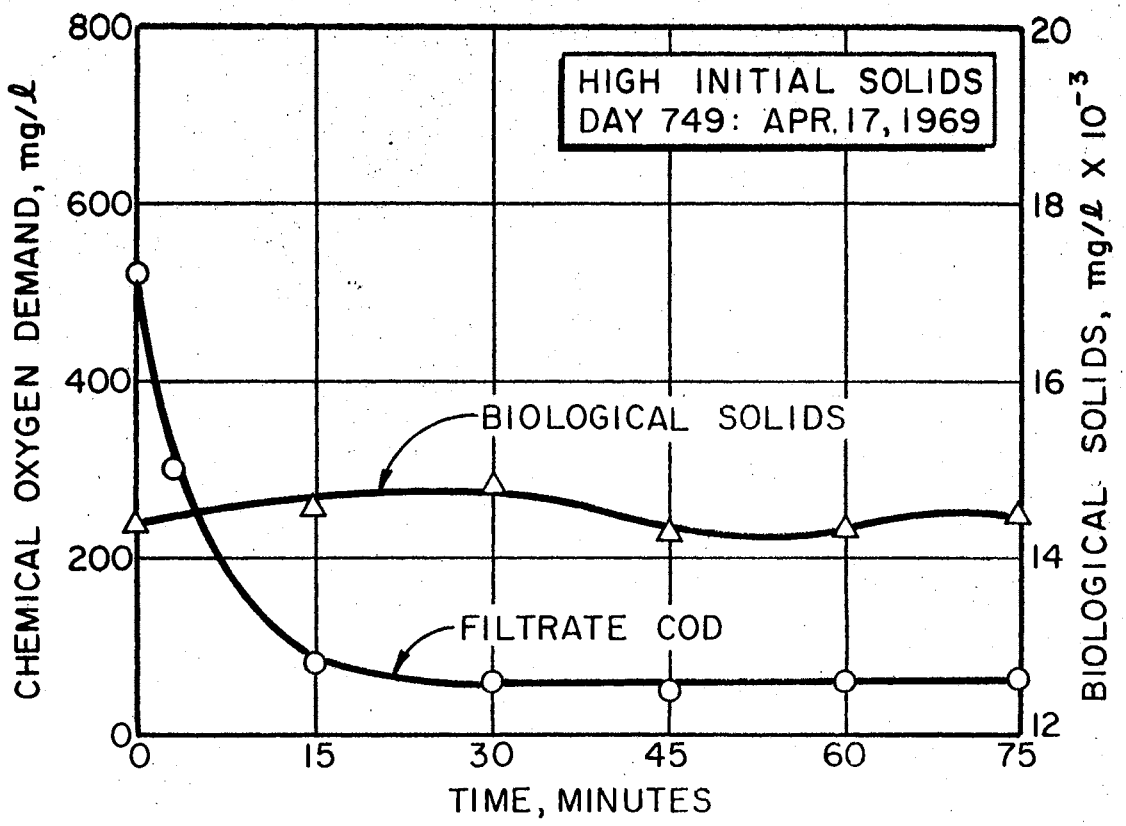
TIME, HOURS
0
200
400
600
800
0
10
20
30
40
50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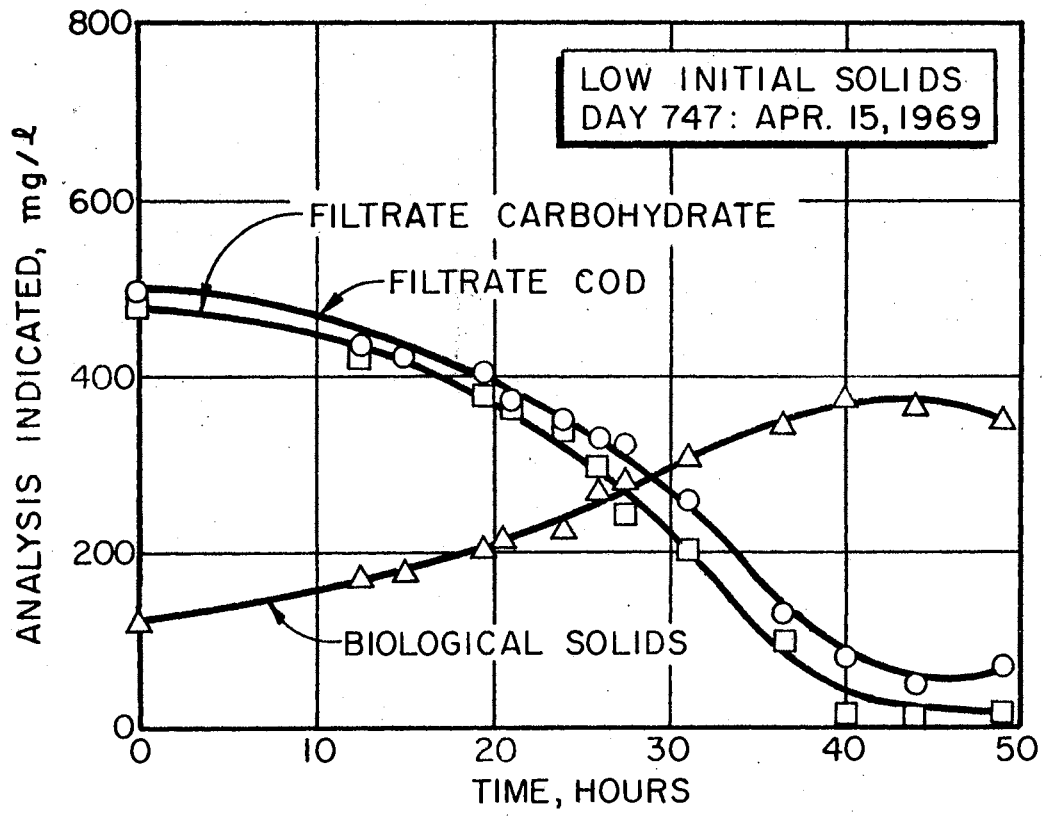
DAY 749: APR. 17, 1969
BIOLOGICAL SOLIDS
FILTRATE COD
CHEMICAL OXYGEN DEMAND, mg/ℓ
BIOLOGICAL SOLIDS, mg/ℓ X 10⁻³
TIME, MINUTES
0
200
400
600
800
12
14
16
18
20
0
15
30
45
60
75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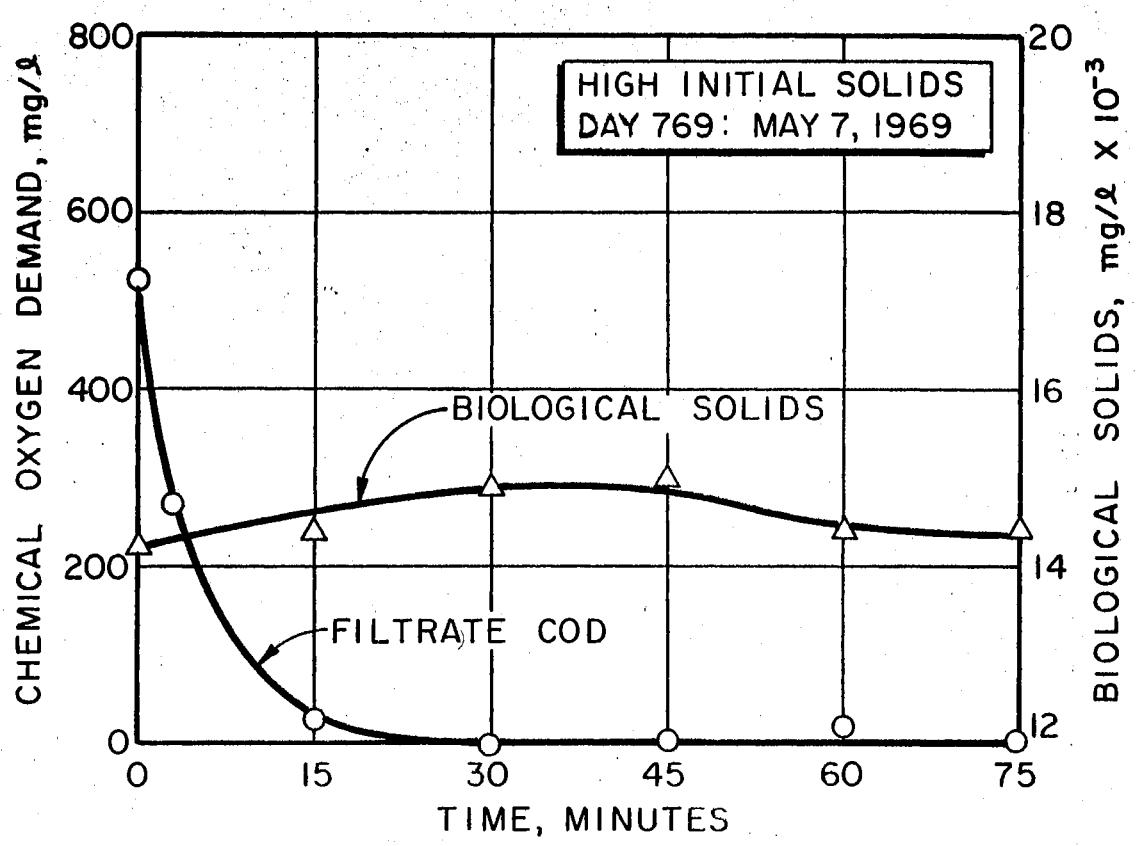
DAY 747: APR. 15, 1969
FILTRATE CARBOHYDRATE
FILTRATE COD
BIOLOGICAL SOLIDS
ANALYSIS INDICATED, mg/ℓ
TIME, HOURS
0
200
400
600
800
0
10
20
30
40
50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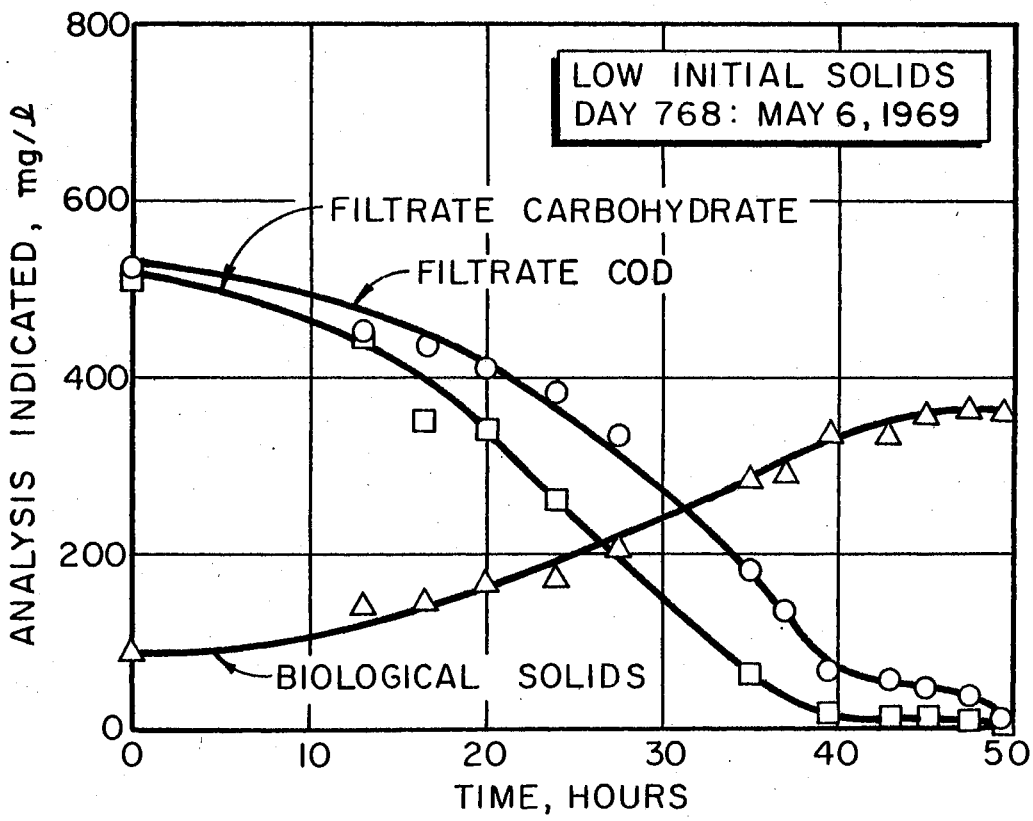
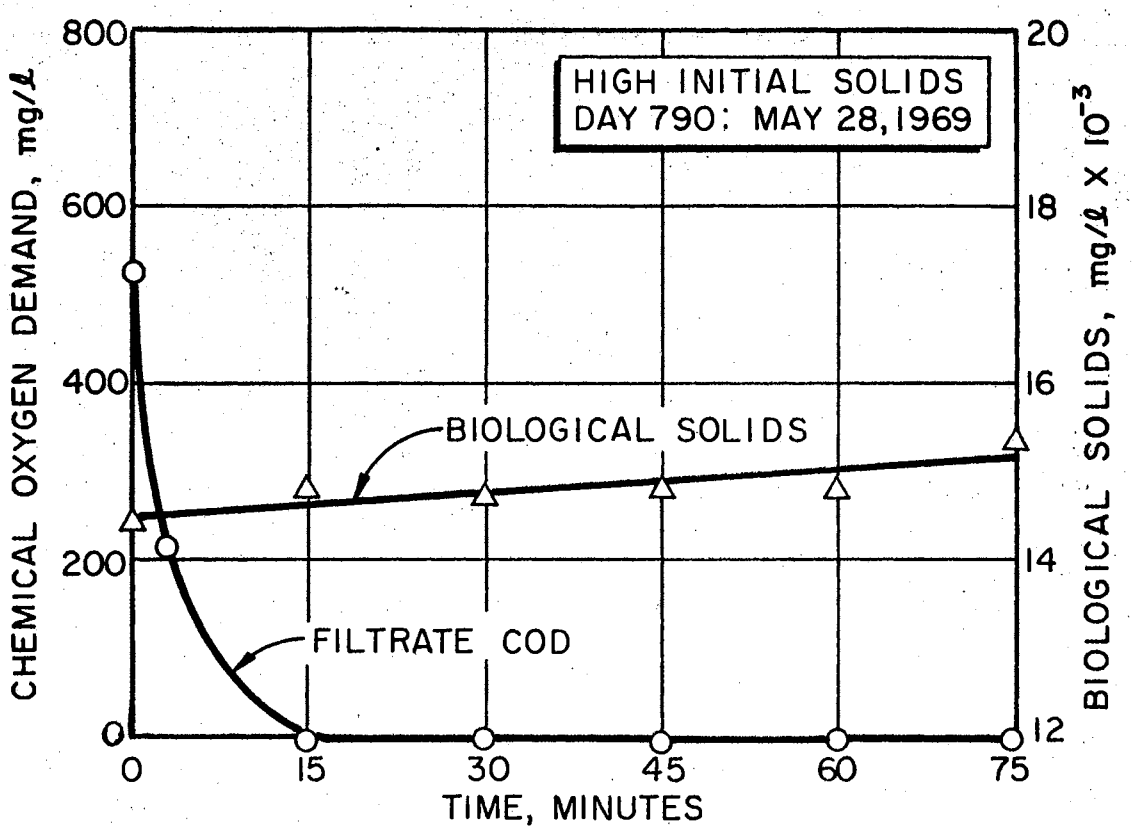
DAY 769: MAY 7, 1969
BIOLOGICAL SOLIDS
FILTRATE COD
CHEMICAL OXYGEN DEMAND, mg/ℓ
BIOLOGICAL SOLIDS, mg/ℓ X 10⁻³
TIME, MINUTES
0
200
400
600
800
12
14
16
18
20
0
15
30
45
60
75


LOW INITIAL SOLIDS
DAY 768: MAY 6, 1969
FILTRATE CARBOHYDRATE
FILTRATE COD
BIOLOGICAL SOLIDS
ANALYSIS INDICATED, mg/ℓ
TIME, HOURS
0
200
400
600
800
0
10
20
30
40
50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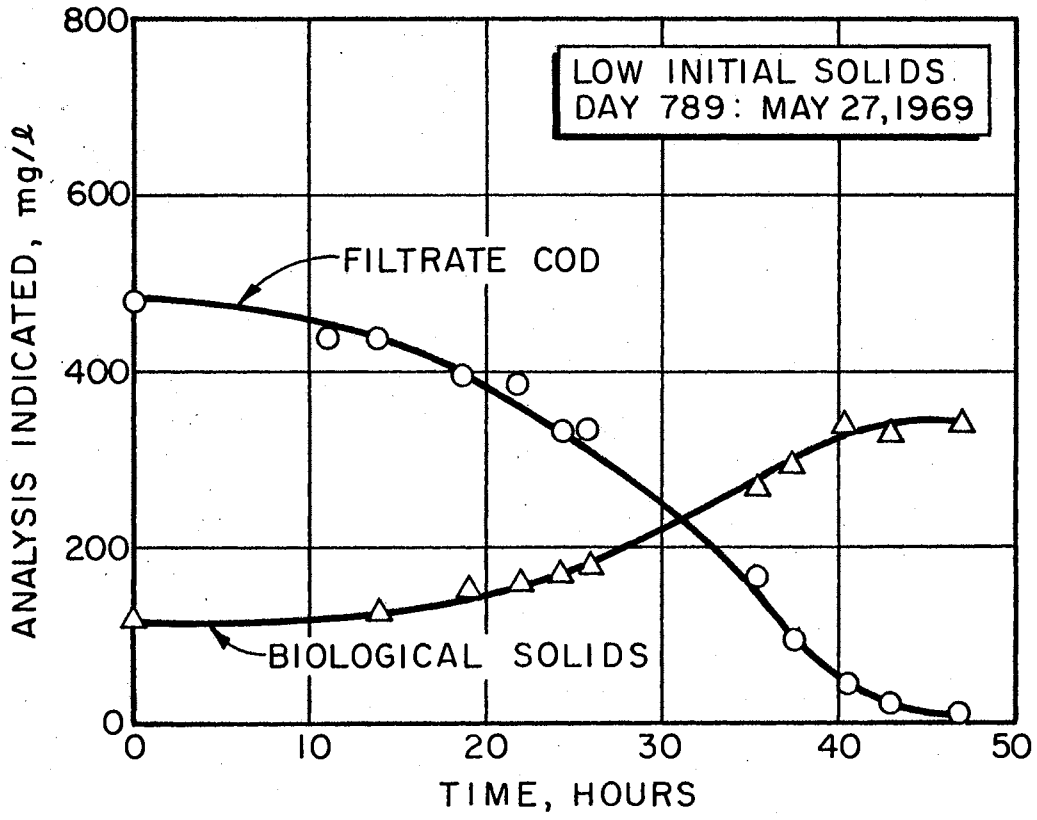
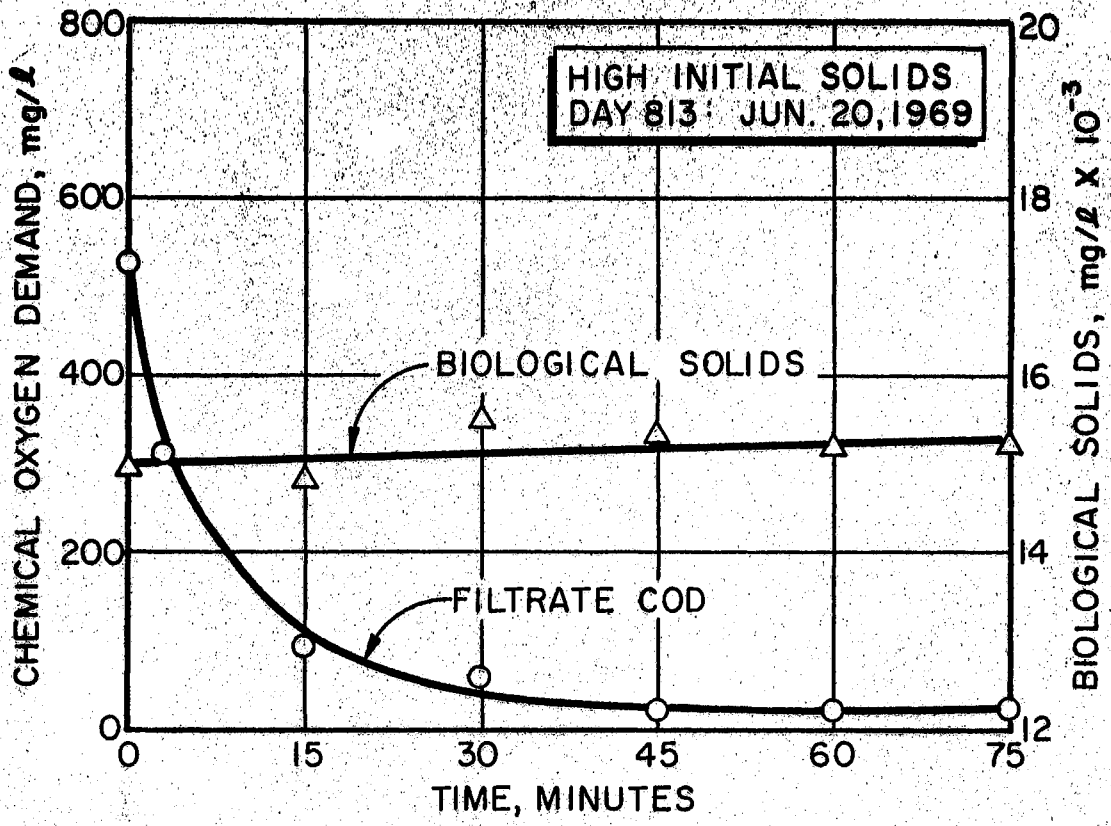
DAY 790: MAY 28, 1969
CHEMICAL OXYGEN DEMAND, mg/ℓ
BIOLOGICAL SOLIDS, mg/ℓ X 10⁻³
BIOLOGICAL SOLIDS
FILTRATE COD
TIME, MINUTES
800
600
400
200
0
20
18
16
14
12
0
15
30
45
60
75


LOW INITIAL SOLIDS
DAY 789: MAY 27, 1969
ANALYSIS INDICATED, mg/ℓ
FILTRATE COD
BIOLOGICAL SOLIDS
TIME, HOURS
800
600
400
200
0
0
10
20
30
40
50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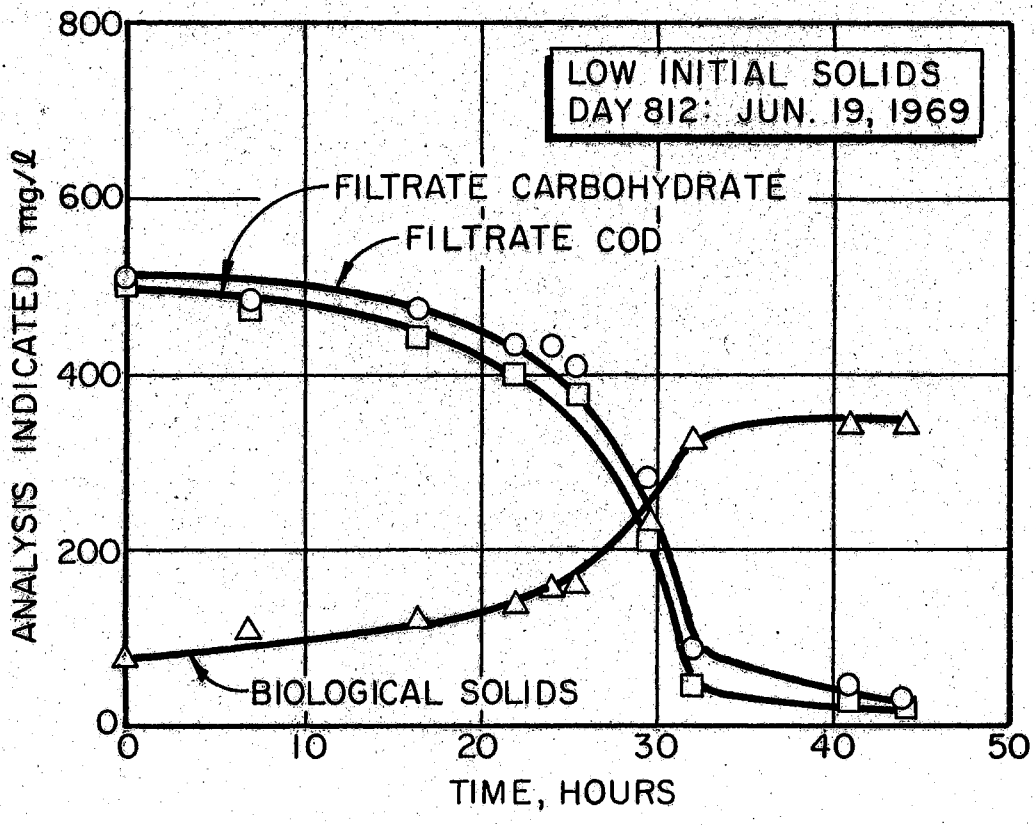
DAY 813: JUN. 20, 1969
CHEMICAL OXYGEN DEMAND, mg/ℓ
BIOLOGICAL SOLIDS, mg/ℓ X 10⁻³
BIOLOGICAL SOLIDS
FILTRATE COD
TIME, MINUTES
0
200
400
600
800
12
14
16
18
20
0
15
30
45
60
75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ANALYSIS INDICATED, mg/ℓ
FILTRATE CARBOHYDRATE
FILTRATE COD
BIOLOGICAL SOLIDS
TIME, HOURS
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400
600
800
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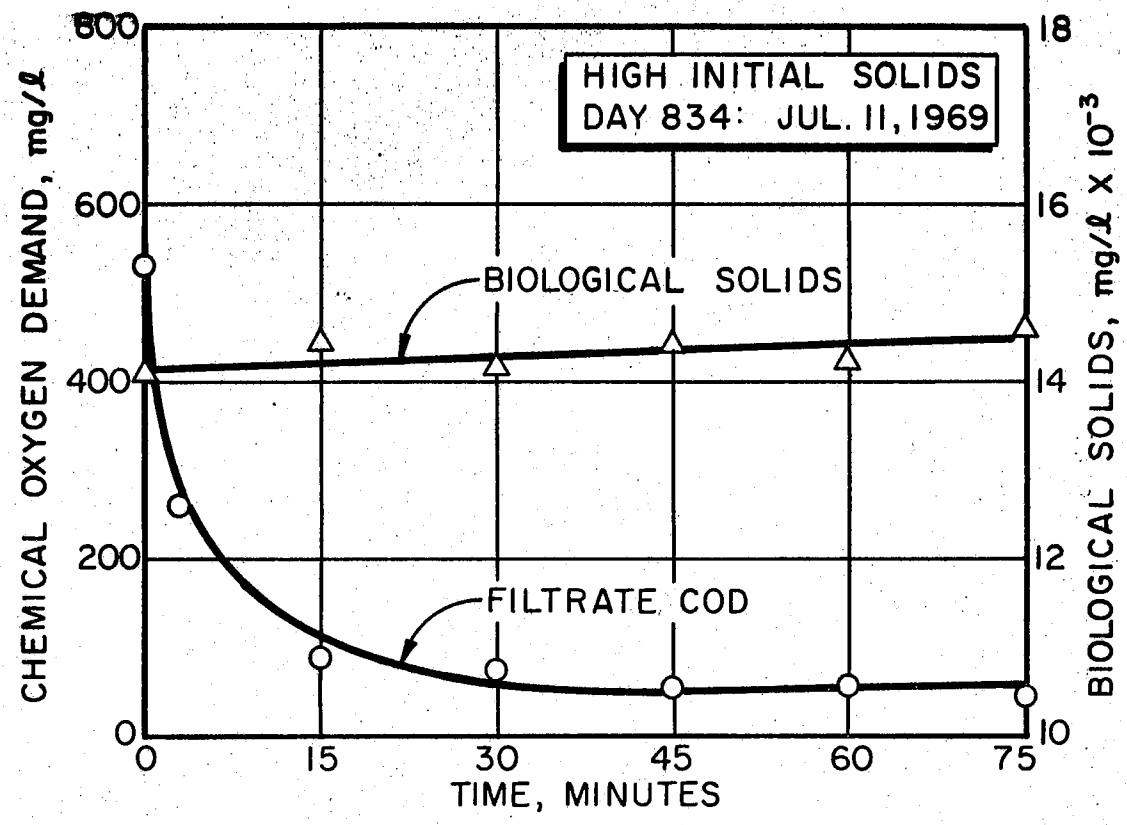
10
20
30
40
50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DAY 834: JUL. 11, 1969
BIOLOGICAL SOLIDS
FILTRATE COD
CHEMICAL OXYGEN DEMAND, mg/ℓ
BIOLOGICAL SOLIDS, mg/ℓ X 10⁻³
TIME, MINUTES
0
200
400
600
800
10
12
14
16
18
0
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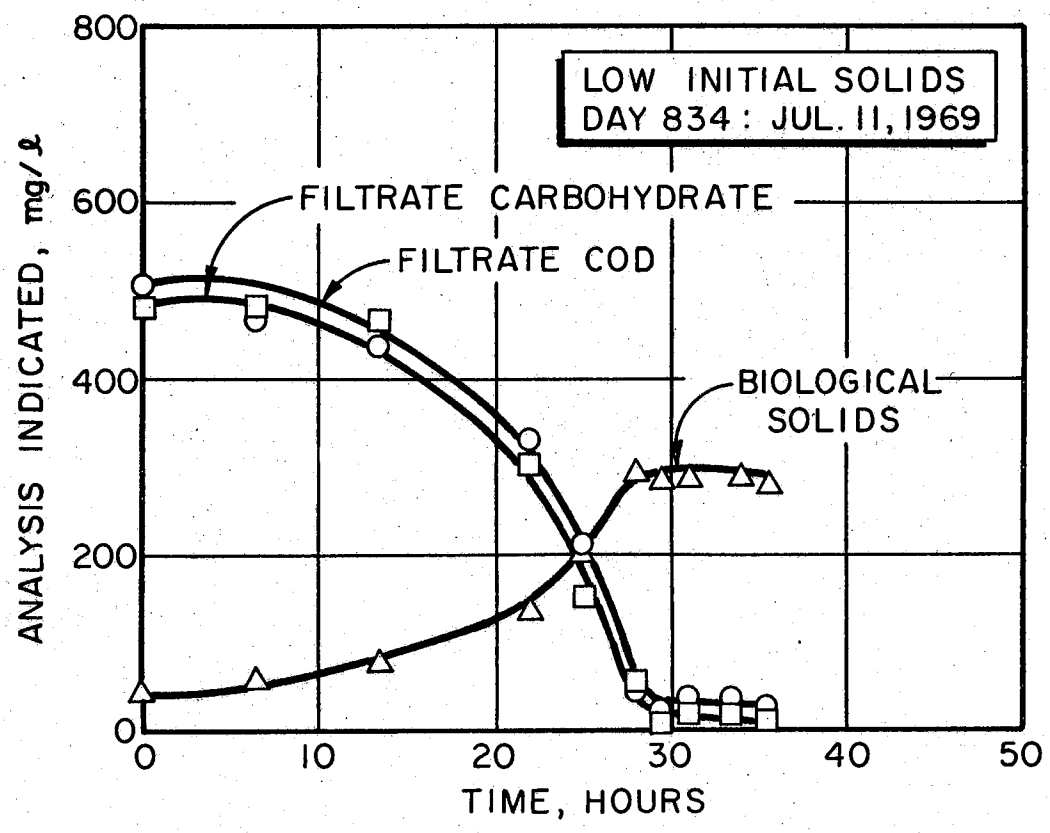
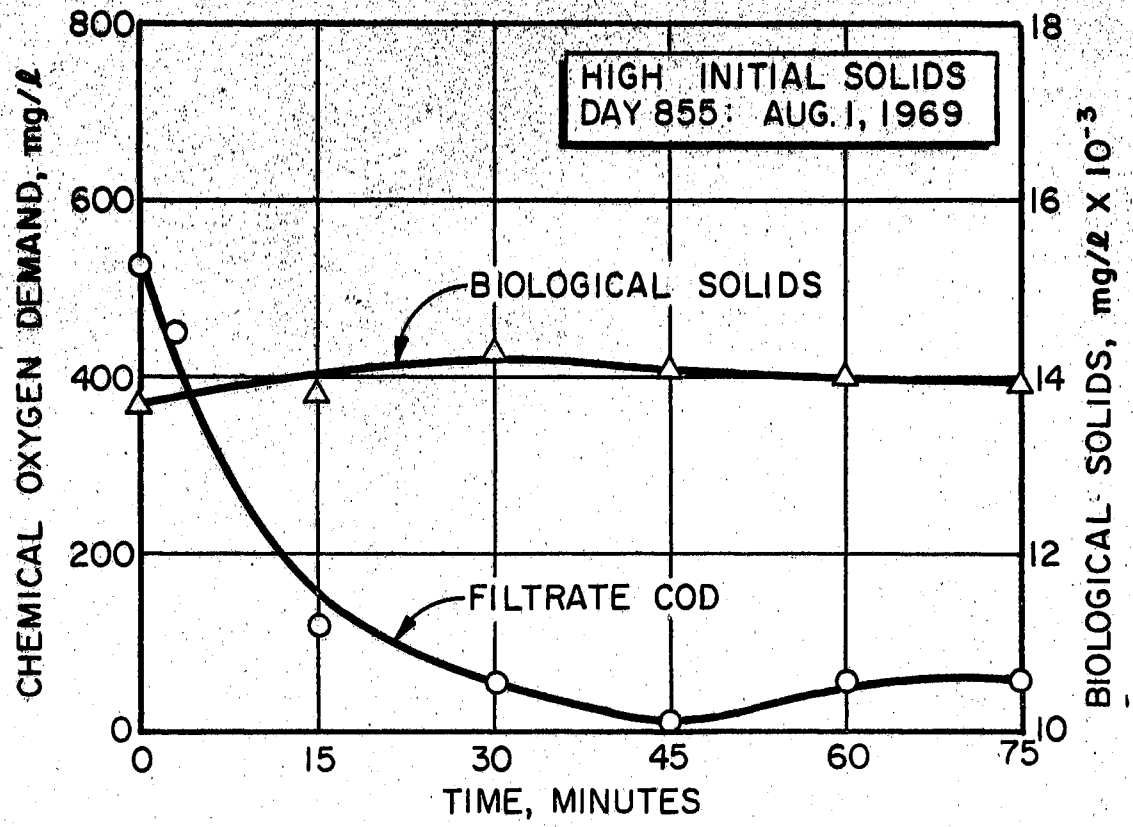
30
45
60
75


LOW INITIAL SOLIDS
DAY 834: JUL. 11, 1969
FILTRATE CARBOHYDRATE
FILTRATE COD
BIOLOGICAL SOLIDS
ANALYSIS INDICATED, mg/ℓ
TIME, HOURS
0
200
400
600
800
0
10
20
30
40
50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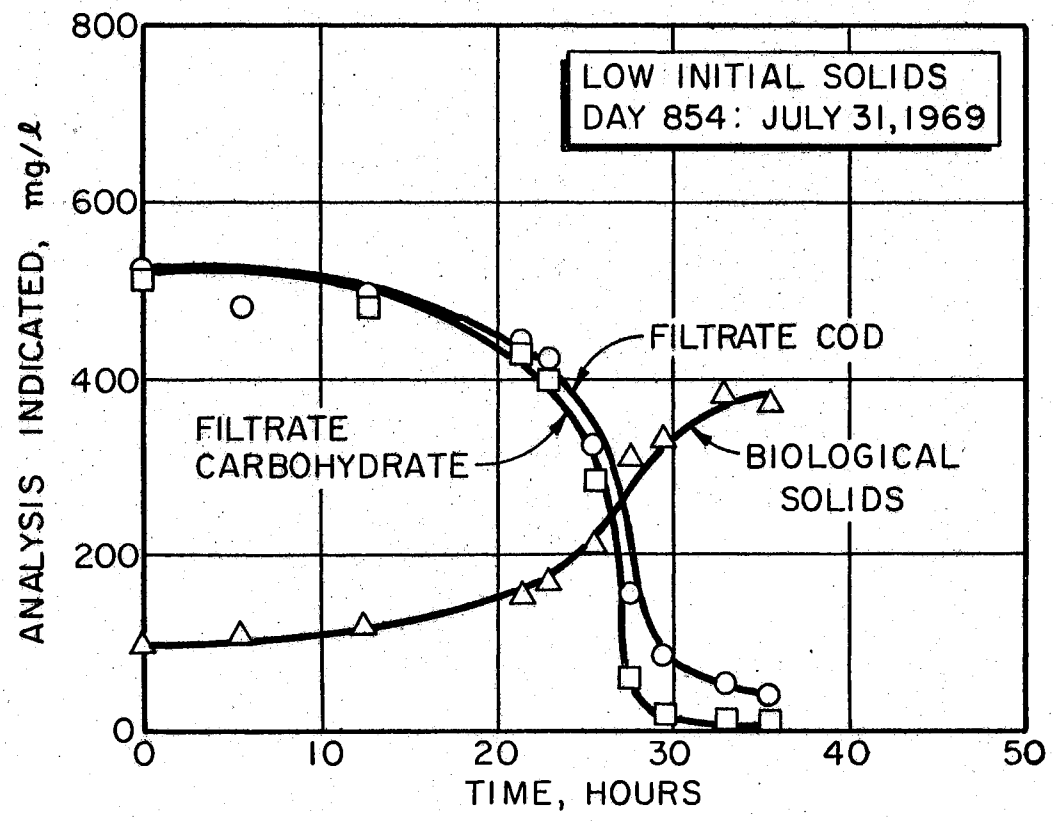
DAY 855: AUG. 1, 1969
BIOLOGICAL SOLIDS
FILTRATE COD
CHEMICAL OXYGEN DEMAND, mg/ℓ
BIOLOGICAL SOLIDS, mg/ℓ X 10⁻³
TIME, MINUTES
0
200
400
600
800
10
12
14
16
18
0
15
30
45
60
75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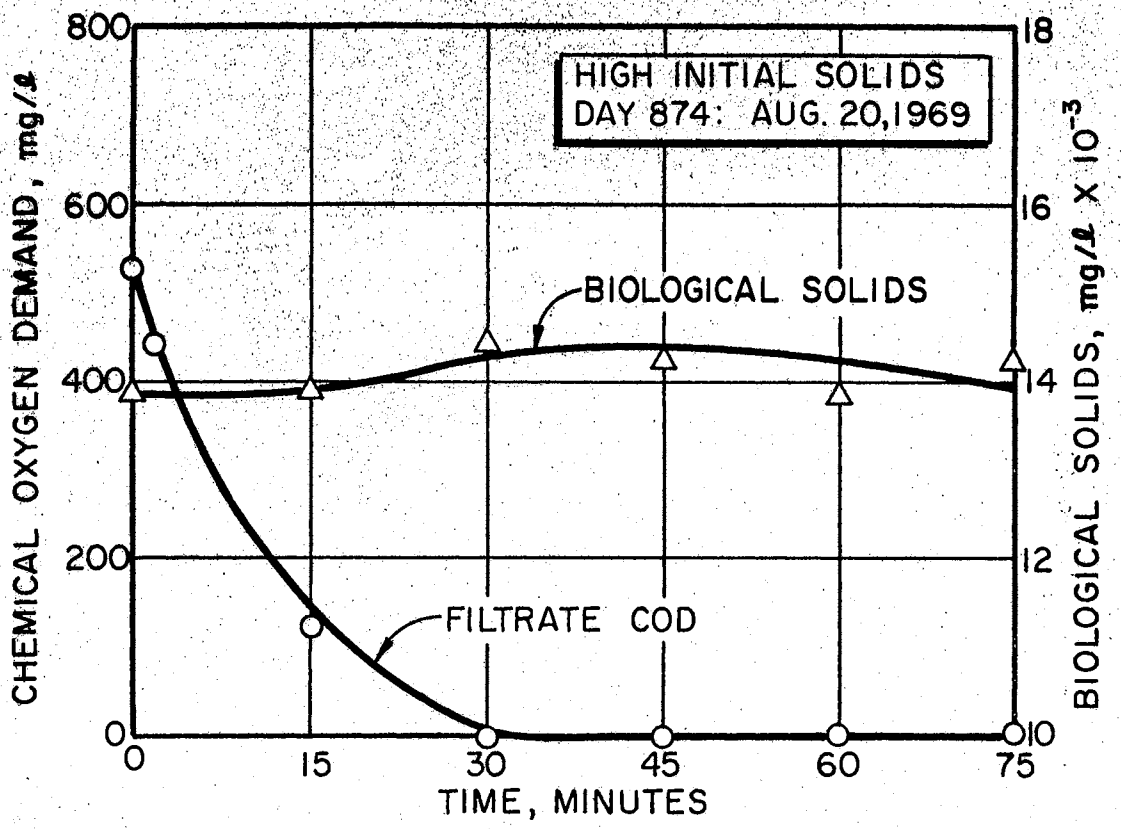
FILTRATE
CARBOHYDRATE
BIOLOGICAL
SOLIDS
ANALYSIS INDICATED, mg/ℓ
TIME, HOURS
0
200
400
600
800
0
10
20
30
40
50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FILTRATE COD
CHEMICAL OXYGEN DEMAND, mg/ℓ
BIOLOGICAL SOLIDS, mg/ℓ X 10⁻³
TIME, MINUTES
0
200
400
600
800
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12
14
16
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15
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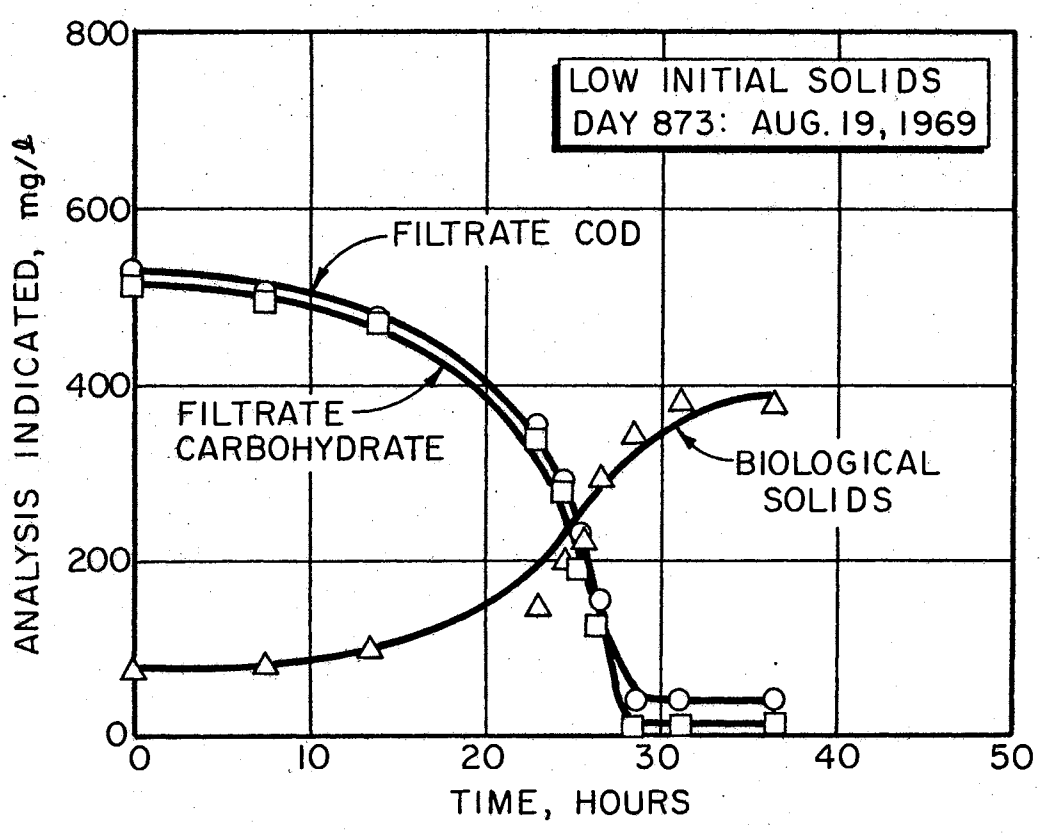
45
60
75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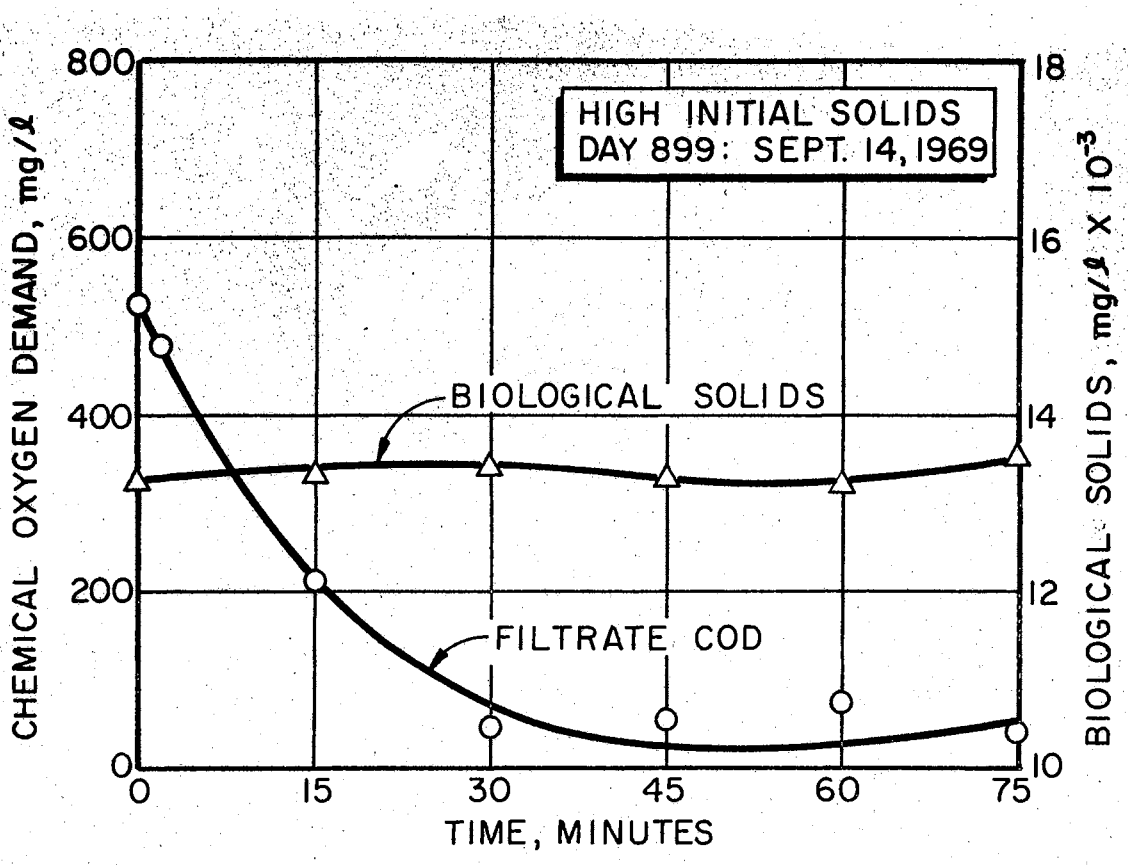
FILTRATE
CARBOHYDRATE
BIOLOGICAL
SOLIDS
ANALYSIS INDICATED, mg/ℓ
TIME, HOURS
0
200
400
600
800
10
20
30
40
50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DAY 899: SEPT. 14, 1969
BIOLOGICAL SOLIDS
FILTRATE COD
CHEMICAL OXYGEN DEMAND, mg/ℓ
BIOLOGICAL SOLIDS, mg/ℓ X 10⁻³
TIME, MINUTES
0
200
400
600
800
10
12
14
16
18
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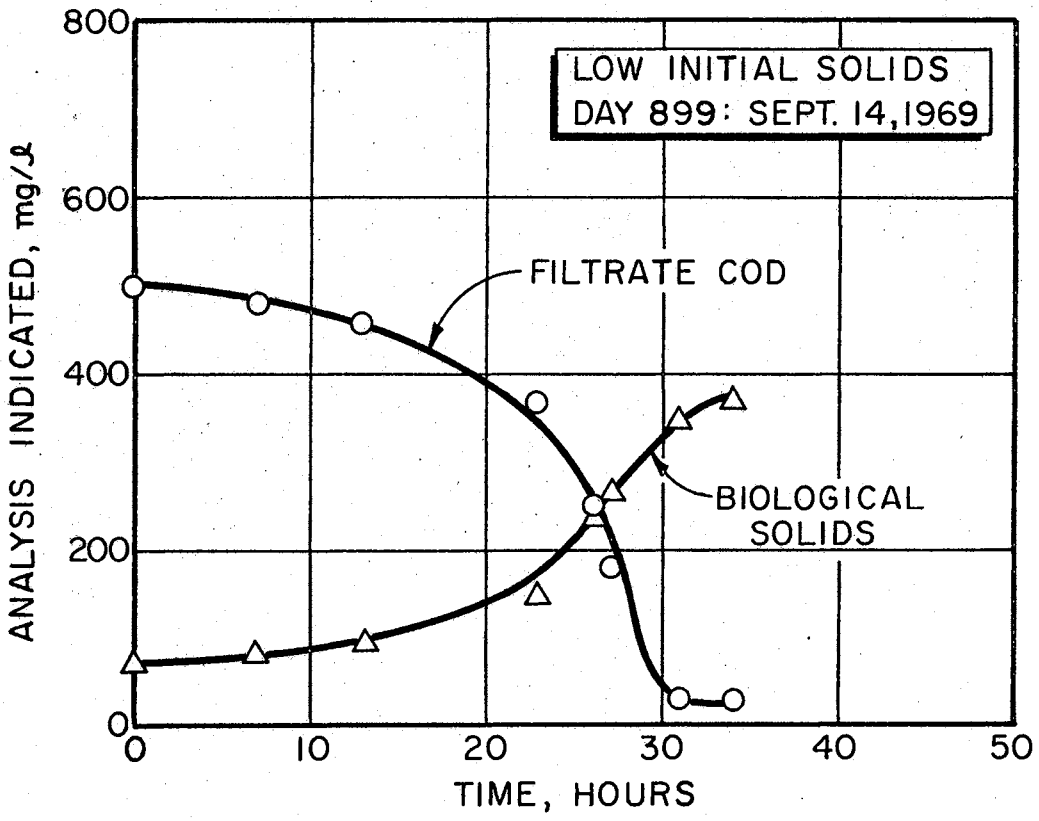
75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FILTRATE COD
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ANALYSIS INDICATED, mg/ℓ
TIME, HOURS
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400
600
800
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40
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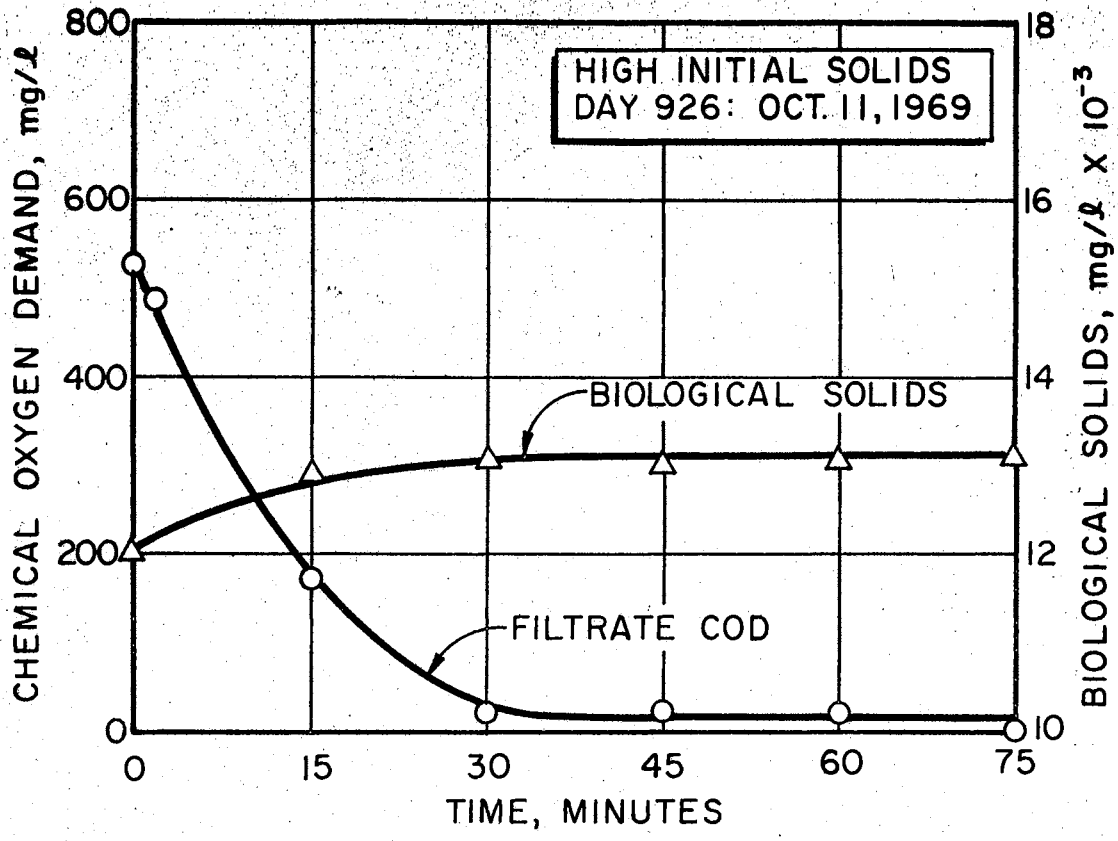
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DAY 926: OCT. 11, 1969
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BIOLOGICAL SOLIDS, mg/ℓ X 10⁻³
BIOLOGICAL SOLIDS
FILTRATE COD
TIME, MINUTES
0
200
400
600
800
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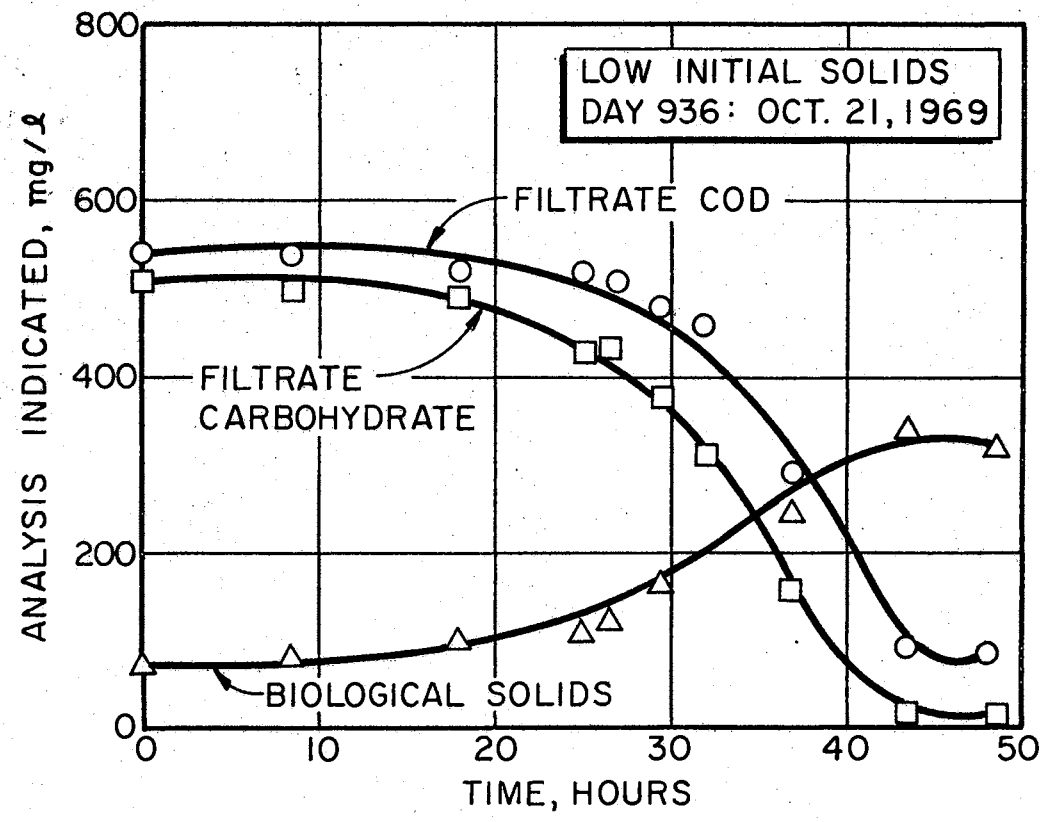
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FILTRATE COD
FILTRATE
CARBOHYDRATE
BIOLOGICAL SOLIDS
TIME, HOURS
0
200
400
600
800
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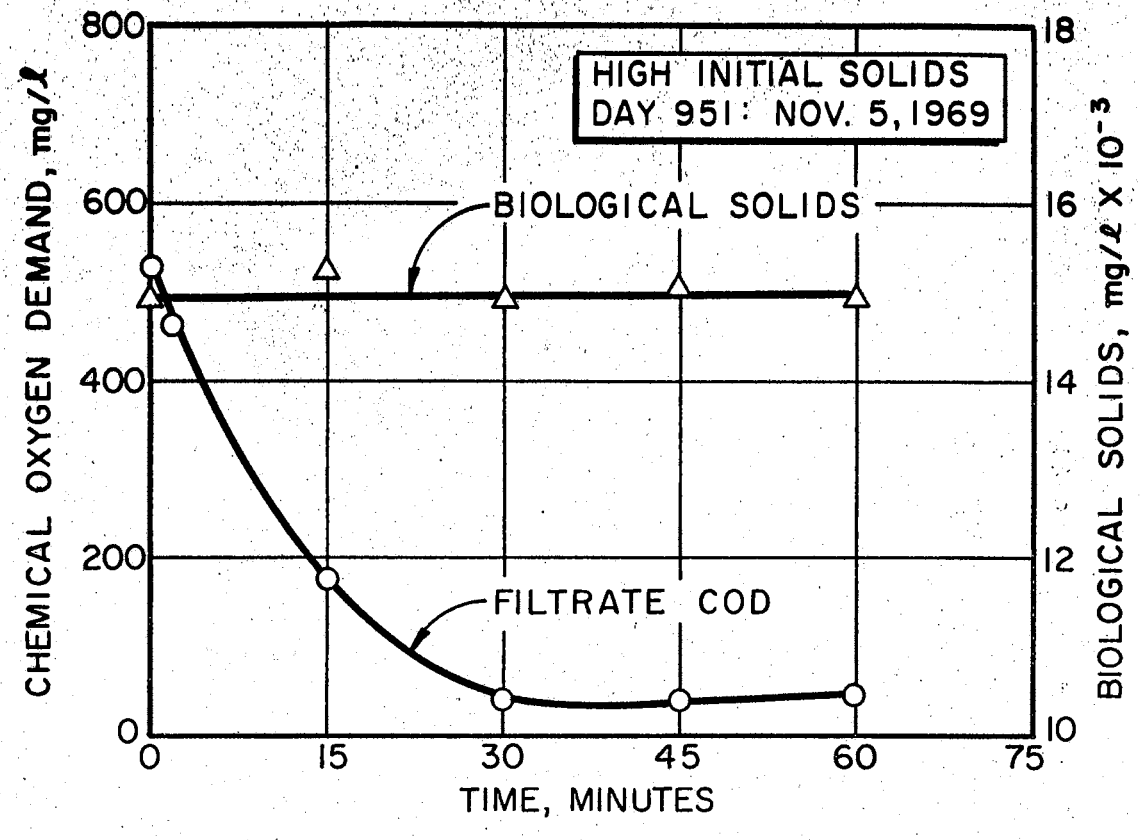
10
20
30
40
50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FILTRATE COD
CHEMICAL OXYGEN DEMAND, mg/ℓ
BIOLOGICAL SOLIDS, mg/ℓ X 10⁻³
TIME, MINUTES
0
200
400
600
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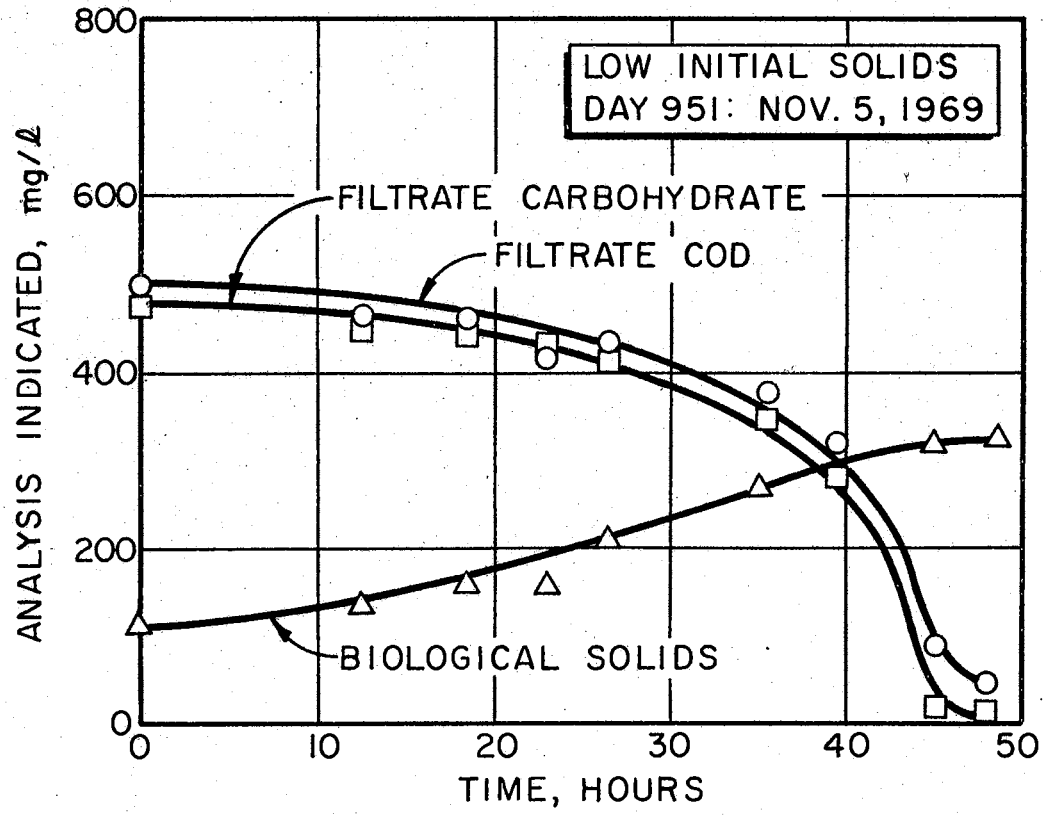
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FILTRATE COD
BIOLOGICAL SOLIDS
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TIME, HOURS
0
200
400
600
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20
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40
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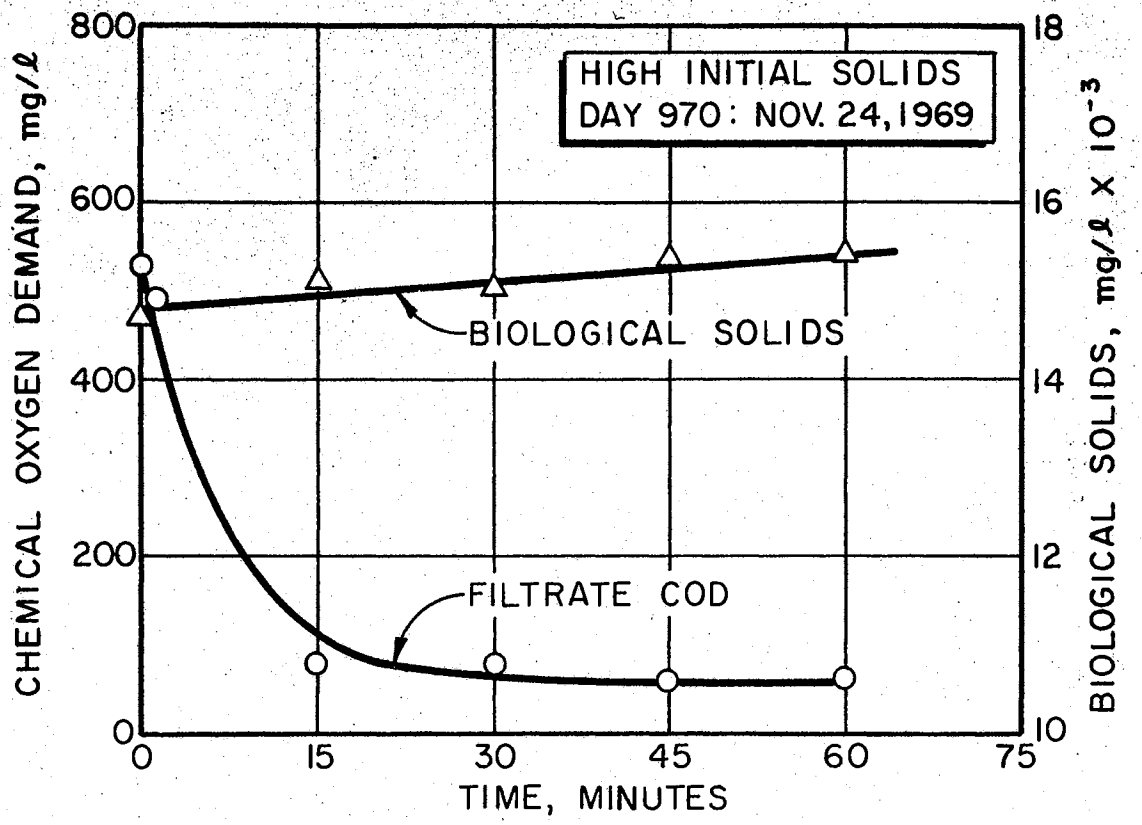
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CHEMICAL OXYGEN DEMAND, mg/ℓ
BIOLOGICAL SOLIDS, mg/ℓ X 10⁻³
TIME, MINUTES
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600
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14
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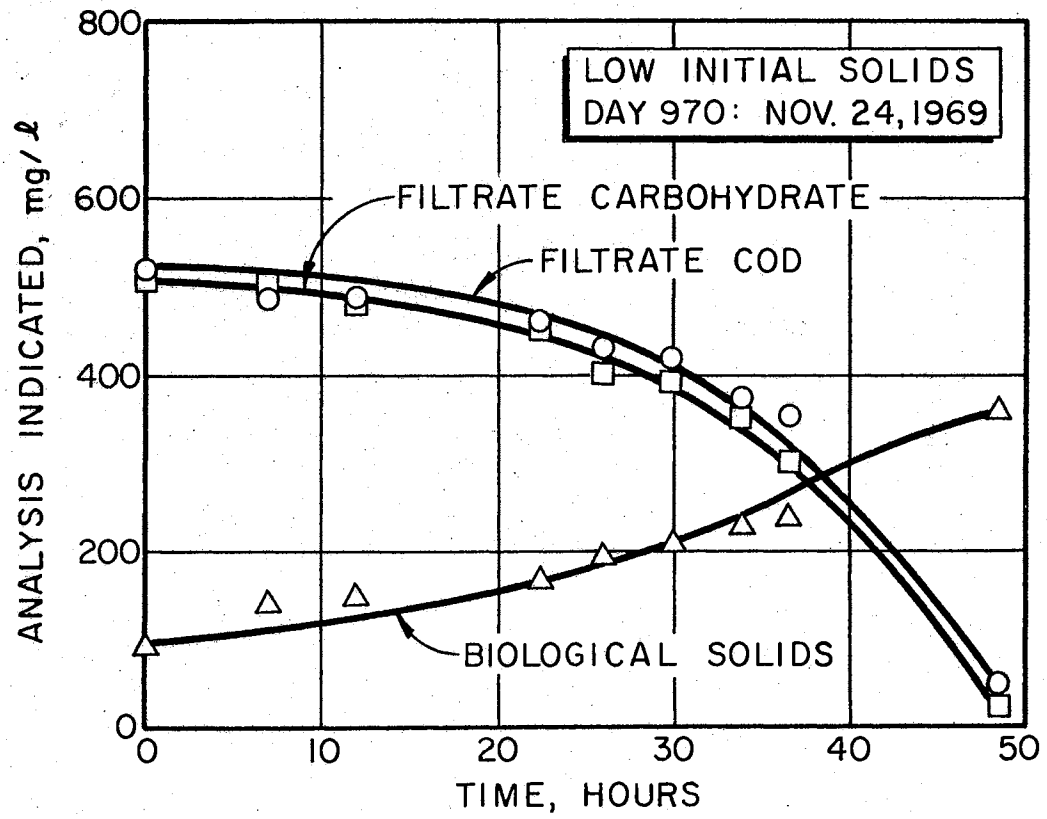
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FILTRATE COD
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TIME, HOURS
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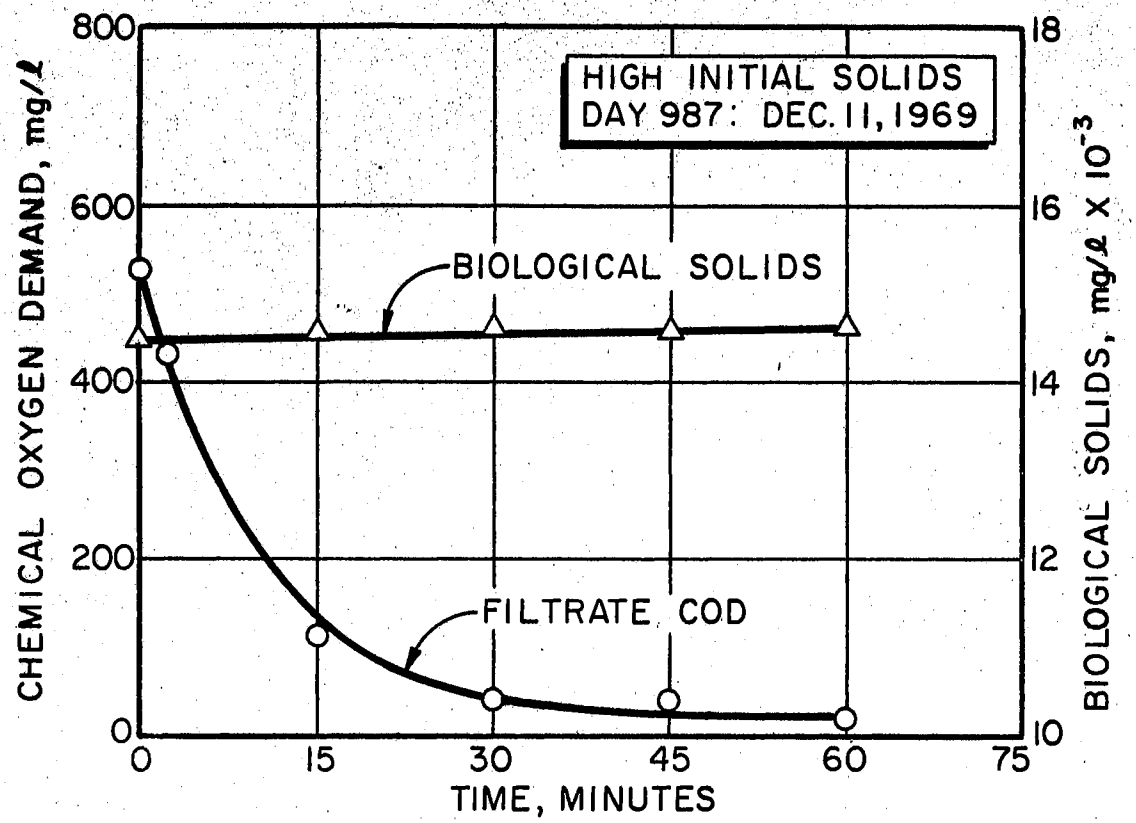
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DAY 987: DEC. 11, 1969
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FILTRATE COD
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BIOLOGICAL SOLIDS, mg/ℓ X 10⁻³
TIME, MINUTES
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200
400
600
800
10
12
14
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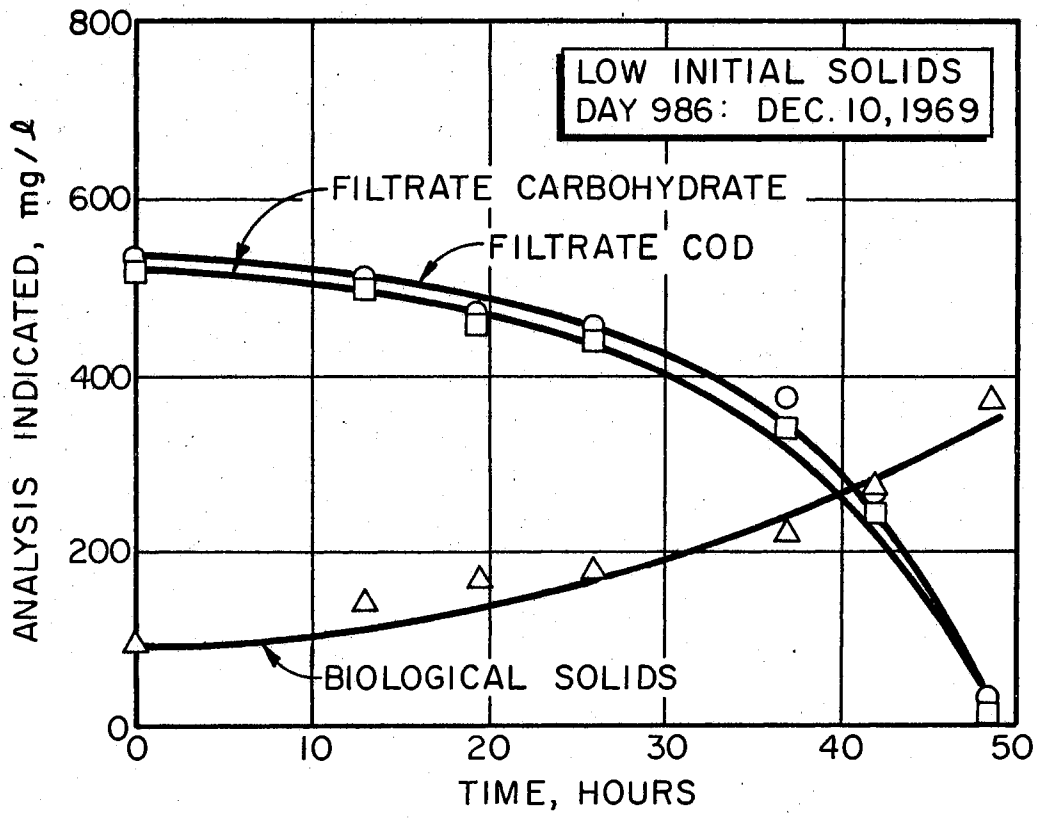
60
75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DAY 986: DEC. 10, 1969
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FILTRATE COD
BIOLOGICAL SOLIDS
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800
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20
30
40
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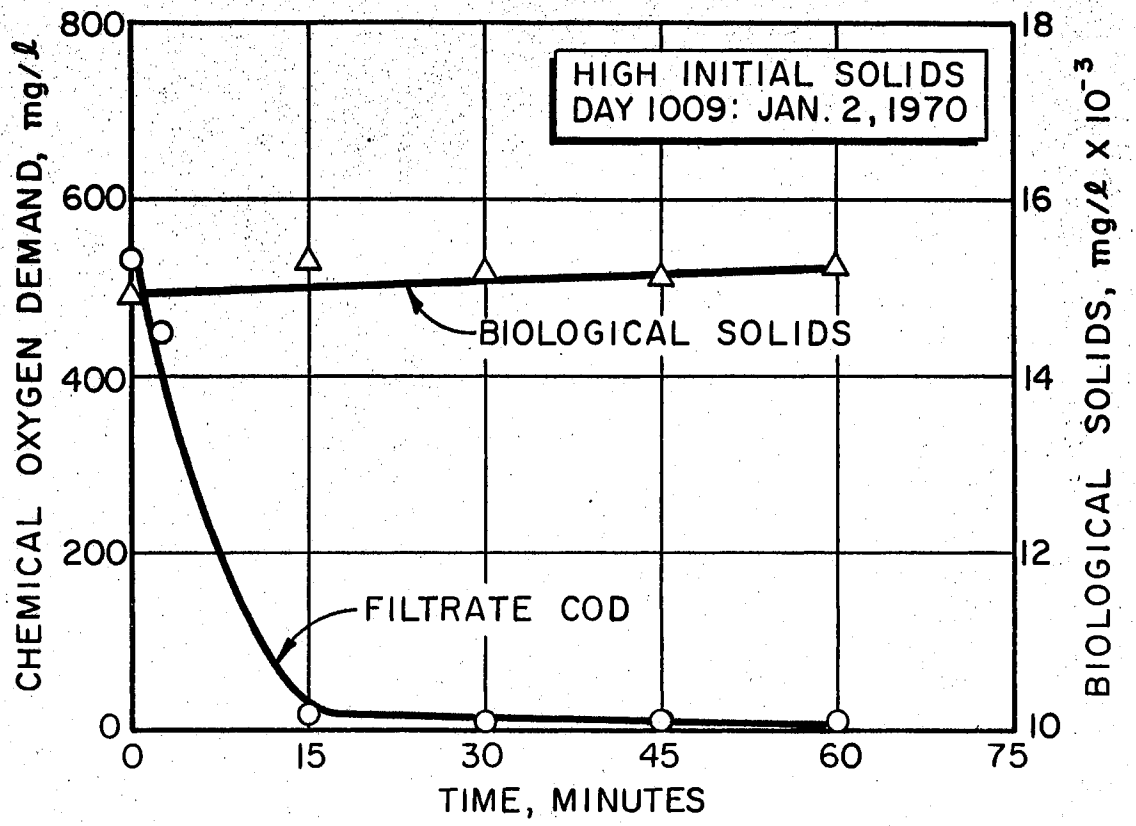
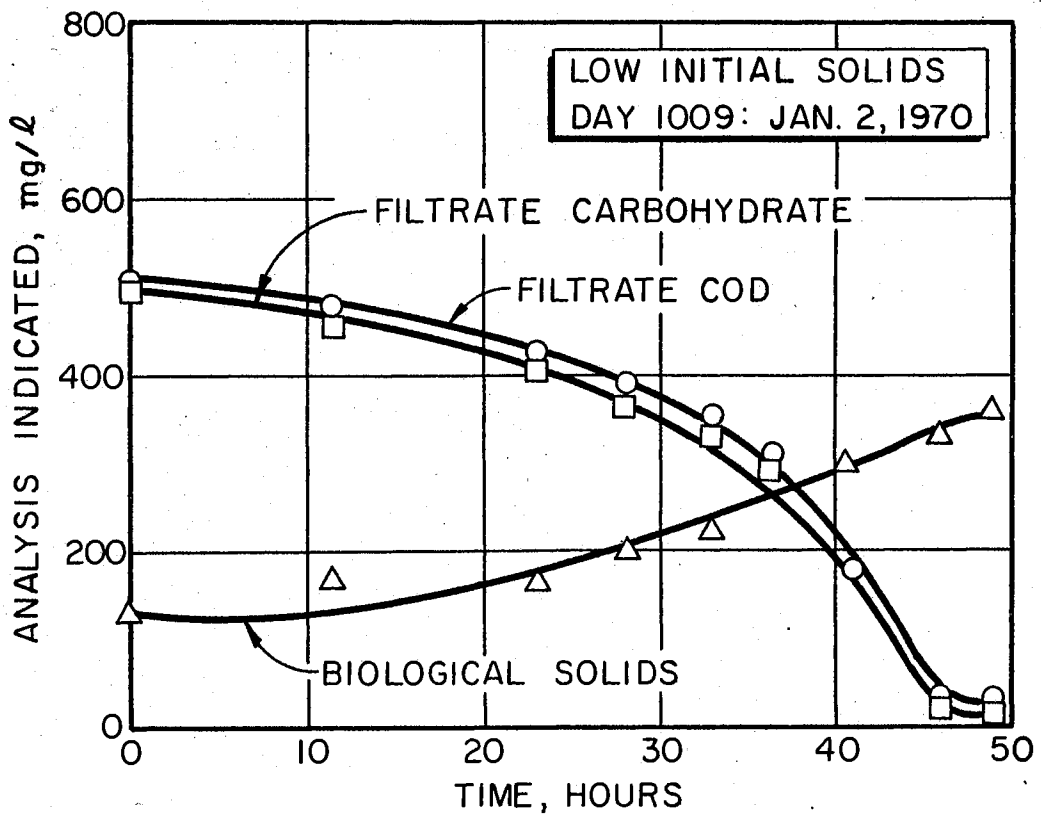
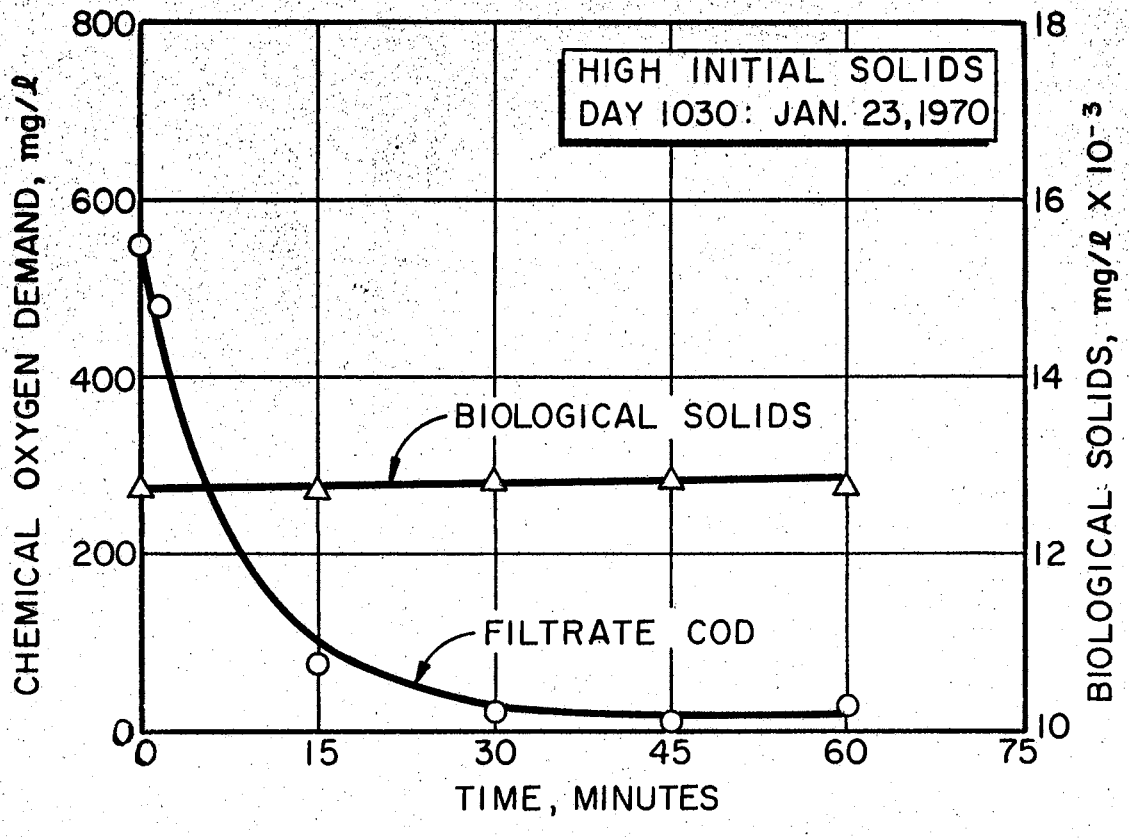
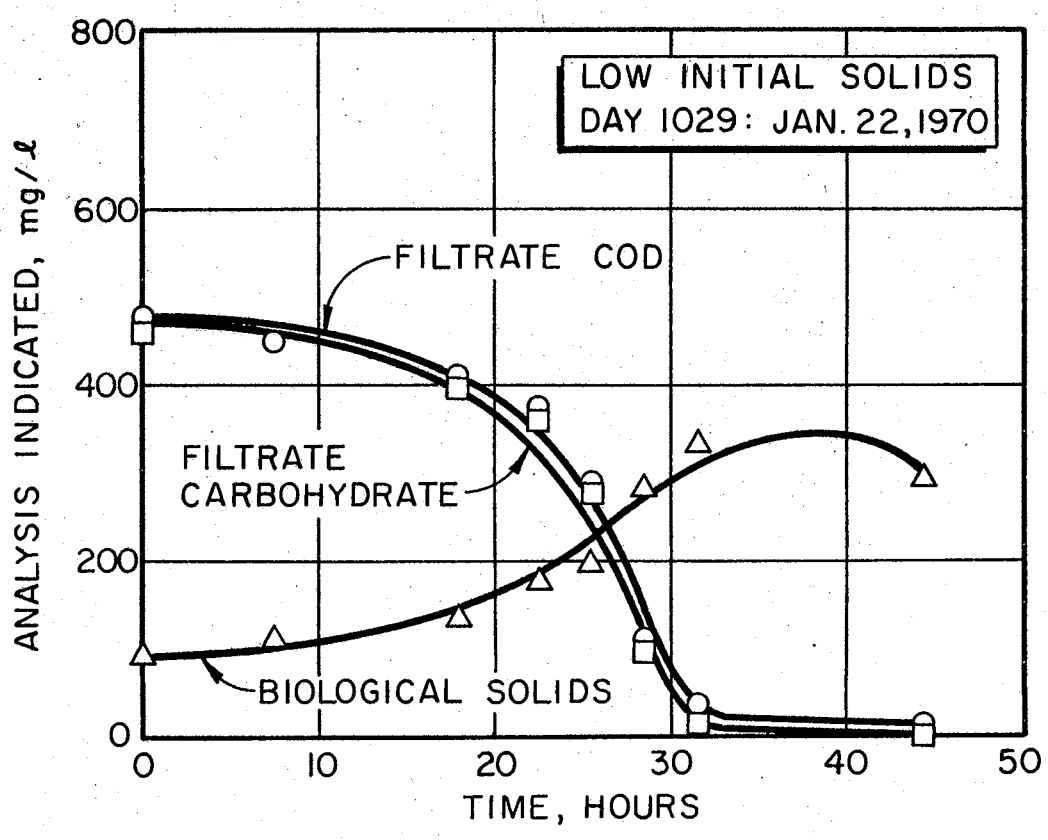
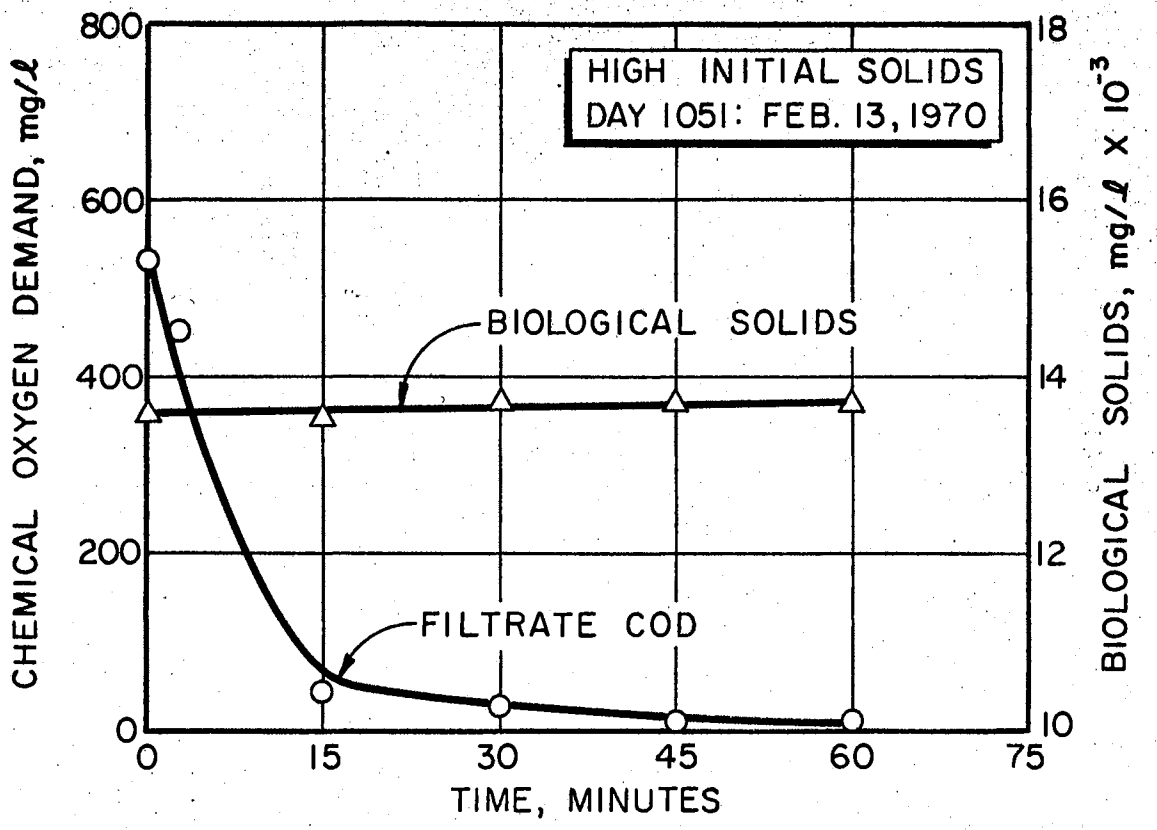
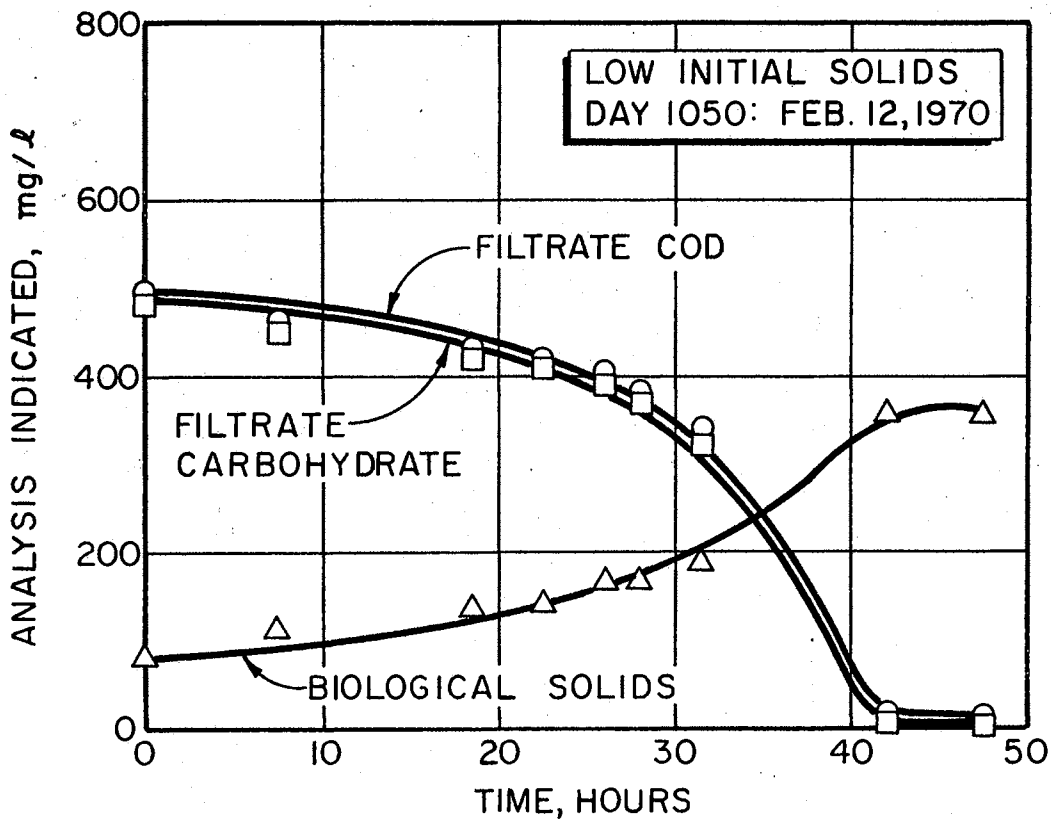

HIGH INITIAL SOLIDS
DAY 1009: JAN. 2, 1970
BIOLOGICAL SOLIDS
FILTRATE COD
CHEMICAL OXYGEN DEMAND, mg/ℓ
BIOLOGICAL SOLIDS, mg/ℓ X 10⁻³
TIME, MINUTES
0
200
400
600
800
10
12
14
16
18
0
15
30
45
60
75

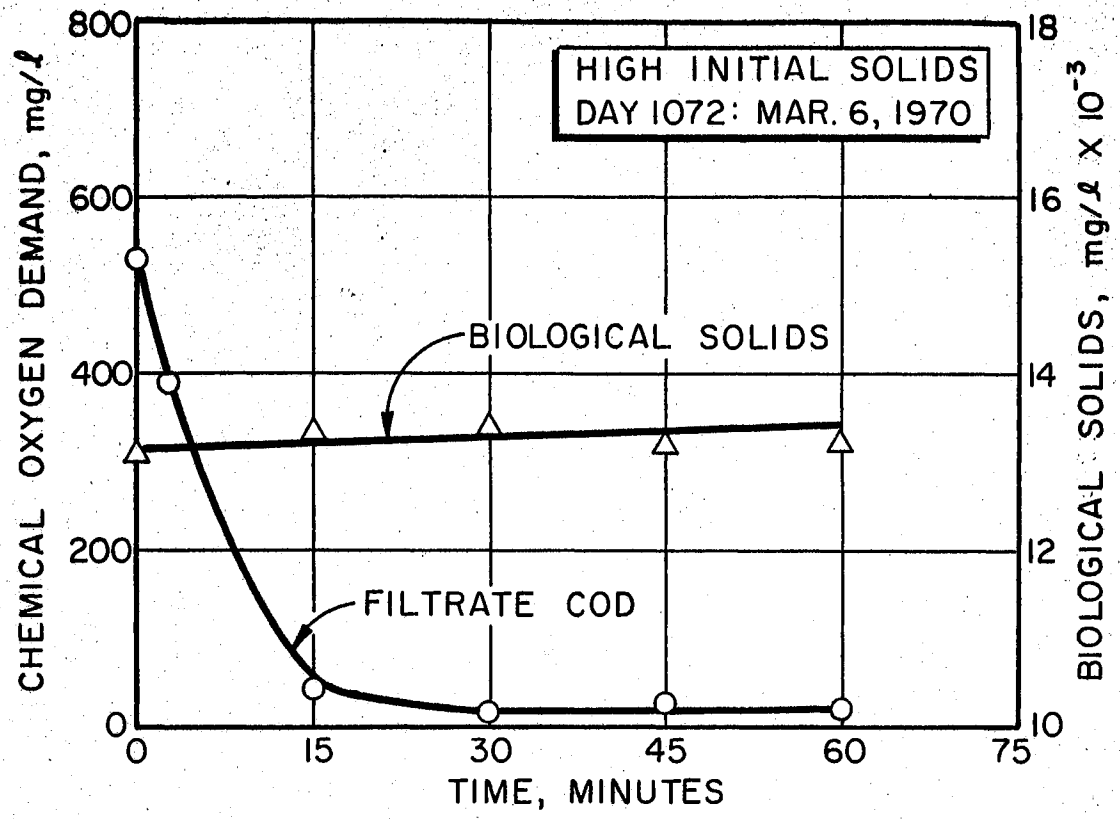

LOW INITIAL SOLIDS
DAY 1009: JAN. 2, 1970
FILTRATE CARBOHYDRATE
FILTRATE COD
BIOLOGICAL SOLIDS
ANALYSIS INDICATED, mg/ℓ
TIME, HOURS
0
200
400
600
800
0
10
20
30
40
50

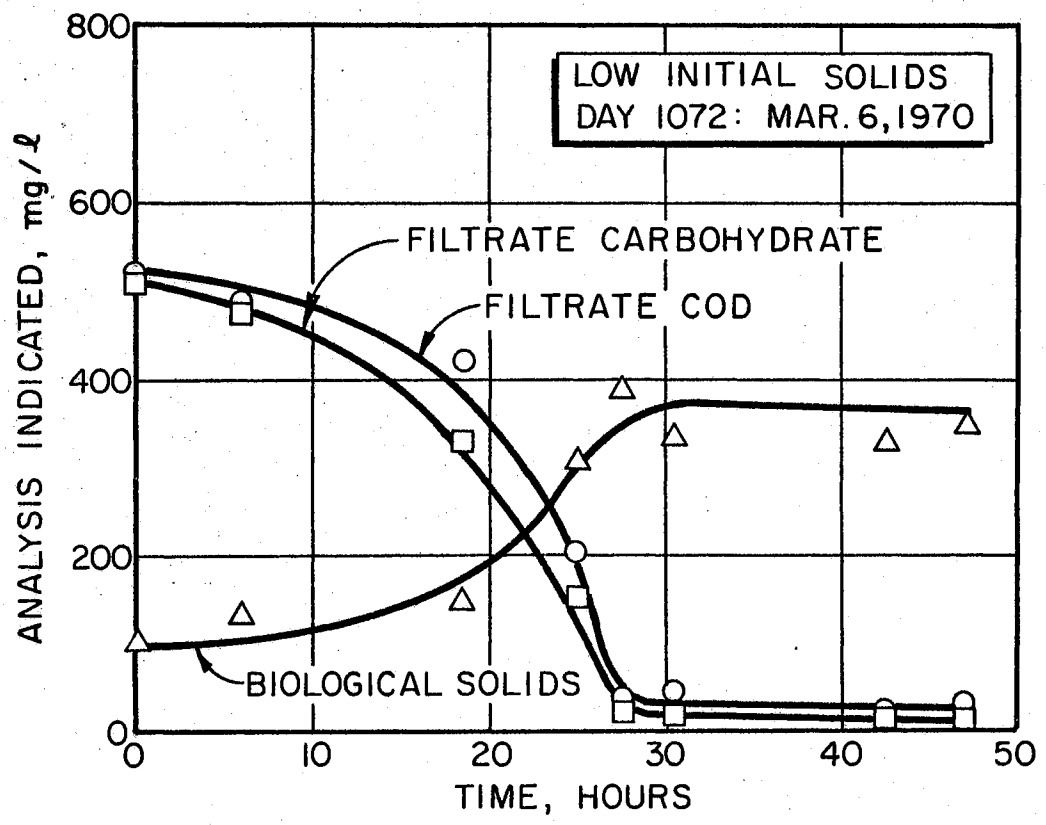

HIGH INITIAL SOLIDS
DAY 1030: JAN. 23, 1970
CHEMICAL OXYGEN DEMAND, mg/ℓ
BIOLOGICAL SOLIDS, mg/ℓ X 10⁻³
BIOLOGICAL SOLIDS
FILTRATE COD
TIME, MINUTES
800
600
400
200
0
18
16
14
12
10
0
15
30
45
60
75

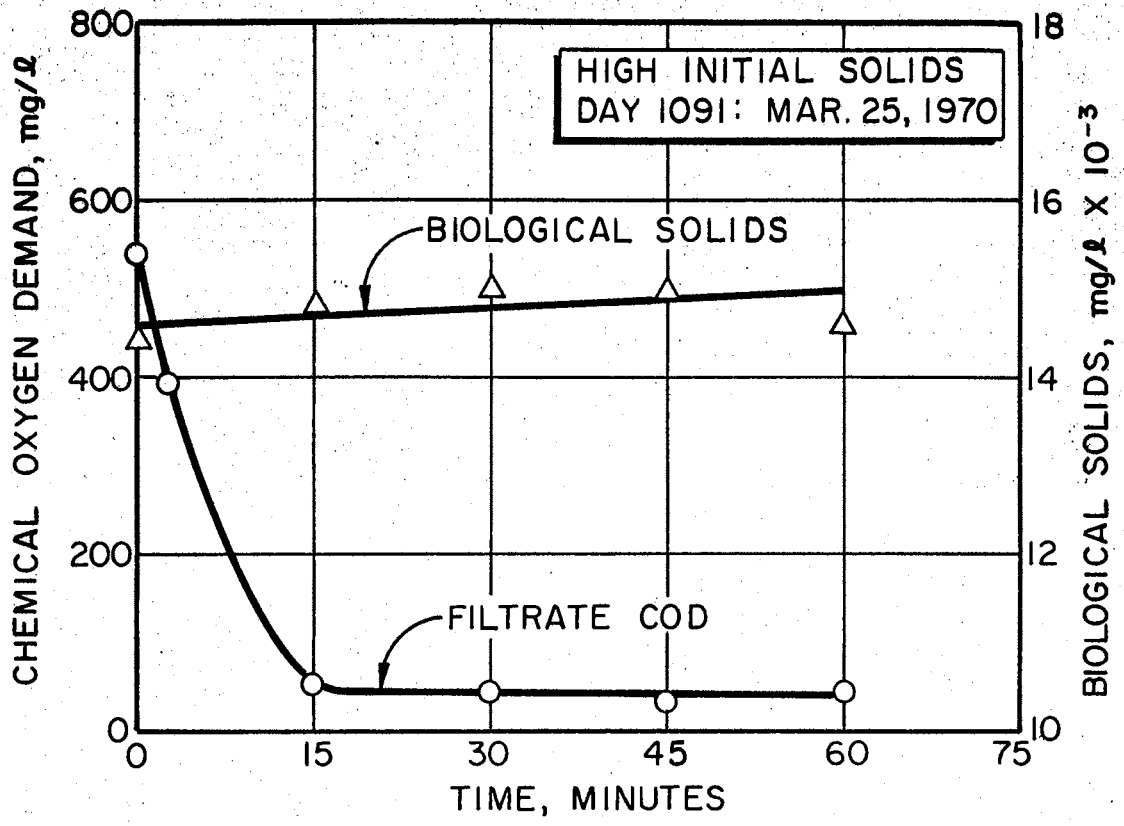

LOW INITIAL SOLIDS
DAY 1029: JAN. 22, 1970
ANALYSIS INDICATED, mg/ℓ
FILTRATE COD
FILTRATE
CARBOHYDRATE
BIOLOGICAL SOLIDS
TIME, HOURS
800
600
400
200
0
0
10
20
30
40
50

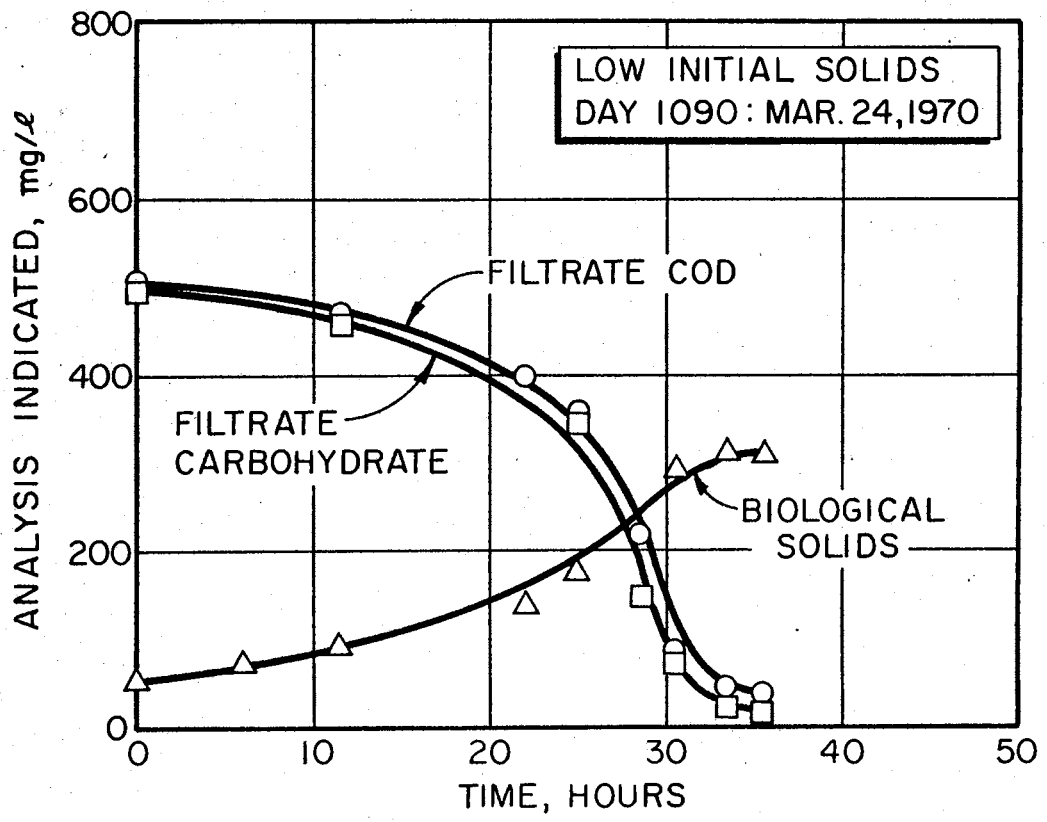

HIGH INITIAL SOLIDS
DAY 1051: FEB. 13, 1970
BIOLOGICAL SOLIDS
FILTRATE COD
CHEMICAL OXYGEN DEMAND, mg/ℓ
BIOLOGICAL SOLIDS, mg/ℓ X 10⁻³
TIME, MINUTES
0
200
400
600
800
10
12
14
16
18
0
15
30
45
60
75


LOW INITIAL SOLIDS
DAY 1050: FEB. 12, 1970
FILTRATE COD
FILTRATE
CARBOHYDRATE
BIOLOGICAL SOLIDS
ANALYSIS INDICATED, mg/ℓ
TIME, HOURS
0
200
400
600
800
0
10
20
30
40
50


HIGH INITIAL SOLIDS
DAY 1072: MAR. 6, 1970
BIOLOGICAL SOLIDS
FILTRATE COD
CHEMICAL OXYGEN DEMAND, mg/ℓ
BIOLOGICAL SOLIDS, mg/ℓ X 10⁻³
TIME, MINUTES
0
200
400
600
800
10
12
14
16
18
0
15
30
45
60
75


LOW INITIAL SOLIDS
DAY 1072: MAR. 6, 1970
FILTRATE CARBOHYDRATE
FILTRATE COD
BIOLOGICAL SOLIDS
ANALYSIS INDICATED, mg/ℓ
TIME, HOURS
0
200
400
600
800
0
10
20
30
40
50


HIGH INITIAL SOLIDS
DAY 1091: MAR. 25, 1970
BIOLOGICAL SOLIDS
FILTRATE COD
CHEMICAL OXYGEN DEMAND, mg/ℓ
BIOLOGICAL SOLIDS, mg/ℓ X 10⁻³
TIME, MINUTES
0
200
400
600
800
10
12
14
16
18
0
15
30
45
60
75


LOW INITIAL SOLIDS
DAY 1090: MAR. 24, 1970
FILTRATE COD
FILTRATE
CARBOHYDRATE
BIOLOGICAL
SOLIDS
ANALYSIS INDICATED, mg/ℓ
TIME, HOURS
0
200
400
600
800
0
10
20
30
40
50

VITA

Ping-Yi Yang

Candidate for the Degree of

Doctor of Philosophy

Thesis: STUDIES ON EXTENDED AERATION ACTIVATED SLUDGE AND A MODIFICATION OF THE PROCESS EMPLOYING CHEMICAL HYDROLYSIS OF PORTIONS OF THE RETURN SLUDGE

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Membership in Honorary Societies: Sigma Xi.

Publications:

Liu, M. T., Chiang, P. C., and Yang, P. Y., "Studies on the Amino Acid Fermentation by Some Microorganisms." Journal of the Chinese Agricultural Chemistry Society 2, No. 3-4, 1964 (Taiwan, R.O.C.).

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