EFFECTS OF AMMONIA NITROGEN CONCENTRATIONS ON GROWTH OF HETEROGENEOUS POPULATIONS DURING PURIFICATION OF SYNTHETIC WASTE WATERS

By

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CHAPTER I

INTRODUCTION

The greatest "war" on water pollution in history is now under way. The reason was stated by President Johnson at the signing of the Water Quality Act of 1952 (1) as follows: "The clear, fresh waters that were our national heritage have become dumping grounds for garbage and filth. They poison our fish, they breed disease, they spoil our landscapes."

Rapid increases of the population, steady increases in per capita use of water, and growth and diversification of industry are the primary reasons for the increased concern about the quality of water. The total fresh water supply in the United States, in the form of stream discharge and surface supply, has been estimated to be 1100 billion gallons per day (BGD) (2). Approximately 300 BGD are now withdrawn for municipal and industrial uses, power generation and irrigation, and approximately 190 BGD are returned to streams in altered form. The water pollution control field is presently concerned with the control of water quality in 14.6 BGD returned from municipalities and 29 BGD returned from industry. It is estimated that by the year

2000, approximately two-thirds of the water will be returned in altered form with municipal returns reaching 39 BGD, and industrial returns 208 BGD, emphasizing the need for increased reuse of water (3).

Conventional primary and secondary sewage treatment usually removes 80-95 per cent of the organic matter, 50 per cent of the nitrogen, and perhaps 20 per cent of the phosphorous. The continuing industrial and population expansion and the intensification of agriculture indicates that a significant increase in water contamination will occur. These contaminants are plant nutrients. The process of increasing the nutrient influx to surface waters is termed "stream enrichment." The presence of excessive amounts of plant nutrients results in the stimulation of algal growth, which in turn leads to increased numbers of other plants and animals such as bacteria, sponges, protozoa, rotifers, crustacea, fungi, and mollusca. Algae are of primary concern, since they can cause tastes and odors, clogged filters and screens, increased color, turbidity, chlorine demand, unwarranted growth in pipes and cooling towers, corrosion, variable pH, floating scum, and toxicity problems in water supplies (4). Heavy growths of algae may form blankets or mats which interfere with the use of some natural waters for recreational purposes. These mats of algae may act as barriers to the penetration of oxygen into the water, thereby suffocating fish. On the other hand, when dispersed in natural waters, algae increase the oxygen concentration

through photosynthesis. Heavy growth of algae may reduce the hardness of water and remove salts which are the causes of brakishness (5). Palmer (6) has reported that algae belonging to the genera *Asterionella*, *Melosira*, *Synedra*, and *Anabaena* are among those most often associated with filter clogging and odor production. Algae belonging to the genera *Nostoc*, *Calothrix*, *Cylindrespermum*, and *Anabaena* can convert atmospheric nitrogen into nitrogenous compounds (5).

The growth of algae is determined to a large extent by the amount of sunlight, carbon dioxide, and inorganic nutrients available. Because the amounts of sunlight and carbon dioxide are relatively constant, the key to the growth of excessive numbers of algae is the availability of inorganic nutrients. The two most significant algal nutrients occurring in limiting concentrations are nitrogen and phosphorous. Algae, as a group, include a large number of individual types of organisms, each of which has somewhat different nutrient requirements. Sawyer (7) reported that an algal nuisance develops in lakes when the concentrations of nitrogen and phosphorous exceed 0.3 and 0.015 mg/l, respectively, at the time of spring turnover. These values are usually referred to as the critical concentrations.

Nitrogen and phosphorous can enter waterways from many sources, either naturally or through the activities of man. Domestic wastes, industrial wastes, rural runoff and drainage, water fowl and the atmosphere are some of the sources

of nutrients.

Phosphorous in domestic sewage results from: (a) human wastes-feces, urine, and waste food disposal; (b) synthetic detergents; (c) carriage waters; and (d) infiltration waters, the first two being the main donors. Human excretion of feces and urine amounts to approximately 1.4 lbs per capita per year as reported by Hawk, Osser, and Summerson (8). The phosphorous contribution from the detergent industry alone was estimated as 1.6 lbs per capita per year in 1950 (9), 1.9 lbs in 1955 (10), and 2.1 lbs in 1958. The total per capita contribution of phosphorous in domestic sewage has been steadily increasing from 1.4 lbs in 1872 to a range of 1.5-4.2 lbs per year in 1959 (11). Some industries do not consume phosphorous or nitrogen but contribute significant quantities of nutrients in their waste; for example, the fertilizer industry. Coke plant wastes and petroleum refinery wastes often contain high concentrations of nitrogen. The condensate from petroleum refineries frequently contains hundreds to thousands of milligrams per liter of ammonia nitrogen. In general, the contribution of nitrogen from petroleum wastes may approach that from domestic and agricultural wastes (11). Also, meat processing industries produce waste rich in nitrogen; the sum of organic and ammonia nitrogen concentrations ranges between 100 and 300 mg/l.

The primary source of nitrogen in domestic waste waters are feces, urine, and waste food. In 1937, approx-

imately 11 lbs per capita per year was estimated to be the contribution of total nitrogen, distributed equally between ammonia and organic nitrogen. Usually fifty per cent of the latter is available to microorganisms as a source of nitrogen (12). Ammonia and organic polyelectrolytes used in water treatment also add a little nitrogen to the wastes. However, the concentrations of these compounds rarely exceed levels of 1.0 and 0.2 mg/1 of nitrogen, respectively. The increased use of fertilizers containing high concentrations of nitrogen and phosphorous also contributes large amounts of nutrients to drainage waters.

It was stated in a report by McCarty et al. (11) that the average municipal sewage treatment plant removes twenty to fifty per cent of incoming nitrogen. For an average waste flow of 100 (gallons per capita per day), the average concentration of nitrogen in the effluent would range between 18 and 28 mg/1. This is considerably higher than the nitrogen concentration cited as being limiting for algal growth.

Many industries produce wastes with very low or no nitrogen content. Examples of this kind include wastes from paper and pulp industries, sugar refineries, and several petrochemical industries. Beychok (13) has pointed out that wastes from catalytic polymerization, desalters, catalytic alkylation processes, and catalytic isomerization are high in COD but do not contain nitrogen. He found that wastes from synthetic rubber manufacturing plants, gas

processing plants, and naphtha crackers are high in chemical oxygen demand, but are essentially devoid of nitrogen.

In order to employ biological treatment for the various nitrogen-deficient wastes, it is necessary to add supplemental nitrogen. It is the usual practice to add the nitrogen (as NH_4^+) to the waste as it enters the aeration tank. The amount of nitrogen to be added generally corresponds to a biochemical oxygen demand (BOD):N ratio of 20:1 (14). The cost of nitrogen supplentation may sometimes run as high as the power cost for the compressed air (15). Often portions of this supplemental nitrogen may be found in the treated effluent. This not only constitutes a wastage of nitrogen, but also contributes to the many stream enrichment problems mentioned earlier. This optimum BOD:N ratio had been suggested for optimum BOD removal in batch experiments with little attention paid to the amount of nitrogen leaking in the treated effluent. With increased emphasis now on the tertiary treatment of waste waters, it has become imperative to re-examine the effect of different nitrogen levels in the activated sludge on the organic removal efficiency and the amount of nitrogen leakage in the effluent. The amount of supplemental nitrogen at which all of the added nitrogen is used up in the process of waste purification need not be the same as that required for optimum organic removal. This necessitates research leading to the information needed to strike a balance between purification efficiency and the nitrogen concentration in the

treated effluent.

The optimum BOD:N ratio suggested by past workers was based on batch experiments. Since that time the continuous flow completely-mixed activated sludge systems have been increasingly used in preference to the traditional activated sludge process. Many experimenters have found that the results of batch studies may not be applicable to these aerators, due to the very significant differences in the two modes of operation. Furthermore, previous studies do not furnish much information on the effect of different nitrogen levels and different dilution rates on the cell composition, which in turn governs the activity of the cell mass. Thus, in order to achieve an insight into the role of nitrogen, its effect on cell composition warrants detailed studies.

As a first step toward the development of a more efficient method for treatment of nitrogen-deficient wastes, it was considered necessary to investigate the factors affecting nitrogen requirements and their effect on cell composition. The first phase of this study deals with this investigation.

Application of basic scientific findings for beneficial use has been the goal of the engineering profession. Much of the value of basic research would be lost if it cannot be used in practice. The second phase of this study deals with the application of previous basic findings to waste purification, which led to a process modification for

treatment of nitrogen-deficient wastes by the activated sludge process. This process could reduce nitrogen supplementation and prevent the leakage of nitrogen in the treated effluent. Such a system has recently been proposed by Komolrit, Goel, and Gaudy (16). The flow sheet is reproduced in Figure 1. The process is dependent upon the ability of protein-rich sludges to remove organic substrate in the absence of exogenous nitrogen, provided the system initially carried a high biological solids concentration. In high solids systems it had been observed that the substrate is first assimilated and stored in the cells. Later, when almost all of the exogenous carbon source has been taken up, part of this stored product is converted to protein if an exogenous nitrogen source is available. The process modification described in the flow sheet represents an attempt to separate physically these two processes. It was reasoned that if successful intracellular conversion of carbohydrate to protein could be accomplished, the protein-rich sludge might be capable of again removing organic matter under nonproliferating conditions. The flow sheet represents a method for treating an organic waste without ever bringing it in contact with exogenous nitrogen. Furthermore, nitrogen is added to only that portion of the sludge which is recycled. It was thought that this could possibly lead to some savings in the cost of supplemental nitrogen. The latter portion of this report describes further studies to assess the mechanistic and operational feasibility of the process.

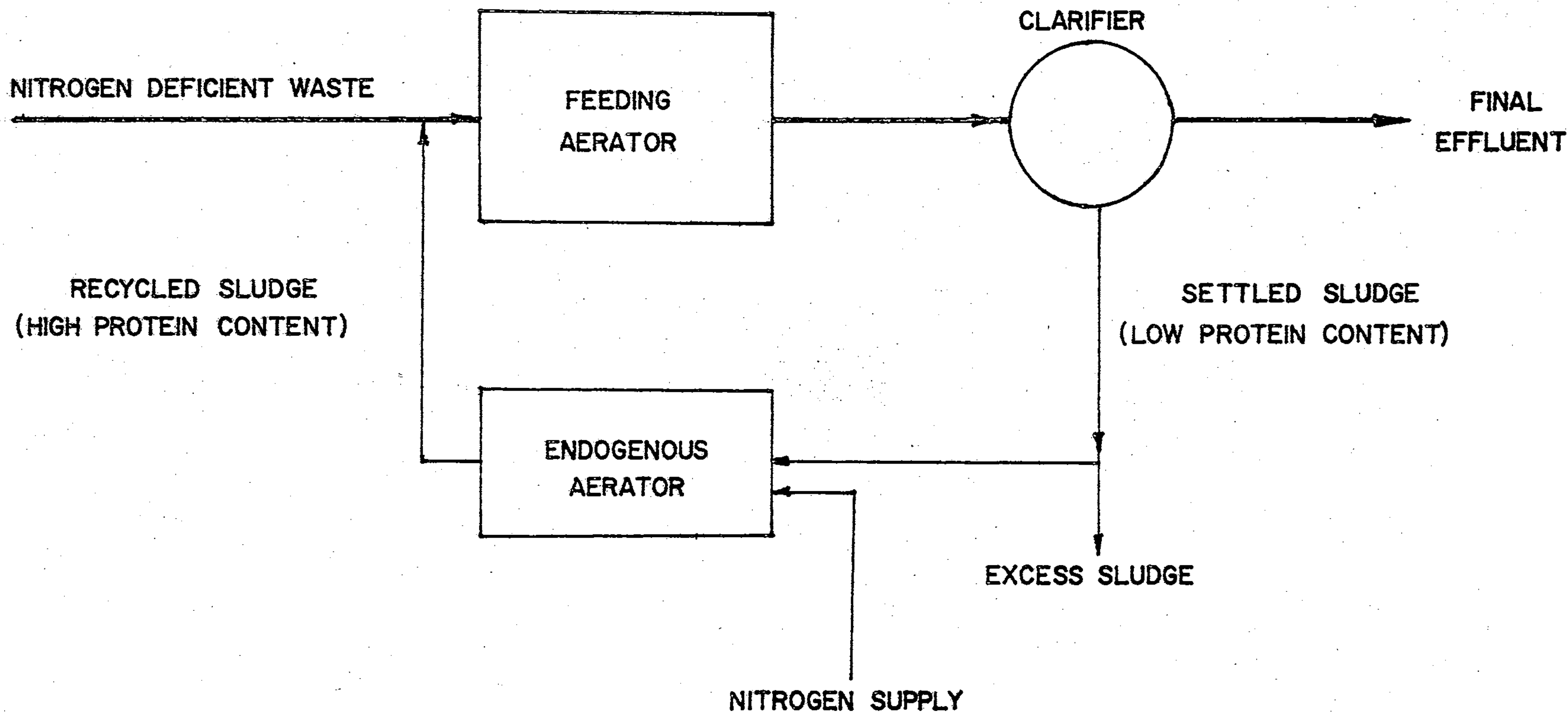


Figure 1. Modified flow sheet for treatment of nitrogen-deficient wastes.

CHAPTER II

LITERATURE REVIEW

A. Activated Sludge Modifications

The activated sludge process is one of the most widely used methods for treatment of domestic and industrial wastes. The process came into being in 1913, when Ardern and Lockett, in the laboratory of the Manchester Sewage Works, discovered that nitrification of sewage occurred at shorter periods of aeration when they retained the accumulated sludge. On April 3, 1914, they reported their findings before the Manchester Section of the Society of Chemical Industry. Ardern and Lockett described their results as follows:

> "The resultant solid matter obtained by prolonged aeration of sludge has the property of enormously increasing the purification effected by simple aeration of sewage; in other words, it greatly intensifies the oxidation process. The extent of the accelerating effect depends upon the intimate manner in which the activated sludge is brought into contact with, and upon its proportion to, the sewage treated."

Their original article and some others describing early thoughts on biological treatment were reprinted (17) on the occasion of the 40th anniversary of the "birth" of the activated sludge process. In these fifty-four years the

process has been employed for treatment of domestic and industrial wastes differing considerably in nature. The application of the process for treatment of industrial wastes resulted in many difficulties, and ways to solve these problems have resulted in numerous modifications of the original process. The development of activated sludge processes has been expertly reviewed by Sawyer (18, 19), Jaffe (20), Babbitt and Baumann (21), Simpson (22), and Mohlman (23). Sawyer (19) attributed many of the difficulties in waste treatment to lack of knowledge regarding the biochemical nature of the process. Many engineers thought that the process was merely application of physical principles. In its very early development, the process involved aerating wastes, along with 2000-3000 mg/l of activated sludge, for six to eight hours.

Sawyer (19) has given the limitations of the conventional process in detail. They are:

> "1) BOD loadings are limited to 35 lbs/1000 cu ft of aeration volume; 2) there is a high initial oxygen demand; 3) there is a tendency to produce bulking sludge; 4) the process is unable to produce an intermediate quality of effluent; 5) high sludge recirculation rates are required for high BOD wastes; 6) solids loadings on the final clarifier are high; and 7) air requirements are high."

Many of the process modifications which have been proposed were devised to overcome some aspect of these limitations. The various modifications of the activated sludge process are: (1) step aeration; (2) tapered aeration; (3) sludge reaeration, or the Mallory process;

(4) the Kraus process; (5) biosorption, or contact stabilization; (6) dispersed aeration; (7) high-rate activated sludge process; (8) bioflocculation, or contact aeration; (9) the Hays process; (10) the Guggenhein process; (11) the Zigerli process; (12) the bio-activation process; (13) the Logan process; (14) two-stage aeration; (15) activated aeration; (16) extended aeration; (17) the spiro-vertex process; (18) the Halmur process; and other various patented processes.

In step aeration, the return sludge or settled sludge may be added to the mixed liquor at various points along its flow through the aeration tank. It is possible to control the concentration of suspended solids entering the final sedimentation tank by controlling the rates and points of application of return sludge to the aeration tank, and the concentration of solids in the return sludge. This practice is believed to allow the maintenance of adequate sludge age while maintaining low suspended solids entering the final settling tanks. The process is used when: (1) there is a persistent increase in the volatile content of the activated sludge; (2) when dissolved oxygen is dropping steadily over a long period; and (3) in case of high *Sphaerotilus* growth (which have been used as an index of over-loading or of poor sludge condition). The process is said to have the advantage, compared with conventional aeration, of requiring as little as one-half of the aerator volume, smaller site area and better control under shock

loads. Gould (24) reported BOD removals from 87 to 97 per cent in five plants in New York City. It is, however, doubtful if this process will give satisfactory results when the waste is high in BOD and low in suspended solids concentration.

Tapered aeration involves the application of air to the aeration tank at a higher rate near the influent end of the tank. It is claimed that 40 to 50 per cent of the air is required in the first two hours, 28 to 31 per cent in the second two hours, and 20 to 29 per cent in the third two hours, for a six-hour aeration period (25). Advantages claimed for tapered aeration include better control of the process, ability to withstand shock loadings consisting of changes in the quality of the influent, and a marked reduction in the cost of operation.

The Mallory process (26) is a patented, proprietary procedure which seeks to separate the adsorption and oxidation stages in the removal of the substrate. The former requires no oxygen supply and, therefore, it is claimed that the process requires less air supply. However, the value of the process is highly questionable, especially in regard to soluble wastes. Furthermore, it requires a more skilled operator to run the plant, as the efficiency of the treatment depends on the maintenance of the "Equilibrium Index" at a value of 100 or more. Mallory (26) defined the "Equilibrium Index" as follows:

$$Eqx = \frac{R^2 D}{B\ M\ K}$$

wherein K = a constant (2A/C - 1); A = aeration period in hours; B = sludge blanket volume (cu ft or gallons); C = sedimentation period in hours; D = clarifier liquid volume (same units as B); M = mixed liquor suspended solids concentration in percent; R = return sludge solids concentration in percent and Eqx = Equilibrium Index.

Difficulties due to bulking of sludge in the secondary clarifier at Peoria, Illinois, led to the development of a modification of the activated sludge process, now called the "Kraus" process (27). The process involves separate aeration of digestion tank supernatant, digested sludge, and activated sludge, to produce a well-nitrified sludge with good settling characteristics. This brings about better settling due to addition of weight and provides nitrates as an additional source of oxygen to maintain aerobic conditions at the inlet end of the tank. Kraus was able to increase the loadings to 175 lb/1000 cu ft aeration volume with BOD removal of 86 to 90 per cent. The successful treatment of high organic loadings would appear to be due to the increased solids concentration in the aerator. However, this higher loading necessitated the use of a "dual" system of aeration where part of the air is admitted through diffusers at the bottom of the aeration tank, and part is distributed near the surface to obtain maximum surface turbulence (28). The process has been patented.

The biosorption process developed by Ulrich and Smith (29) at Austin, Texas, is essentially a high rate activated

sludge process in which sludge from the secondary clarifier is "reactivated" by aeration in a separate tank so that it can be reused under intense short term aeration (15 to 40 minutes) in the mixed-liquor aeration tank. The aeration time used for reaeration of the concentrated sludge from the secondary clarifier is approximately 90 minutes. The return sludge volume is very high, being about 40 per cent. The process has the advantages of eliminating primary sedimentation and requiring smaller aeration tanks and is especially useful if the waste is colloidal in nature. However, it suffers from the disadvantage that difficulties are experienced in compaction of the sludge. The BOD loading used in the process is 150 lb/1000 cu ft aeration volume.

A modification of the biosorption process has been termed "contact stabilization." In this process, a primary sedimentation tank is included in the flow sheet. Several plants have been successfully converted to this process (30). It is claimed that the process offers greater flexibility of operation and better protection against shock loadings imposed by discharge of industrial wastes. The mechanism of the process was originally considered to be adsorption, but Gaudy and Engelbrecht (31) showed that the mechanism of rapid removal of soluble carbohydrate wastes does not involve adsorption but does entail a metabolic assimilation of the substrate. According to Komolrit, Goel, and Gaudy (16), the very short period of contact in the biosorption process does not provide opportunity for a

large amount of protein synthesis in the first aerator, and the so-called regeneration or reactivating basin is simply one which provides for the conversion of stored products to protein.

Dispersed aeration and high-rate activated sludge processes are used for producing an intermediate quality effluent. In the dispersed aeration process, the aim is removal of substrate by oxidation alone, i.e., not by both partial growth of microorganisms and oxidation of soluble substrate as in the activated sludge treatment. This is suitable when only 30 to 50 per cent purification is desired, and is especially suited for wastes containing soluble organics. The relatively low organic removal efficiency is due to non-settleability of the sludge. The need for sludge disposal facilities is eliminated. However, a considerable amount of the residual oxygen demand of the waste (due to the high microbial content) is exerted in the receiving stream. This process can be recommended only in cases where partial treatment is warranted.

High-rate activated sludge treatment refers to aeration for shorter than standard periods of time, with the return of a smaller than standard percentage of sludge from the settling tank. Setter and Edwards (32) called the process "modified sewage aeration," and recommended it for partial sewage purification. They also claimed that any degree of purification can be obtained by controlling the air supply, the aeration period, and the amount of return solids.

Chase (33) recommended the use of spiral-circulation tanks with two or three hours' retention period and return of 10 to 25 per cent of the sludge. Torpey and Chasick (34) pointed out that: (1) only two-thirds as much total tank volume; (2) a lower sludge volume; (3) lower air ratios; and (4) a smaller area for the treatment plant site are required as compared to the conventional plants, and the digester gas yield is higher. This process, however, does not produce a very well clarified effluent. According to Wuhrman (35), BOD loadings of 190 lb/1000 cu ft of aeration volume have been treated with 80 per cent removal efficiency. He also pointed out that addition of asbestos in the aerator had no merit.

According to Heukelekian (36), in the bioflocculation or the contact aeration process, a large surface area for attachment of microorganisms is exposed to induce adsorption at the same time that air is diffused gently through the sewage to induce oxidation. Bioflocculation is the coalescence of finely divided suspended matter in sewage under the action of biological agencies. A contact aerator is a water-tight tank filled with material that exposes a large surface to the sewage in the tank, such as crates, corrugated metal sheets or wire netting. Bacterial growth forms a zoogleal mass on the material submerged in the sewage. The principal advantage of the process lies in the reduced air requirements; however, a considerable amount of mechanical equipment is required.

The Hays process is an application of contact aeration in two stages and consists of preliminary sedimentation, first-stage contact aeration, intermediate sedimentation, second-stage contact aeration, and final sedimentation (37). It was developed between 1930 and 1938 at Waco, Texas, and the first municipal plant was constructed in 1939. The aeration tanks are shallower than conventional tanks and contain thin plates made of asbestos cement sheets. Under favorable conditions, particularly in relatively small installations such as army camps, satisfactory results have been obtained. The air consumption was found to be relatively high (38).

The biochemical process is the trade name applied to the Guggenhein process, which employs the addition of a coagulant, iron salts or alum in the activated sludge process, the coagulant being added to the returned sludge. It is claimed that addition of the coagulant decreases the aeration time and increases the removal ability. Advantages include applicability to treatment of industrial wastes with either high or low pH. It requires skilled operation. The cost of operation is also high.

The Zigerli process involves the addition of approximately 1 mg/l of asbestos to increase the weight of the sludge, which in turn hastens precipitation and also shortens aeration time (21).

The bioactivation process employs presedimentation followed by trickling filtration, and a short secondary

settling period. This is followed by activated sludge treatment and final sedimentation. The purpose of using both a trickling filter and an activated sludge aeration tank is to treat, rapidly, domestic sewage at high BOD loadings and wastes with a high suspended solids concentration with high removal efficiency. The process produces excellent effluent using lower air volumes than standard activated sludge plants. It is claimed that this process withstands shock loads better than most conventional plants. However, the construction cost is higher.

The Logan process employs direct recirculation of the aerator effluent and also the recirculation of sludge from the final sedimentation tank. As large volumes of aerator effluent are returned, the sludge is kept more active and efficient. The detention time is approximately three hours. It has been recommended for treatment of domestic wastes with low BOD and suspended solids in large plants. The removal efficiency of this process is not very high.

The two-stage aeration process was devised primarily to alleviate overloaded conventional activated sludge plants and, although the amount of overall removal is somewhat reduced, the effluent quality still remains sufficiently high. It consists of dividing the treatment plant into two operational systems in series, with a provision for a first-stage effluent bypass. The arrangement exploits the treatment potential of the excess sludge. The process can yield any degree of partial purification and

the excess sludge contains less water, thus has a smaller volume.

Activated aeration is a form of two-stage treatment in which the excess sludge from the first stage is pumped to the second stage and sludge is wasted only from the second stage. The two plants operate in parallel, each using the same settled sewage as the influent. The process provides an effluent of intermediate quality at savings in power costs as compared to the conventional activated sludge plants (34).

The elimination of primary sedimentation and sludge digestion and provision for a longer aeration period of approximately twenty-four hours, with 100 per cent recirculation of settled sludge, has been found useful in small installations. BOD loadings of 30 lb/day/1000 cu ft have been employed. This process has been termed "extended aeration." Sometimes a pond is provided to polish the effluent because, at times, the plant will unload "ash" which may form sludge banks in the receiving stream. The cost of air supply is very high due to the extended aeration period. Opinions differ considerably on the feasibility of oxidizing the sludge completely (39, 40, 41, 42).

The Halmur process (22) is basically an activated sludge process except for modification in equipment arrangement and flow sheet. There is no primary sedimentation prior to aeration. The detention time is also low.

It involves three major units--two combination settling tanks and a digester. The aeration of mixed liquor and sedimentation of activated sludge are carried out in the same structural unit. A sewage treatment plant at Troy, Pennsylvania, using a detention time of only forty-three minutes, gave a BOD purification efficiency of 89 per cent. The cost of construction is low, due to elimination of the primary settling tank and provision of the short detention time. Varying degrees of purification can be obtained by adjusting the air flow.

While the modifications above tend, in some respects, to overcome the shortcomings of the conventional activated sludge, they do not meet the optimum conditions for best operation. Busch and Kalinske (43) summarized these optimum conditions as: (1) presence of young flocculent sludge in the logarithmic stage of growth; (2) the maintenance of logarithmic growth rate by controlled sludge wasting; (3) continuous organic loadings to the organisms; and (4) elimination of anaerobic conditions at any point in the oxidative treatment.

McKinney, et al. (44) believed that the fundamental problem with activated sludge is the continually oscillating biological populations. The biological population was found to be undergoing a feed-rest-feed-rest cycle. These authors also stated that, if the microorganisms were to operate at maximum efficiency at all times, they should be maintained in a constant state of growth. They developed a

new method of hydraulic operation of the activated sludge, called the "complete mixing" system, incorporating the optimum conditions cited above. Complete mixing is primarily a new hydraulic condition, compared with the old plug flow type reactors, and can be employed for any of the process modifications mentioned earlier. In this system the inflow nutrient is instantaneously and thoroughly mixed in the reactor. The waste is introduced along the length of the tank just above the air diffusers so as to take advantage of mixing by the diffused air. The effluent is removed from the opposite side. To do this, a longitudinal baffle was placed along the aeration tank, dividing the tank into an aeration section and a parallel section. The design implied that the activated sludge would flow from the aeration section up into the sedimentation section, where it would be separated from the liquid effluent and settle back into the aeration section by gravity. These authors designed a completely mixed continuous flow process for treatment of cotton textile plant wastes, and found that BOD loadings up to 60 lb/day/1000 cu ft of aeration volume could be applied successfully, even without pre-neutralization of the highly alkaline waste. They estimated that the cost of construction for such a plant was one-third that of the conventional plant.

In 1959, Busch (45) developed a bench-scale continuous flow unit at Rice Institute. The basic housing is a six-liter capacity percolator tube with a two-liter percolator

suspended inside to provide concentric recirculation pathways. Four porous stone diffusers and an influent tube are placed in a disc in the lower portion of the percolator. A cylindrical lucite tube serves as the clarification compartment. Substrate is pumped from the reservoir into recirculating mixed liquor. Clarified effluent is aspirated from the surface of the sedimentation chamber. Mixed liquor is wasted cyclically through the influent tube connection and is controlled by a valve.

The following advantages have been claimed for the completely mixed systems over the conventional activated sludge systems: (1) the aertor acts as a surge tank and thus reduces the ill effects of shock loads; (2) uniform effluent characteristics; (3) reduction in plant size for same volume of waste; (4) elimination of pretreatment of some toxic and alkaline wastes; (5) ease of control in operation; (6) maintenance of the same physiological state of the microorganisms over an extended period; and (7) ease of obtaining research data on a small scale model for development of system kinetics.

Pilot plant studies by Eidsness (46) indicated that an effluent with 25 mg/l BOD can be produced by the aero-accelerator after 2.5 hours' aeration.

Ludzack and Ettinger (47) showed that the main sources of nitrogen leakage in treated effluent are: (1) fixed nitrogen in the effluent, which includes most of that not retained as sludge solids; (2) digested solids removal;

(3) the nitrogen fraction in the digester gas; (4) denitrification in the secondary clarifier; (5) denitrification losses during aeration. These authors also suggested a semi-aerobic activated sludge operation using circulated mixed liquor from later stages of aeration to provide dissolved nitrite and nitrate oxygen to compensate for reduced aeration in the influent zone. This process modification claimed the advantage of minimizing nitrogen leakage in the effluent. It was, however,recognized by the authors that dilute wastes or wastes with high BOD:N ratios decrease the advantage of the process.

The oxidative assimilation of substrates in the absence of exogenous nitrogen has recently been employed in two process modifications for treatment of nitrogen-deficient wastes. RamaRao, Speece, and Engelbrecht (15) proposed a flow sheet in which the effluent from the primary settling tank was divided into two parts and channelled to activated sludge plants operated in parallel as practiced in activated aeration systems. One waste stream was supplemented with 500 mg/l of ammonium chloride for 1000 mg/l COD, and was treated in the conventional manner. A portion of the sludge from the settling tank of this conventional unit was used as return sludge for the aerator, and the remaining sludge was mixed with a portion of the waste to which no nitrogen was added, and thence channelled to the second activated sludge plant. All sludge from the second plant was wasted for final disposal. The quantity of waste

treated in the second aerator varied from 33 to 48 per cent of the total waste volume. However, in analyzing these experiments, one can conclude that the quantity of nitrogen added in the first aerator was considerably more than is normally used in field practice. Considering that the quantity of waste treated in the second aerator was half of that treated in the first aerator, the net nitrogen supplementation amounts to 500 mg/l ammonium chloride for 1500 mg/l COD, which gives the COD:N ratio of 11.4:1 for the entire system. On this basis, it cannot be said that this modification results in a saving in the cost of nitrogen supplementation. It is possible that nitrogen was escaping from the first conventional system to the second system, along with the settled sludge. Thus, the second aerator may have functioned essentially in the same manner as the first aerator.

Another modification, using the oxidative assimilation capability of the sludge in the absence of nitrogen, has been proposed by Komolrit, Goel, and Gaudy (16). This modification will be discussed elsewhere in detail in this dissertation as it forms a major part of the research herein reported.

B. Nutritional Aspects in Waste Treatment

It is widely recognized that the removal of organic matter from waste waters is accomplished primarily by the bacteria which use the organic matter as a source of energy. Various other essential nutrients are needed, but nitrogen

plays a pivotal role since it is required for the synthesis of proteins and nucleic acids. If nitrogen is totally lacking in a biological waste water treatment system, net synthesis of new cells is prevented. It is therefore a common practice in waste water treatment operations to maintain the nitrogen supply at a BOD:N ratio of 20:1 by addition of nitrogen in the incoming waste stream (14).

The need for nitrogen supplementation in biological systems has long been recognized. In determining the 5-day BOD of certain organic compounds, Eldridge (48) found that the quantity of microbial seed employed greatly affected the value obtained. He believed that there was some optimum quantity of seed above which correct results were obtained. He found that the ultimate (20-day) BOD appeared to reach about the same value with all quantities of seeding.

In 1935, Holderby and Lea (49) explained the results of Eldrige (48). They found that a change in carbon: nitrogen from 287 to 5.7 resulted in more than a three-fold increase in the apparent BOD of 0.1 per cent lactose solution (from an average of 169 mg/l to an average of 537 mg/l). They also observed relatively little change in the BOD results when C:N ratios were above 120, whereas below this value the change was marked, becoming increasingly rapid as the extreme low values were approached.

Lea and Nichols (50) found that the 5-day BOD value of a 0.1 per cent glucose solution was only 129 mg/l when the dilution water did not contain any nitrogen or phosphorous.

The addition of 2.66 mg/l of ammonium sulfate and 0.066 mg/l of KH_2PO_4 to the bicarbonate dilution water resulted in a more nearly normal rate of oxidation of glucose, as reflected by the increase in BOD to 438 mg/l. Further addition of four salts resulted in a BOD of 482 mg/l for the same waste. They concluded that the dilution water should contain 2.5 mg/l of ammonium sulfate. This corresponds to BOD:N ratio of 16:1, and a BOD:P ratio of 400:1.

These authors later (51) showed that the BOD of paper mill waste increased 2.4 and 4.6 times with the addition of phosphorous and nitrogen to the dilution water. They also suggested that the dilution water should contain 0.2 to 0.5 mg/l of nitrogen, since these quantities are also found in stream waters. However, Lea (52) later found that the concentration of phosphorous which he had previously advocated was insufficient and recommended a ten-fold increase to a BOD:P ratio of 40:1.

Sawyer and Williamson (53) indicated that the fortified dilution water proposed by Lea and Nicols (50) and modified by Lea (52) provided an ideal dilution water.

Meanwhile, difficulties were encountered by Sawyer (54) in the joint treatment of nitrogen-deficient sulfite liquors and domestic wastes. The rate of BOD removal from waste-sewage mixtures by activated sludge was restricted when deficiencies of nitrogen and phosphorous occurred. He found that approximately 3 mg/l of NH_3-N and 1 mg/l of phosphorous were utilized in the stabilization of 45 mg/l of

5-day BOD.

Sawyer (55) also determined the BOD to ammonia nitrogen ratio in five different sewages and found it to vary between 8.2 and 21.0. The sludge operating on BOD:N of 8.2:1 developed the greatest ability to oxidize nitrogen while the sludges fed on diets containing 16 or more mg/l of BOD per unit of ammonia nitrogen lost most of their ability to oxidize nitrogen.

Helmers, et al. (56) have reported studies made with cotton kiering, rope kiering, and brewery wastes, where the nitrogen in the system was varied by changing the amounts of domestic waste mixed with these wastes. They defined the nitrogen requirement as the amount of nitrogen "utilized" by the activated sludge in the stabilization of a substrate. The nitrogen requirements were classified as the "maximum" and the "critical." The maximum nitrogen requirement is the amount of nitrogen utilized when an excess of the nutrient is available in the feed mixture and the critical requirement was defined as the amount of nutrient which must be available for satisfactory treatment plant operation.

These authors used two methods for making the nitrogen balance. They recommended the equation

$$I = E + Gn/200$$

where I = nutrient element in the influent feed mixture in mg/l; G = rate of sludge growth in mg/l/day; n = percentage of nutrient element in dry sludge, and E = nutrient element in the effluent in mg/l. The nutrient requirement was

calculated by these authors: (1) by differences in influent and effluent analyses, and (2) by measuring the mineral nutrient contained in the sludges wasted to maintain uniform aeration solids concentration (1500 mg/l). In their study they employed batch feeding at 8-hour intervals.

In a subsequent study, Helmers, et al. (57) found the "maximum" nitrogen requirement to vary from a BOD to N ratio of 13:1 to as high as 26:1, with six of the nine values ranging from 16 to 19 parts of BOD to one part of nitrogen. The temperature did not have a definite consistent effect. Presence of less than 2 mg/l inorganic nitrogen in the final effluent was considered to be indicative of nitrogen deficiency. Their data also indicated that considerable variation exists in the availability of organic nitrogen from different wastes. Only 9 to 23 per cent of the organic nitrogen contained in rag rope waste became available as compared with 55 to 78 per cent of the organic nitrogen contained in brewery and domestic wastes.

In a later study Helmers, et al. (58) observed that the most marked effect of nitrogen supplementation is the pronounced increase in the nitrogen content of the activated sludge. The nitrogen content of the sludge varied from 3 per cent in the system which received no supplementation to 8 per cent in the system with maximum nitrogen supplementation. Addition of the supplementary nitrogen also resulted in better settling sludges as indicated by lower sludge volume indices. They found that efficient BOD

removal can be observed even when the "maximum" nitrogen requirement is not supplied. They suggested that a minimum of 7 per cent nitrogen be present in the volatile portion of the sludge to maintain desirable qualities. A value below 7 per cent was considered as indicative of a critical deficiency. The nitrogen requirements based on BOD removal were found to be 3, 4, and 3 lb nitrogen per 100 lb BOD removed at 10°, 20°, and $30^{o}C$, respectively. Their data would indicate that as little as 1.1 lb of nitrogen and 0.19 lb of phosphorous would suffice for the removal of 100 lb of BOD. However, the BOD removals accomplished on such limited nutrients were unsatisfactory in the established aeration time. Elemental analysis indicated a formula of $C_{118}H_{170}O_{51}N_{17}P$ for the activated sludge. Ignoring the phosphorous content, this formula becomes

$C_7H_{10}N\ O_3$.

Sawyer (12) concluded from the works of Helmers, et al. (56, 57, 58) that a BOD to available N ratio of approximately 17 to 1 is optimum for stabilization of such wastes in combination with domestic wastes, so as to produce a biological growth with maximum nitrogen content. However, he recognized that when it is desired to accomplish stabilization of waste with the minimum amount of mineral nutrients, the ratio of 5-day BOD to nitrogen and phosphorous can be increased to 32 to 1 and 150 to 1, respectively. He also concluded from Weinberger's work that nitrogen present as NH_4^+ can be counted upon as being 100 per cent

available; nitrogen in the form of urea may become 100 per cent available but only after hydrolysis by the enzyme urease. He suggested that for estimation purposes, a value of 50 per cent may be used and adjustments made later, based upon experience.

The composition of sludges grown on milk was determined by Hoover and Porges (59). The statistical average values for C, H, N, O, and ash were found to be 47.26, 5.69, 11.27, 27.0, and 8.61 per cent of the dry weight, yielding $C_5H_7NO_2$ as a close approximation of the resultant empirical formula for the sludge. It was pointed out that this formula expresses only the statistical average proportions of the major atoms of the organic constituents. It may be seen that the percentage of nitrogen in these cells was considerably higher than that obtained by Helmers, et al. (58).

Symons and McKinney (60) studied the biochemistry of nitrogen and the effect of nitrogen deficiency. They also studied the effect of the concentration and form of nitrogen on the growth of sludge. They postulated that, when the cell dies, it lyses and releases the nitrogenous material into solution. This organic bound nitrogen is then available for futher use by other bacteria for synthesis. It was found that from the COD removal point of view, the nitrogen requirement is 1.17 lb per 100 lb of COD, which corresponds to a COD:N ratio of 85:1. These conclusions were based on batch studies with 100 per cent

recycling of sludge, using sodium acetate as the sole carbon source. The cells were originally grown on a medium containing 50 mg/l nitrogen and 1000 mg/l of sodium acetate and then split into five parts. These portions of the sludge were then fed the same organic loading but differing predetermined amounts of nitrogen; one received no nitrogen. They observed that "all five of the units operated well throughout the five weeks of testing, as shown by low COD in the effluent on each day of testing." Since one of these five systems did not contain any nitrogen, it would appear that a satisfactory COD removal was obtained by them even in the system with no nitrogen supplementation. This is somewhat doubtful. These authors found that a decrease in the nitrogen in the system was usually accompanied by a buildup of non-degradable biological solids; it was found that this material was not utilized during a long period of endogenous respiration and was slowly accumulated in the sludge mass throughout the run when the systems were operated with no sludge wasting. Microscopic examination with Alcian Blue stain revealed that such sludge possessed a high extracellular polysaccharide content.

Hattingh (61) suggested a value of BOD:N of 19:1 for maximum nitrogen content in the sludge. Later (62) he also found that when the BOD:N ratio was greater than 37:1, bulking of sludge occurred. Simpson (63) found that nitrogen requirement could be reduced to one-fifth of the amounts proposed by Sawyer (12) in extended aeration

systems. Eckenfelder and McCabe (64) found that pulp and paper mill waste can be treated with no nitrogen supplementation in aerated lagoons due to the extended period of aeration which permitted reuse of nitrogen after auto-oxidation. Eckenfelder and Weston (65) have summarized the chemical equations for the synthesis of cellular material, oxidation of organic substrate, and auto-oxidation of the cellular material. Ludzack, Schaffer and Ettinger (66) found an increase in ammonia concentration in the effluent when detention time or temperature or mixed liquor suspended solids were increased.

A comprehensive study on the biochemistry of activated sludge synthesis was conducted by Symons and co-workers (67). They found that the feeding schedule, batch or continuous, had a strong influence on the resultant solids production, while the sludge wasting method, batch or continuous, had very little influence on the resultant solids production. Higher yields of sludges were obtained for carbohydrate wastes. In conventional batch activated sludge, the new cell yield in mass units was estimated to be 0.6 to 0.7 of the COD removed, while the inert mass production was approximately 16 per cent of the COD removed. These ratios were 0.5 and 9 per cent for the completely mixed systems. The per cent nitrogen in the mixed liquor volatile solids declined as the COD:N ratio was increased. The lowest limit of cellular nitrogen was found to be four per cent and approximately 80 per cent COD removal was obtained at

Komolrit (70) observed that nitrogen-deficient systems had poorer capability of withstanding qualitative shock loads. He indicated the necessity of the presence of nitrogen for acclimation purposes. The preferential substrate utilization or substrate interaction phenomenon was found to be more apparent in nitrogen-limited systems.

Krishnan (71) also studied the effect of short-term shock loading under nitrogen deficiency in continuous flow systems. In systems operated without cell feedback, a shock load of nitrogen-deficient waste was characterized by the release of intermediates which were not subsequently rapidly utilized. These systems showed a marked decrease in protein content with an accompanying increase in carbohydrate content. Using solids recirculation, he found that the biochemical composition of cells did not vary much under the shock load of a nitrogen-deficient waste. Organic nitrogen of less than 7.5 per cent in the cell did not necessarily correlate with poor substrate removal efficiency. With respect to substrate removal, the continuous flow units were more sensitive to nitrogen deficiency than were batch systems.

Krishnan and Gaudy (72) found that in many systems utilizing a single substrate such as glucose, considerable amounts of COD remained after glucose, as measured by anthrone or glucose oxidase, had been removed. Further analysis showed that volatile acids, primarily acetic acid, may constitute a large fraction of this COD (71). These

metabolic intermediates or end products were found during active growth or under nonproliferating conditions. If adequate nitrogen was available they were metabolized after the original carbon source was removed. Similar results of partial oxidations of glucose were reported by Clifton in pure culture studies with *Bacillus megaterium* (73) and *Escherichia coli* (74).

By observing cell growth in flasks with different amounts of ammonium sulfate but the same amount of organic carbon source, Komolrit (70) estimated that approximately 150 mg/l ammonium sulfate would be required for metabolizing 500 mg/l of either glucose or sorbitol. This corresponds to a COD:N ratio of 16.6:1. He also observed that the nitrogen-limited conditions did not seem to affect the growth rate of the systems, but affected the total yield of the population. However, the smallest concentration of nitrogen used by him corresponded to a COD:N ratio of 50:1.

By measuring absorption in the UV region, Holme and co-workers (75, 76) showed that glucose residues accumulate as glycogen during assimilation of this compound in *Escherichia coli B*; those incorporated last were the first to be split off during a subsequent endogenous period, i.e., when the glycogen was utilized. Addition of ammonium chloride to the washed cells started the division of the cells immediately. In order to obtain a cell concentration of approximately 5 x 10^8 cells per ml during the period of endogenous respiration, the initial concentration of

ammonium chloride in the medium had to be 0.75×10^{-3}m (77).

Holme (78) conducted continuous flow experiments using nitrogen as the limiting growth factor. Specific growth rates of 0.13 - 0.94 $hour^{-1}$ were established. It was found that glycogen-glucose measured as percentage of dry weight reduced from 21.9 to 2.3 when the dilution rate increased from 0.18 to 0.94, whereas total nitrogen, measured also as percentage of the dry weight, increased from 11.2 to 14.1. However, when the carbon source was used as the limiting factor, no significant variation in the glycogen content of the cells was observed at different dilution rates. The yield was found to increase as the growth rate decreased.

Ramanathan (79) also observed that the carbohydrate content of the sludge remains unchanged in carbon-limited systems at various dilution rates.

In *Escherichia coli*, polysaccharides can account for as little as 4 per cent and as much as 25 per cent of the total dry weight. It has been shown that intracellular polysaccharide consists of granules, ranging from 50-100 mμ in diameter (80). Holme (78) found an increase in the rate of glycogen synthesis as the growth rate approached zero, i.e., the slower the cells produce daughter cells, the faster they produce glycogen. He stated that a rapidly growing culture consists of cells that, on the average, possess a larger volume and greater mass and more DNA, more protein, and more RNA than the average cell in a culture of

the same organism growing slowly.

Marr and Ingraham (81) found that cells grown in a chemostat limited by the nitrogen source show a much higher content of unsaturated fatty acids than cells from corresponding batch cultures. Wright and Lockhart (82) found that cells grown in a limited nitrogen medium contain larger amounts of nucleic acids per cell as compared to a carbon-limited system. In *Escherichia coli* an increase in cell size of four to six times was observed with growth rate was changed from 0.1 to 0.5 generations per hour. Organisms grown under a nitrogen limitation were almost twice as large as those grown under carbon-limitation for the same growth rate. Protein content of nitrogen-limited cells expressed as protein per cell was found to be higher than that in cells grown in carbon-limited medium. Nitrogen-limited cells contained up to twice as much free amino acids as did carbon-limited cells.

Wilkinson (83) found that as the level of nitrogen source in the medium was gradually lowered, until it became limiting, the amount of polysaccharide produced per cell rose to a maximal level. The increase was reflected in both extracellular and intracellular polysaccharides.

However, Pipes (84) felt that if the sludge has no opportunity to oxidize most of the organic matter which it removes from the waste, or if nitrogen is lacking in the system, the filamentous organisms accumulate large amounts of lipid materials as a food reserve.

Ecker and Lockhart (85) found that the sequence of physiological events during the process of growth termination depends on the nature of the factors limiting the growth. Cell division and mass synthesis were found to terminate simultaneously in glucose-limited cultures of Escherichia coli, whereas in populations fed on nitrogen-limited medium the termination of mass synthesis was coincident with the exhaustion of nitrogen source. However, the energy source continued to be utilized beyond the point of exhaustion of nitrogen. Average cell size was found to be one-fourth of that at the point when the "limiting" nutrient had exhausted. When exogenous nitrogen was completely utilized earlier than the carbon source, the system was classified as nitrogen-limited. Their data would indicate that a system with COD:N of 5:1 was carbon-limited whereas the system with COD:N equal to 15:1 was nitrogen-limited.

Tempest and Herbert (86) grew Torula utilis in a chemostat at several rates in an NH_4^+-limited medium, with glucose as the carbon source. The concentration of ammonium sulfate used by them was 1.8×10^{-2}M, and glucose was 5 per cent (w/v). These amounts of ammonium sulfate and glucose correspond to a COD:N ratio of 105:1. They found that the glucose-oxidizing ability of a nitrogen-limited system did vary markedly with growth rate. They also observed increased cell yield at low growth rates, and argued that the increase in cell carbohydrate was responsible for the

yield of biological solids.

Herbert (87, 88) obtained quantitative agreement with Monod's equations for growth rate in his study on continuous cultures of *Aerobacter aerogenes* growing on a glycerol-salts medium with ammonia as the growth-limiting factor. The medium contained M/20 NH_3 and 2 per cent glycerol, which gives a COD:N ratio of 30:1. There was no tendency in this organism to accumulate polysaccharides at any dilution rate. In reply to a question by Holme, Herbert explained this phenomenon by saying that it was due to the fact that glycerol was used as the carbon source (89). He predicted that if glucose were used as carbon source, probably the organism would have accumulated polysaccharides. However, Herbert also pointed out (88) that unpublished work of Herbert and Tempest with *Torula utilis* showed that with ammonia as the limiting nutrient, the carbohydrate content increased with decreasing growth rate in the same manner as Holme (78) had observed with *Escherichia coli*. The medium used by Herbert and Tempest in this unpublished work contained 500 μg NH_3-N/ml and 3 per cent glucose. The COD:N ratio for this medium is 64:1. They found a decrease in yield at low growth rates.

C. Oxidative Assimilation

When a carbon-energy source is added to a suspension of washed aerobic bacteria it is metabolized, oxygen is consumed, and carbon dioxide is evolved. If no exogenous nitrogen is included in the medium, multiplication of cells

does not take place and yet there may be a gain in the dry weight of the organisms. To this phenomenon is applied the term "oxidative assimilation." Siegel and Clifton (90) showed the assimilation of succinate, fumerate, lactate, pyruvate, and glycerol in Escherichia coli by making carbon balances. The carbon content of the cells was analyzed before and after the assimilation and the difference was considered as the carbon stored.

Assimilation of glucose in the absence of an exogenous nitrogen source has been shown to occur in pure cultures of microorganisms by Barker (91) and later by Clifton (92).

Marino and Clifton (93) showed that autotrophically-grown cells assimilate less carbon from organic substrates than do heterotrophically-grown ones, and they are more limited in their ability to utilize different substrates.

Wuhrman (94) observed that substrate could be removed in the absence of exogenous nitrogen. He washed a heterogeneous culture of microorganisms grown on glucose, to remove any extenal nitrogen, fed the substrate again and found good organic removal. Only 15 to 18 per cent of the substrate was oxidized and the remainder was used for the synthesis of new cell material.

Clifton and Sobek (95) found that approximately 50 per cent of the exogenously-supplied glucose is oxidatively assimilated by Bacillus cereus. Duncan and Campbell (96) showed oxidative assimilation of glucose by Pseudomonas aeruginosa. Using radioactive tracers, Clifton (97) found

that exogenous glucose is taken up rapidly by washed cells of Bacillus cereus and apparently enters a metabolic pool. From this pool the label passes rapidly into other cellular fractions. Assimilation in part, at least, was believed by him to be at the expense of cellular reserves which appear to be replenished or replaced by the assimilated substrate.

Meanwhile in 1959, Gaudy (98) had also observed the phenomonon of substrate removal in the absence of exogenous nitrogen in heterogeneous populations. He studied the major differences in system behavior for nitrogen-deficient and nitrogen-balanced systems. These systems were termed respiring and growing systems, respectively. It was found that the sludge increase in the two systems was comparable in amount and that the rate of COD removal was also essentially the same in both systems. However, it was found that initially there was an increase in carbohydrate content of the cells in both systems. Part of the cellular carbohydrate was later converted to protein in the growth system (after the exhaustion of the substrate). In 2.5 hours all glucose had been removed and converted as follows: 17 per cent oxidized, 46 per cent stored as carbohydrates, and approximately 13 per cent synthesized into protein.

Low percentages of respiration were also observed by Placak and Ruchhoft (99), Porges, et al. (100), Washington and Symons (41), and van Gils (101).

Clifton (73) observed the oxidative assimilation of glucose by Bacillus megaterium. However, pyruvate and

acetate were oxidized very rapidly by these cells. He also showed that nutrient-agar grown cells of Escherichia coli assimilated about 50 per cent of the glucose whereas cells grown on glucose-agar medium assimilated only 20 to 30 per cent of the glucose (74).

The removal of substrates other than glucose in the absence of exogenous nitrogen has also been shown to occur in heterogeneous cultures. Rama Rao, Speece, and Engelbrecht (15) showed that phenol and acetic acid could be utilized in the absence of nitrogen. van Gils (101) also observed assimilation of organic acids such as acetic and lactic acids as polysaccharides. Unpublished work of Thabaraj (103) also has indicated the possibility of removal of sucrose and glycerol in the absence of exogenous nitrogen. Growth and substrate removal rates for growing and respiring systems are significantly different at low initial biological solids concentration, but the difference between the two systems reduces considerably with an increse in solids concentration. It may be added that in all studies thus far mentioned at relatively high biological solids concentration the substrate removal is found to be linear, whereas at relatively low initial solids concentration the substrate removal is found to follow first order kinetics.

The mechanism of substrate removal and solids production in systems with high and low initial biological solids concentration is very different and has been delineated by

Rao and Gaudy (104) and Gaudy and Gaudy (105). It was postulated that at initially low solids concentrations the course of substrate removal depends on cell replication and therefore follows an autocatalytic path. At high solids concentration solids production and substrate removal occur in what may be termed a "lag" period of growth--a lag in the sense that cell replication as indiated by protein synthesis lags the synthesis of carbohydrate. At high initial solids concentrations the system essentially behaves as a nonproliferating system, i.e., one to which no exogenous nitrogen is added, and in such systems generally linear or zero order kinetics are observed. It was found that even in the presence of an ample source of exogenous nitrogen the substrate is channelled largely into the synthesis of carbohydrates and possibly other storage products, and the most significant portion of the new cell production, as indicated by net protein synthesis, occurs at the expense of the stored product which has already been incorporated into the sludge. This hypothesis is supported by the work of McWhorter and Heukelekian (106), who found an increase in the nitrogen content of the cells even after the biological solids production inthe system had reached its maximum value and the exogenous substrate had been exhausted.

D. Endogenous Metabolism

The term "endogenous metabolism" has been defined differently by different workers. Dawes and Ribbons (107) have defined it as the metabolic reactions that occur within

the living cell when it is held in the absence of compounds which may serve specifically as exogenous substrate. They stated that the functions of endogenous respiration are to provide energy, to provide carbon substrates for resynthesis of degraded cellular constituents, and to perform special duties such as furnishing a source of reducing power in some chemoautotrophic and phototrophic bacteria or a source of phosphorous or sulfur in organisms that store volutin or sulfur granules.

Porges, et al. (108) and Wilson and Harrison (109) have used the term "endogenous respiration" to refer to a slow, continuous oxygen uptake for utilization of protoplasm in the absence of exogenous substrate. Wilson and Harrison (109) believed that the true endogenous phase was not reached until intracellular storage products were exhausted. McKinney (110) used the term "endogenous phase" to indicate the phase of declining cell mass in which the bacteria must draw on their own food reserves. Lamanna and Mallette (111) have defined "endogenous metabolism" as the oxidation of various stored materials accumulated during the period when exogenous food was present, and believed that it furnishes energy of maintenance by which microorganisms maintain their status quo. Eckenfelder and Weston (65) have termed the phase of declining sludge solids as the "endogenous" phase. McWhorter and Heukelekian (106) suggested two possible definitions for endogenous respiration, i.e., the point at

which there is a substantial decrease in the rate of oxygen utilization. Rahn (112) suggested that the level of exogenous energy just sufficient to replace the energy used endogenously but without inducing growth be termed "energy of maintenance." McGrew and Mallette (113) reasoned that there was a need for metabolic energy to meet the demand of chemical and physical wear and tear, and have defined the energy required to maintain the status quo as the energy of maintenance. Marr, Nilson, and Clark (114) have modified the above definition and suggested that "specific maintenance" represents the consumption of the source of carbon and energy for purposes that are not a function of the rate of growth. They believed that some reactions might be expected to require an expenditure of energy per cell per unit time, independent of the rate of growth. Such a requirement for energy demands a diversion of part of the carbon source. A large portion of the specific maintenance is required for providing the energy necessary for the turnover of protein and nucleic acids. Levine (115) defines the intracellular protein turnover as the reutilization by one cell of amino acids derived from the protein of another cell. For *Escherichia coli* he estimated a rate of 0.16 to 0.18 per cent per hour in non-growing cultures. Experiments conducted by Mallette (116) appear to show the existence of energy of maintenance for *Escherichia coli*. However, Monod (117) had concluded that *Escherichia coli* and *Bacillus subtilis* did not require

energies of maintenance. Lamanna and Mallette (111) criticized Monod's work on the ground that there is no reason *a priori* for believing that an external food source must serve as the oxidizable substrate for energy of maintenance. Energy of maintenance, in their opinion, may be derived only from endogenous substrate by way of endogenous respiration.

The concept of maintenance implies that some threshold concentration of substrate is required for growth and should such low nutrient levels be fed, bacteria will be able to keep themselves alive without growth for longer times. This concept has been proven by Lamanna and Mallette (111). They showed that such threshold concentrations of carbon source are required for maintenance. However, the existence of a threshold concentration for other nutrients like nitrogen or phosphate was not observed.

Mandelstam (118) observed that in *Escherichia coli* in a growing culture there is little or no degradation of protein. In non-growing conditions in *Escherichia coli* protein was degraded at the rate of approximately 5 per cent per hour for several hours, the rate being measured by release of ^{14}C from previously labelled cells. In *Bacillus cereus* he observed appreciable degradation of protein even in a growth system. Degradation of RNA followed the same pattern as protein. He believed that the degradation of protein and nucleic acid is carred out enzymatically. He also believed that all organisms have a latent tendency to

degrade protein and nucleic acid, yet the activity of proteases is not observed in growth systems due to the inhibitory effect of Mg^{++} on these enzymes. When Mg^{++} is removed, the enzymes are activated. He observed no accumulation of intracellular free amino acids, or of purines or pyrimidines, under carbon starvation conditions.

The question of whether reactions characteristic of endogenous metabolism continue at the same rate or at reduced rates during the growth period has received considerable attention during the last few years. Clifton (119) showed that the endogenous metabolism of Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Escherichia coli, Azobacter agilis, and Hydrogenomonas facilis was not influenced to a marked extent in the presence of glucose. Campbell, Gronlund, and Duncan (120) observed that the rates of endogenous respiration of glucose-grown cells continues unabated in the presence of an exogenous supply of glucose. Ribbons and Dawes (121) reported that Ramsey also found that in Staphlococcus aureus endogenous respiration proceeded uninterrupted at the same rate as in the presence of an exogenous source of carbon. However, they observed that endogenous respiration occurs at a slightly reduced rate during the oxidation of exogenous carbon source. This view is shared by Gronlund and Campbell (122), who observed 93 per cent suppression of endogenous $^{14}CO_2$ evolution during growth. Keshvan, et al. (123) believe that endogenous respiration does not necessarily take place

under growth conditions. In further complication of the problem, some workers have concluded that endogenous respiration is increased in the presence of exogenous substrate. Einer, Gest, and Kamen (124) concluded that endogenous metabolism was accelerated by the presence of glucose or acetate as substrate. Endogenous activity was found to be stimulated in the case of baker's yeast by exogenous catabolism (111).

Still another concept has been put forward by Danforth and Wilson (125). They suggest that endogenous metabolism of Euglena gracilis is the direct source of energy for growth and essential cellular activities and that exogenous substrates are used only to replenish or increase the supply of endogenous reserves. They assumed that endogenous metabolism continues at the same rate during growth as in washed suspensions.

In some cases the observed oxygen consumption, uncorrected for endogenous respiration, has been observed to exceed the theoretical uptake for complete oxidation of the substrate added. In such cases endogenous respiration was believed to be functioning inthe presence of the substrate. Results of this kind are frequently encountered when the carbon source is acetate (107).

The nature of the endogenous reserve has received the attention of many workers. Storage products observed include glycogen, poly-β-hydroxy butyrate, and nitrogenous products. What a cell can be is determined by its genetic

endowment; what a cell is, is the resultant of many environmental forces. Frequently, environmental forces appear to affect the chemical composition of cells (80). Since the endogenous metabolism of microorganisms is necessarily a function of their chemical composition, it follows that the nature and extent of endogenous metabolic activities is markedly affected by environmental and growth conditions. Escherichia coli has been found to store glycogen when grown in glucose-salts medium. However, when the same organism was grown in tryptone or succinate-salts medium, it did not accumulate glycogen. As a result, endogenous catabolism in the latter case draws on nitrogen compounds immediately (111). In Aerobacter aerogenes, possession of glycogen reserves was noted by Strange, Dark, and Ness (126). Possession of this reserve was found to favor the survival of this organism. Azotobacter agilis was found to accumulate poly-β-hydroxybutyrate (PHB). Cells with high initial PHB levels were also observed to survive longer (127). Sierra and Gibbons (128) found that Micrococcus halodenitrificans also accumulates PHB. Bacillus megaterium is also believed to depend on PHB as the endogenous reserve (111). Ribbons and Dawes (121) found that the free amino acid and peptide pool constituted the main endogenous substrates for Sarcina lutea, whether grown on peptone or glucose-peptone medium. They also showed that glycogen is the primary endogenous substrate and that, only when this is exhausted, does the net degradation of other endogenous

material occur (129). Protein is degraded to provide amino acids. RNA is also degraded, but DNA is relatively stable. Postgate and Hunter (130) also found that degradation of protein occurs only when glycogen is almost completely exhausted. Viability was observed only when glycogen was present as endogenous reserve.

Holme and Palmstierna (75) allowed stationary phase cells from nitrogen-deficient cultures to assimilate ^{14}C-glucose into glycogen. When the cells were subsequently incubated with a nitrogen source but no carbon source, ^{14}C flowed from the glycogen to protein. A breakdown of some 25 per cent of the total glycogen sufficed for the label to be lost, indicating that the last formed glycogen is the first to be degraded. The conversion of stored carbohydrate to protein in the endogenous phase in the presence of an exogenous supply of nitrogen has also been noted in heterogeneous cultures by Komolrit, Goel, and Gaudy (16), and Gaudy, Goel, and Freedman (131).

E. Applications of the Completely-Mixed Continuous Flow Process

The development of completely-mixed continuous flow systems has been reviewed by Gaudy, Engelbrecht, and DeMoss (132). According to them, the development of this technique has taken place in three major areas--physical; biochemical and microbiological; and sanitary engineering research. These authors have cited Denbigh as the first scientist to use the technique of continuous flow systems. Denbigh

employed continuous flow units to study the kinetics of steady state polymerization.

Since the pioneer work of Utenkov in Russia and Malek in Czechoslovakia, the completely mixed continuous flow process has been used extensively in the field of biochemical and microbiological research, fermentation industries, waste water purification, and several other industries. These industrial applications have been extensively reviewed by Malek (133). Applications of this process in water pollution control have been discussed by Krishnan (71) and Ramanathan (79). Much of the material described below has been derived from these three sources.

The completely-mixed activated sludge system is credited with treatment of several industrial wastes, which could not have been treated in the conventional activated sludge treatment process. Completely mixed aerators have been employed for treatment of: (a) highly alkaline textile wastes without preneutralization, (b) toxic wastes, (c) petroleum wastes containing hydrocarbons, phenols, carboxylic acids, nitrogen and sulfur compounds, (d) complex wastes from orlon manufacturing, (e) wastes from synthetic resin manufacture, (f) pharmaceutical wastes, (g) food processing wastes, (h) paper mill wastes, (i) dairy wastes, (j) wastes containing high salt concentrations, (k) wastes containing aniline, nitrobenzol, phenol, and 2,4 -dichlorophenol, antibiotics, synthetic vitamins and cortisone, and (l) coke and oven wastes containing phenols, thiosulfates,

and cyanides.

Among other applications, the following have been reported in the international symposia on continuous cultivation of microorganisms: (a) production of baker's yeast, (b) production of chlorotetracycline and pigments by Streptomyces aureofaciens, (c) studies of growth and influence of pH, substrate, quality and flow-rate on the age of fibrous actinomycetes, (d) quantitative analysis of the propagation of microorganisms on sources of energy, (e) preparation and analysis of antigenic fractions of different microorganisms, (f) genetic studies, (g) study of limitation with nitrogen material in pure cultures, (h) conversion of azauracil to azauracil riboside by means of Escherichia coli in multi-stage systems, (i) production of antibiotics in multistage systems, (j) transformations of steroids by using multistage aerators, (k) decomposition and conversion of nutrients by soil microflora, (l) production of alcohols, (m) decontamination or recovery of various industrial wastes and effluents such as production of protein biomass, and vitamin B_{12}, and (n) wine production.

CHAPTER III

THEORETICAL CONCEPTS

A. Concept of Steady State Kinetics

In a typical growth experiment using a very small inoculum, bacteria are not observed to divide instantaneously. The number of cells remains constant for some time. After cell replication begins, it proceeds slowly in the beginning, then more rapidly until the rate of division becomes constant; this latter phase is termed "exponential growth." The exponential growth phase comes to an end when the food concentration has dropped to a limiting value or when toxic metabolic products accumulate in sufficient quantity to upset the dynamic steady state inside the cell. There is no inherent reason why this growth phase should not go on indefinitely if the food supply is maintained and the toxic products of metabolism eliminated. Such steady state cultures have indeed been achieved, if not *ad infinitum*, at least for some days.

A continuous culture can be defined to be a culture of cells of any type into which there is a continuous addition of fresh medium for the purpose of maintaining the culture's steady level of growth. Such systems consist of a reactor

into which the reactants are allowed to pass at a predetermined rate and out of which the products emerge. In some cases the cells are retained in a semi-permeable reactor; however, the liquid portion is allowed to escape at a rate equal to the inflow rate. Such reactors can be either completely-mixed or piston flow type. In the former, the inflow nutrient is instantaneously and thoroughly mixed in the reactor. Under idealized conditions the effluent from the reactor is identical in character to the mixed liquor in the reactor. In piston flow or tubular reactors, plug flow conditions are maintained. The nutrient solution and the microorganisms are mixed before entering the reactor and this mixture passes through successive sections of the reactor without mixing during passage of the mixture through the reactor. The products increase and food reserves decrease as the slug of mixed liquor passes through the reactor. Every element remains in the reactor for the same period of time, which is the detention time as determined by flow rate and reactor volume. In most cases the completely mixed reactors are more efficient than the piston-flow types (44).

The inoculation of a single bacterial cell into a medium containing nutrients adequate for its growth will result in division of that cell into two daughter cells, with subsequent replications. The time elapsing between the formation of a cell and its division is termed the "generation" time. The number of bacteria (x) in any

generation is:

First generation: $x = 1 \times 2 = 2$ (1)

Second generation: $x = 1 \times 2 \times 2 = 2^2$ (2)

Third generation: $x = 1 \times 2 \times 2 \times 2 = 2^3$ (3)

Thus, if the number of bacteria present at a time t_0 is x_0, then the number of bacteria for any generation "n" after a time "t" is given by

$$x = x_0 2^n \tag{4}$$

where

$$n = \frac{t}{T_d} \text{ and } T_d \text{ is the doubling time} \tag{5}$$

or

$$\log_e x = \log_e x_0 + n \log_e 2 \tag{6}$$

or

$$\log_e x = \log_e x_0 + \frac{\log_e 2 (t)}{T_d} \tag{7}$$

Differentiating with respect to t,

$$\frac{1}{x} \frac{dx}{dt} = 0 + \frac{\log_e 2}{T_d} \tag{8}$$

Thus

$$\mu = \frac{1}{x} \cdot \frac{dx}{dt} = \frac{0.693}{T_d} \tag{9}$$

where μ = specific growth rate which is defined as the rate of increase of organisms per unit of concentration of biological solids.

Monod (117) was first to show that the specific growth rate μ is a function of substrate concentration. He found that μ increases with increase in substrate concentration

up to a limiting saturation value at high substrate concentration. According to Monod:

$$\mu = \left(\mu_{max}\left(\frac{S}{K_s + S}\right)\right) \quad (10)$$

where S = substrate concentration, μ_{max} = maximum value of μ, and K_s = saturation constant, which is numerically equal to the substrate concentration at which $\mu = 0.5\,\mu_{max}$.

Since the production of a new cell may be considered to involve the consumption of a definite amount of substrate, a straight line relationship between the growth and the utilization of the substrate is usually assumed, when only one limiting growth factor is used:

$$\frac{dx}{dt} = - Y \frac{ds}{dt} \quad (11)$$

where $\frac{dx}{dt}$ is the rate of change of cell concentration, $-\frac{dS}{dt}$ is the rate of substrate consumption, and Y is known as the yield coefficient which is assumed to be constant for a particular culture and substrate. Integration of Equation 11 leads to the following expression:

$$Y(S_0 - S) = (x - x_0) \quad (12)$$

where x_0 and S_0 refer to the initial concentrations of cells and substrate, respecitively. This equation also shows that

$$Y = \frac{\text{weight of bacteria produced}}{\text{weight of substrate consumed}} \quad (13)$$

The assumption that yield is constant is not completely correct for heterogeneous populations. Rao and Gaudy (104) found that yield coefficient varied between 0.48 and 0.82. They concluded that the variation in yield might be due to predominance or selection of bacterial species. Servizi and Bogan (134) related the yield coefficient to moles of ATP produced in the oxidation of a particular substrate. However, ATP yield is different for different pathways, and a single organism can metabolize a compound through more than one pathway. Therefore, the yield cannot be directly related to the COD unless the biochemical pathways of the compounds are known under the experimental conditions.

Equations 9, 10, and 11 above allow a relatively complete quantitative description of growth in the log phase, if μ_{max}, K_s and Y are known. These three parameters can be determined in batch or continuous flow experiments.

Gaudy, Engelbrecht and DeMoss (132) have shown that the mathematical simplicity of completely-mixed continuous systems makes them more attractive than batch systems for studies on bacterial growth.

<u>Completely-Mixed Systems Without Recirculation</u>

The following kinetics are, in general, taken from those derived by Herbert, Elsworth, and Telling (135). If F and V (Figure 2) represent the flow rate and the volume of the reactor, respectively, $\frac{F}{V}$ represents the number of complete turnovers per unit time. This ratio has been termed "dilution rate" D. Thus

$$D = \frac{F}{V} \tag{14}$$

making material balances for substrate and cell material, the performance of the system can be predicted.

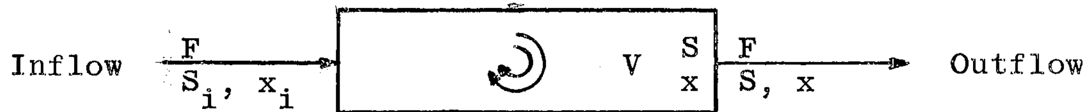


Figure 2. Schematic representation of a single stage completely-mixed reactor.

Substrate Balance

Since the flow is the same in all portions of the system,

[rate of change of substrate in the reactor] = [rate of inflow] - [rate of outflow] - [rate of consumption]

$$V \frac{dS}{dt} = F\ S_i - F\ S - \frac{\mu x}{Y} \cdot V \tag{15}$$

where S_i is the substrate concentration in the inflow, or

$$\frac{dS}{dt} = \frac{F}{V}(S_i - S) - \frac{\mu x}{Y} \tag{16}$$

or

$$\frac{dS}{dt} = D(S_i - S) - \frac{\mu x}{Y} \tag{17}$$

Cell Material Balance

$$\begin{bmatrix}\text{rate of change}\\ \text{of cell material}\\ \text{in the reactor}\end{bmatrix} = \begin{bmatrix}\text{rate of inflow + rate of growth}\end{bmatrix} - \begin{bmatrix}\text{rate at which cells leave reactor}\end{bmatrix}$$

$$V \frac{dx}{dt} = F\, x_i + \mu xV - Fx \qquad (18)$$

where x_i is the concentration of cell material in the inflow or

$$\frac{dx}{dt} = Dx_i + \mu x - Dx \qquad (19)$$

As sterile medium is used in most studies, no viable cells are assumed to be present in the incoming feed, i.e., x_i equals zero in such cases. Thus the above equation reduces to

$$\frac{dx}{dt} = \mu x - Dx \qquad (20)$$

Under steady state conditions there is theoretically no net change in cell concentration in the reactor, i.e., $\frac{dx}{dt} = 0$. Therefore

$$\mu = D \qquad (21)$$

i.e., the specific growth rate is equal to the dilution rate. Since the dilution rate, D, equals the inflow rate, F, divided by the volume, V, which is constant for any unit, it can be seen that under steady state conditions the specific growth rate is controlled mainly by the feed inflow rate. The steady state values of substrate concentration $\bar{S}$ and the cell concentration $\bar{x}$ in the aerator can thus be obtained from Equations 15 and 21.

$$\mu = D = \frac{\mu_{max} \cdot \bar{S}}{K_s + S} \tag{22}$$

or

$$\frac{\bar{S} + K_s}{\mu_{max} \cdot S} = \frac{1}{D} \tag{23}$$

or

$$S = \frac{K_s D}{\mu_{max} - D} \tag{24}$$

If D is greater than μ_{max}, cells are washed out of the reactor. Therefore, D is always maintained lower than μ_{max}. At steady state conditions, $\frac{dS}{dt} = 0$.

$$(S_i - S)D = \frac{\mu \bar{x}}{Y} \tag{25}$$

Since $\mu = D$, the equation can be written as follows:

$$\bar{x} = Y(S_i - \bar{S}) \tag{26}$$

From Equation 24 it is seen that the substrate concentration in the reactor or in the effluent is independent of the substrate concentration in the influent until D approaches μ_{max}. It is also seen that a high value of K_s leads to higher concentration of substrate in the effluent. From Equations 24 and 26 the steady state substrate concentration and cell concentration in the reactor can be found.

The critical dilution rate (D_c) which represents the dilution rate above which the microorganism will be completely washed out of the system is obtained by substituting the substrate concentration in the aerator, S, by that in the inflow, S_i, in Equation 24:

$$S_i = K_s \left(\frac{D_c}{\mu_{max} - D_c} \right) \tag{27}$$

Re-arranging the above equation, we get

$$D_c = \mu_{max} \left(\frac{S_i}{K_s + S_i} \right) \tag{28}$$

The kinetic expressions described above are essentially those derived by Herbert, Elsworth, and Telling (135). Several modifications have since been proposed. The relationship between specific growth rate and the substrate concentration proposed by Monod (117) was modified by Moser (136) as follows:

$$\mu = \mu_{max} \left(\frac{1}{1 + K_s S^{-\lambda}} \right) \tag{29}$$

This expression is very similar to Monod's equation, except for the component λ. When $\lambda = 1$, the two equations become the same.

Deindoerfer (137) discussed an equation proposed by Teissier:

$$\mu = \mu_{max} \left(1 - e^{-S/K_s} \right) \tag{30}$$

Either expression can be used for μ, but it should be pointed out that Gaudy, Ramanathan, and Rao (138) have found that for heterogeneous populations, Monod's equation provides the best fit to the experimental data.

The realization that the activated sludge process is essentially a completely mixed flow process came in 1943 when Sawyer and Rohlich (139) found that the concentrations

of biological solids in the effluent and inlet were the same, regardless of the position of the return sludge addition. In 1952, Garrett and Sawyer (140) proposed a kinetic theory for the conventional activated sludge process based on the completely mixed system.

McKinney (110) has also proposed a theory for a continuous flow activated sludge process incorporating endogenous respiration. Whether endogenous respiration proceeds in the presence of exogenous substrate is a controversial point. This aspect has been discussed in detail above. According to McKinney, the growth of a microbial population in continuous culture is a function of limiting nutrient concentration but independent of cell concentration, i.e.,

$$\frac{dx}{dt} = K_s S \tag{31}$$

Keshvan, et al. (123) also proposed kinetic formulations for activated sludge systems, omitting the endogenous respiration. These authors regarded the reaction between bacteria and substrate as unidirectional, resulting in the growth of new cells. According to these authors, bacteria + substrate $\longrightarrow$ increased number of bacteria + oxidized products. The rate of the forward reaction is proportional to the concentration of bacteria as well as substrate.

$$\frac{dx}{dt} = \mu \, x \, S \tag{32}$$

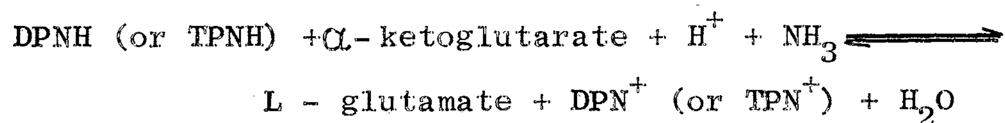
This equation is very similar to that proposed by Garrett and Sawyer (140) and used by Eckenfelder (141).

Sack and Schulze (142) observed that the concentration of biological solids inside the aerator is higher than in the effluent. They proposed a set of kinetic formulations using Teissier's equation. It appears that the difference in the solids concentration inside the aerator and in the effluent was primarily because the reactor was not completely mixed. In an idealized condition, the concentrations of substrate and biological solids inside the reactor and in the effluent are equal.

B. Assimilation of Inorganic Nitrogen

Most commonly, ammonia is fixed by three major reactions, the syntheses of glutamic acid, glutamine and carbamyl phosphate (143). In some microorganisms, alanine or aspartic acid formation may substitute for glutamic acid. Glutamic acid, glutamine, and carbamyl phosphate serve as nitrogen donors in the synthesis of all other amino acids, and participate in formation of purines, pyrimidines, and other nitrogenous compounds.

Glutamic acid is synthesized by the following reaction catalyzed by glutamic acid dehydrogenase:



$$\text{DPNH (or TPNH)} + \alpha\text{-ketoglutarate} + H^+ + NH_3 \longrightarrow \text{L - glutamate} + DPN^+ \text{ (or } TPN^+) + H_2O$$

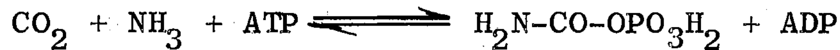
The thermodynamic equilibrium strongly favors the synthesis of glutamate.

The synthesis of glutamine takes place by the following reaction:

$$\text{glutamic acid} + \text{ATP} + NH_3 \xrightarrow{Mg^{++}} \text{glutamine} + \text{ADP} + P_i$$

This reaction is catalyzed by glutamine synthetase.

In bacteria, carbamyl phosphate synthetase catalyzes the following reaction:

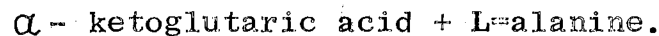


$$CO_2 + NH_3 + \text{ATP} \rightleftharpoons H_2N\text{-}CO\text{-}OPO_3H_2 + \text{ADP}$$

Having fixed NH_3 into organic linkages by the three reactions described above, amino acids are then fabricated from carbon compounds which are available as products of carbohydrate metabolism, e.g., pyruvate and oxaloacetate.

As pointed out earlier, aspartic acid and alanine may be produced directly in some microorganisms, but most commonly these two amino acids are formed from glutamic acid by transamination with oxaloacetic acid and pyruvic acid, respectively. These reactions are as follows:

$$\text{L - glutamic acid = oxaloacetate} \xrightarrow{\text{transaminase}} \alpha\text{- ketoglutaric acid + L-aspartic acid}$$



$$\text{L-glutamic acid + pyruvic acid} \rightleftharpoons \alpha\text{- ketoglutaric acid + L-alanine.}$$

Transamination thus provides a mechanism for redistribution of nitrogen.

CHAPTER IV

MATERIALS AND METHODS

A. Experimental Design

1. Effects of Different Nitrogen Levels in a Continuous Flow Activated Sludge Process

Continuous flow, completely mixed bench scale pilot plants were run at five different detention times. The detention times employed in the studies were 12, 8, 4, 2, and 1 hours. No sludge was recirculated in these studies. At each detention time the effect of three different nitrogen levels was studied. The three nitrogen levels employed in the study corresponded to COD:N ratios of 25:1, 40:1, and 70:1. Glucose was employed as the sole carbon source in the synthetic waste because a considerable amount of work has been published by other workers using it as substrate and, therefore, the use of glucose as substrate affords the opportunity of comparing the results with those previously published by other researchers. The source of nitrogen was ammonium sulfate.

For most experiments four continuous flow units were operated at one time from the same feed bottle containing the synthetic waste. Studies at the one-hour detention

time required independent operation due to the very large quantities of waste involved. The units were run at each dilution rate for a relatively long time, in order to detect possible changes in the "steady state" parameters. Furthermore, maintenance of the units for at least twenty days (except the unit with one hour detention time) permitted enough time for changes in predominance to occur.

The parameters investigated include chemical oxygen demand (COD) of the feed and the filtered effluent, biological solids concentration in the aeration tank, carbohydrate concentration in the filtered effluent, and ammonia nitrogen in the filtered effluent. Washed cells from the mixed liquors were resuspended in distilled water, made up to a known volume and stored in a freezer maintained at $-20^{o}C$ for subsequent analysis of the carbohydrate and protein content. Periodically, samples were taken from the effluent and the mixed liquor in the reactor in order to check for complete mixing conditions. The stored samples were also analyzed for volatile acids.

The growth parameters μ_{max}, Y, and K_s were determined for conditions where the limiting growth factor was nitrogen. This involved shaker-flask experiments. The flasks were supplied with identical growth media and seed but different amounts of nitrogen source. The seed for these studies was taken from the continuous flow units mentioned earlier. The growth was followed by measuring the optical density of the mixed liquor at 540 mμ, and the O.D.'s were

converted to concentrations of biological solids by a standard curve. At the end of the experiments, when the growth had stopped, the COD and ammonia nitrogen of the filtrates from the mixed liquor were determined to measure the extent of carbon and nitrogen utilization.

2. <u>Effect of Different Nitrogen Levels in Batch Operated Activated Sludge</u>

Five activated sludges were developed in laboratory scale bench units using a sewage seed obtained from the Stillwater municipal sewage treatment plant. The units were operated in accordance with the following procedure: Daily, one-third (500 ml) of the mixed liquor was wasted, the remainder was settled for one hour, and 500 ml of the supernatant was discarded. The volume of the aerator was made up to the 1500 ml volume with synthetic waste, the composition of which is given in Table I. The feed in all five units was identical except for the amount of ammonium sulfate. The amount of ammonium sulfate was varied so that the units contained nitrogen corresponding to COD:N ratios of 10:1, 25:1, 40:1, 55:1, and 70:1. After approximately twenty days of operation, during which time the units came into solids balance, the sludge was harvested and washed in buffer-salts medium, devoid of any carbon and nitrogen source, and used in the batch experiments. The sludge from the five units after washing was resuspended in five different batch units containing salts and buffer. The optical density of all mixed liquors was made identical by diluting

TABLE I

COMPOSITION OF GROWTH MEDIUM FOR 1000 mg/l GLUCOSE AS GROWTH-LIMITING SUBSTRATE

Constituents	Concentration
Glucose	1000 mg/l
Magnesium sulfate, $MgSO_4 \cdot 7H_2O$	100 mg/l
Ferric chloride, $FeCl_3 \cdot 6H_2O$	0.50 mg/l
Manganous sulfate, $MnSO_4 \cdot H_2O$	10.00 mg/l
Calcium chloride, $CaCl_2$	7.50 mg/l
KH_2PO_4	527.00 mg/l
K_2HPO_4	1070.00 mg/l
Tap water	100 ml/l
Ammonium sulfate, $(NH_4)_2SO_4$	variable

with the salts-buffer medium; 1.5 liters of the mixed liquor was then retained in each unit and the excess was wasted. Each unit was then supplied with the same amount of glucose, but different amounts of nitrogen source, the ammonium sulfate added being equal to that which was supplied to them during the previous twenty days. The course of COD removal and biological growth was followed by taking samples at different times.

3. Mechanistic Feasibility of a New Process Modification for Treatment of Nitrogen-Deficient Wastes

In order to test the mechanistic feasibility of the flow sheet proposed by Komolrit, Goel, and Gaudy (16), batch experiments which simulated the various unit operations were conducted. Activated sludge was developed in a batch unit in the manner outlined above, keeping the nitrogen supply in the feed at a level corresponding to a COD:N ratio of 10:1. After one month of such operation during which the unit came into solids balance the sludges were harvested, washed twice in buffer-salts medium devoid of substrate and nitrogen source, and used in a three-phase batch experiment designed to test the feasibility of the flow sheet shown in Figure 1.

a. Feeding Phase

The sludge first underwent a feeding phase. The sludge was divided into two equal portions; to one of them nitrogen source was added (growth system); nitrogen was not supplied to the second batch unit (nonproliferating). Both

systems were aerated identically. After ascertaining that the substrate had been almost completely exhausted in the growth system, both the systems were aerated for a few extra hours in order to allow the nonproliferating system to utilize the substrate if it had not been keeping pace with the growth system.

b. Endogenous Phase

The sludges from both systems were harvested separately, washed, and resuspended in buffer-salts medium devoid of any exogenous carbon or nitrogen source. This suspension was then subdivided into two parts. Thus, four systems were obtained, two from the cells which underwent growth conditions in the feeding phase and two from the system under nonproliferating conditions in the feeding phase. To one of the subdivisions from each of these two major categories (growth and nonproliferating), nitrogen was added, while the other was deprived of nitrogen. All four systems were aerated for several hours, during which samples were taken to detemine the turnover of intracellular carbohydrate to protein.

c. Refeeding Phase

The sludges from the four systems described above were harvested, washed, and then re-fed with the medium containing the carbon source but no nitrogen (nonproliferating conditions) in order to determine the degree of restoration of the purification efficiency of the cells.

It will thus be seen that the three phases of this experimental design simulated the path of the sludge shown in Figure 1. In all three phases the parameters investigated included COD of the mixed liquor filtrate, biological solids concentrations, carbohydrate and protein content of the sludges. The respiratory activity of the systems was followed using the Warburg apparatus.

The substrate employed for this mechanistic study was acetic acid. It may be mentioned that the original experiments were carried out using glucose as the sole carbon source (16).

4. <u>Operational Feasibility of the New Process Modification using Continuous Flow Studies</u>

A schematic drawing of the continuous flow bench scale pilot plant used in the study is shown in Figure 3. Synthetic waste was continually supplied to the feeding aerator at a predetermined rate; the mixed liquor from the aerator overflowed to a settling tank. The supernatant from the settling tank was continuously discharged. The settled sludge was harvested from the bottom of the settling tank at 12-hour intervals. The sludge was then diluted with salts-buffer medium devoid of carbon and nitrogen source to a predetermined optical density. Twenty-five hundred ml of this diluted mixed liquor was retained in the endogenous aerator and the excess was wasted. A predetermined quantity of ammonium sulfate was added to this batch-operated endogenous tank and the system was aerated for twelve hours.

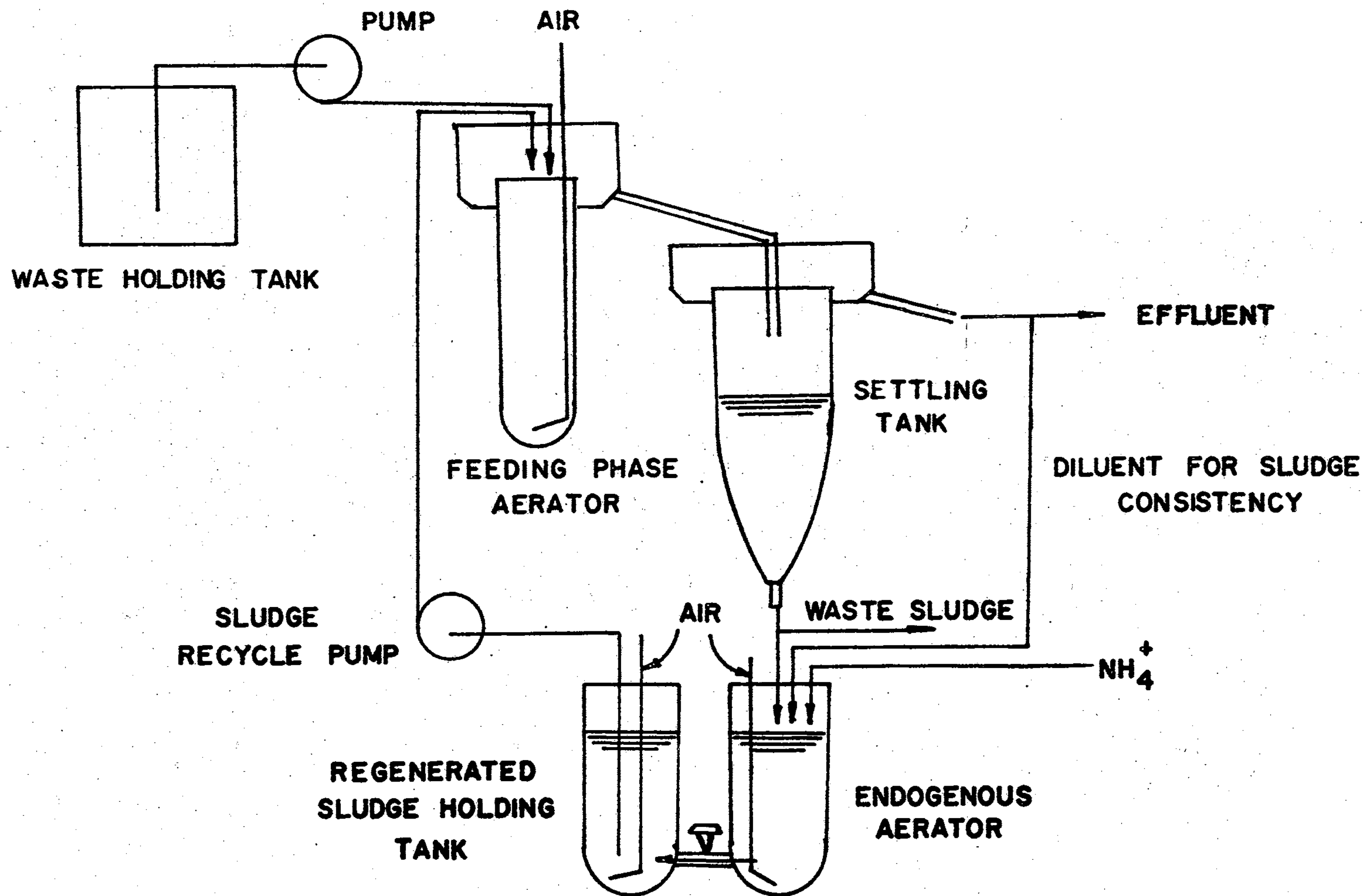


Figure 3. Laboratory scale pilot plant employed in operational feasibility studies.

The sludge was then recycled at a predetermined rate. During the time the sludge was being recycled from the holding tank another batch was undergoing endogenous metabolism in the presence of nitrogen. Thus, regenerated sludge was continuously available. In all studies the concentration of carbon source in the feed was 1000 mg/l. In all studies the flow to the feeding aerator was 400 ml/hour of the fresh incoming waste and 200 ml/hour of recycled sludge. The volume of the mixed liquor under air in the feeding aerator was 2400 ml, thereby yielding a detention time of four hours in the feeding aerator, calculated on the basis of a total flow of 600 ml/hour. The amount of ammonium sulfate was calculated on the basis of the total COD supplied to the feeding aerator each twelve hours (12 hours x 400 ml/hour x 1.06 mg/ml = 5088 mg). Thus, for a COD:N ratio of 10:1, 508.8 mg of ammonia nitrogen was supplied to the endogenous aerator every twelve hours. This quantity of ammonia nitrogen is contained in 2422 mg of ammonium sulfate added every twelve hours. This procedure allowed measurement of nitrogen usage in this process in terms of COD:N ratio so that the magnitude of this parameter could be compared with its magnitude in normal activated sludge processes.

The temperature in all studies was measured as $25^{o} \pm 3^{o}C$. The parameters investigated included COD of the effluent (filtered and unfiltered) and mixed-liquor filtrate from the recycle sludge holding tank, biological solids

concentration in the feeding aerator and the recycled sludge, carbohydrate and protein content of the sludges in the feeding aerator and the recycle sludge and the ammonia nitrogen in the filtrates from the feeding aerator and regeneration aerator.

In order to gain an insight into the optimum detention time in the endogenous aerator, a batch experiment was conducted. The settled sludge from the bottom of the settling tank was washed and then used in the endogenous aerator as usual. Samples were taken periodically during the 12-hour aeration period in the endogenous tank and analyzed for COD, biological solids concentration and sludge composition with respect to carbohydrate and protein content.

This operational feasibility study was carried out in separate experiments, one using glucose and one using acetic acid as the sole carbon source. Five different nitrogen levels corresponding to COD:N ratios of 10:1, 30:1, 40:1, 50:1, and 70:1 were employed for the glucose system whereas COD:N ratios used for acetic acid systems were 30:1, 50:1, and 70:1. These nitrogen levels were employed with a view to finding the critical nitrogen requirements for this new process and to gain more insight into the design parameters involved.

B. Analytical Techniques

The chemical oxygen demand of the samples from continuous flow experiments was determined in accordance with the

procedure recommended in Standard Methods (144). The volume of 0.25 N potassium dichromate solution used in the analysis was 10 ml. The approximate normality of the ferrous ammonium sulfate solution was 0.1 N. In batch experiments described under part 2 of the experimental design, procedures outlined in the previous edition of Standard Methods (145) were followed. The determination of carbohydrate was carried out by the anthrone test, as suggested by Gaudy (146). Biological solids concentration was determined by the membrane filter technique using HA filters, 0.45 μ pore size (Millipore Filter Co., Bedford, Mass.) The protein and carbohydrate contents of the sludges were determined by the methods suggested by Gaudy (146). The ammonia determination was carried out initially by the micro-kjeldahl technique but later a method developed by Niss and reported by Ecker and Lockhart (85) was employed. This method involves use of two reagents; the first of these is prepared as follows: sodium citrate, 4.7 gm; citric acid, 1.7 gm; phenol, 9.6 gm; distilled water to 480 ml. The second reagent contains boric acid, 6.0 gm; sodium hydroxide, 8 gm; Chlorox, 30 ml; and distilled water to 200 ml. Samples were taken so that they contained 2 to 20 μg of ammonia nitrogen per ml, and these were made up to one ml with distilled water. To these samples 5 ml of the first reagent and 2 ml of the second reagent were added. Samples were mixed thoroughly, heated in a boiling water bath for five minutes then cooled, and their optical

densities were compared with those of standards at 615 mμ wave length using distilled water blanks.

The individual volatile acids were determined by gas chromatography (F & M Scientific Company, Model 810). The separation of the components is achieved due to their differing partition coefficients between the stationary phase consisting of high boiling liquid and a mobile phase consisting of inert carrier gas. The sample is injected into the carrier gas stream through the injection port. This sample is immediately carried into the column, where its various components move at different velocities, depending upon their partition coefficients between the liquid phase and the carrier gas.

The column used in the experiments consisted of a glass tube, 3/16 inch internal diameter, hand packed with "poly pak 2," a polymer which is reported to be stable up to 300°C in an oxygen-free atmosphere. To pack the column, suction was applied at one end of the glass column while the packing material was admitted from the other end. Trapping of air along the length of the column was avoided. After packing the two ends of the column were plugged with cotton to prevent loss of the packing material.

The "poly pak 2" has a surface area of 300 m^2/gm or 120 m^2/cc, density = 0.4 gm/cc, mesh size = 80/120, and is white in appearance. This material is capable of detecting air, carbon monoxide, carbon-dioxide, water, ketones, volatile fatty acids, aromatic and cyclic aliphatic hycrocarbons.

Helium was used as the carrier gas. A flow rate corresponding to a rotometer reading of 2.0 was used. The pressures of compressed air, helium, and hydrogen gas were 33, 60, and 20 psi, respectively. The oven temperatures at the injection port and detector, respectively, were 220°C and 250°C.

CHAPTER V

RESULTS

Phase I

A. Effect of Different Nitrogen Levels in Continuous Flow Activated Sludge Process

1. Steady State Parameters (COD:N = 70:1 in the feed)

Figure 4 shows the daily values of feed COD, biological solids concentration in the aerator, COD and carbohydrate in the mixed liquor filtrate and temperature in the reactor for continuous flow studies for which the detention time in the unit was twelve hours, and the COD:N ratio was 70:1. It is seen that there was some variation in the feed COD; however, the differences were small. The biological solids concentration fluctuated between 610 mg/l and 740 mg/l, with an average value of 694 mg/l. The temperature varied between 21° and $23^{o}C$. The effluent COD was fairly constant. The range of effluent COD values was 180 mg/l to 130 mg/l with an average of 150 mg/l. The average carbohydrate content in the effluent was found to be 60 mg/l. The average biochemical purification efficiency was found to be 86 per cent. Average values for all parameters are summarized in Table II. No ammonia nitrogen was detected

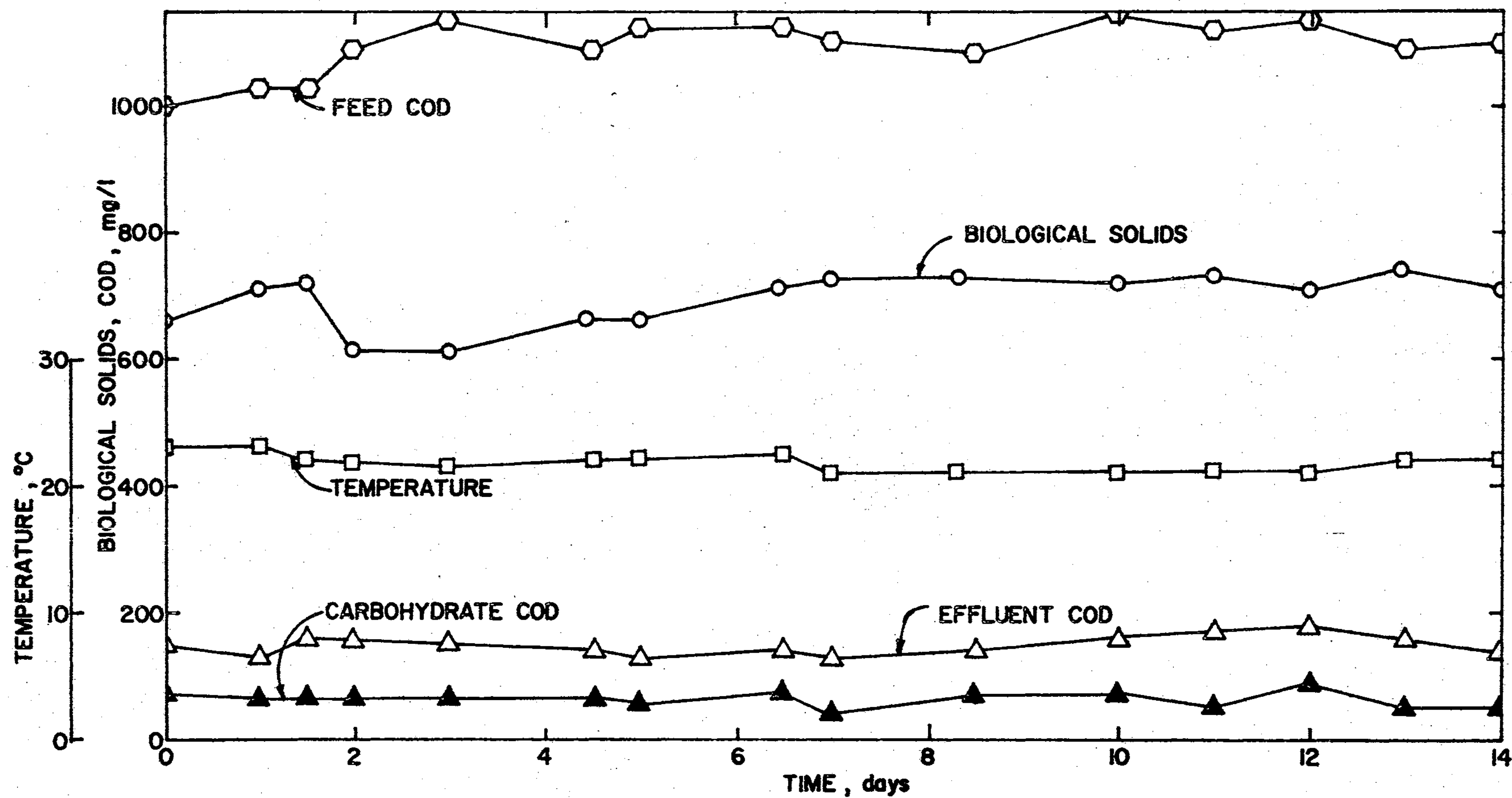


Figure 4. "Steady state" parameters at D = 1/12 $hour^{-1}$ and COD:N = 70:1.

TABLE II

SUBSTRATE REMOVAL, GROWTH AND SLUDGE COMPOSITION FOR HETEROGENEOUS MICROBIAL POPULATIONS GROWN IN A CONTINUOUS FLOW COMPLETELY MIXED REACTOR AT VARIOUS CONCENTRATIONS OF AMMONIA NITROGEN

Detention Time Hours	Dilution Rate $Hour^{-1}$	Effluent COD mg/l	COD Removal Efficiency %	Biological Solids mg/l	Yield %	Sludge mg/l	Carbohydrate %	Sludge mg/l	Protein %	NH_3-N in Effluent mg/l	NH_3-N Utilized %
				COD:N in the feed = 1060:15.1 = 70:1							
1	1.0	998	6	40	64						
2	0.5	823	22	222	80	110	50	80	36	0.0	100
4	0.25	582	45	284	55	131	46	80	28	0.0	100
8	0.125	240	77	690	80	266	39	85	12	0.0	100
12	0.083	150	86	694	82	314	46	80	12	0.0	100
				COD:N in the feed = 1060:26.5 = 40:1							
1	1.0	923	13	88	69						
2	0.5	373	65	336	48	122	36	130	39	2.2	92
4	0.25	180	83	371	43	128	35	140	38	1.9	93
8	0.125	127	88	510	55	159	31	125	26	2.2	92
12	0.083	81	92	709	73	218	31	130	18	2.7	90
				COD:N in the feed = 1060:42.4 = 25:1							
1	1.0	895	16	55	53						
2	0.5	294	72	422	60	128	30	215	51	2.7	94
4	0.25	130	88	400	46	113	28	200	50	3.8	91
8	0.125	63	94	394	42	97	25	190	48	6.2	86
12	0.083	57	95	583	62	147	25	175	30	8.6	80
				COD:N in the feed = 1060:106 (Data taken from M. Ramanthan Ph.D. Thesis)							
2	0.5	874	17	101	54	32	31	41	40.75		
4	0.25	92	91	661	68	162	25	392	59.37		
12	0.083	45	95	655	65	145	22	415	63.4		

in the effluent. The average carbohydrate and protein contents in the biological solids were 314 and 80 mg/l, respectively. These correspond to average carbohydrate and protein contents of the sludge of 46 and 12 per cent. The yield of biological solids was 82 per cent.

Similar plots for systems operated at 8, 4, 2, and 1-hour detention times with a COD:N ratio of 70:1 are shown in Figures 5 through 8. The variations in COD values of the effluent and in biological solids concentration are greater than those observed for the unit run at the 12-hour detention time. The average biological solids concentrations in the units with 8, 4, 2, and 1-hour detention times were found to be 690, 284, 222, and 40 mg/l respectively, indicating that at this COD:N ratio cells begin to dilute out of the system at a detention time between eight hours and four hours.

The average COD values for the effluent were 240, 582, 823, and 998 mg/l for systems with 8, 4, 2, and 1-hour detention times. The average carbohydrate contents in the effluents for these four units were 70, 78, 85, and 150 mg/l, respectively. Using average biological solids concentrations and effluent COD values, the yields of biological solids were 0.8, 0.55, 0.8, and 0.64 for 8, 4, 2, and 1-hour systems. These yield figures would seem to indicate that detention time is not a major factor in determining the yield of biological solids. However, it is apparent that the yields are, in general, higher than those obtained in

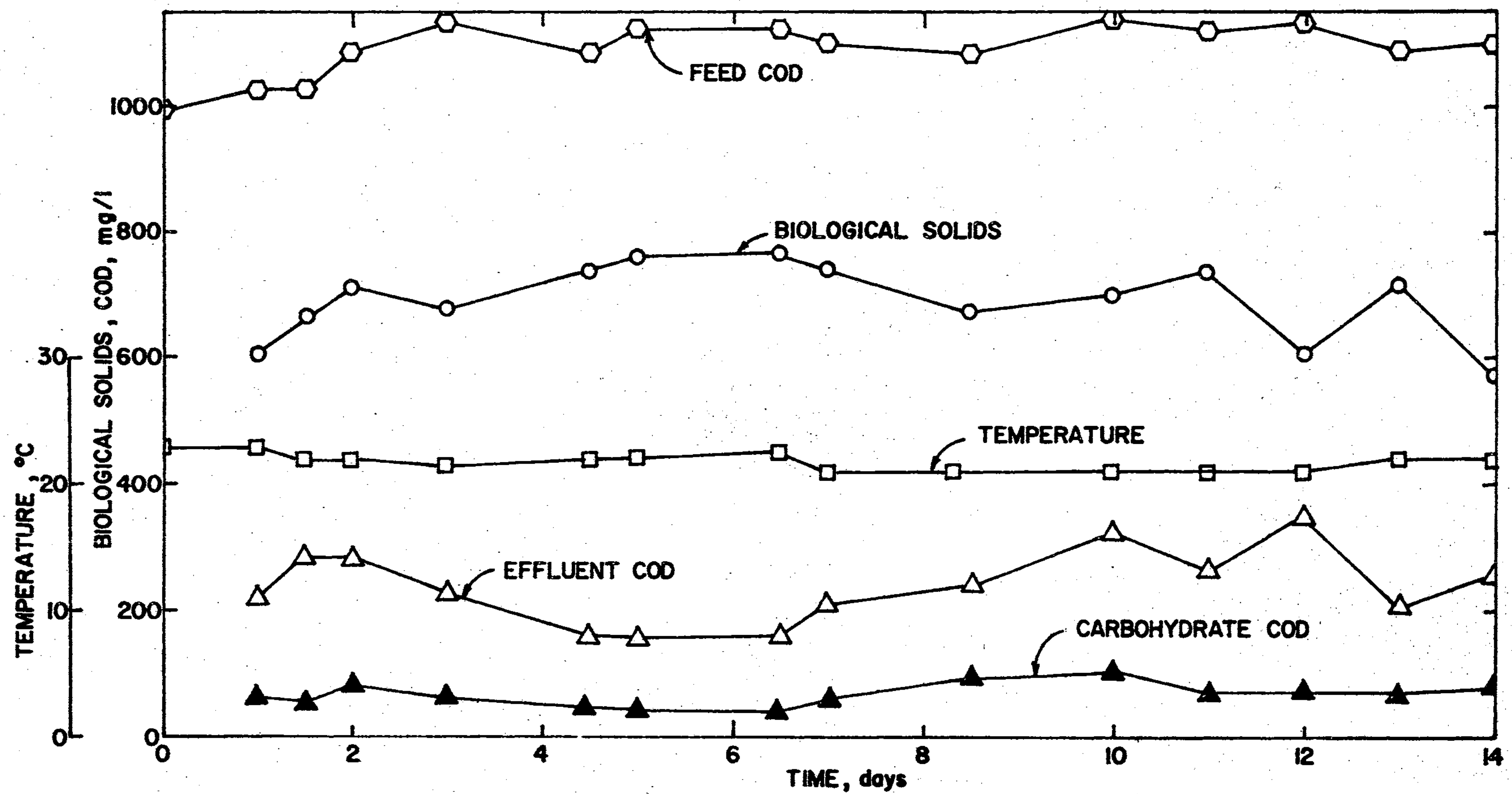


Figure 5. "Steady state" parameters at D = 1/8 $hour^{-1}$ and COD:N = 70:1.

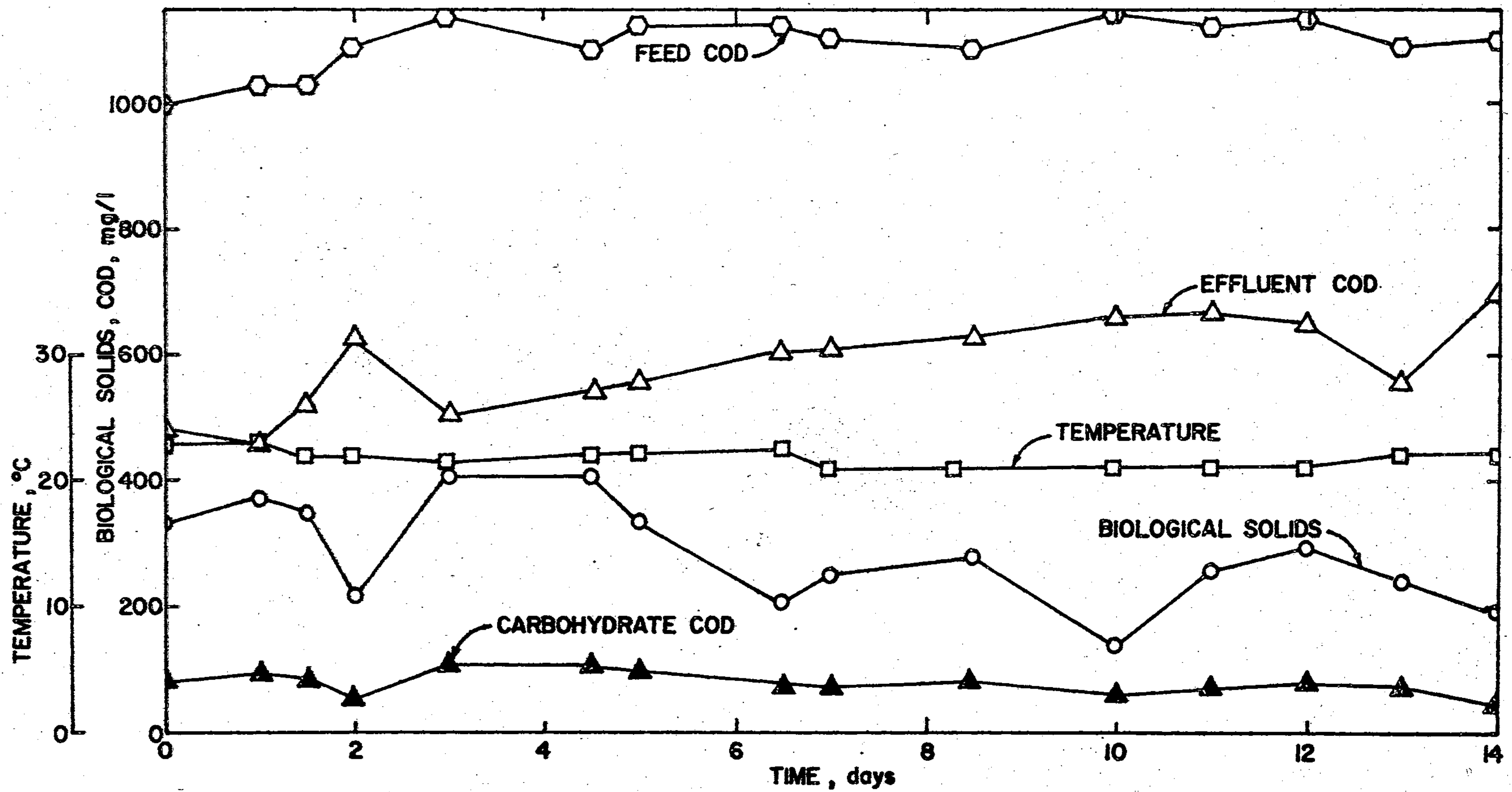


Figure 6. "Steady state" parameters at D = 1/4 $hour^{-1}$ and COD:N = 70:1.

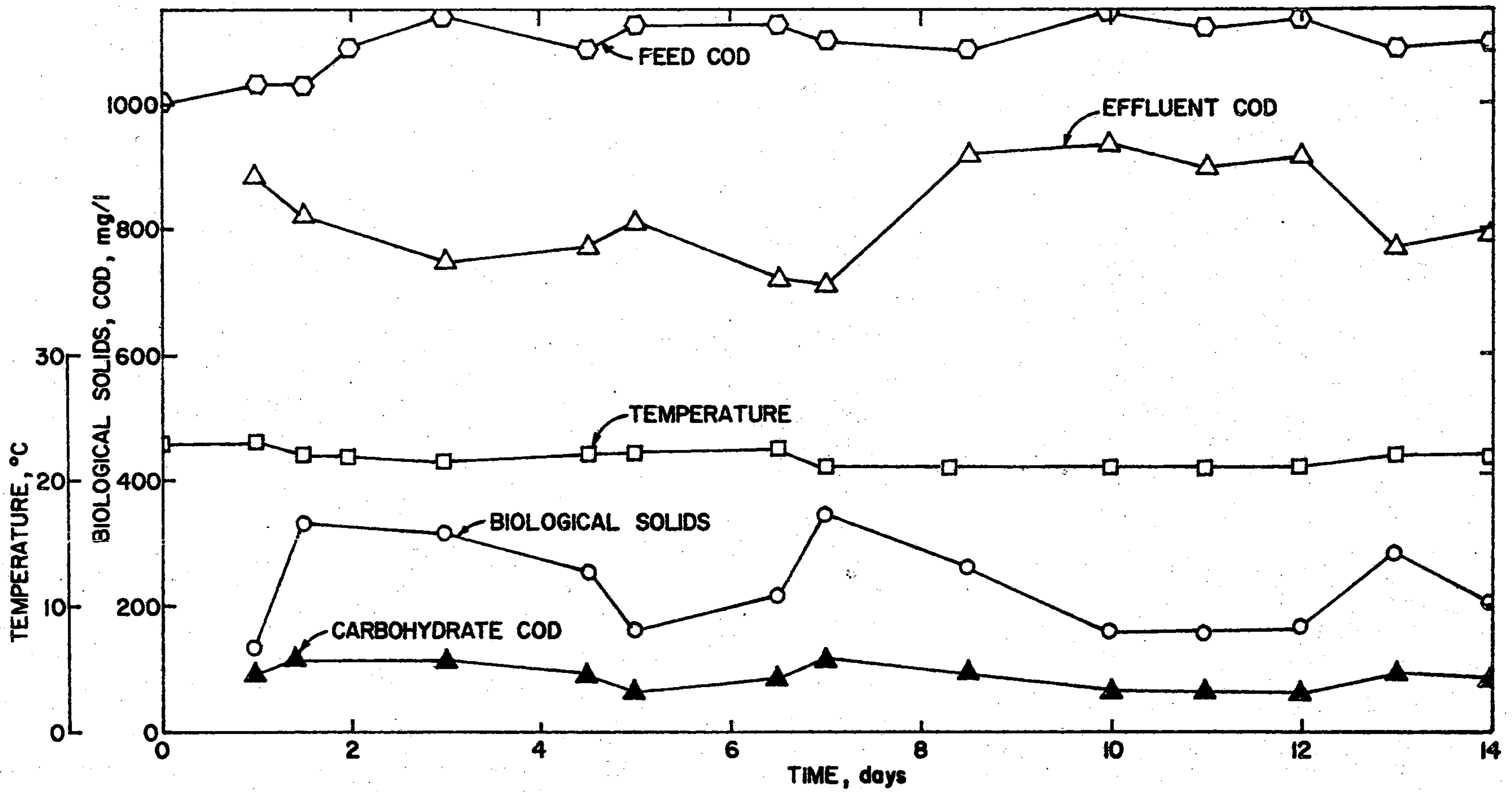


Figure 7. "Steady state" parameters at $D = 1/2 \text{ hour}^{-1}$ and COD:N = 70:1.

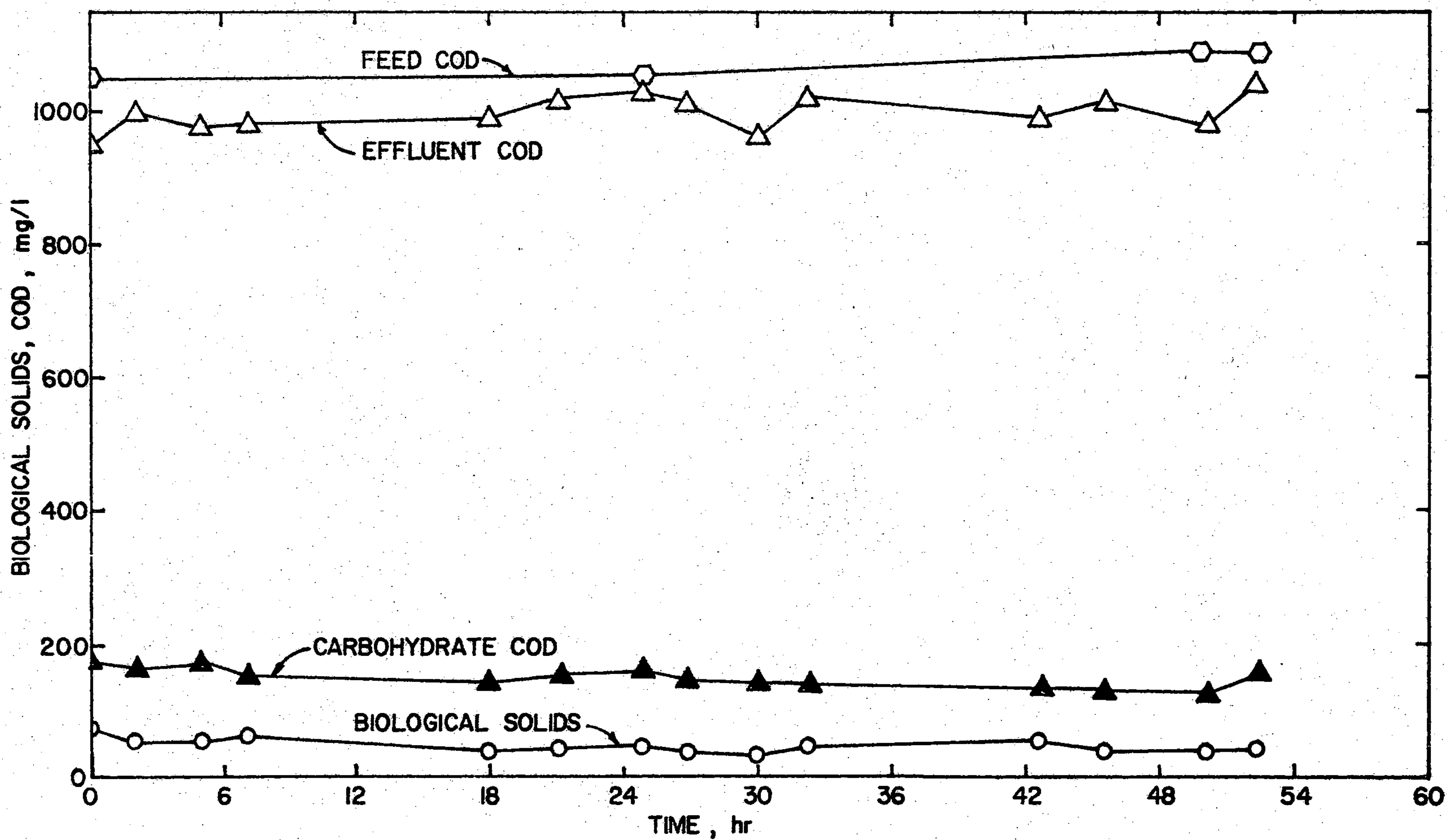


Figure 8. "Steady state" parameters at D = 1 $hour^{-1}$ and COD:N = 70:1.

conventional systems where the waste carries higher nitrogen concentrations. The carbohydrate content of the sludges was found to be 266, 131, and 110 mg/l for the systems operated at 8, 4, and 2-hour detention times, respectively, which amounts to 39, 46, and 50 per cent carbohydrate content of the dry solids weight. It is thus seen that generally the carbohydrate content of the sludges was higher at lower detention time (except for the system with a 12-hour aeration period). The average protein content in the sludges ranged between 80 to 85 mg/l for all systems at this nitrogen level. This finding indicates that the detention time was not a factor in the synthesis of protein at this low nitrogen level; however, the protein content of sludges, measured as the fraction of dry weight of solids, was found to fluctuate considerably, depending upon the concentration of the biological solids in the aerator. In general, it was higher at the lower detention times.

Absence of any ammonia nitrogen in the effluent provides some indication that all of the nitrogen in the medium was used by the cells for production of protein or nucleic acids. This tends to explain the constant level of protein in all systems, i.e., protein synthesis was limited to the amount observed due to the ammonia nitrogen concentration in the feed. It thus appears that a 2-hour aeration period was sufficient for the cells to attain the maximum amount of protein synthesis (80 mg/l) in these systems.

2. Steady State Parameters (COD:N = 40:1 in the Feed)

Figures 9 through 13 show the daily values of feed COD, biological solids concentration in the aerator, COD and carbohydrate content of the mixed liquor filtrate, and temperature for the continuous flow units operated at 12, 8, 4, 2, and 1-hour detention times, when the feed contained nitrogen at a COD:N ratio of 40:1. It is seen that the different parameters are not absolutely constant, but oscillate to some extent. The phenomenon of oscillation was observed to be more pronounced in the system with the 2-hour detention time.

The data in Table II show that the efficiency of COD removal as well as the biological solids concentration increased as the detention time in the aerator increased. The cell yield seemed to be independent of detention time employed and varied between 0.43 and 0.73. The cellular carbohydrate increased with detention time, although the carbohydrate percentage of the dry weight of solids decreased with increase in detention time. The protein in the system varied slightly (between 125 mg/l and 140 mg/l) and can, for all practical purposes, be regarded as constant at all detention times employed. The protein content of the dry solids, expressed as a percentage, was found to increase as the detention time decreased. Ammonia-nitrogen analysis showed that 90 per cent or more of the supplemental nitrogen was utilized by the cells. The concentration of ammonia-nitrogen leaking into the effluent

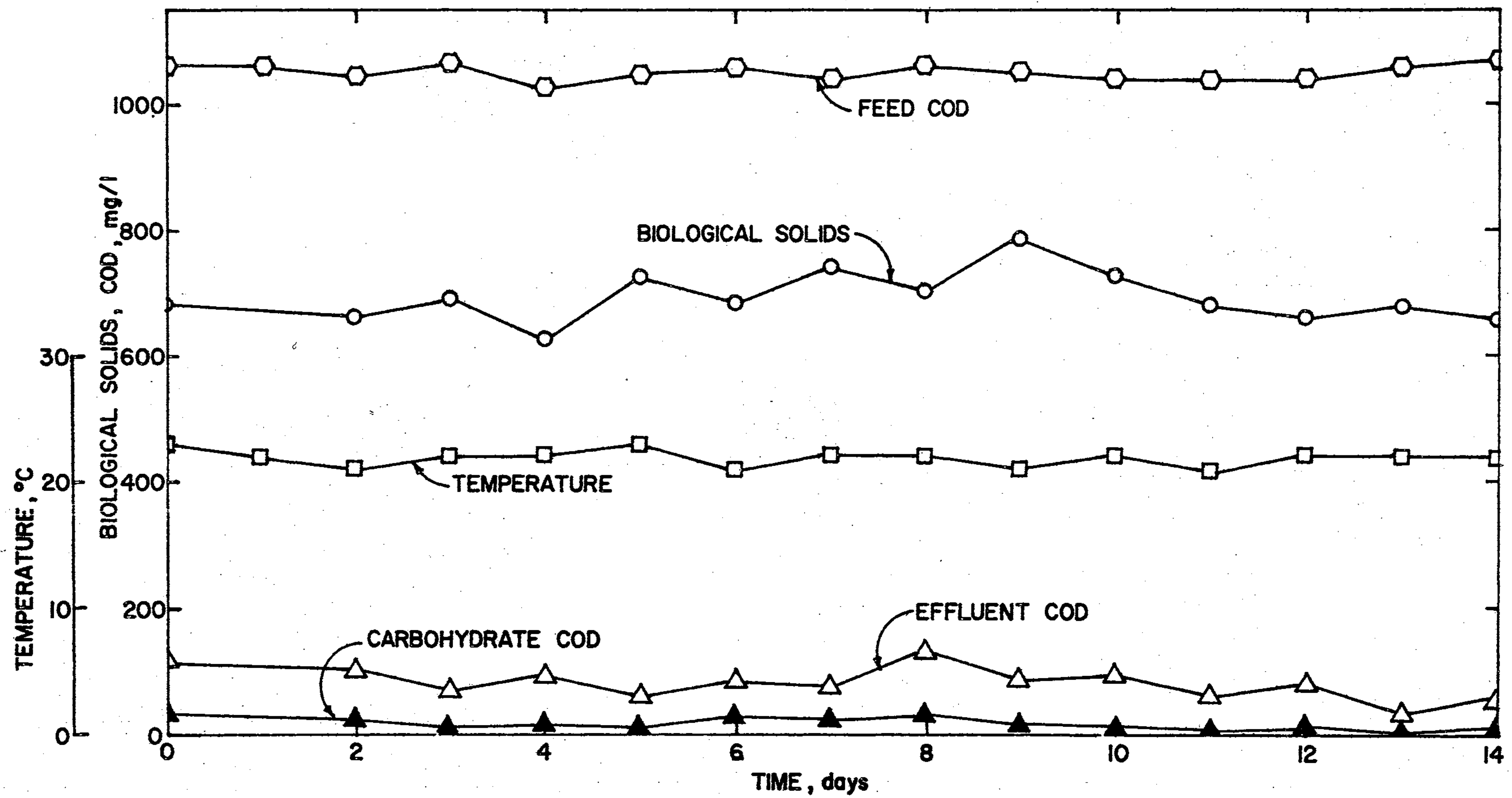


Figure 9. "Steady state" parameters at D = 1/12 $hour^{-1}$ and COD:N = 40:1.

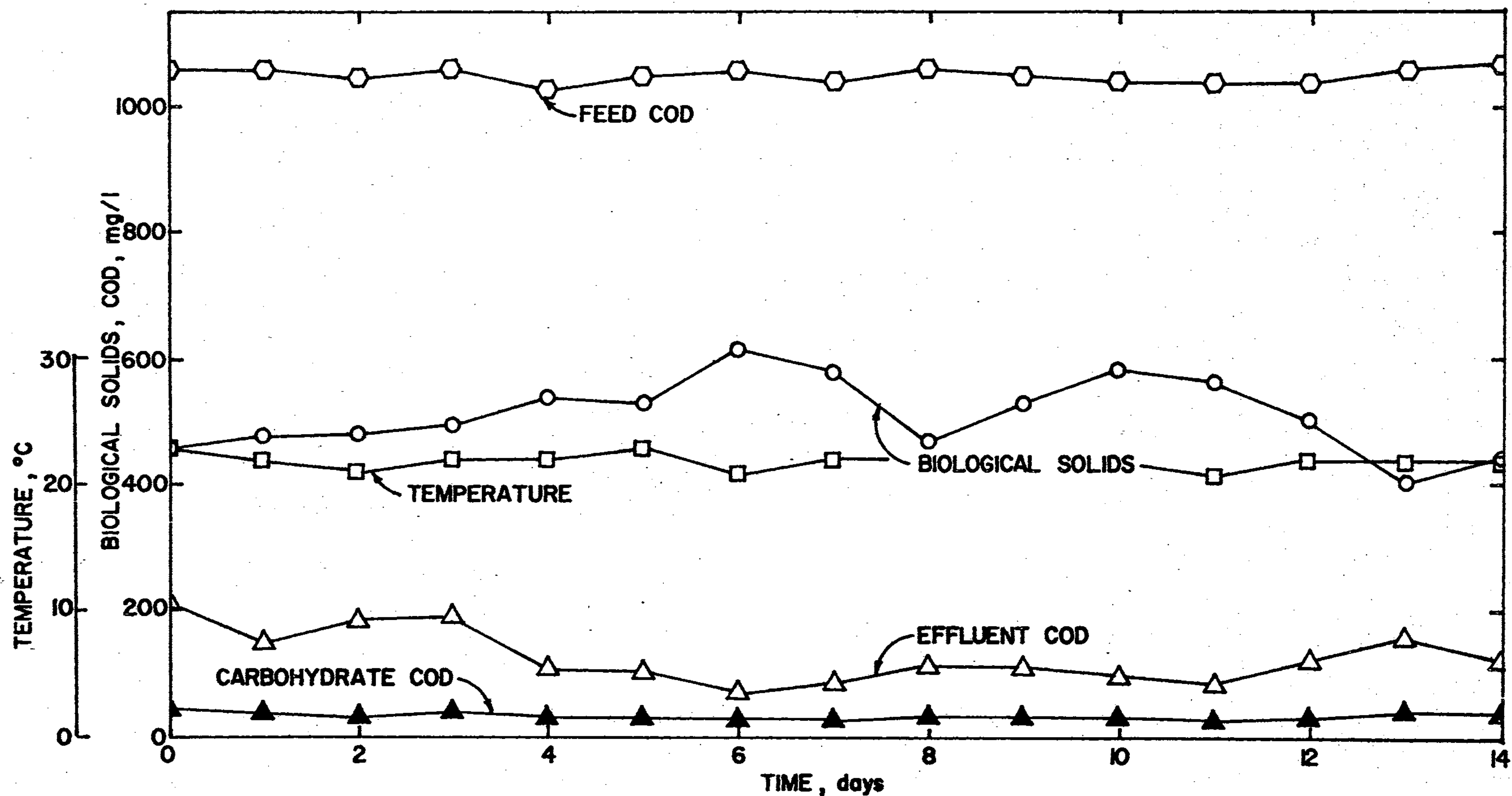


Figure 10. "Steady state" parameters at D = 1/8 $hour^{-1}$ and COD:N = 40:1.

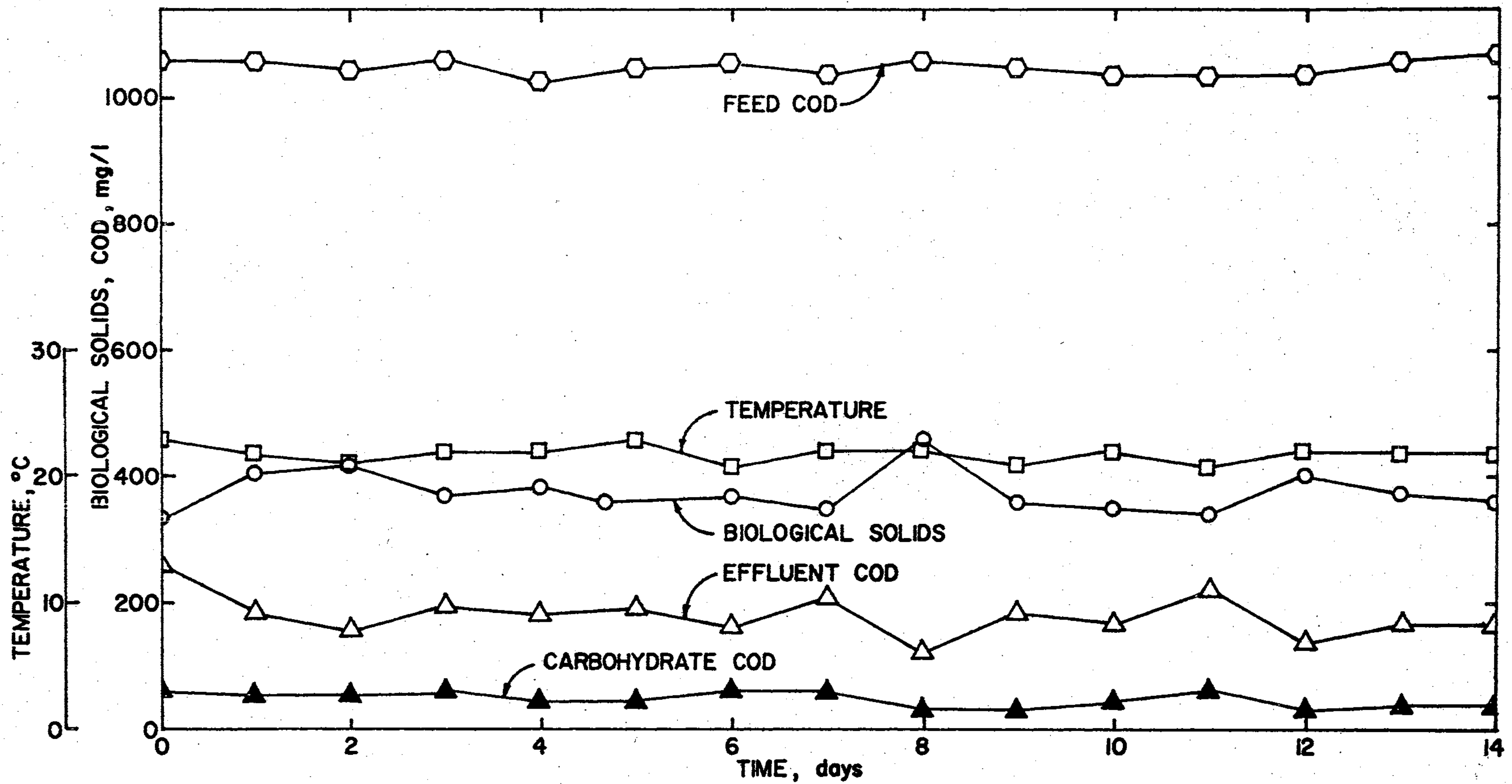


Figure 11. "Steady state" parameters at D = 1/4 $hour^{-1}$ and COD:N = 40:1.

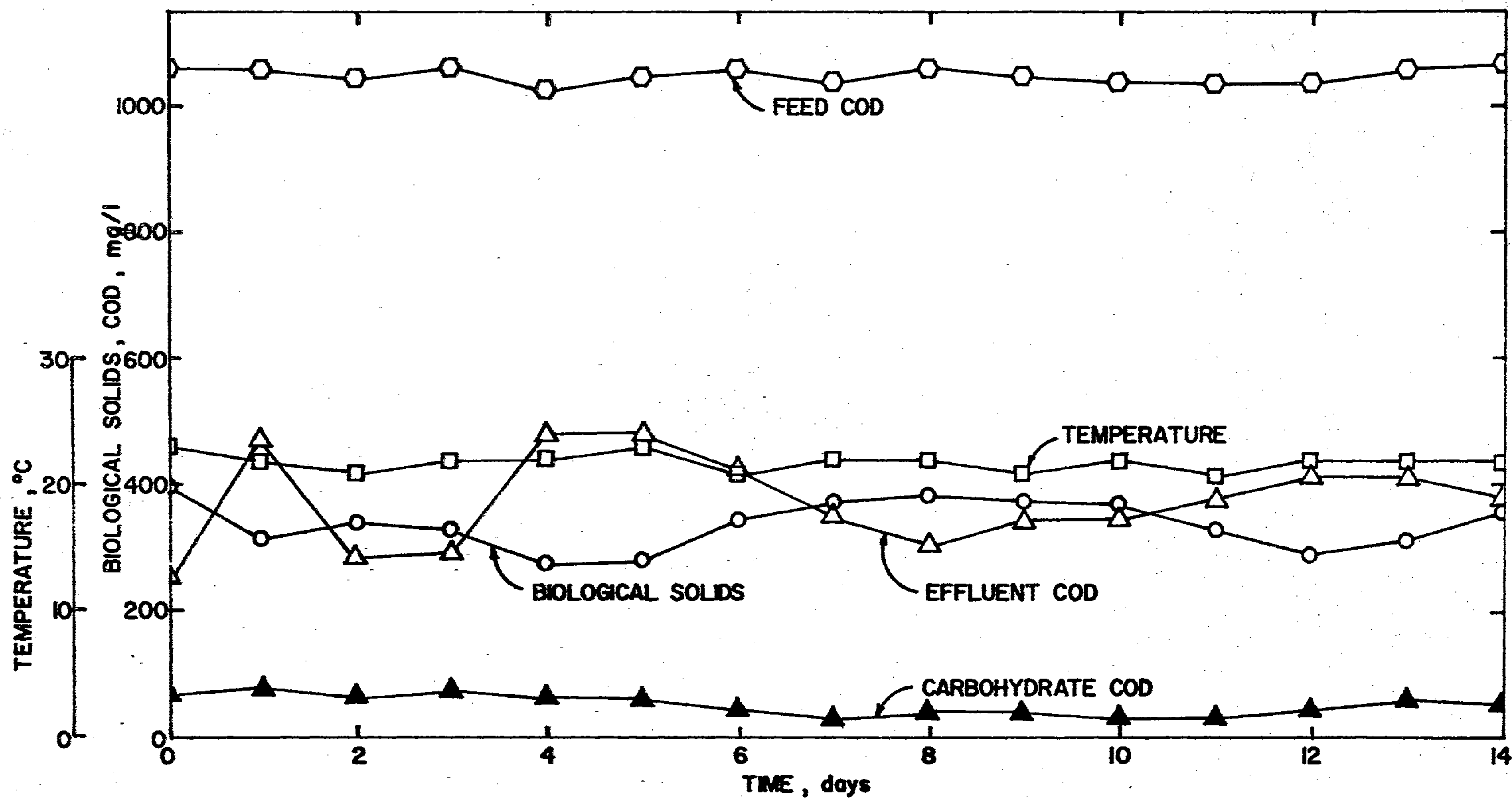


Figure 12. "Steady state" parameters at D = 1/2 hour^{-1} and COD:N = 40:1.

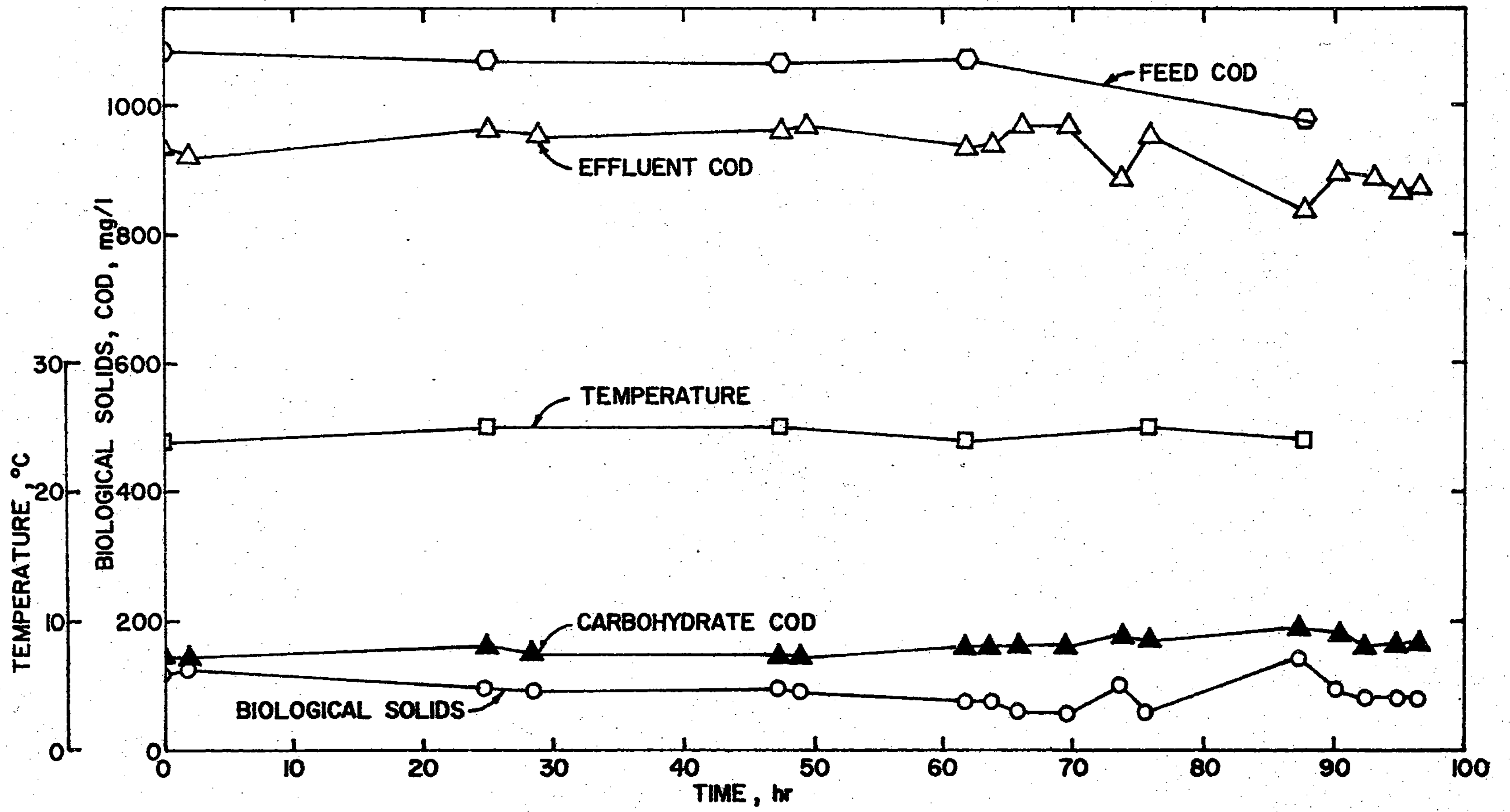


Figure 13. "Steady state" parameters at D = 1 $hour^{-1}$ and COD:N = 40:1.

was found to be very small; the maximum and the minimum values for the four systems were 1.85 and 2.7 mg/l.

3. Steady State Parameters (COD:N = 25:1 in the feed)

Figures 14 through 18 show the daily values of feed COD, biological solids concentration in the aerator, COD, and carbohydrate contents of the mixed liquor filtrate, and temperature for the five continuous flow units when the feed contained ammonia nitrogen corresponding to a COD:N ratio of 25:1. The aerator detention times employed were 12, 8, 4, 2, and 1 hours.

From Figures 14 and 17 it is seen that the biological solids in the aerator fluctuated considerably, although the effluent COD remained fairly constant. The yield of biological solids does not depend, necessarily, on the type of substrate and the metabolic pathway for its degradation, but also on the microorganisms present. In a heterogeneous culture the predominating bacterial species are likely to change, and this may explain some of the variations in the cell yields. The oscillating nature of the biological solids is not uncommon in continuous flow aerators using heterogeneous cultures (138).

The average values for the various parameters are shown in Table II. The average effluent COD values for the systems operated at 12, 8, 4, 2, and 1-hour detention times were observed to be 57, 63, 130, 294, and 895 mg/l; whereas the mean solids concentrations were found to be 584, 394, 400, 422, and 55 mg/l, respectively. These figures show

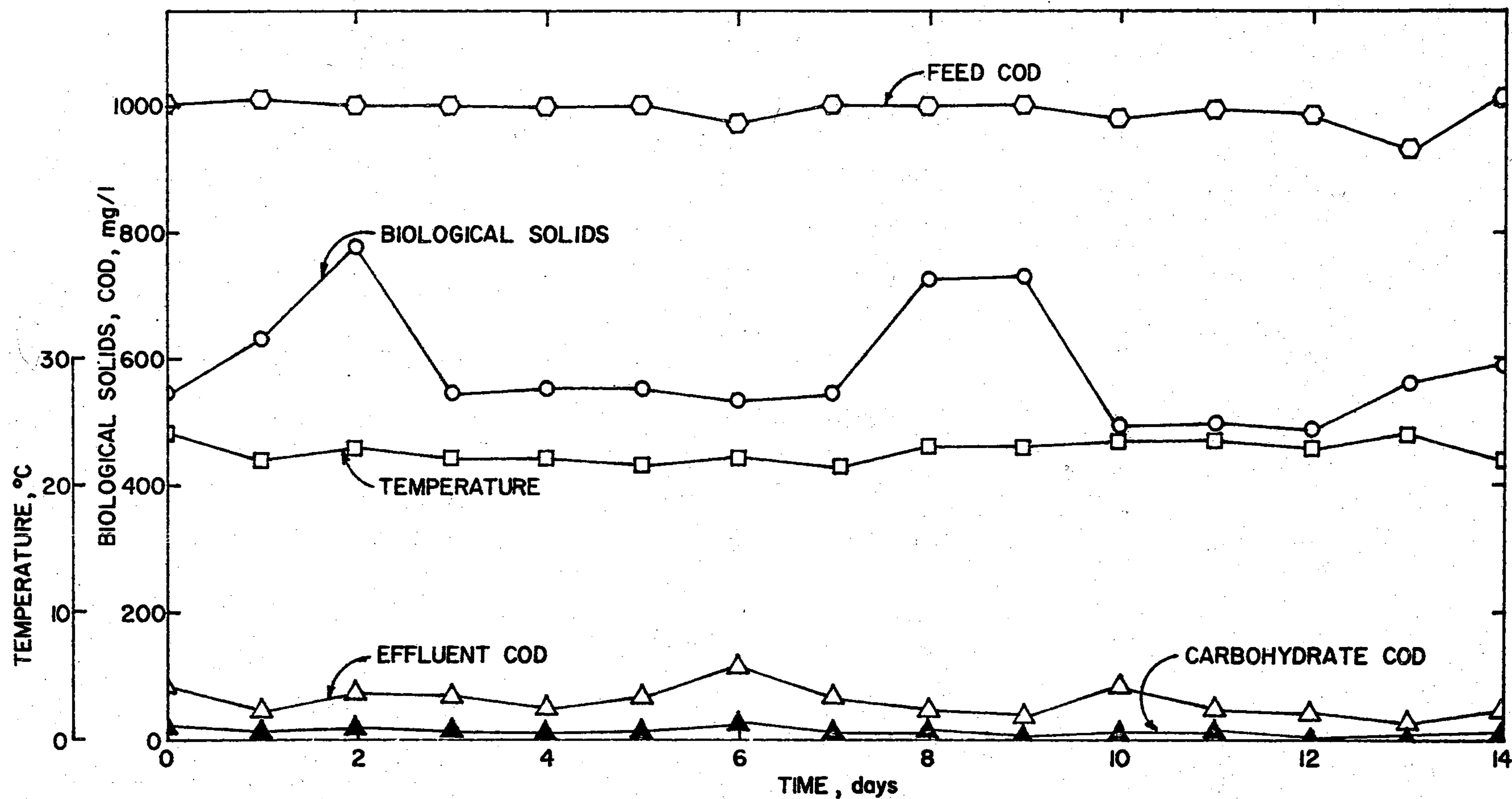


Figure 14. "Steady state" parameters at D = 1/12 hour $^{-1}$ and COD:N = 25:1.

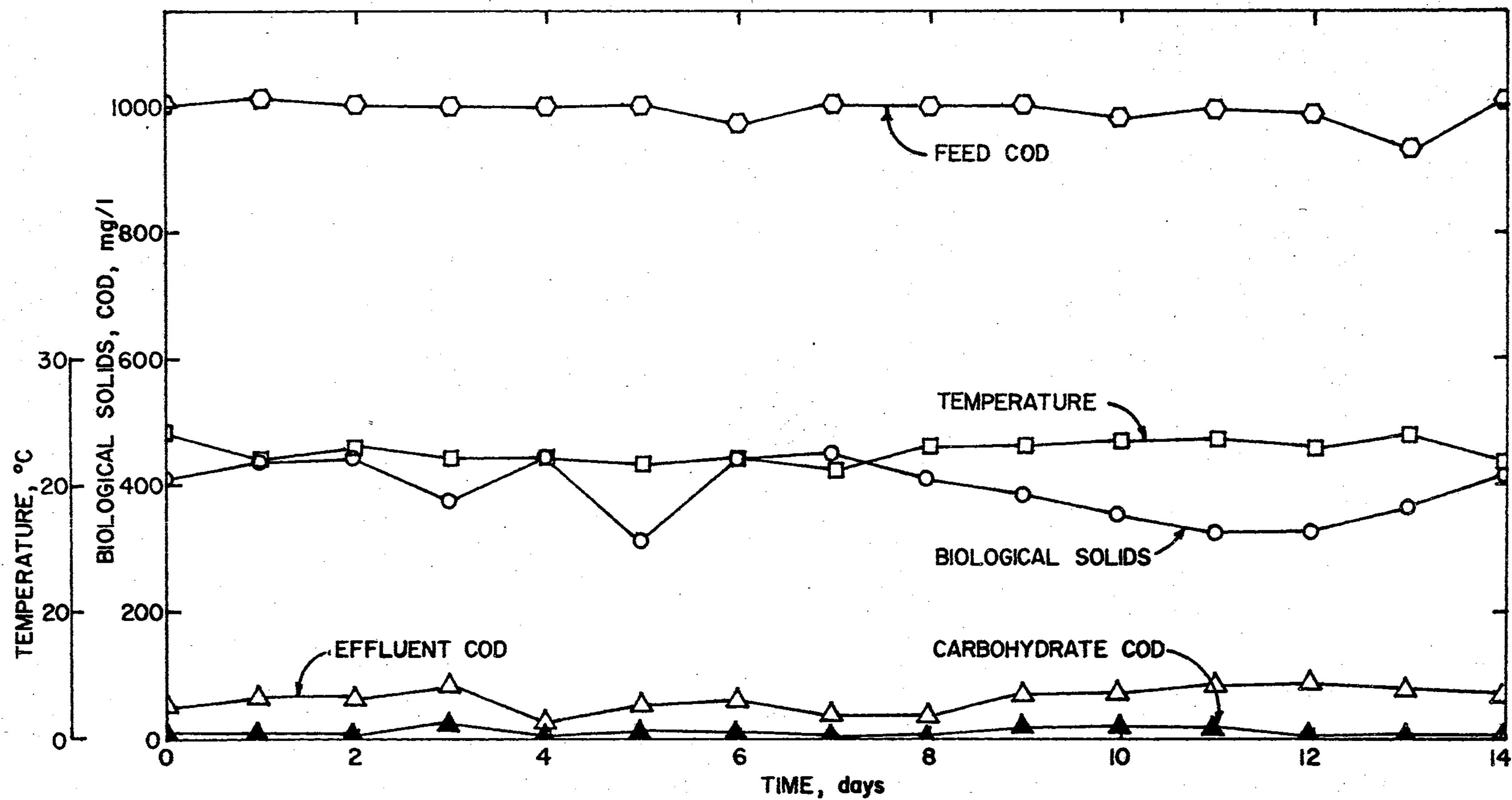


Figure 15. "Steady state" parameters at $D = 1/8 \text{ hour}^{-1}$ and COD:N = 25:1.

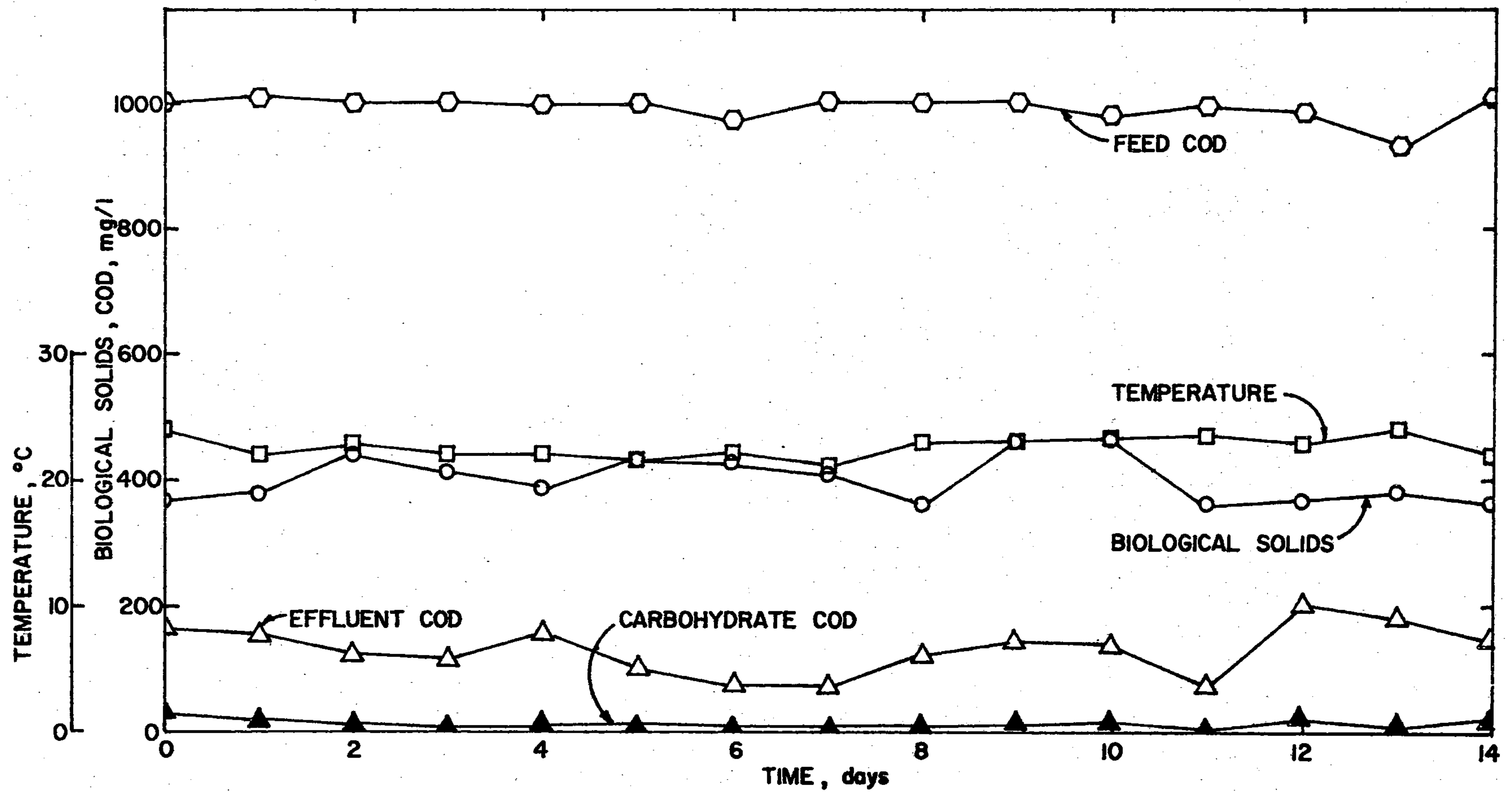


Figure 16. "Steady state" parameters at D = 1/4 $hour^{-1}$ and COD:N = 25:1.

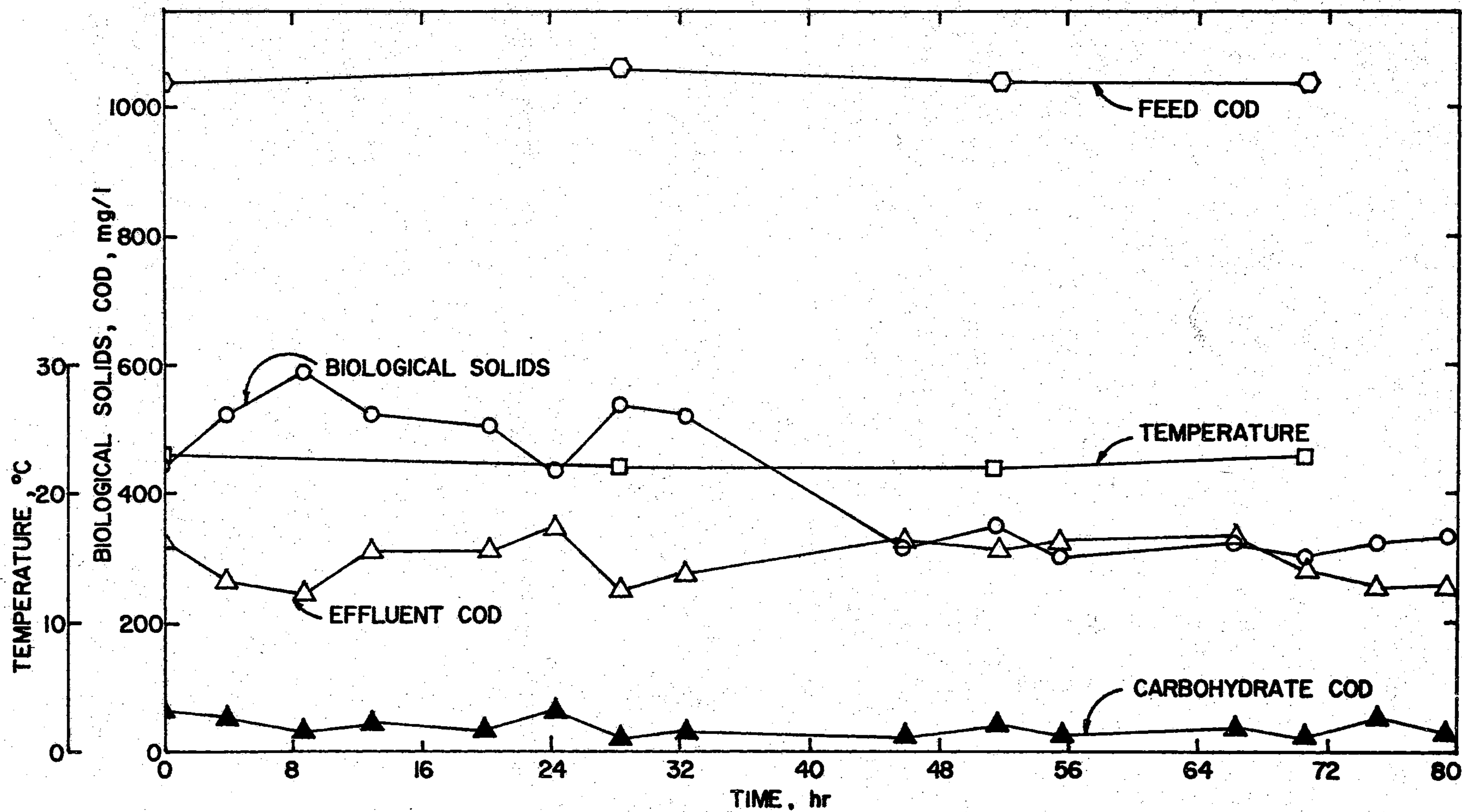


Figure 17. "Steady state" parameters at D = 1/2 hour^{-1} and COD:N = 25:1.

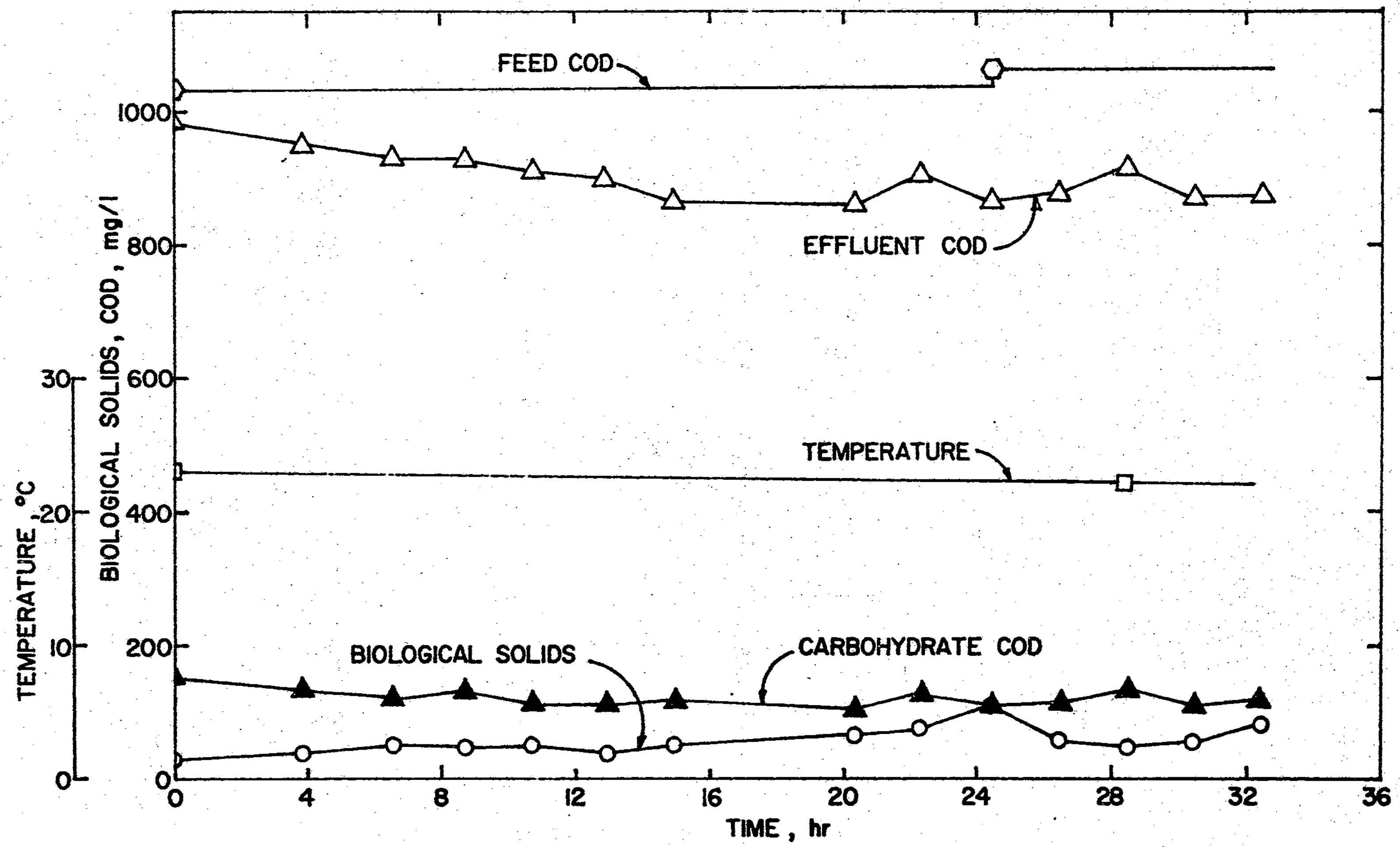


Figure 18. "Steady state" parameters at D = 1 $hour^{-1}$ and COD:N = 25:1.

that the increase in detention time caused an increase in the purification efficiency. The yield was found to be independent of the detention time; it ranged between 0.42 and 0.62. A gradual lowering of solids was observed in the systems operated with 2-hour detention times. This could possibly be due to a change in the predominance of bacterial species. The carbohydrate content of the sludge was between 25 per cent and 30 per cent, being higher at very low detention times. The protein content of the sludge ranged between 30 and 51 per cent, being higher at lower detention times. The protein concentration of the system was found to vary between 175 and 215 mg/l, being higher at low detention times. It will be recalled that the protein in the system remained constant at various detention times in systems with COD:N ratios of 70:1 and 40:1. The percentages of nitrogen utilization were 93.5 and 80 for 2-hour and 12-hour detention times, respectively. This result indicates that the fast-growing organisms, i.e., those being grown at low detention times, incorporated more of the available nitrogen. It is also noted that not all of the supplemental nitrogen was incorporated in the sludge. In the system operated at a detention time of twelve hours, as much as 20 per cent of the added nitrogen leaked in the final effluent.

To allow comparison of data obtained in this study with those obtained previously in a similar unit operated with excess nitrogen, data from M. Ramanathan's Ph.D.

thesis (79) were included in Table II.

4. Release of Metabolic Intermediates

The results of substrate analyses by the anthrone test and the COD test on the mixed liquor filtrates (shown in Figures 4 through 18), revealed that not all of the effluent COD was composed of carbohydrates, i.e., there was evidence for the production of metabolic intermediates and/or end products. Using gas-liquid chromatographic methods, attempts were made to determine the nature of the compounds which were produced during the metabolism of the substrate. The filtrates were analyzed for metabolic products using a column packed with "poly pak 2," and the results are shown in Table III. It is seen that acetic acid, ethanol, and propionic acid were the only compounds detected. Significant quantities of acetic acids were found in the system at the 2-hour detention time with a COD:N ratio of 70:1. At this COD:N ratio, acetic acid was detected in systems operated at 4 and 8-hour detention times. No metabolic intermediate was detected at the 12-hour detention time.

In systems with higher nitrogen supplementation, small quantities of acetic acids were found for the 2-hour detention time.

The total quantities of metabolic intermediates as calculated from the difference between the COD and anthrone COD are also shown in Table III. It is seen that the sum of acetic and propionic acids is considerably lower than the total metabolic intermediates, indicating the presence

TABLE III

DETERMINATION OF METABOLIC INTERMEDIATES

COD:N	1 Detention Time Hours	2 Effluent COD mg/l	3 Anthrone COD mg/l	4 Acetic Acid mg/l	5 Propionic Acid mg/l	6 Ethanol (approx) mg/l	7 Total of Columns 4, 5, 6	8 Total Intermediates 2-3 mg/l
70:1	2	823	94	86	7	50	143	729
	4	582	90	15	0	5	20	492
	8	240	72	15	0	5	20	168
	12	150	60	0	0	0	0	90
40:1	2	373	60	17	0	0	17	313
	4	180	50	0	0	0	0	130
	8	127	32	0	0	0	0	95
	12	81	20	0	0	0	0	61
25:1	2	294	42	15	0	0	15	252
	4	130	15	0	0	0	0	115
	8	63	15	0	0	0	0	48
	12	57	10	0	0	0	0	47

of some other metabolic intermediates or end products. It may be added that aerobic conditions were prevalent at all times in the aerator, and therefore the compounds detected by gas chromatography were not fermentation products.

5. <u>Growth Parameters Under Nitrogen-Limiting Conditions</u>

The growth parameters μ_{max}, Y, and K_s were determined where the limiting growth factor was nitrogen, using shaker flask experiments. The flasks were supplied with identical growth media and seed, but differing amounts of nitrogen source. The seed for these studies was taken from the continuous flow units mentioned under sections 1, 2, 3 above.

A typical case is presented in Figure 19; twelve similar studies were conducted in all. Figure 19 shows the pattern of biological growth. The seed was taken from a unit operating at the 2-hour detention time with a COD:N ratio of 40:1. It is seen that although each flask contained the same concentration of organic substrate, the biological growth was not the same in all flasks. Since all constitutents of the medium were identical except for the concentration of NH_4^+, the growth in the flasks which contained NH_4^+ concentrations of 10 mg/l or less was limited by nitrogen concentration. The maximum solids concentrations in flasks containing 1, 2, and 10 mg/l NH_4^+ were 22, 38, and 105 mg/l, respectively, whereas in all flasks with NH_4^+ concentrations above 10 mg/l, the maximum solids concentration was close to 275 mg/l. These data show that provision of 20 mg/l NH_4^+ ensured adequate unhindered growth

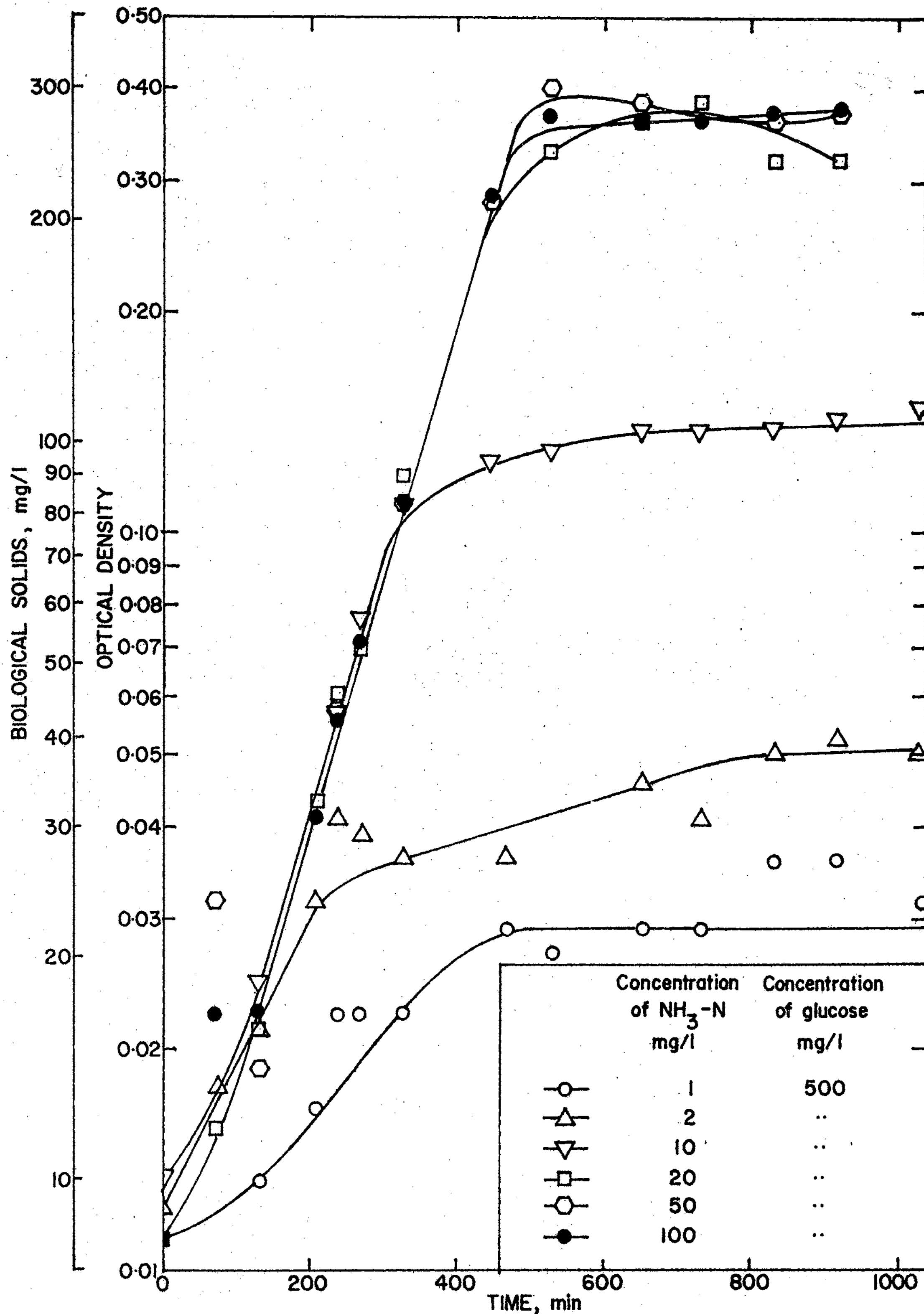


Figure 19. Growth pattern at different nitrogen levels. Seed taken from continuous flow unit operated at a detention time of 2 hours and COD:N = 40:1.

on 500 mg/l glucose. Below 20 mg/l NH_4^+ concentration, the cell production was restricted due to inadequacy of nitrogen.

The COD and ammonia nitrogen in the filtrates after cessation of growth are shown in Table IV. The table also shows the growth rates observed for various nitrogen concentrations in the flasks. It is seen that the COD removal in flasks seeded with cells taken from the continuous flow units with COD:N ratios of 25:1 and 40:1 and containing 10 mg/l NH_4^+ ranged from 46 to 79 per cent, whereas it was found to vary between 73 and 93 per cent in flasks containing 20 mg/l NH_4^+. The COD removal did not seem to increase significantly when the nitrogen level was increased beyond 20 mg/l NH_4^+. This concentration of NH_4^+ corresponds to a COD:N ratio of 32:1. Except for one case, the ammonia-nitrogen concentration in the mixed liquor after the cessation of growth was very small (less than 3 mg/l) when the initial ammonia concentration was 20 mg/l or less. For higher initial nitrogen concentrations there was an increase in the ammonia-nitrogen in the mixed liquor after the growth had reached its peak. It seems evident that the growth in the flasks which initially contained NH_4^+ in excess of 20 mg/l was not limited by lack of nitrogen.

A plot of ammonia concentration versus growth rate for cells harvested from the system operated at a COD:N ratio of 70:1 is shown in Figure 20. This was a preliminary experiment. Due to the slimy nature of the microorganisms,

TABLE IV

SUBSTRATE REMOVAL AND GROWTH CHARACTERISTICS FOR HETEROGENEOUS POPULATIONS GROWN IN SHAKER FLASKS AND SEEDED WITH EFFLUENT FROM CONTINUOUS FLOW UNITS OPERATED AT VARIOUS CONCENTRATIONS OF AMMONIA NITROGEN

Glucose Concentration = 500 mg/l

	D = 1/2 $Hour^{-1}$				D = 1/4 $Hour^{-1}$				D = 1/8 $Hour^{-1}$				D = 1/12 $Hour^{-1}$			
Initial Concentration of NH_4^+	COD utilized mg/l	COD utilized %	NH_3-N after Cessation of Growth mg/l	Specific Growth Rate $Hour^{-1}$	COD utilized mg/l	COD utilized %	NH_3-N after Cessation of Growth mg/l	Specific Growth Rate $Hour^{-1}$	COD utilized mg/l	COD utilized %	NH_3-N after Cessation of Growth mg/l	Specific Growth Rate $Hour^{-1}$	COD utilized mg/l	COD utilized %	NH_3-N after Cessation of Growth mg/l	Specific Growth Rate $Hour^{-1}$
COD:N = 40:1																
2 μg/l	49	8.5	0	-	15	2.6	<3		63	9.5	<3		30	4.8	<3	
10 "	89	16	0	-	20	3.6	<3		15	2.3	<3		15	2.4	<3	
20 "	113	20.2	0	-	11	2.0	<3		66	10.0	<3		19	3.1	<3	
50 "	226	40.0	0	-	21	3.8	<3		30	4.5	<3		15	2.4	<3	
0.1 mg/l	228	41	0	-	18	3.6	<3		35	5.3	<3		30	4.8	<3	
0.2 "	245	44	0	-	00	0	<3		50	7.6	<3		23	2.7	<3	
1.0 "	255	46	0	0.16	57	10.6	<3	0.23	26	3.9	<3	0.04	59	9.5	<3	0.12
2.0 "	267	48	0	0.24	104	20.5	<3	1.22	230	34.8	<3	0.10	98	15.8	<3	0.39
10.0 "	404	72.0	0	0.45	348	62	<3	0.41	524	79.3	<3	0.67	333	53.1	<3	0.35
20.0 "	523	93.3	<3	0.45	455	81.1	3	0.41	573	86.6	<3	0.59	449	74.4	<3	0.39
50.0 "	527	94	12.5	0.45	479	87	20	0.36	525	79.5	35	0.67	477	76.9	36	0.43
100.0 "	521	93.0	>50	0.45	446	83.2	>50	0.39	612	92.5	>50	0.67	445	71.5	>50	0.39
COD:N = 25:1																
2 μg/l	20	3.9	<3		65	11.0	<3		31	5.4	<3		6	1.1	<3	
10 "	23	4.5	<3		14	2.3	<3		40	7.0	<3		15	2.7	<3	
20 "	10	1.9	<3		10	1.7	<3		0	0	<3		6	1.1	<3	
50 "	26	5.1	<3		41	6.9	<3		40	7.0	<3		39	7.1	<3	
0.1 mg/l	23	4.5	<3		65	11.0	<3						39	7.1	<3	
0.2 "	20	3.9	<3		53	8.9	<3		71	12.4	<3		35	6.4	<3	
1.0 "	39	7.6	<3	0.14	89	15	<3	0.20	63	10.9	<3	0.17	94	17.1	<3	0.15
2.0 "	82	15.9	<3	0.29	80	13.3	<3		138	24.0	<3	0.49	117	21.2	<3	
10.0 "	318	61.7	<3	0.40	407	68.0	<3	0.37	264	45.9	<3	0.59	424	77.0	<3	0.52
20.0 "	377	73.1	<3	0.42	525	87.5	<3	0.37	418	72.5	<3	0.59	475	86.1	5	0.52
30.0 "			5	0.48									479	87.0	11	0.64
40.0 "	389	75.5	15	0.37									487	88.6	18	0.58
50.0 "	369	71.6	26	0.40	521	87.0	35	0.29	465	80.6	35	0.70	479	87.0	34	0.58
100.0 "	377	73.1	≈75	0.44	506	84.3	>60	0.28	441	76.5	>60	0.70	444	80.5	≈75	0.58

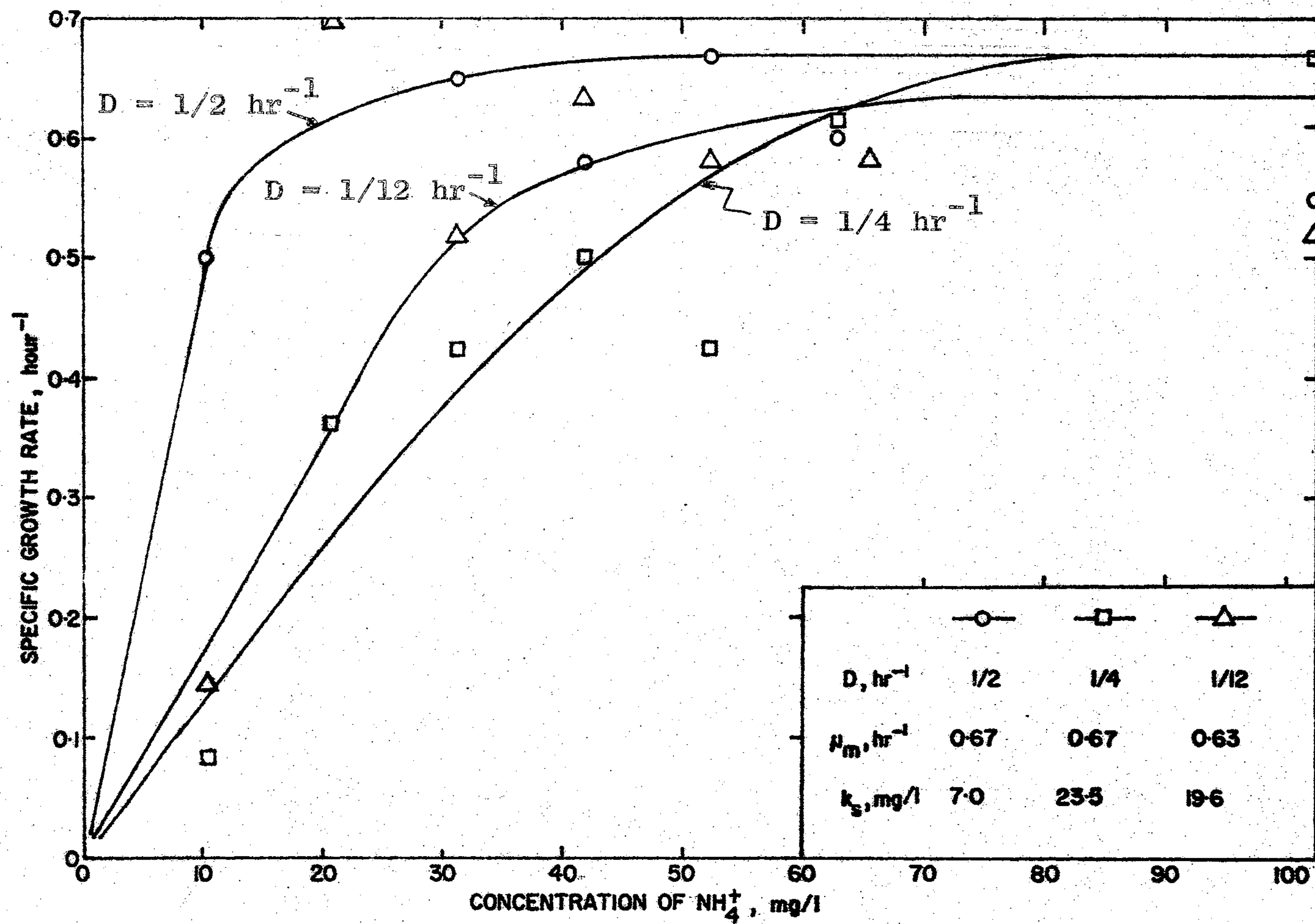


Figure 20. Relationship between specific growth rate and NH_4^+ concentrations. Seed taken from continuous flow units operated at COD:N = 70:1.

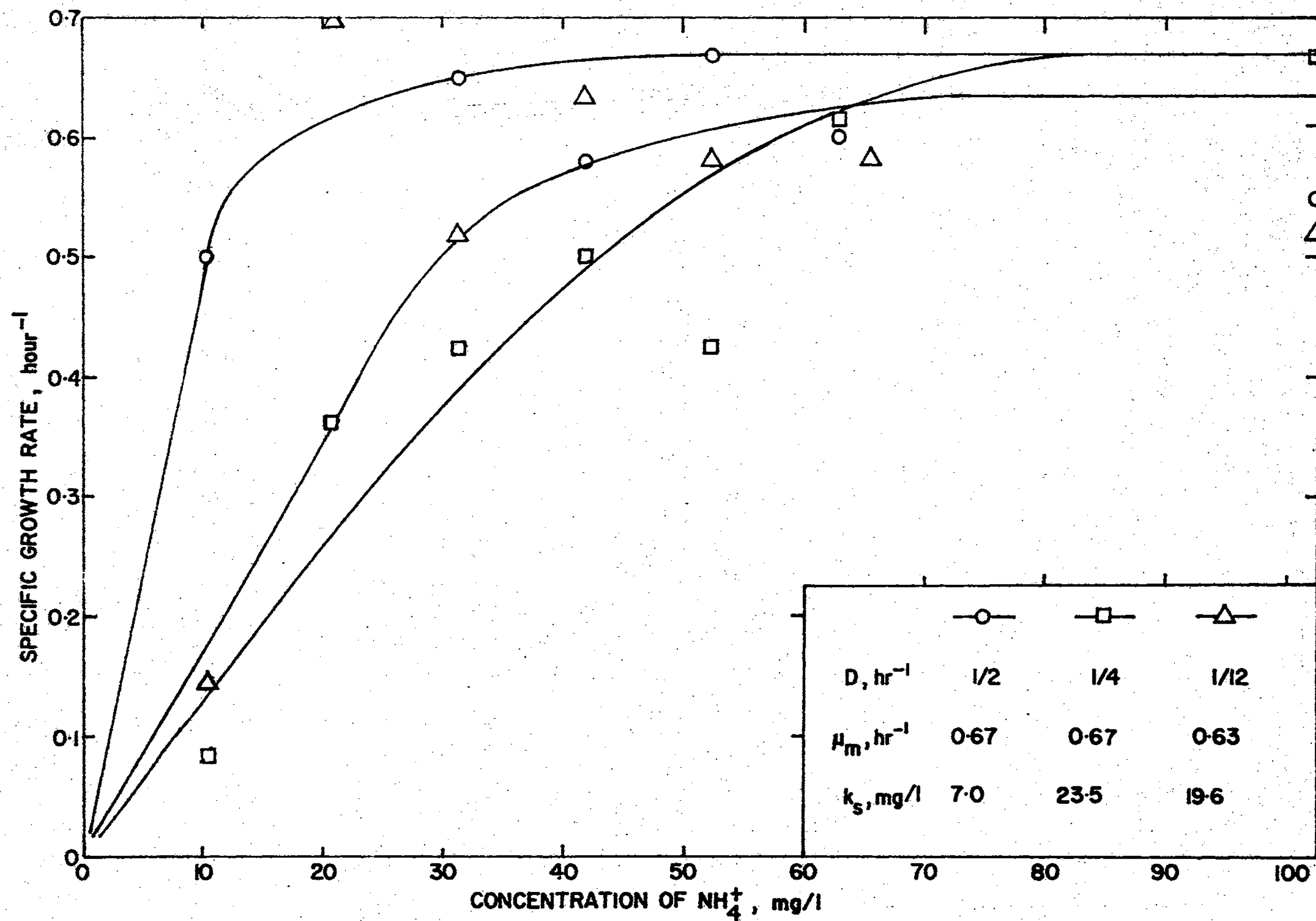


Figure 20. Relationship between specific growth rate and NH_4^+ concentrations. Seed taken from continuous flow units operated at COD:N = 70:1.

considerable scattering of data was observed. Therefore this experiment was not considered in deriving conclusions. Similar plots for the cells from systems operated with a COD:N ratio of 40:1 are shown in Figure 21. It is seen that for cells taken from the continuous flow unit operated at a 2-hour detention time (i.e., a dilution rate of 0.50) the maximum growth rate was 0.45 hr^{-1}, and that it occurred at ammonia-nitrogen concentrations of 10 mg/l or more, whereas at lower concentrations the growth rate decreased with decreasing concentrations of NH_4^+. Theoretically, the growth rate cannot be less than the dilution rate; however, the maximum growth rate obtained by the batch experiment was found to be 0.45. Although the observed value of μ_{max} was slightly lower than D, the dilution rate, the difference in their magnitudes was too small to be of concern. The value of K_s was found to be 1.75 mg/l NH_4^+ in this case. The presence of 10 mg/l NH_4^+ ensured unrestricted growth rate in this system which contained 500 mg/l glucose. Similar results were obtained when the seed was taken from continuous flow units operated at the same COD:N ratio (40:1) but at detention times of 4, 8, and 12 hours (Figure 21). Values of μ_{max} varied between 0.41 and 0.67, while K_s fluctuated between 1.3 and 5.1 mg/l NH_4^+.

The results of growth rate studies of cells harvested from continuous flow units operated at a COD:N ratio of 25:1 are shown in Figure 22. The growth rates of cells taken from the unit operated at a 4-hour detention time

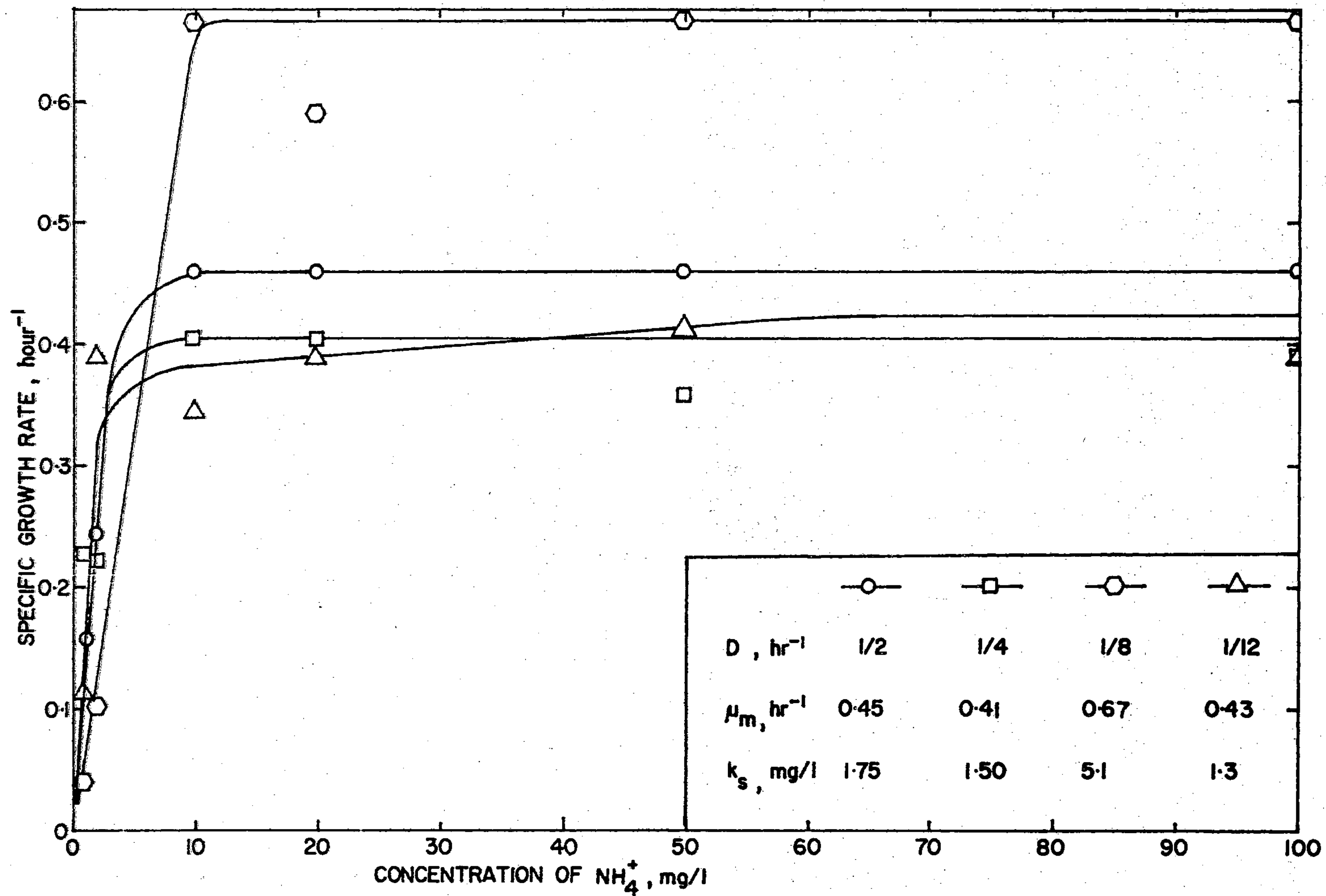


Figure 21. Relationship between specific growth rate and NH_4^+ concentrations. Seed taken from continuous flow units operated at COD:N = 40:1.

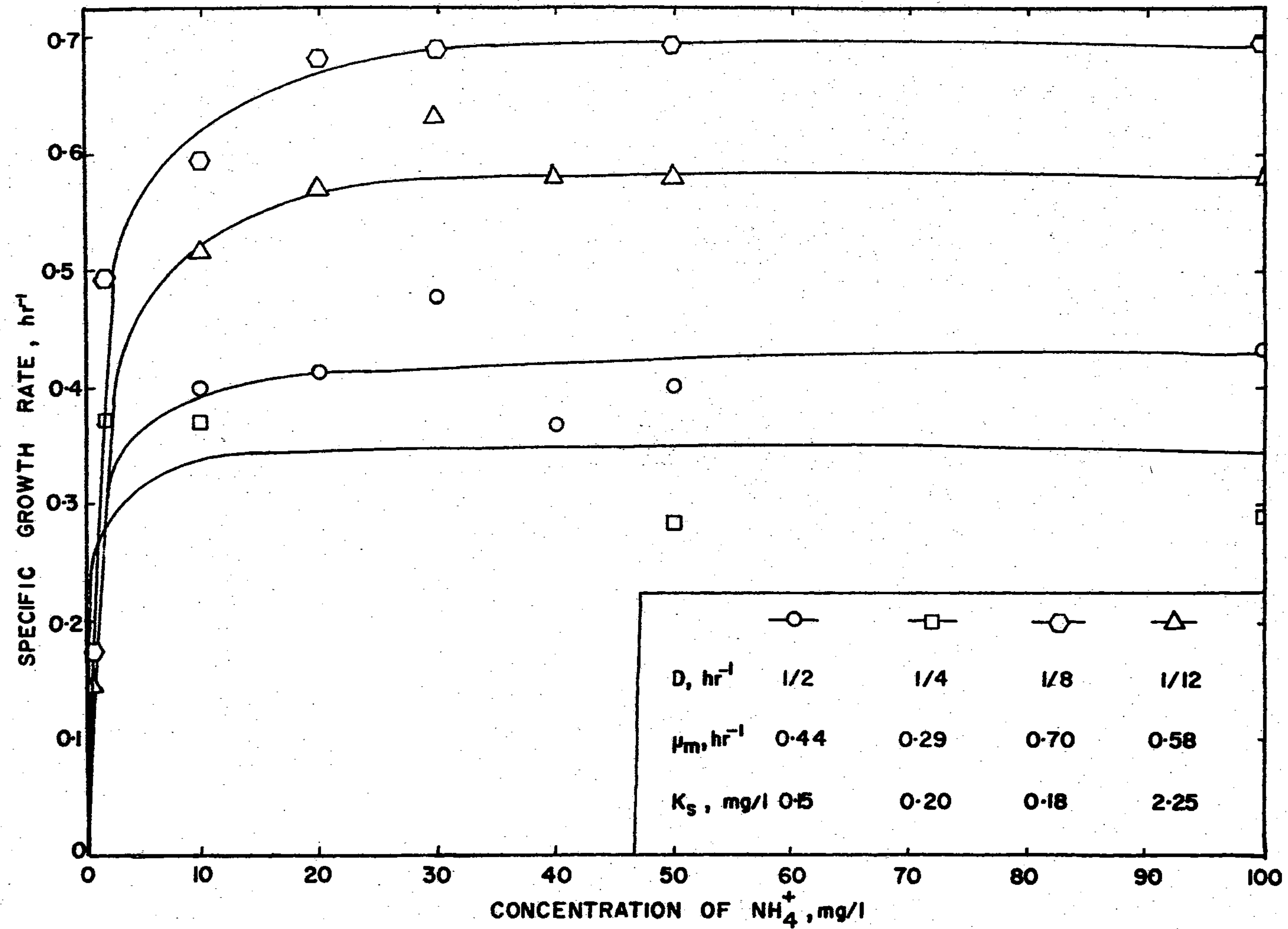


Figure 22. Relationship between specific growth rate and NH_4^+ concentrations. Seed taken from continuous flow units operated at COD:N = 25:1.

showed considerable fluctuation and, therefore, have not been considered in deriving conclusions. The μ_{max} varied between 0.29 and 0.70, while K_s fluctuated between 0.15 and 2.25 mg/l NH_4^+. Provision of 20 mg/l NH_4^+ seemed to ensure unhindered growth rate in these cells.

The μ_{max} seems to be independent of the source of the seed, i.e., it did not matter whether the seed was taken from a continuous flow unit with low or high nitrogen concentration or from a unit with high or low detention time.

B. Effect of Different Nitrogen Levels in Batch-Operated Activated Sludge Systems

The results of the batch studies using nitrogen levels corresponding to COD:N ratios of 10:1, 25:1, 40:1, 55:1, and 70:1 are shown in Figure 23. These systems were operated at the corresponding nitrogen level for a period of fifteen days prior to this experiment in order to assure acclimation. Figure 23 shows the patterns of COD removal in these five systems. All five systems were able to purify the waste; however, the time taken by the different systems for COD removal was significantly different. The 10:1 and 25:1 systems permitted a faster purification rate than did the other systems. The times taken by the 10:1, 25:1, 40:1, 55:1, and 70:1 systems to bring the COD level to 150 mg/l was found to be 1.7, 1.7, 7.0, 13.2, and 15.0 hours, respectively. It is thus seen that 10:1 and 25:1 systems behaved essentially in the same manner, whereas 55:1 and 70:1 systems required quite long detention periods.

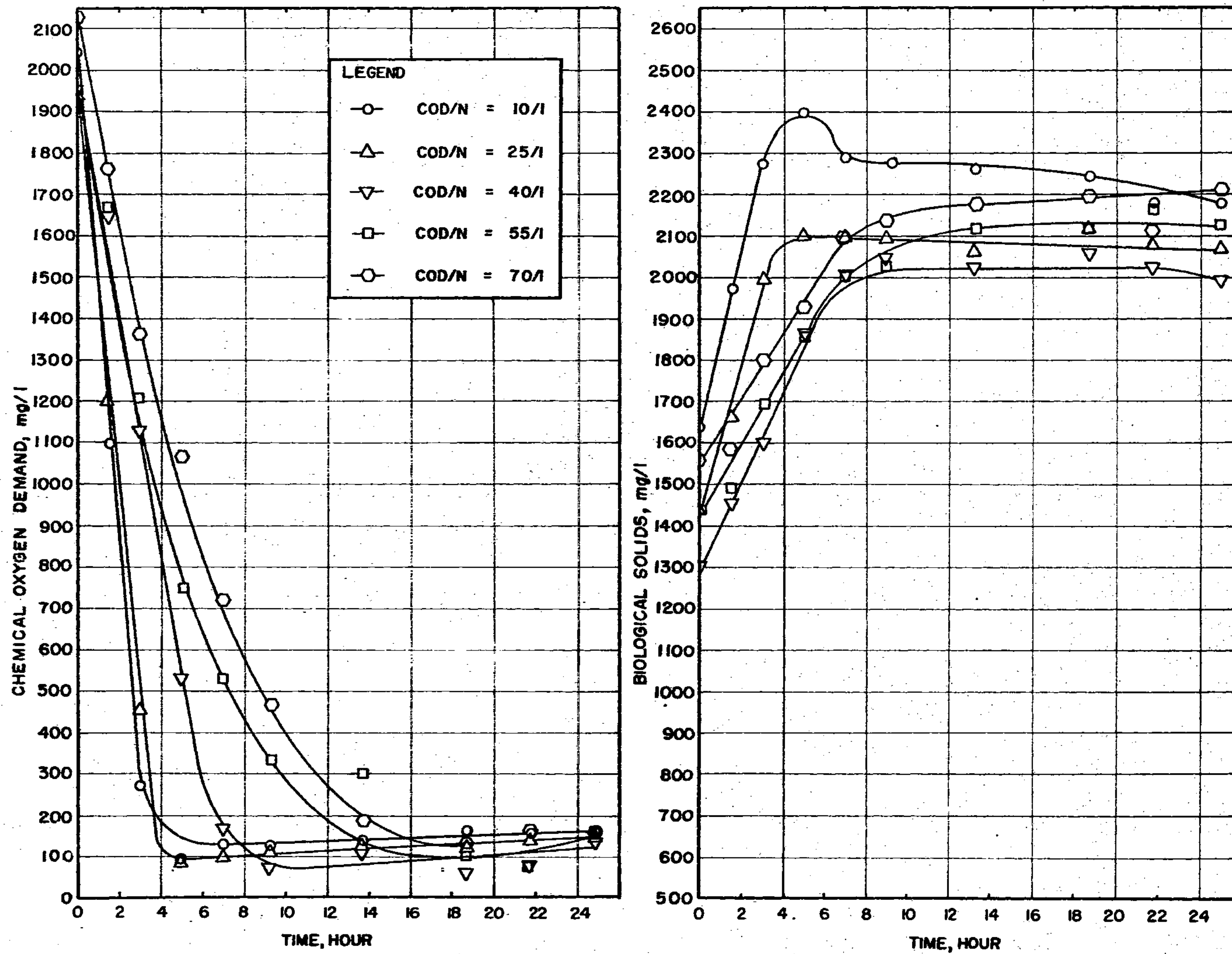


Figure 23. System performance of "old cells" acclimated to various nitrogen levels.

Figure 23 also shows the patterns of biological growth in the five batch units described above. Linear biological growth was observed in all five systems. The systems produced 760, 670, 900, 690, and 660 mg/l biological solids (arranged in order of decreasing nitrogen levels). Biological sludge production accounts for 39.0, 36.1, 48.0, 37.4, and 33.6 per cent of the COD removed. This range of yield values is somewhat low but is not uncommon in the waste water purification field.

Phase II

A Modification of the Activated Sludge Process for Nitrogen-Deficient Wastes

A. Batch Experiment

Acetic Acid: The batch experiments pertaining to the proposed process modification consisted of an initial feeding phase and an endogenous phase, followed by a refeeding phase under nonproliferating conditions.

Feeding Phase: Figure 24 shows the performance of the systems under growth and under nonproliferating conditions; the rates of COD removal were approximately the same. In approximately four hours, 90 per cent of the COD was removed in both cases. The biological solids production followed a linear path and approximately 48 per cent of the substrate was channelled to solids production in both cases. The cell yield on acetate was somewhat lower than for a comparable experiment using glucose (16). The biological solids

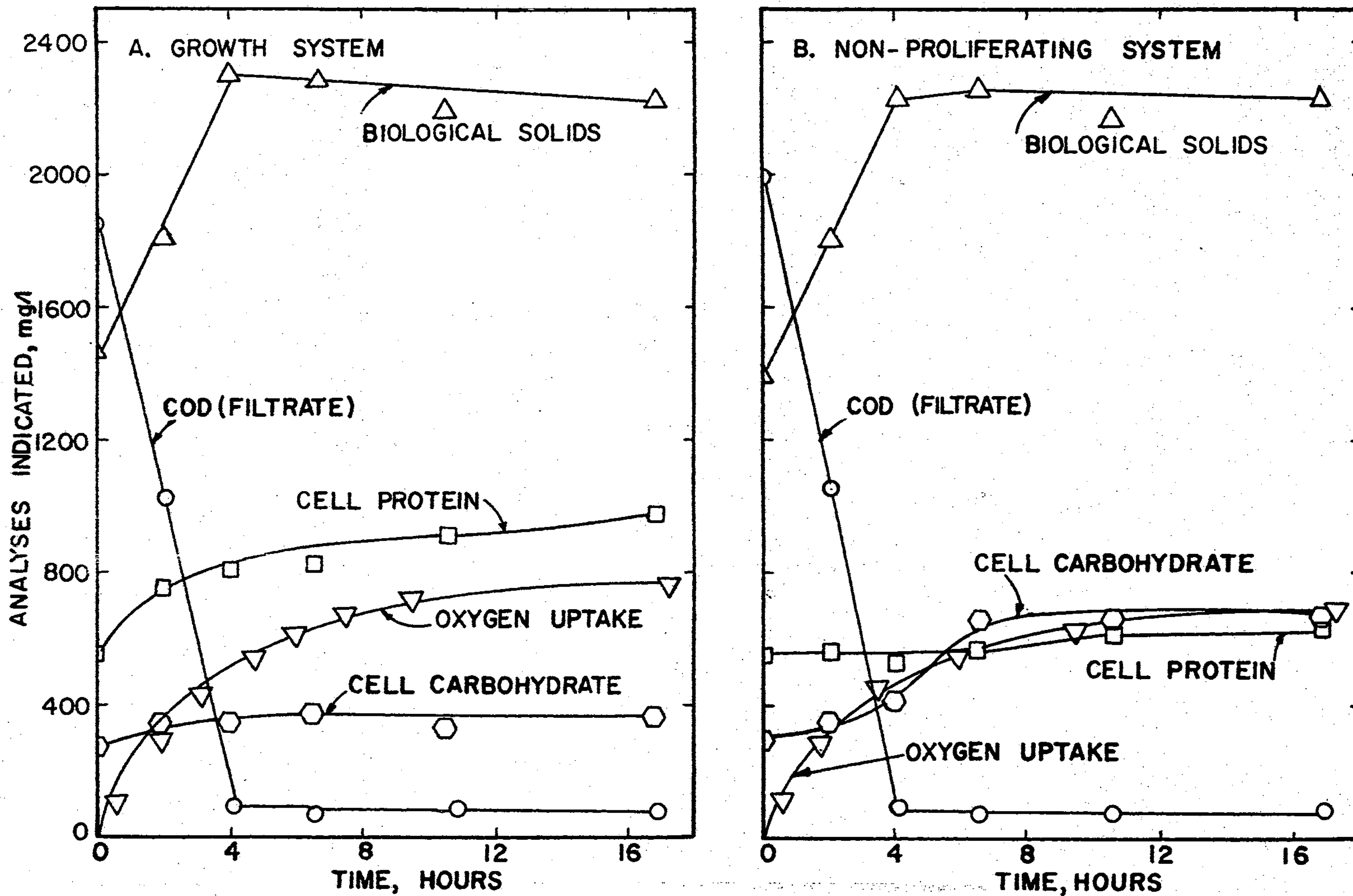


Figure 24. Comparison of system characteristics in the feeding phase.

production reached a maximum at the point of exhaustion of substrate. The oxygen uptakes for both system were also comparable.

The cell composition analyses show that the carbohydrate content of the cells under growth conditions was initially 18.5 per cent of the total dry weight, decreased to 15 per cent at the point of substrate removal and was 16.5 per cent at the end of the feeding phase. Under nonproliferating conditions, carbohydrate content increased from 20 per cent to 29 per cent of the total dry weight of solids. In general, the carbohydrate content of cells grown on acetic acid was lower than those grown on glucose (16). It will also be seen that the total carbohydrate content increased from 290 mg/l to 690 mg/l. The protein content increased from 38 per cent to 46 per cent in seventeen hours under growth conditions while it decreased from 40 per cent to 30 per cent in the nonproliferating system. It is noted that the protein content of these cells was considerably higher than those grown on glucose (16).

Endogenous Phase: The endogenous phase for all cells really began after four hours from the beginning of the feeding phase, because almost all of the substrate had been exhausted at that time. During the period from four to seventeen hours (Figure 24) the system undergoing endogenous metabolism in the presence of nitrogen showed an increase in protein content from 840 mg/l to 980 mg/l, while carbohydrate content remained essentially unchanged.

From Figure 25 it is seen that three of the four systems did not show any change during the endogenous phase. However, the system which received nitrogen but had undergone nonproliferating conditions in the feeding phase showed a small increase in protein content. Even so, the carbohydrate content remained essentially unchanged. It would appear that the expected increase in protein did not occur. The biological solids concentration remained essentially unchanged.

Refeeding Phase: The results of refeeding phase experiments under nonproliferating conditions are shown in Figure 26. It is seen that three of the four systems exhibited excellent capability for removing organic matter. However, the system which did not receive any nitrogen in the initial feeding phase or in the endogenous phase was not very efficient in removing COD.

B. Continuous Flow Experiments

1. Glucose

The results of previous batch studies (16) had proved the mechanistic feasibility of the mode of operation; however, there was no experimental evidence that this process would work on a continuous flow basis, and it was necessary to undertake experiments under this condition. The results of the continuous flow experiments using glucose as substrate are shown in Figures 27 through 40, and summarized in Table V for the various COD:N ratios employed. The COD:N ratio refers to the ratio of the total COD of the

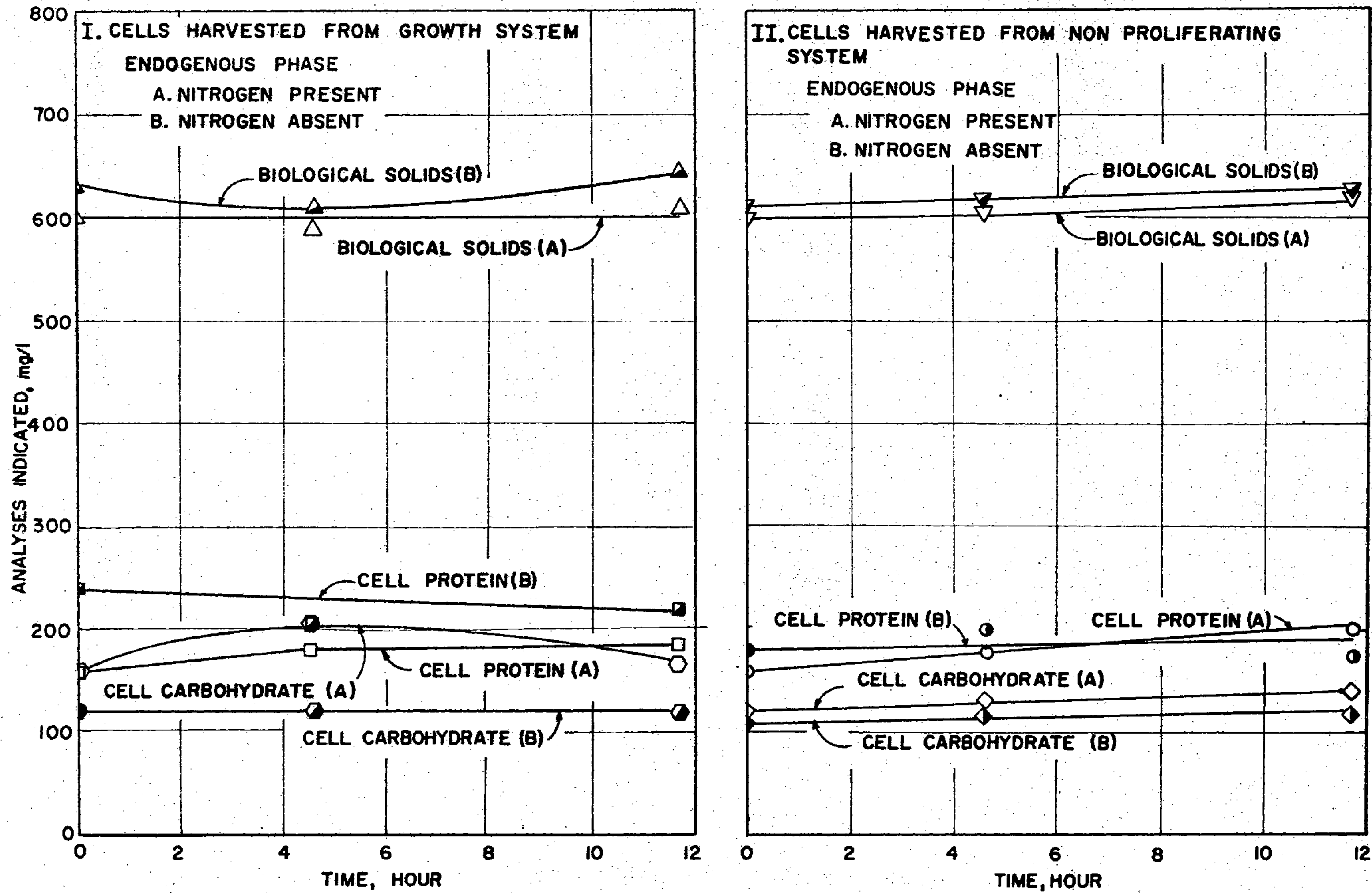


Figure 25. Comparison of system characteristics in the endogenous phase.

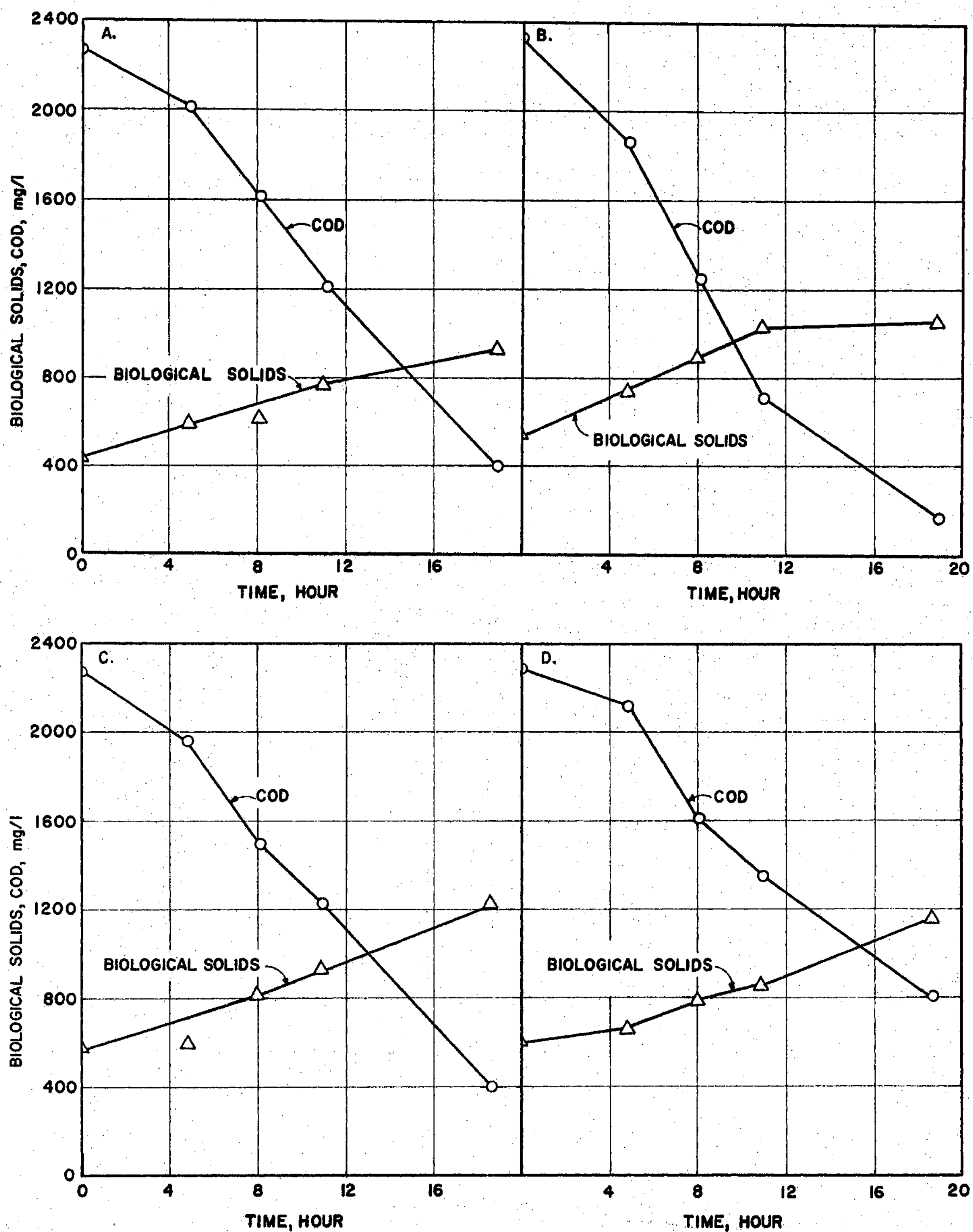


Figure 26. Comparison of substrate (acetic acid) removal under nonproliferating conditions in the refeeding phase.

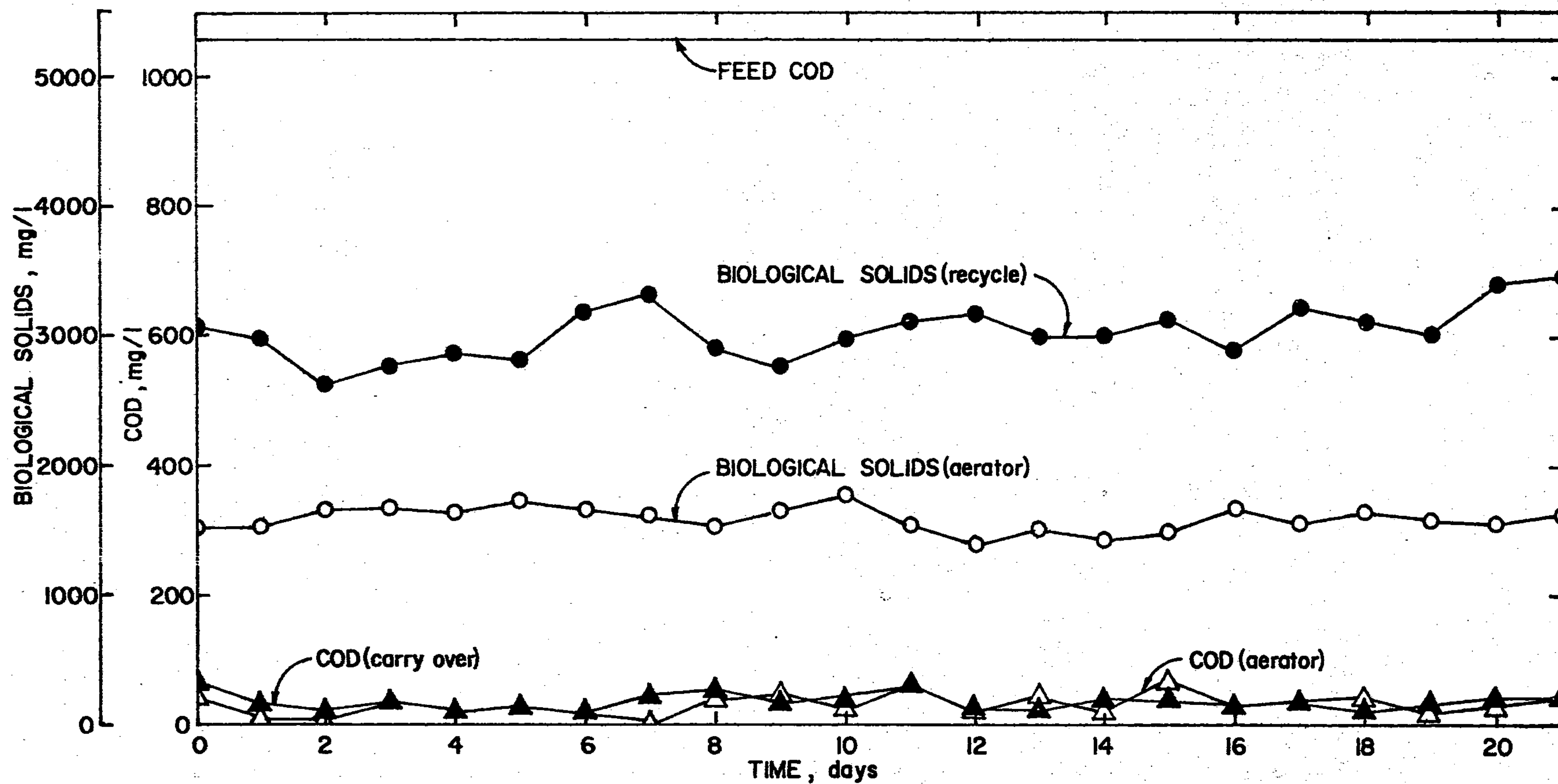


Figure 27. System characteristics at COD:N = 10:1, and D = 1/4 $hour^{-1}$ (0 to 21 days).

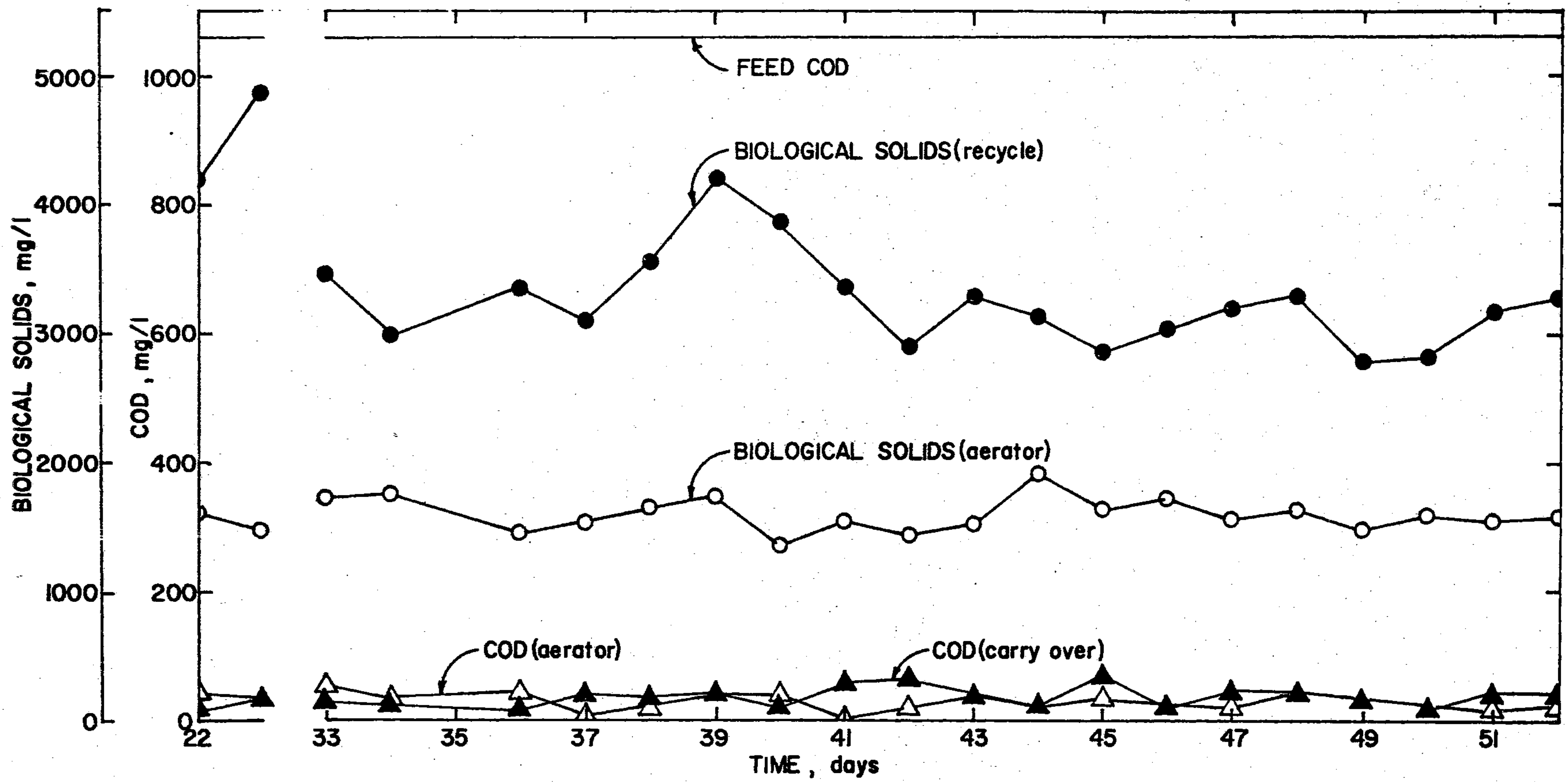


Figure 28. System characteristics at COD:N = 10:1, and D = 1/4 $hour^{-1}$ (22 to 52 days).

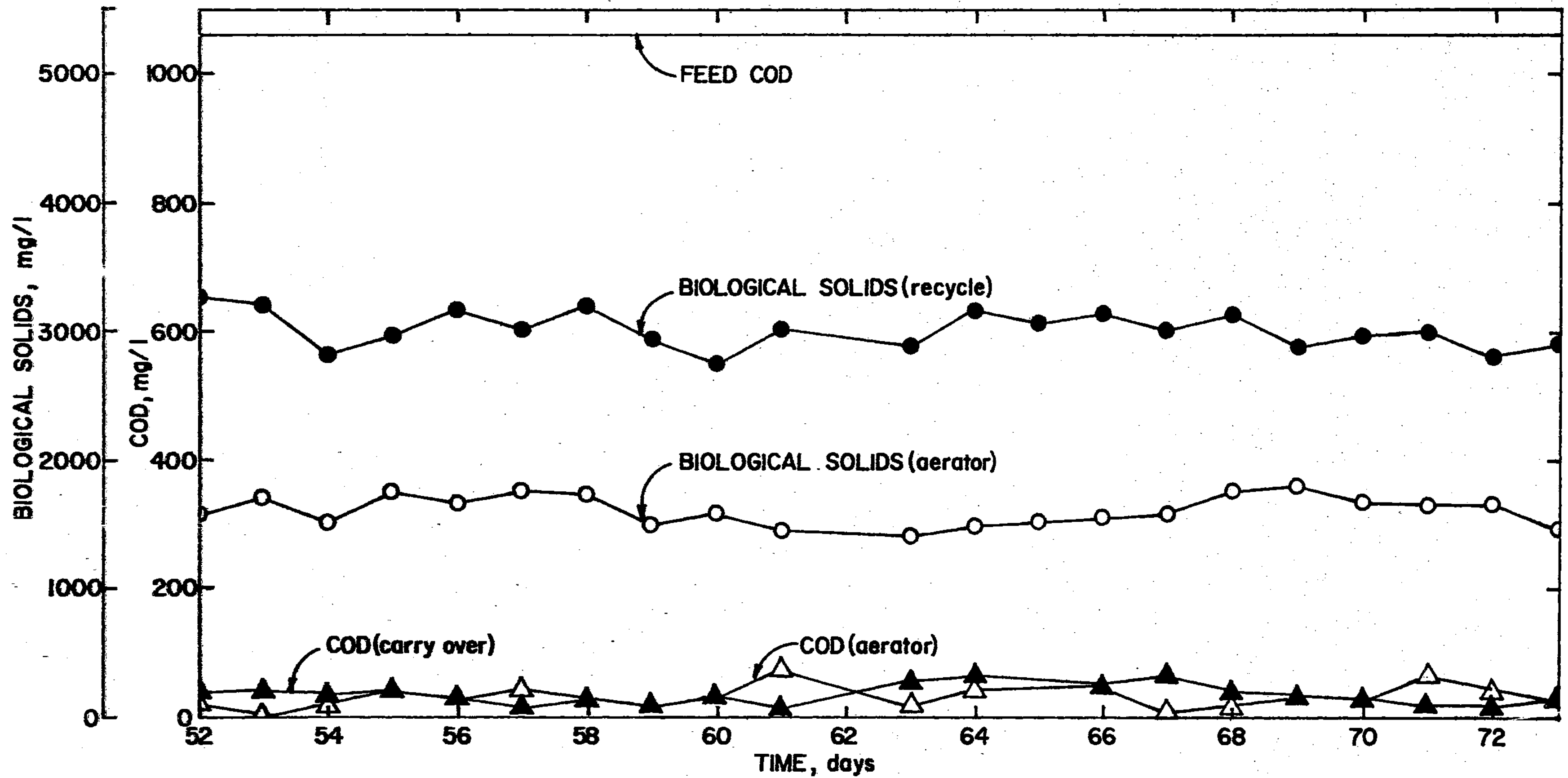


Figure 29. System characteristics at COD:N = 10:1, and D = 1/4 hour^{-1} (52 to 73 days)

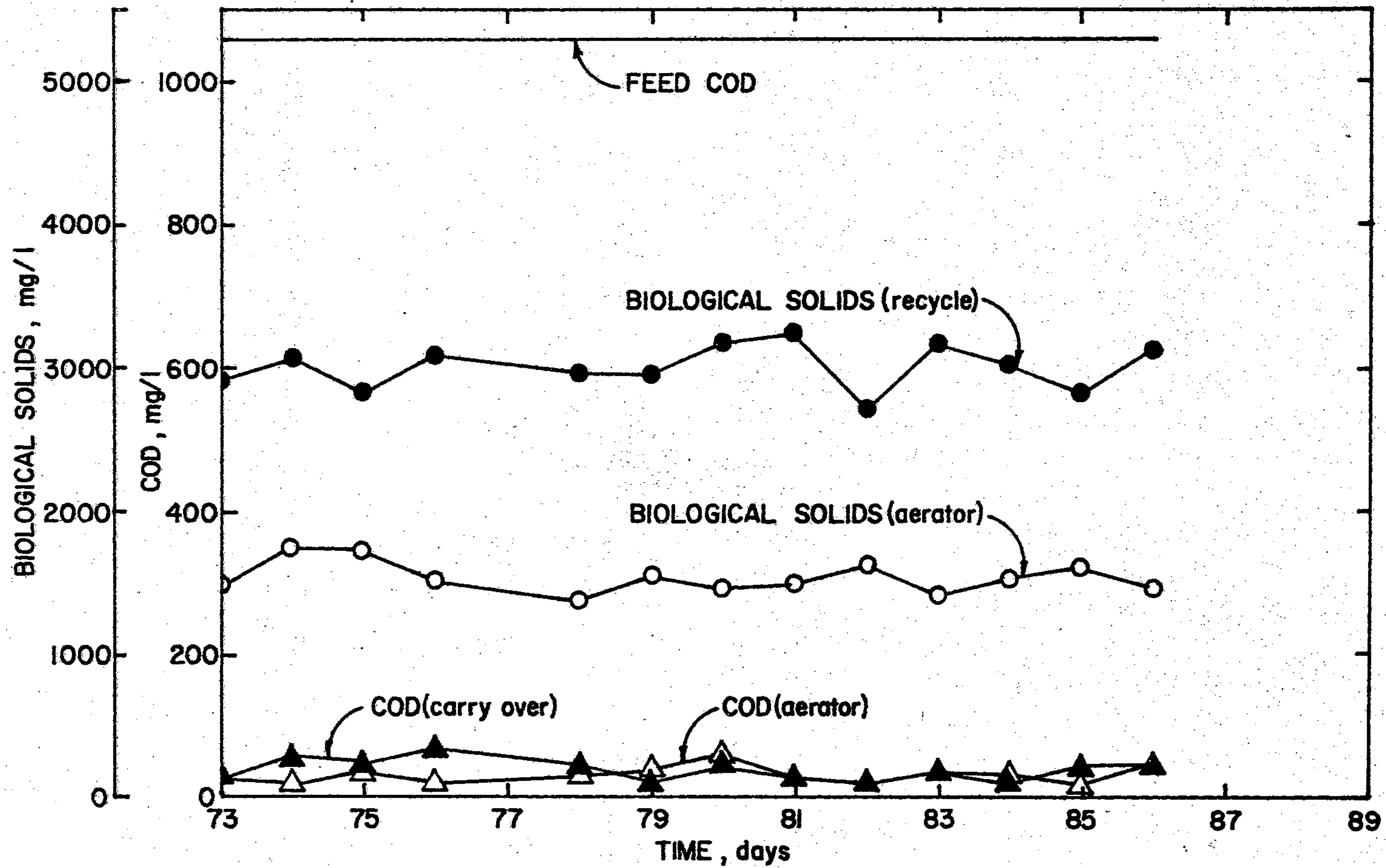


Figure 30. System characteristics at COD:N = 10:1, and D = 1/4 $hour^{-1}$ (73 to 86 days).

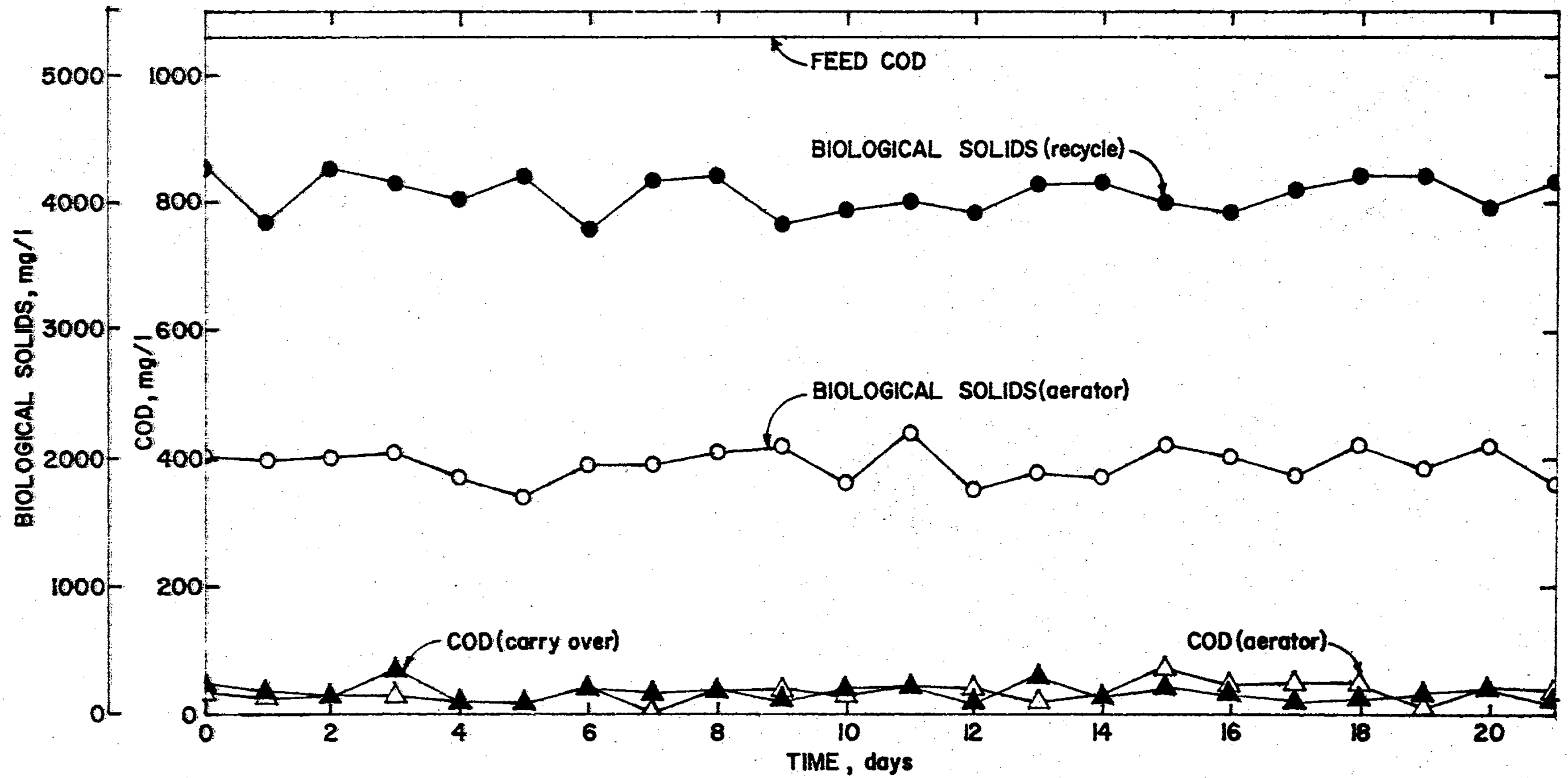


Figure 31. System characteristics at COD:N = 30:1, and D = 1/4 $hour^{-1}$ (0 to 21 days).

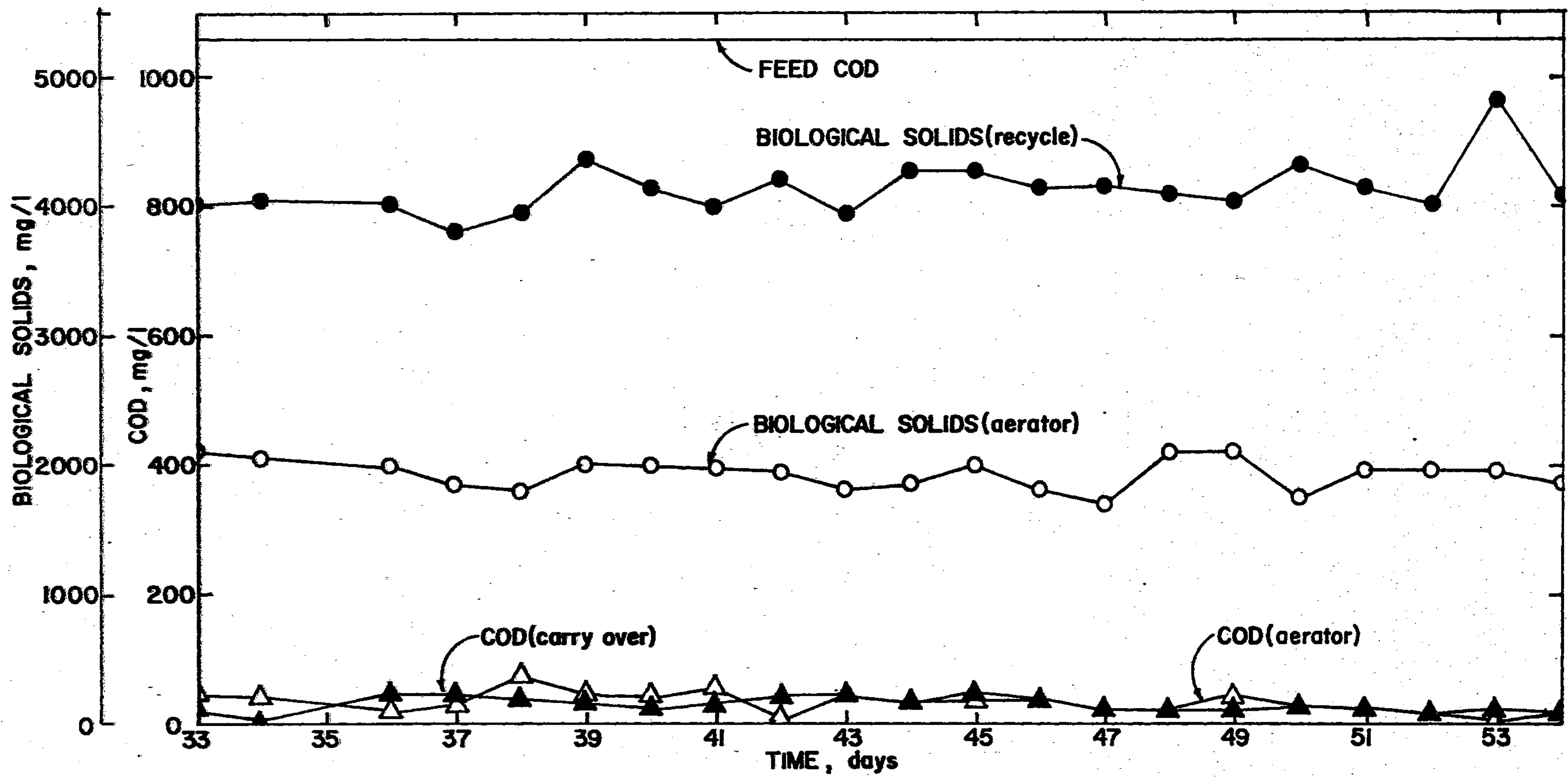


Figure 32. System characteristics at COD:N = 30:1, and D = 1/4 $hour^{-1}$ (33 to 54 days).

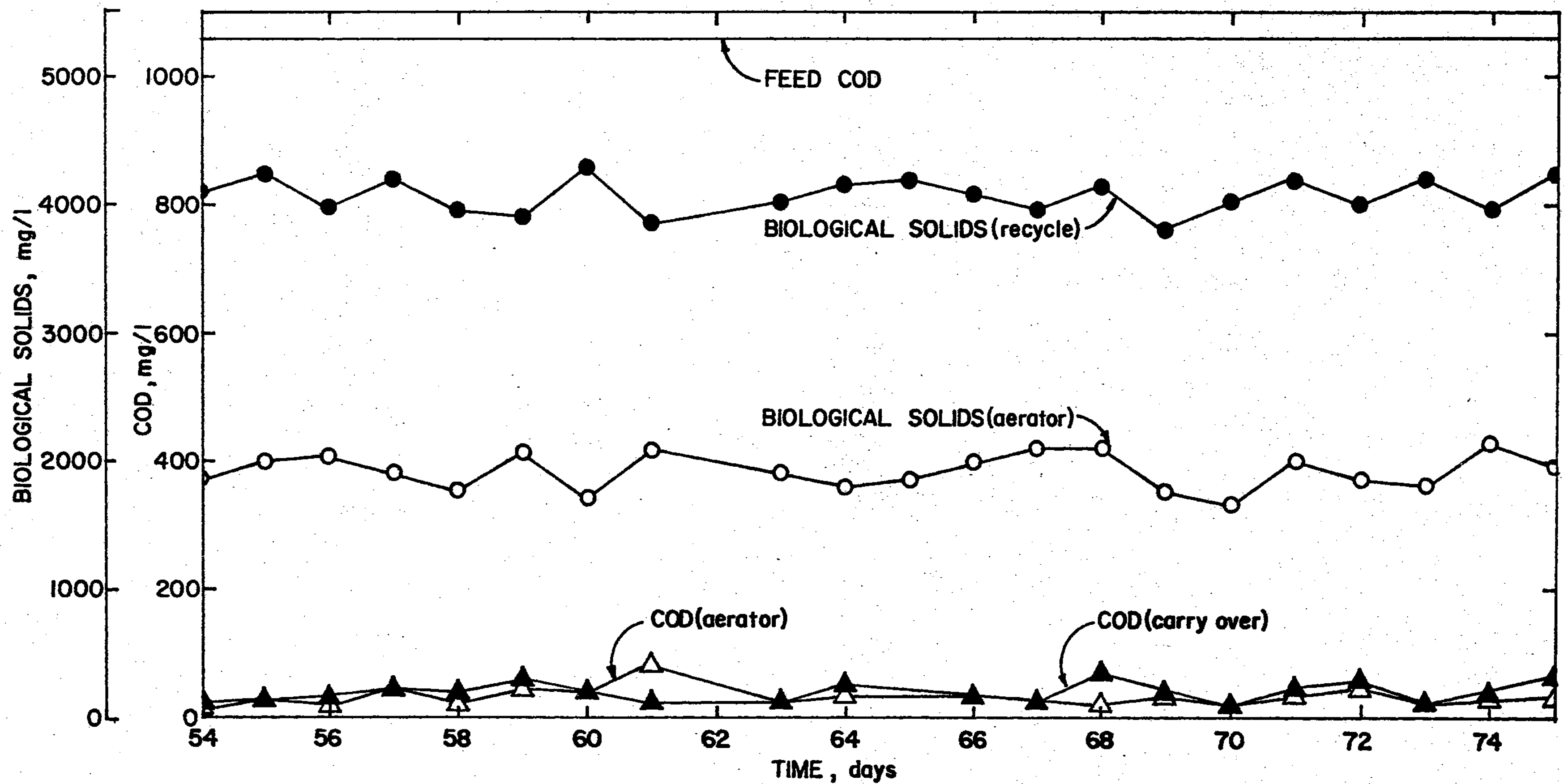


Figure 33. System characteristics at COD:N = 30:1, and D = 1/4 $hour^{-1}$ (54 to 75 days).

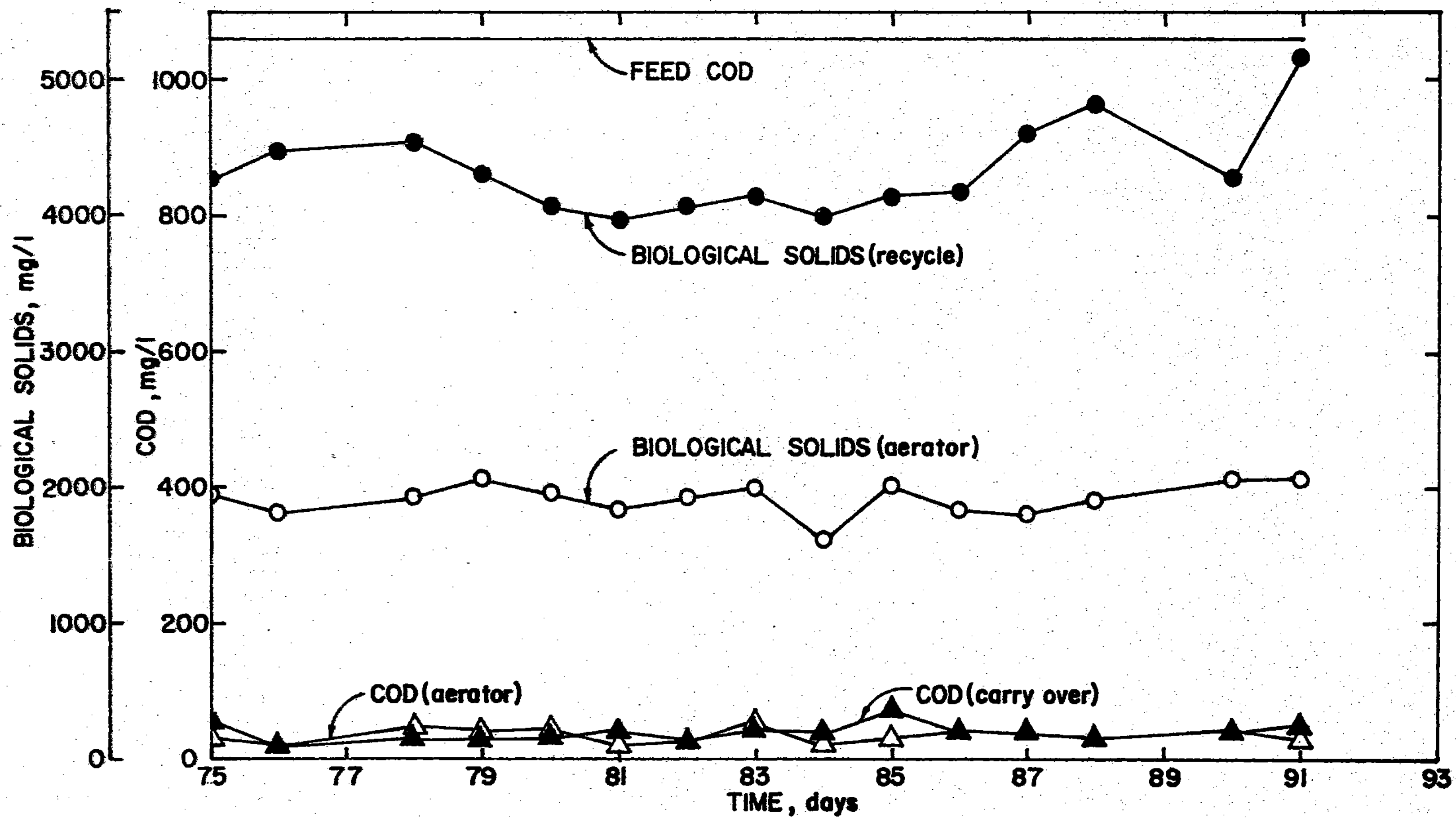


Figure 34. System characteristics at COD:N = 30:1, and D = 1/4 $hour^{-1}$ (75 to 91 days).

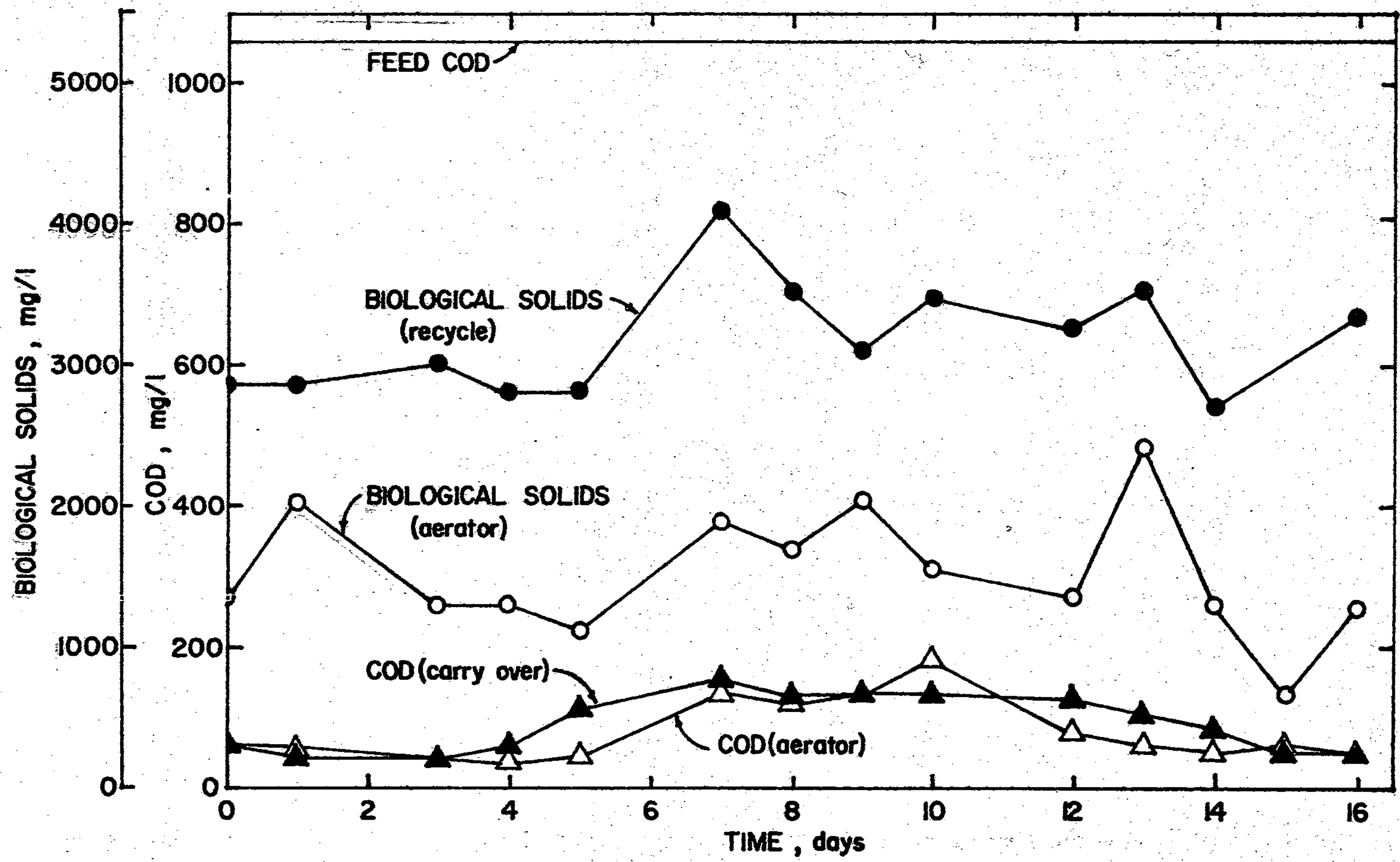


Figure 35. System characteristics at COD:N = 40:1, and D = 1/4 $hour^{-1}$.

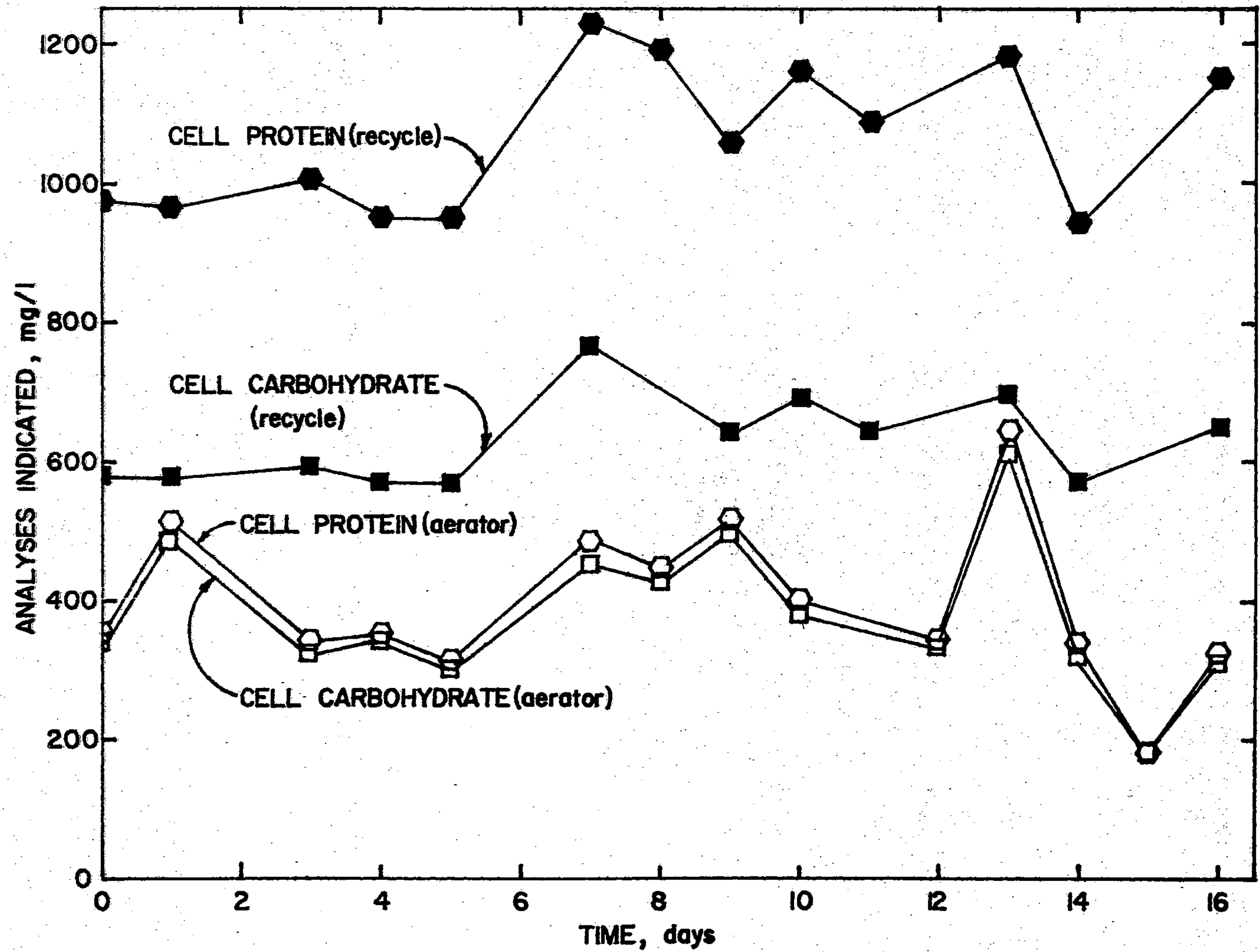


Figure 36. Biological solids composition at COD:N = 40:1, and $D = 1/4$ $hour^{-1}$.

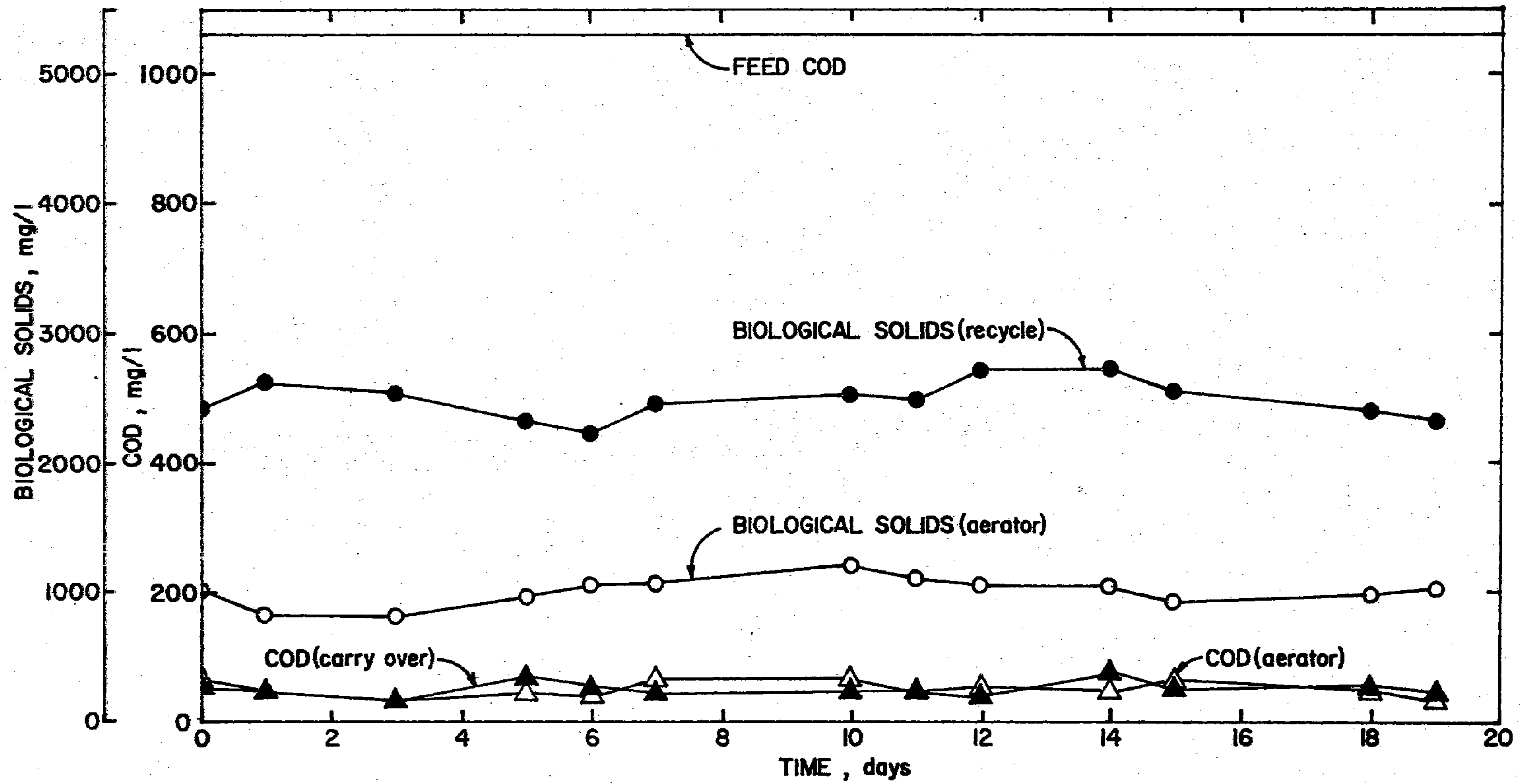


Figure 37. System characteristics at COD:N = 50:1, and D = 1/4 $hour^{-1}$.

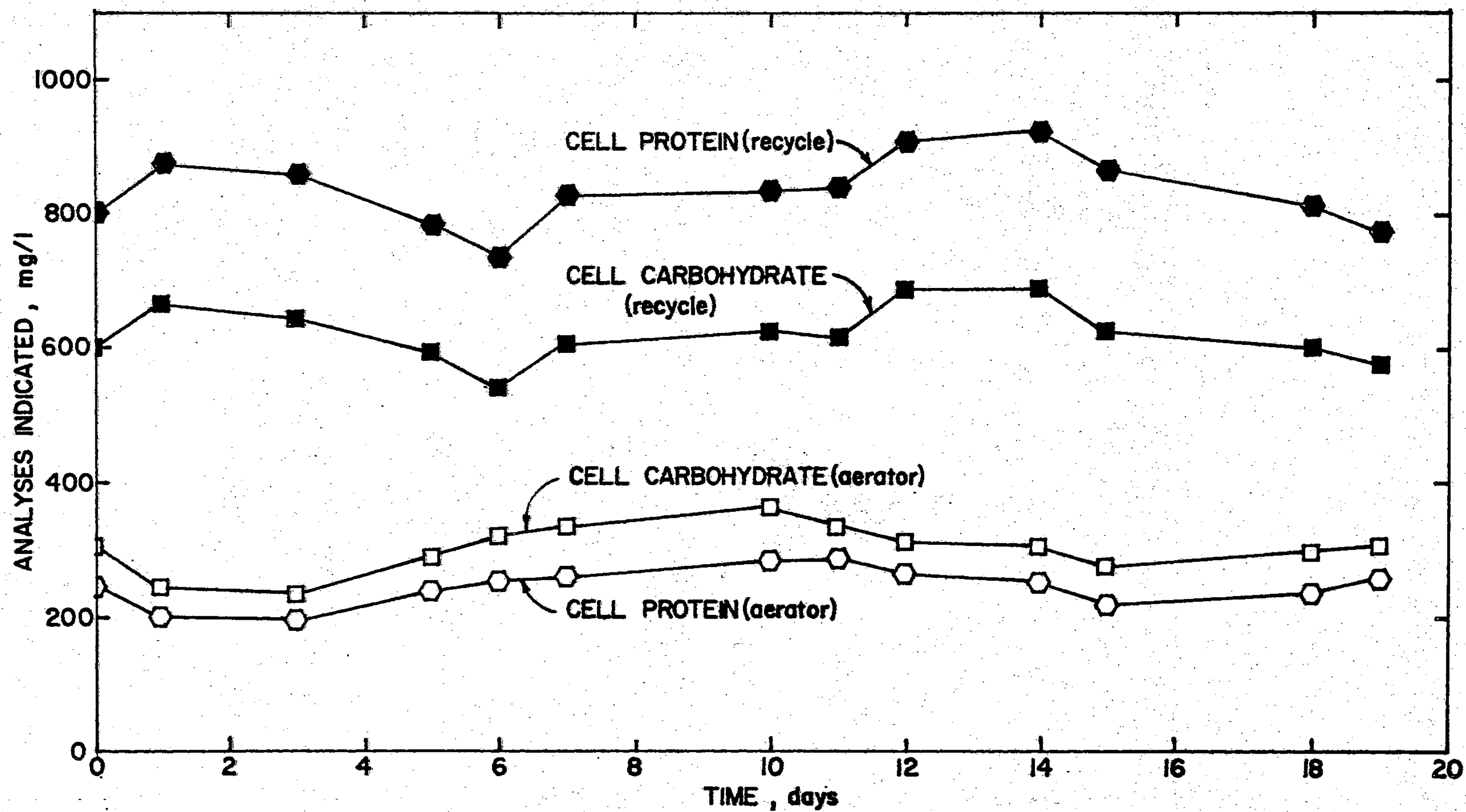


Figure 38. Biological solids composition at COD:N = 50:1, and D = 1/4 $hour^{-1}$.

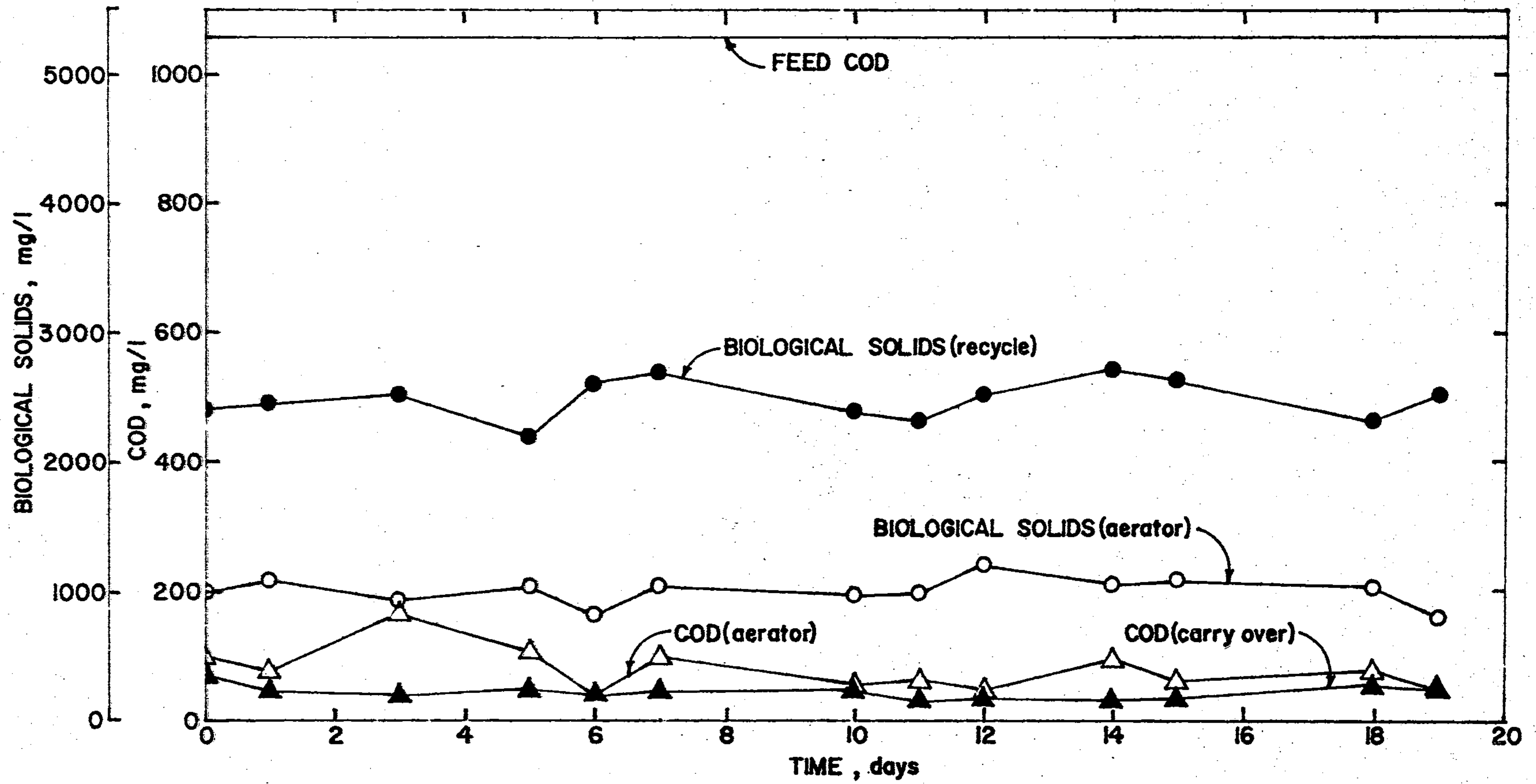


Figure 39. System characteristics at COD:N = 70:1, and D = 1/4 $hour^{-1}$.

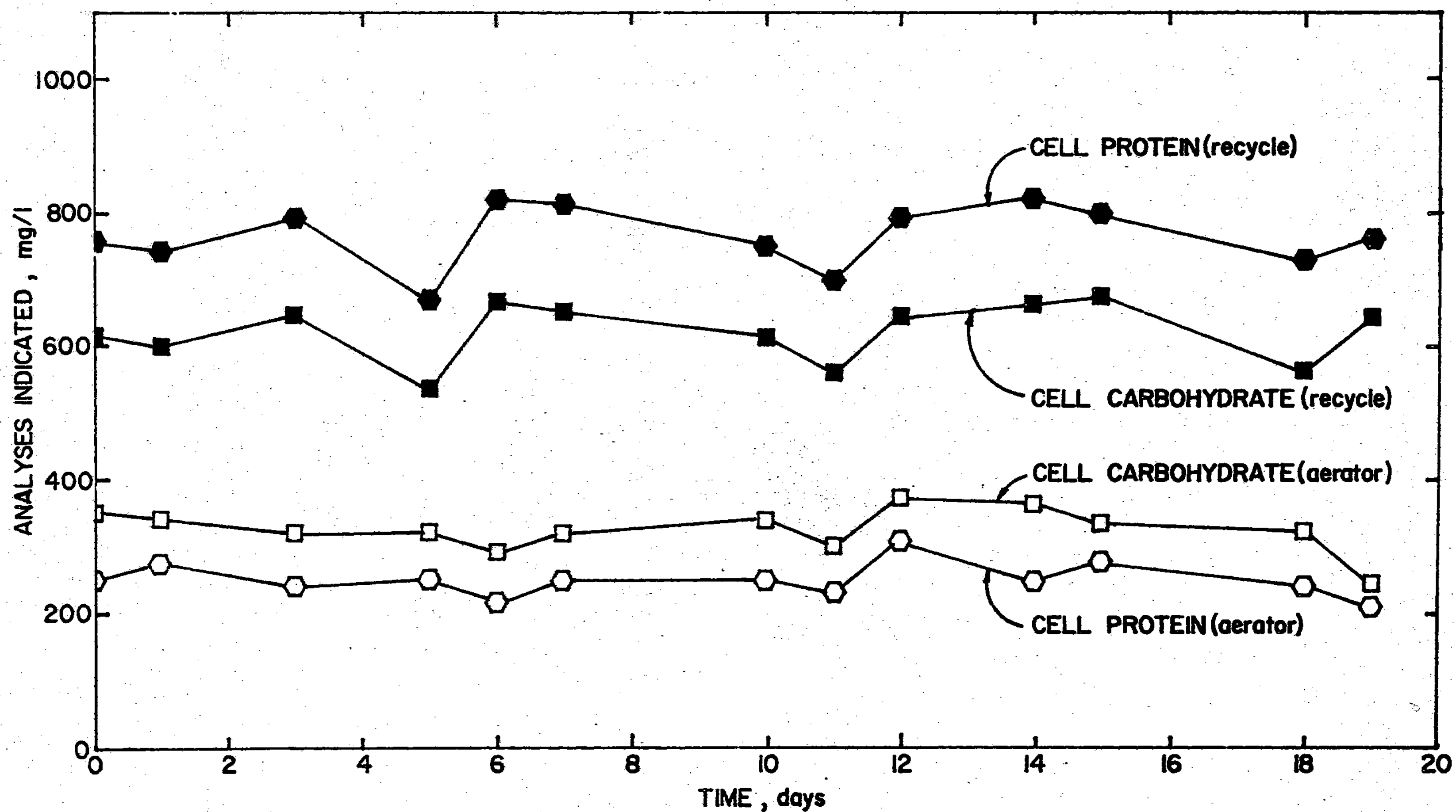


Figure 40. Biological solids composition at COD:N = 70:1, and D = 1/4 $hour^{-1}$.

TABLE V

AVERAGE VALUES FOR ANALYSES OF EFFLUENT FROM THE FEEDING AND ENDOGENOUS AERATORS

DURING CONTINUOUS OPERATION AT VARIOUS COD:N RATIOS

($D = 0.25\ hr^{-1}$; Carbon Source = 1000 mg/l Glucose = 1060 mg/l COD in the Feed)

	Effluent from Feeding Aerator											Effluent from Endogenous Aerator				
COD:N	Filtrate COD		Supernatant COD (Unfiltered)		NH_3-N in Final Effluent	Sludge	Sludge Yield	Sludge Protein	Sludge Carbo-hydrate	Sludge Wasted	Carry-over COD	NH_3-N In Carry-Over	Sludge	Sludge Protein	Sludge Carbo-hydrate
	mg/l	% Removal	mg/l	% Removal	mg/l	mg/l	%	%	%	%	mg/l	mg/l	mg/l	%	%
10:1	32	97	60	94	25	1600	80	28	22	34	43	160	3160	31	20
30:1	34	97	62	94	1	1940	82	28	24	29	43	45	4130	31	20
40:1	78	93	105	90	0	1520	70	26	25	30	86	34	3190	29	20
50:1	50	95	102	90	0	1046	32	24	30	21	66	5	2480	32	25
70:1	50	95	105	90	0	1007	26	24	30	17	60	0	2490	31	25

waste pumped into the aerator during a 12-hour period to the amount of nitrogen contained in ammonium sulfate solution added to the endogenous aerator at each 12-hour interval. The continuous flow experiment at a COD:N ratio of 10:1 was carried out for 86 days. There was a period of 10 days during which it was not possible to take samples. However, arrangement for feeding the unit was made during this time, and operation was not interrupted. Although no regular sampling of the feed was carried out, several spot checks were made. The results of these checks showed that the feed COD was very close to the theoretical value of 1060 mg/l.

It may be seen that considerable variation in sludge concentration occurred in both the feeding and endogenous aerators. However, the COD of the effluent was fairly constant at all times. The average filtrate COD of the effluent was found to be 32 mg/l, whereas the unfiltered effluent had a COD of 60 mg/l. This shows a purification efficiency of 97 and 94 per cent, respectiverly. The difference in the two COD values is due to the biological solids in the supernatant and is a measure of effectiveness of the sludge settleability. In this case the difference amounted to 28 mg/l. Assuming the COD of bacterial cells to be 1.37 times the cell weight (147), 28 mg/l of COD is equivalent to 20 mg/l of biological solids. Thus, it may be concluded that the sludge exhibited excellent settling properties.

The average concentrations of biological solids in the feeding aerator and the endogenous aerator were found to be 1600 and 3160 mg/l, respectively. Since 200 ml/hr was the rate of recycle, and 400 ml/hr was the rate of pumping of fresh feed, the recycle sludge was diluted to one-third its concentration in the feeding aerator. Thus, on the average, 1053 mg/l biological solids were contributed by the recycle sludge to the feeding aerator. Therefore, the net synthesis of new cells amounted to 547 mg/l. Similarly, the feed was diluted to two-thirds in the feeding aerator.

The yield of biological solids was calculated by making a materials balance for the feeding aerator consideraing all inputs and outputs on a per-hour basis. The input of substrate is from two sources: fresh incoming feed, and COD in the recycle sludge. The rate of flow of incoming feed and recycle sludge was 400 ml/hr and 200 ml/hr, respectively. The COD of feed and recycle being 1060 mg/l and 42 mg/l, the total input of COD in one hour amounted to 1060 x 0.4 + 42 x 0.2 = 432 mg. The soluble COD of the effluent was 32 mg/l and the rate of discharge was 600 ml/hr. Thus, the effluent was 0.6 x 32 = 19 mg COD per hour. Thus, in one hour the COD utilized by the system amounted to 432 - 19 = 413 mg.

Similarly, the solids carried in the mixed liquor effluent from the aerator in one hour amounted to 0.6 x 1600 = 960 mg, while the input to the aerator was 0.2 x 3160 = 632 mg. Thus, in one hour 960 - 632 = 328 mg

biological solids were produced as a result of the utilization of 413 mg COD; therefore, the sludge yield was 328/413 = 0.795.

The average protein contents of the sludge in the feeding aerator and the endogenous aerators were 28 per cent and 31 per cent, respectively, which shows that the addition of nitrogen to the endogenous tank increased the protein content of the sludge by 3 per cent. However, the values for the protein content are, in general, lower than those observed in conventional systems. This may be due to the mode of operation of the system, which is basically different from the conventional methods of operation of the activated sludge process.

The carbohydrate contents of the sludge were found to be 22 and 20 per cent in the feeding aerator and the endogenous aerator, respectively. Thus, a decrease in the carbohydrate content of the sludge was obtained in the endogenous aerator. This decrease is apparently due to intracellular conversion to protein.

Ammonia analyses showed that the nitrogen source was present in the medium in excess concentration at the COD:N ratio of 10:1, since the final effluent contained about 25 mg/l ammonia nitrogen. However, a considerable amount of the supplemental nitrogen escaped to the feeding aerator along with the recycled sludge. Thus, while this experiment proved that the system was feasible on the continuous flow basis, it did not help to prove or disprove the

advantages of the system with respect to nitrogen leakage in the effluent.

The average sludge wasting was estimated to be approximately one-third of the sludge collected at the bottom of the settling tank; however, the amount of sludge wasted daily fluctuated considerably, depending on the sludge produced each day.

The results of the study conducted at a COD:N ratio of 30:1 were shown in Figures 31 to 34. As observed for the previous system (COD:N = 10:1), the concentration of biological solids fluctuated from time to time, although the effluent COD was fairly constant. The fluctuation in solid concentration was found to affect the carbohydrate and protein concentrations in the reactor, so that the carbohydrate and protein fractions of the sludge dry weight remained fairly constant.

The average filtrate COD of the effluent was found to be 34 mg/l while it was 62 mg/l for the unfiltered effluent. These correspond to 97 and 94 per cent, biochemical and overall purification efficiency, respectively. The average biological sludge concentrations in the feeding and endogenous aerators were 1940 and 4130 mg/l. It is thus seen that the concentration of biological solids obtained in the 30:1 system was higher than at 10:1, whereas the purification efficiencies were found to be the same. The sludge yield was 0.82. This is rather high. Ramanathan (79) had obtained a maximum sludge yield of 0.785.

The average protein content of the sludge rose from 28 per cent in the feeding aerator to 31 per cent in the endogenous aerator after twelve hours of aeration. The carbohydrate content of the cells decreased from 24 per cent in the feeding aerator to 20 per cent in the recycle sludge. It will be seen that the recycled sludge carried approximately 45 mg/l ammonia nitrogen, whereas the average ammonia concentration in the final effluent was only 1 mg/l. The average amount of sludge wasted was estimated to be approximately 29 per cent.

The results of studies conducted at COD:N ratios of 40:1, 50:1 and 70:1 were shown in Figures 35 to 40. Results very similar to those observed at 10:1 and 30:1 were obtained. The average values of the various operational parameters are given in Table V. It is seen that even in the system operated at a COD:N ratio as low as 70:1, biochemical and overall purification efficiencies of 95 per cent and 90 per cent were obtained. These results clearly show that this type of system offers a method of treating waste with a considerable saving in the cost of supplemental nitrogen. The concentration of biological solids in the two aerators had to be maintained at lower levels at lower nitrogen levels, due to reduction in sludge yield. It is seen that the sludge yield dropped from 0.82 at the COD:N ratio of 30:1 to 0.26 at the COD:N ratio of 70:1. The low yield reduced the amount of sludge wasting to approximately 17 per cent in the 70:1 system.

The protein content in the system operated at a COD:N ratio of 70:1 was found to increase from 24.2 per cent to 33.5 per cent during the 12-hour aeration in contact with nitrogen, whereas carbohydrate content decreased from 30.2 per cent to 24.9 per cent. These findings indicate that the increase in protein content in the endogenous tank was due not only to conversion of stored carbohydrate to protein, but also to conversion of some other cellular components to protein. All of the added nitrogen was taken up by the sludge in the endogenous aerator, and neither the recycled sludge nor the final effluent contained any ammonia nitrogen.

The effect of nitrogen levels on COD removal, sludge yield and sludge composition are shown graphically in Figure 41.

The results of the "turnover" experiment are shown in Figure 42. It is seen that the biological solids concentration decreased slightly during the 12-hour period. The protein content increased from 700 mg/l to 840 mg/l, whereas the carbohydrate content decreased from 810 mg/l to approximately 655 mg/l. It would appear that a detention time of nine hours or less is sufficient for intracellular turnover.

2. Acetic Acid

The results of continuous flow experiments using glucose as the sole carbon source provided definite evidence that the process was mechanistically and operationally feasible for such a waste. The batch experiment using

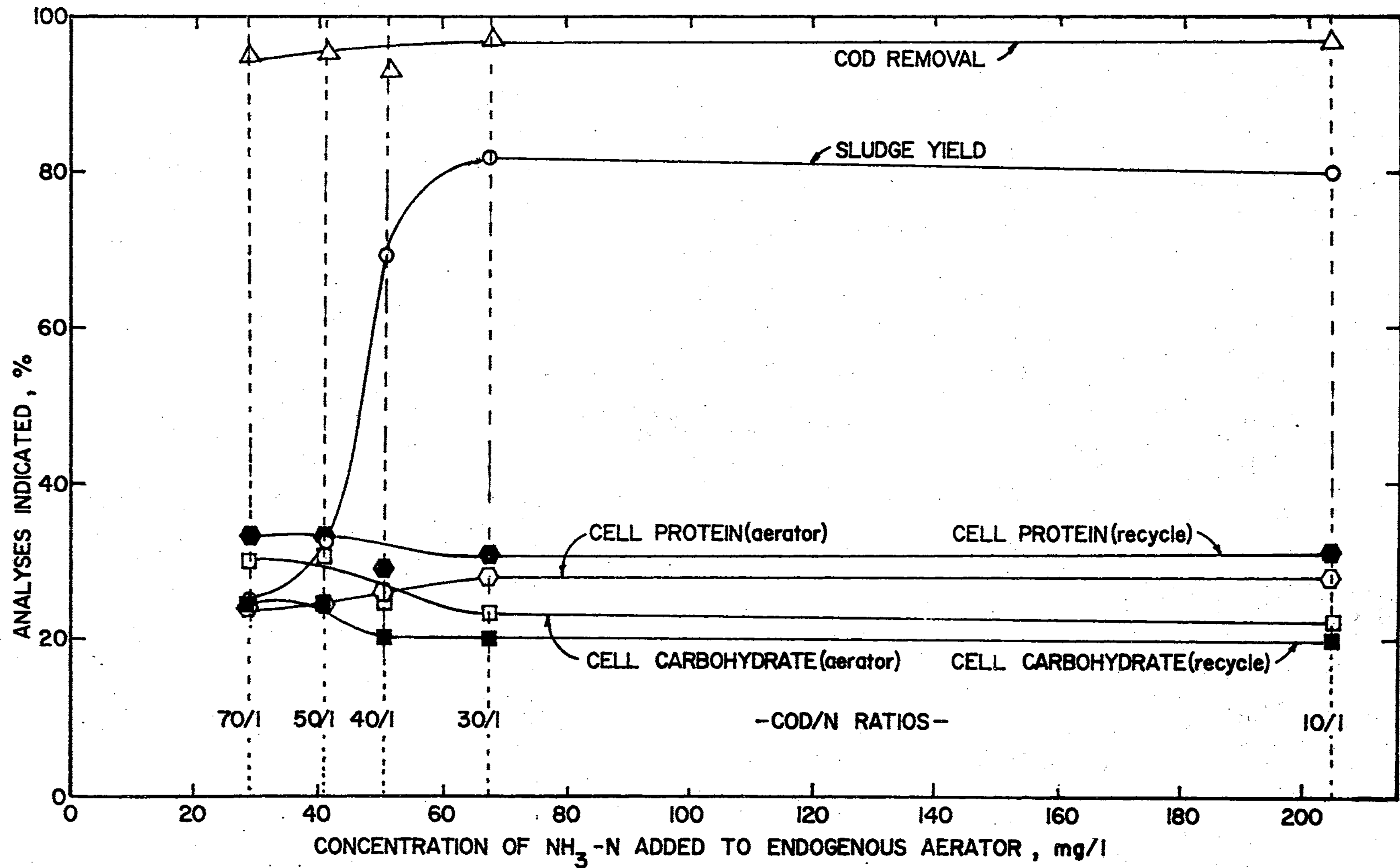


Figure 41. System performance at different nitrogen levels.

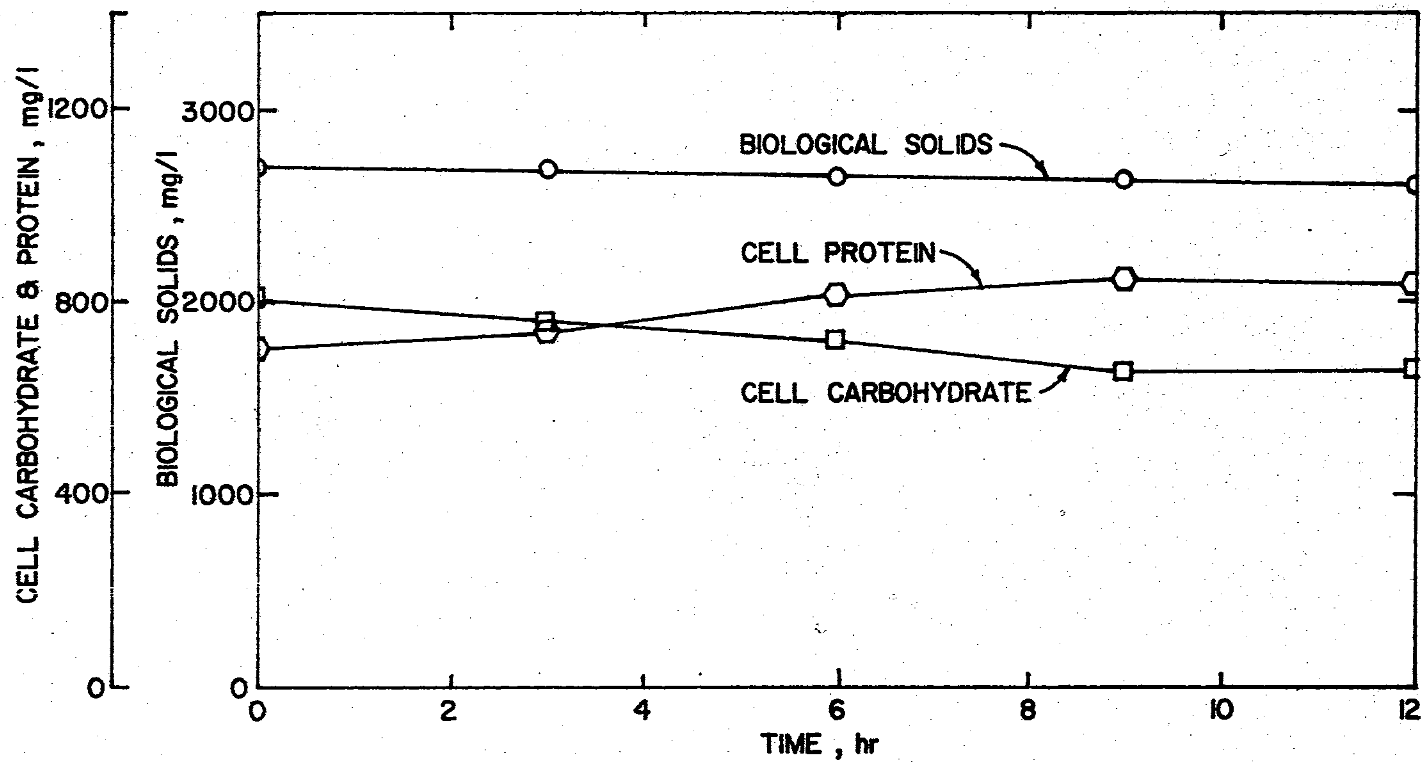


Figure 42. Biochemical transformations in the endogenous aerator, COD:N = 50:1.

acetic acid as the sole carbon source indicated the mechanistic feasibility of treating a non-carbohydrate waste. The operational feasibility of a continuous flow system using acetic acid was examined in this section of the study.

The results of the study using COD:N ratios of 30:1, 50:1, and 70:1 are shown in Figures 43 to 48; all results are summarized in Table VI and Figure 49. It is seen that at a COD:N ratio of 30:1, both the feeding aerator and the endogenous aerator solids concentrations fluctuated considerably. This was due to the fact that the concentration of biological solids in the endogenous aerator was not rigidly controlled. In fact, the conditions represented for the COD:N ratio of 30:1 are closer to those which might be observed in practice. In later experiments the solids in the endogenous aerators were more rigidly controlled by adjustment to a predetermined optical density.

From Table VI it is seen that the lowest average COD removals based on filtered and unfiltered effluent COD values occurred in the systems operated at a COD:N ratio of 70:1, and were 89.6 per cent and 87 per cent. The yield of biological solids ranged between 0.4 and 0.48. The mean biological solids concentrations in the feeding aerator were 822, 758, and 706 mg/l for systems operated at COD:N ratios of 30:1, 50:1, and 70:1. Sludge wasting ranged between 33 per cent and 43 per cent. Small concentrations of ammonia nitrogen were detected in the final effluents from the systems operated at COD:N ratios of 30:1 and 50:1;

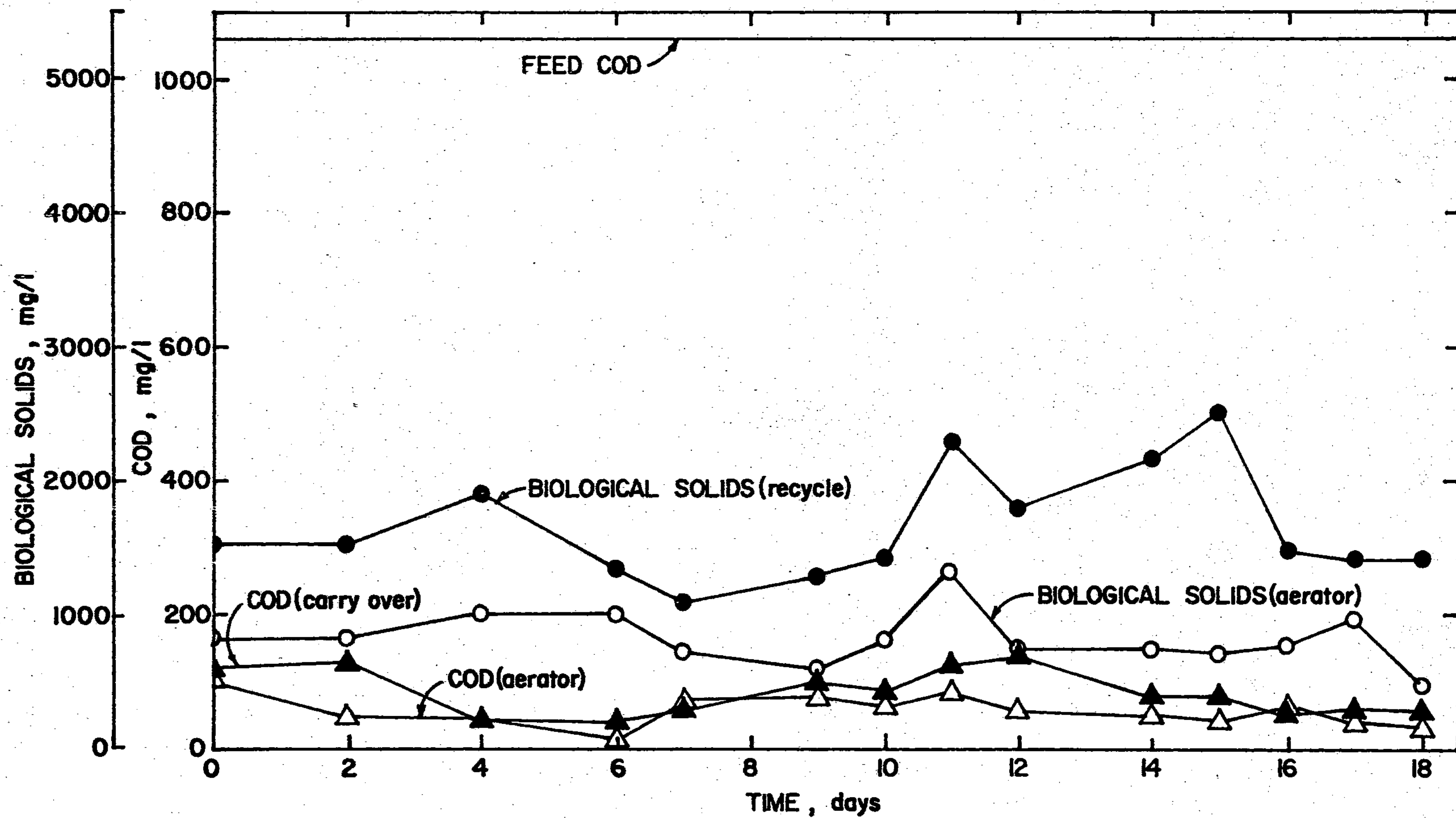


Figure 43. System characteristics at COD:N = 30:1, and D = 1/4 $hour^{-1}$.

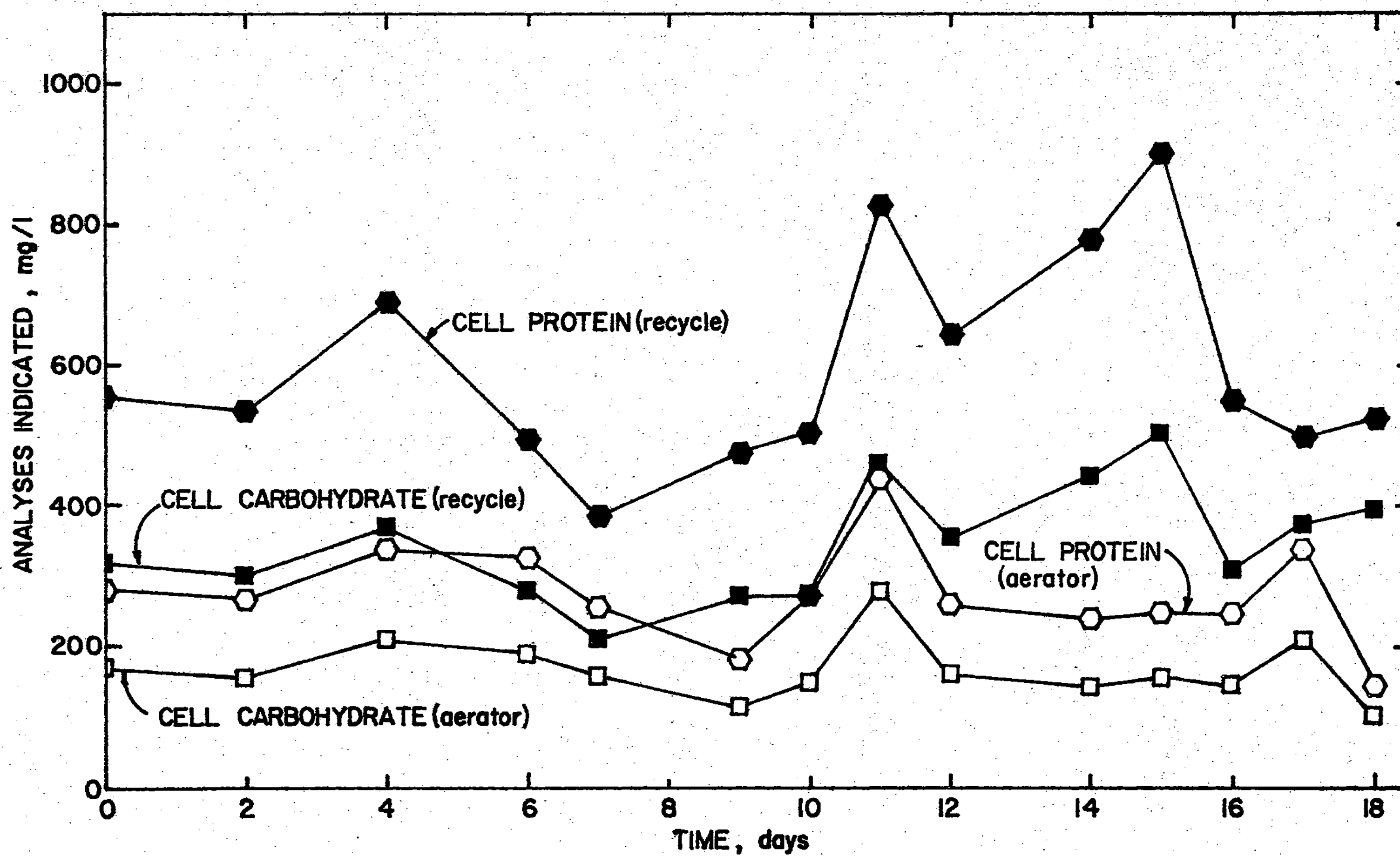


Figure 44. Biological solids composition at COD:N = 30:1, and D = 1/4 $hour^{-1}$.

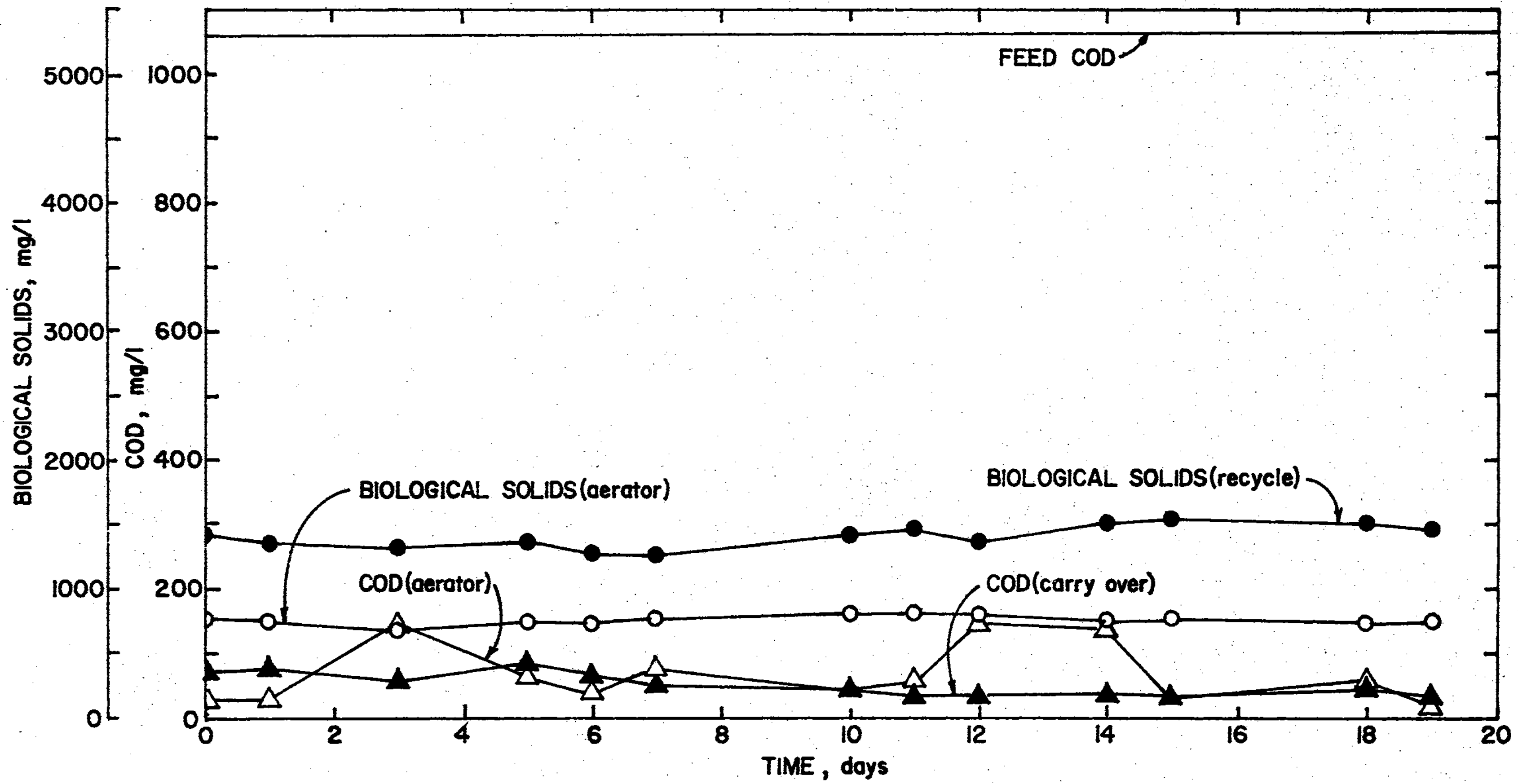


Figure 45. System characteristics at COD:N = 50:1, and D = 1/4 $hour^{-1}$.

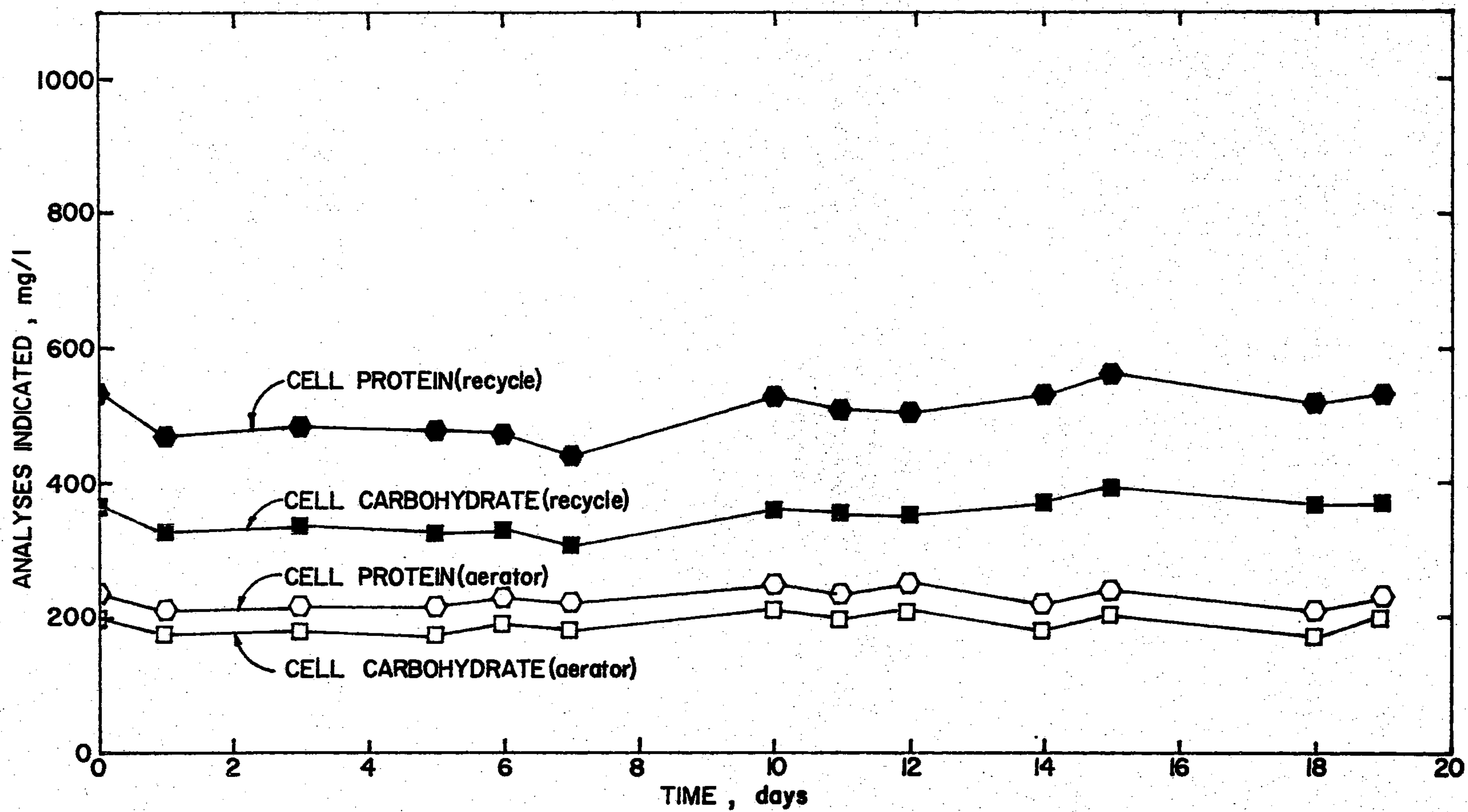


Figure 46. Biological solids composition at COD:N = 50:1, and D = 1/4 $hour^{-1}$.

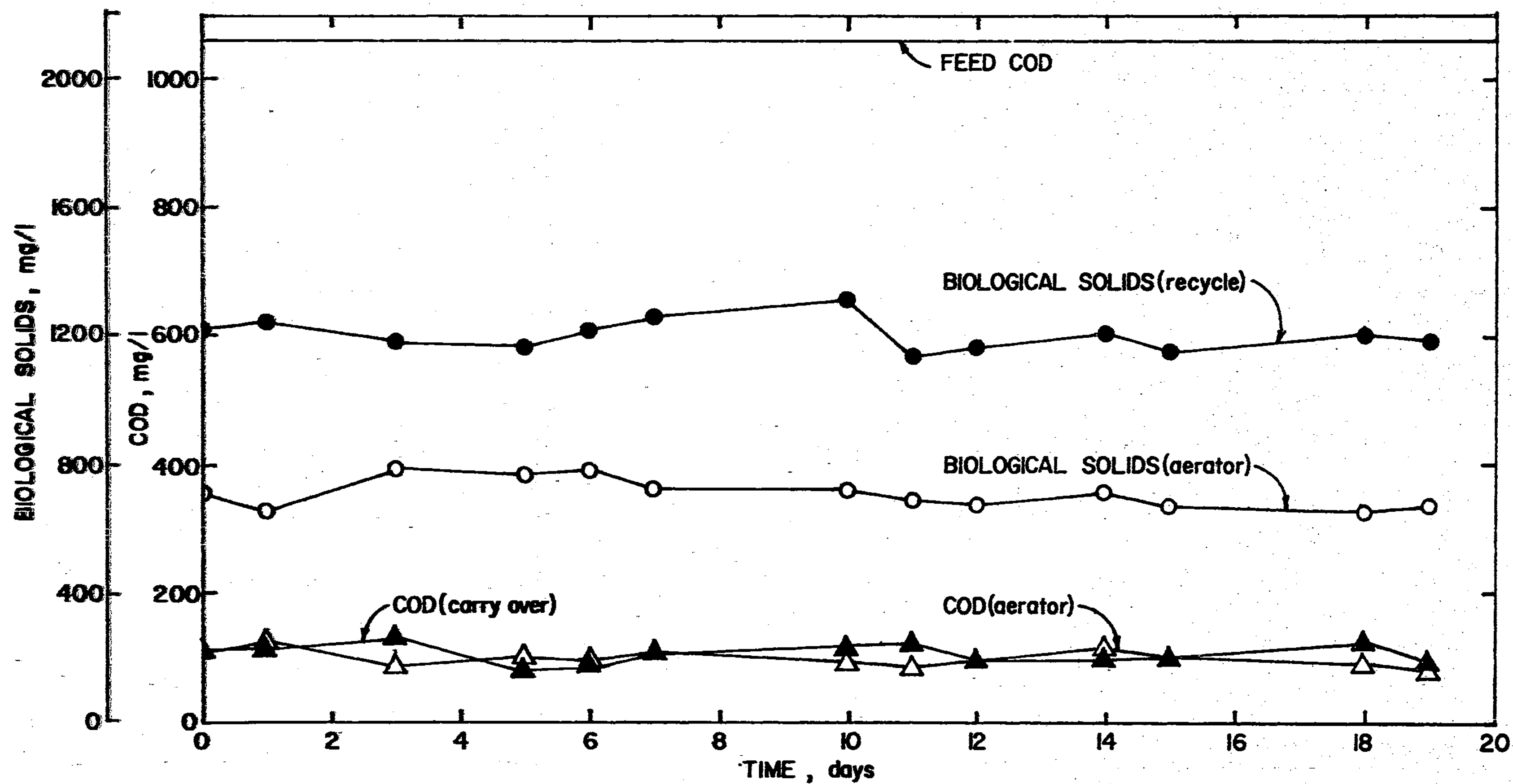


Figure 47. System characteristics at COD:N = 70:1, and D = 1/4 $hour^{-1}$.

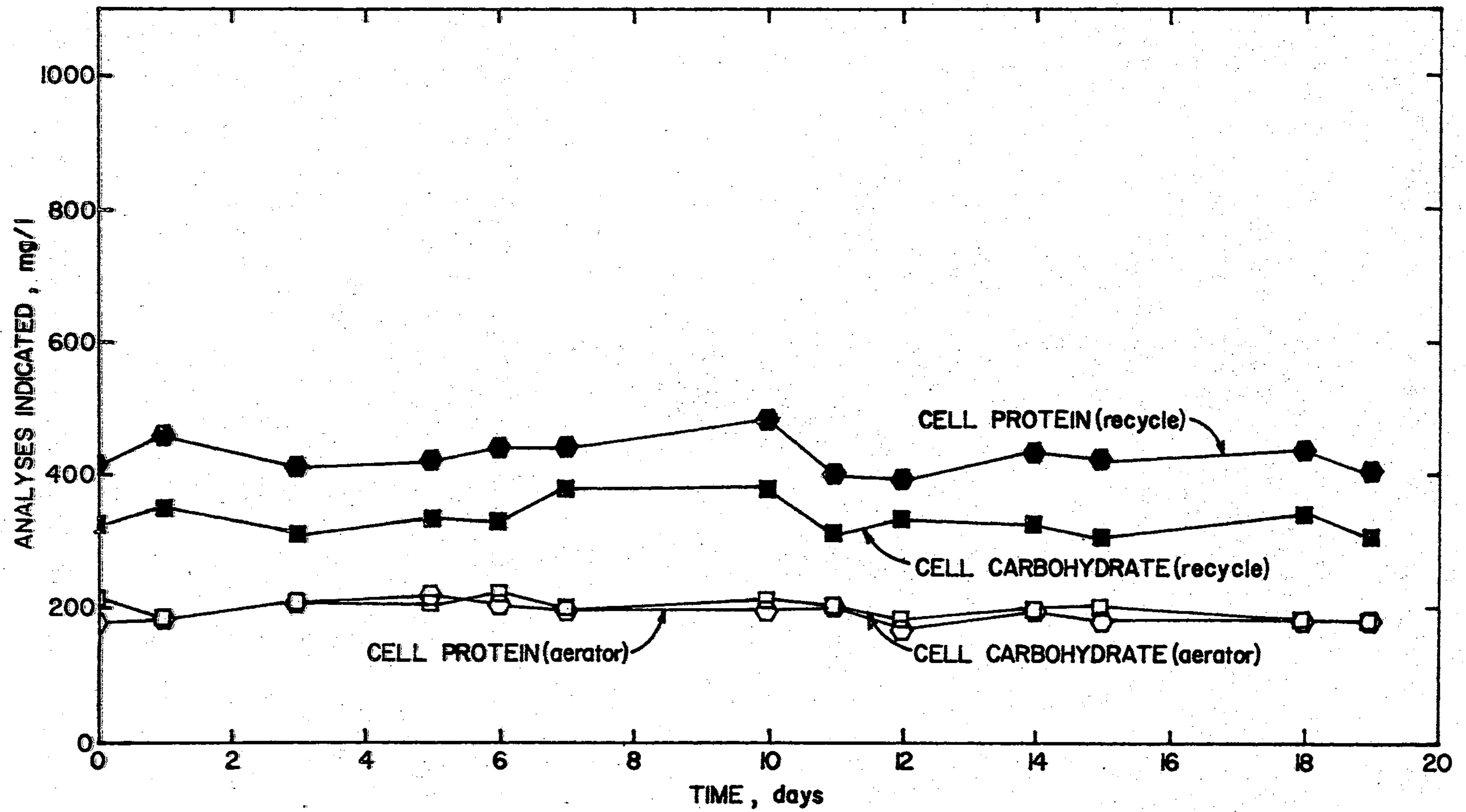


Figure 48. Biological solids composition at COD:N = 70:1, and D = 1/4 $hour^{-1}$.

TABLE VI

AVERAGE VALUES FOR ANALYSES OF EFFLUENT FROM THE FEEDING AND ENDOGENOUS AERATORS

DURING CONTINUOUS OPERATION AT VARIOUS COD:N RATIOS

(D = 0.25 hr^{-1}; Carbon Source = 1000 mg/l Acetic Acid = 1060 mg/l COD in the Feed)

	Effluent from Feeding Aerator											Effluent from Endogenous Aerator				
COD:N	Filtrate COD		Supernatant COD (Unfiltered)		NH_3-N in Final Effluent	Sludge	Sludge Yield	Sludge Protein	Sludge Carbohydrate	Sludge Wasted	Carry-over COD	NH_3-N Carry-over	Sludge	Sludge Protein	Sludge Carbohydrate
	mg/l	% Removal	mg/l	% Removal	mg/l	mg/l	%	%	%	%	mg/l	mg/l	mg/l	%	%
30:1	56	94.5	95	91	4	822	40	33.3	20.3	33	65	55	1659	36	20
50:1	67	93.6	108	90	1	758	48	30	25	41	76	24	1317	37.4	26.7
70:1	98	89.6	140	87	-	706	47.4	24.7	28.3	43	110	-	1205	35.7	27.6

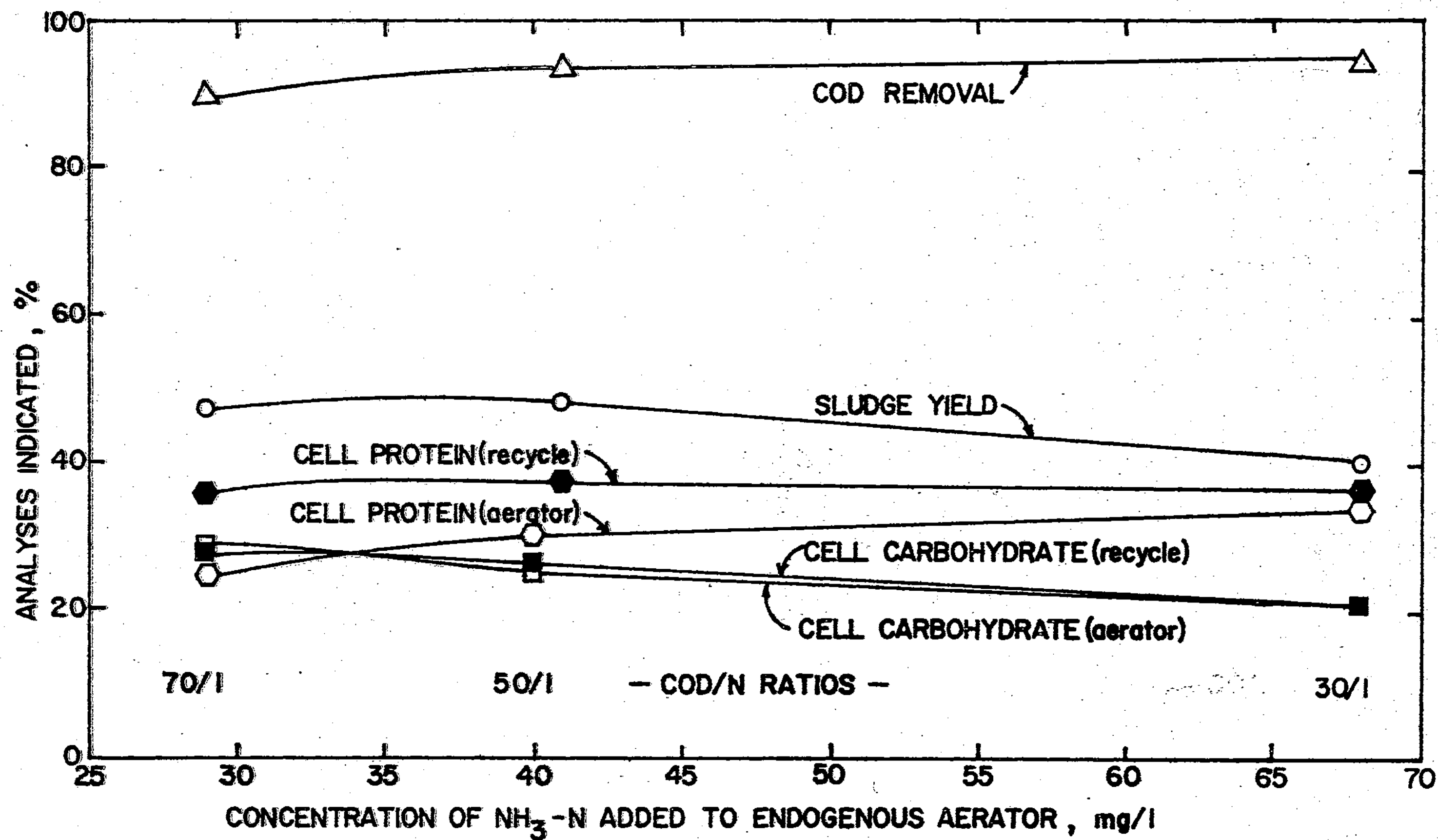


Figure 49. System performance at different nitrogen levels.

however, no ammonia nitrogen was detected in the final effluent from the unit operated at a COD:N ratio of 70:1. At this nitrogen level the entire nitrogen supplement was consumed in the endogenous aerator.

Analysis for sludge composition showed that the average protein content in the feeding aerator decreased as the nitrogen supplementation was reduced. The maximum and minimum concentrations were 33.3 per cent and 24.7 per cent. However, the protein content of the recycled sludge was practically the same at all nitrogen levels. It fluctuated between 35.7 per cent and 37.4 per cent. This finding indicates that the increase in the protein content, i.e., protein production, in the endogenous aerator was greater when lower nitrogen levels were maintained.

The carbohydrate content of the sludge in the feeding aerator increased from 20.3 per cent to 28.3 per cent as the nitrogen level was reduced from COD:N ratio of 30:1 to 70:1. The carbohydrate content of the sludge remained essentially unchanged in the endogenous aerator.

The results of the "turnover" experiment are shown in Figure 50. It is seen that the biological solids concentration remained essentially unchanged during the 12-hour aeration period. The protein content of the sludge increased from 335 mg/l to 440 mg/l, whereas the cell carbohydrate decreased very slightly (from 380 mg/l to 350 mg/l). No significant change in the protein content was observed after nine hours' aeration. Therefore it is believed that nine hours' aeration period in the endogenous aerator is adequate.

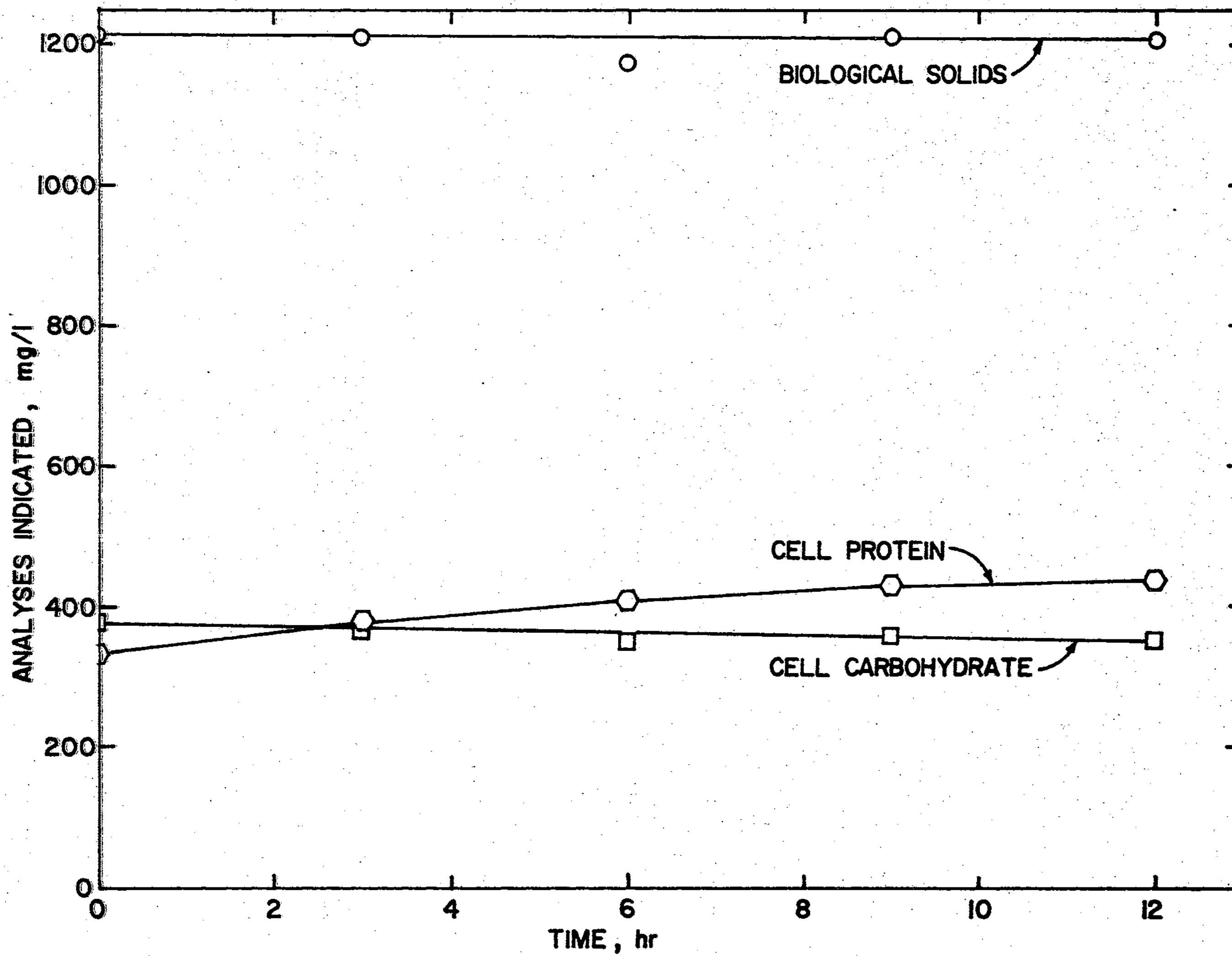


Figure 50. Biochemical transformations in the endogenous aerator, COD:N = 70:1.

CHAPTER VI

DISCUSSION OF RESULTS

Phase I

A. Effect of Different Nitrogen Levels in Continuous Flow Process

1. Effect of Detention Time on Steady State Parameters (COD:N = 70:1)

The effects of detention time on the "steady state" parameters at a nitrogen level corresponding to a COD:N ratio of 70:1 are summarized in the "dilute out" curves shown in Figure 51. It is seen that the biological solids concentration in the reactor ranged between 700 mg/l at a 12-hour detention time and 40 mg/l at a 1-hour detention time. The concentration of biological solids dropped off from 700 mg/l to 285 mg/l when the detention time was changed from 8 hours to 4 hours. In studies with carbon-limited systems, Ramanathan (79) observed that the reduction of biological solids occurred when the dilution rate was higher than 0.33. This dilution rate corresponds to a detention time of three hours. This indicates that nitrogen-limited systems may require longer detention times if it is desired to maintain the biological solids

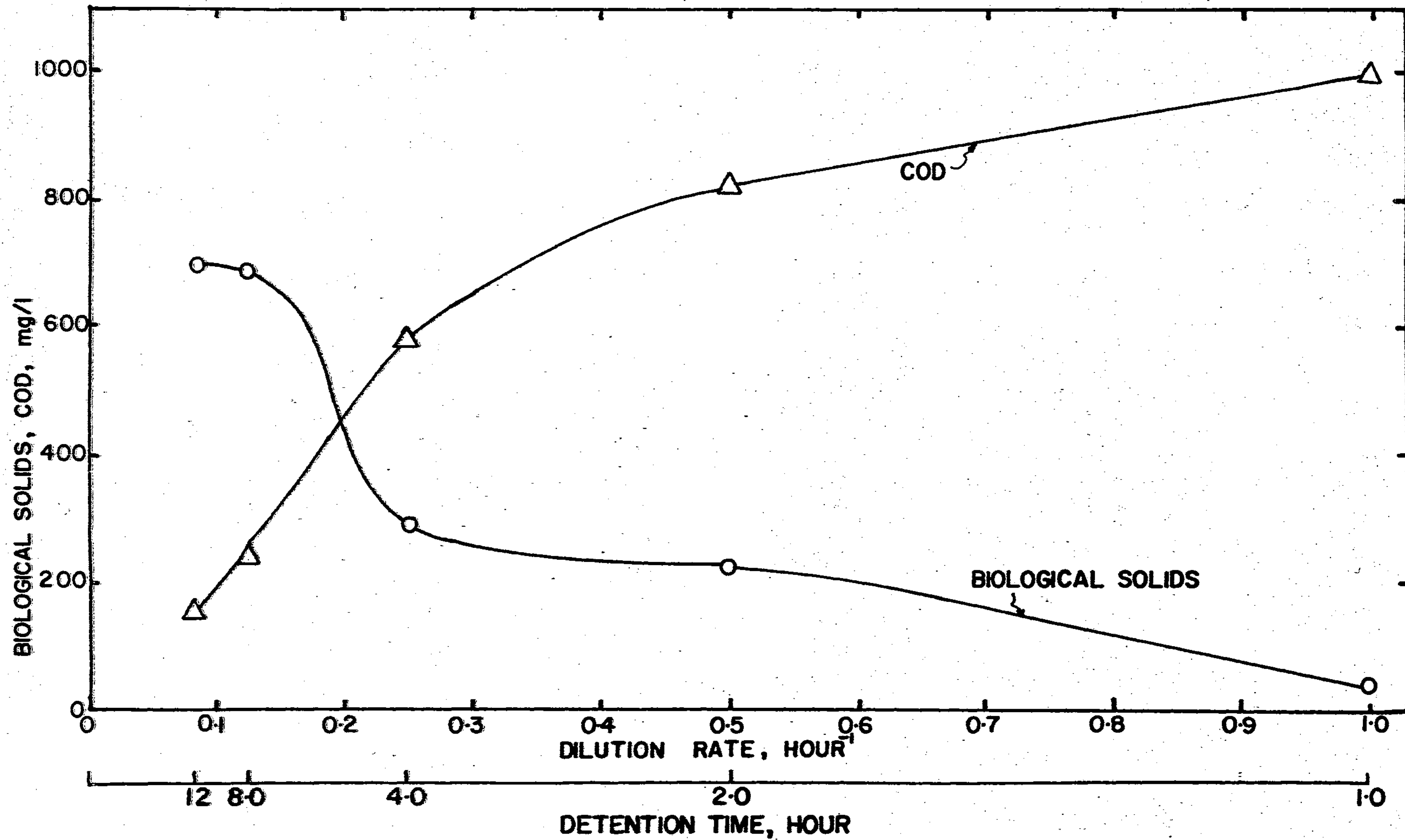


Figure 51. Dilute-out curves for biological solids and substrate with S_i = 1000 mg/l glucose and COD:N = 70:1.

concentration at a fairly constant level. It would appear that the cells are more sensitive to changes in dilution rate of the system when the nitrogen source is the limiting nutrient. Complete wash-out of cells did not occur even at the 1-hour detention time. Possible reasons for this "tailing off" may lie in the fact that the biological population was of a heterogeneous nature. The system has an inherent tendency to select faster-growing species as the detention time is decreased (138).

The effluent COD (Figure 51) increased with decreasing detention time. The effluent COD was 150 mg/l at the 12-hour detention time, yielding a biochemical purification efficiency of 86.4 per cent. The purification efficiencies at 8, 4, 2, and 1-hour detention times were 78.2, 47, 26.2, and 9.4 per cent. It is thus seen that if the nitrogen level is to be maintained at a COD:N ratio of 70:1, a detention time of approximately twelve hours would be required to obtain 85 per cent COD removal. Aerator detention times of eight hours or less are not adequate at this nitrogen level. Decreased biochemical purification efficiency with very short aeration periods is also observed in carbon-limited systems. Ramanathan (79) observed that wash-out did not begin until the detention time was reduced below three hours. The present studies show that in nitrogen-limited systems, wash-out is initiated at higher detention times than in carbon-limited systems, thereby necessitating higher aeration periods for comparable substrate

utilization efficiency.

Ammonia nitrogen was absent in the treated effluent, suggesting that the entire amount of added nitrogen was utilized by the system. In continuous flow systems operated at long aeration periods with low nitrogen supplementation (up to a COD:N ratio of 500:1), Symons and co-workers (67) observed that 80 per cent of the added nitrogen was discharged every day in the form of organic nitrogen in the wasted solids. The results of the present study do not seem to follow the pattern observed by these authors. It may be added that 100 per cent sludge wasting was practiced in this portion of the present study. Thus, at this COD:N ratio all supplemental nitrogen which found its way to the effluent was in the form of cellular nitrogen. It is possible that the nitrogen detected by Symons and co-workers (67) was due to cell lysing,(since the nitrogen was in organic form), caused by very long aeration periods. In the present study all of the nitrogen was incorporated into the cells. In the absence of analyses for nitrate and nitrite, the possibility of their presence in the treated effluent is not completely ruled out. However, nitrification would not be expected to occur at the detention times and substrate loadings employed in these studies. Also, it should be pointed out that the mode of operation employed by Symons and co-workers (67) was different from that employed in the present study. Their studies employed recirculation of sludge, with low sludge wasting. This

resulted in increased sludge age. The detention time employed by these workers was also very long.

The yield of biological solids was found to vary between 0.55 and 0.82. No definite trend with respect to the detention time was observed. However, the yields were, in general, higher than those usually observed in carbon-limited systems. In systems with identical organic loadings, Ramanathan (79) observed yields between 0.552 and 0.785; these two values, incidentally, were observed for 24 and 1.5 hours retention, respectively. His studies also do not show any definite relation between the detention time and the yield.

The percentage of carbohydrate in the dry weight of sludge was found to vary between 39 per cent and 50 per cent. In general, the carbohydrate content of the sludge was higher at the low detention times. A similar trend was also observed in Ramanathan's studies (79), the carbohydrate content being approximately 11 per cent at 12-hours detention time, increasing to 36 per cent at 1.5 hours aeration time. However, Herbert (88) found that the carbohydrate content of *Torula utilis* was higher at higher detention time when the carbon source was glucose and nitrogen source was the limiting growth factor. It is interesting to note that, when he (87) used glycerol as the source of carbon, and nitrogen was the limiting growth factor, the carbohydrate content of *Aerobacter aerogenes* remained fairly constant at different detention times.

The cellular carbohydrate in the mixed liquor increased from 110 mg/l at the 2-hour detention time to 314 mg/l at the 12-hour detention time. Thus, it seems that a greater amount of carbohydrate was synthesized at higher detention times. However, the increase in production of biological solids at higher detention times was greater than the increase in carbohydrate synthesized. Thus, the fraction of carbohydrate, expressed as the percentage of the dry solids weight, decreased slightly at higher detention times.

The relatively constant level of cellular protein at different dilution rates (80 mg/l) and complete absence of ammonia nitrogen in the effluent indicates that either the entire amount of supplemental nitrogen was utilized for protein synthesis or a constant fraction of the added nitrogen was used for the synthesis of protein. Any difference in the sludge concentration at different detention times affects the percentage of the protein in the dry weight of the cell mass. In other words, as the total protein in the system was constant, any increase in the biological solids concentration reduces the fraction of protein in the dry weight of the cell mass. Ramanathan (79) and Herbert (88) had observed a decrease in the protein content at low detention times in carbon-limited systems. It was argued that low detention times do not allow adequate time for the microorganisms to complete the complex process of protein synthesis. This may be true when the system carries a considerable amount of nitrogen; however, in the present

studies the amount of nitrogen in the feed was rather low, and a detention time of two hours may be adequate for protein synthesis from this lower amount of nitrogen.

If the nitrogen content in the protein is assumed to be 16 per cent (143), the maximum protein that could be synthesized from the nitrogen added in the feed can be calculated to be 94 mg/l. The protein actually synthesized was 80 mg/l in most cases, which shows that approximately 85 per cent of the nitrogen fed to the system was incorporated into the cellular protein. In the absence of RNA or DNA analyses, the fate of the remaining 15 per cent nitrogen is not known. However, it appears reasonable to expect that this was used in the synthesis of RNA and DNA. The RNA content of sludges can vary between 3 and 30 per cent, depending upon the environment (88). Herbert (88) observed higher concentrations of RNA at lower detention times.

It is significant to note that satisfactory COD removals were obtained with sludges containing as little as 11.5 per cent protein. This amount of protein indicates a nitrogen content of the sludge of only 1.84 per cent. It will be recalled that Helmers, et al. (58) had advocated a minimum of 7 per cent nitrogen in the sludge for satisfactory performance. Krishnan (71) also obtained satisfactory COD removals when the sludge nitrogen content was far below 7 per cent. The results of this study and those of Krishnan (71) and Bechir (69) show that this criterion (a minimum of 7 per cent nitrogen) for satisfactory organic

removals is not necessarily one of general applicability and may be a somewhat conservative one.

2. Effects of Detention Time on Steady State Parameters (COD:N = 40:1)

The effects of detention time on the "steady state" parameters at a nitrogen level corresponding to a COD:N ratio of 40:1 were shown in Figures 9 through 13, and are summarized in the "dilute out" curve shown in Figure 52. It is seen that the biological solids concentration decreased as the aeration time was decreased from 12 to 1 hours. The biological solids concentration at the 12-hour detention time was greater than at the 8-hour detention time. Thus, it is not known whether the solids concentration observed at 12-hour aeration time is the maximum value of solids concentration for any detention time. Unlike the systems with COD:N equal to 70:1, there was considerable difference in the biological solids concentraions at 8 and 12-hour detention times. Complete wash-out of cells was not observed even at the 1-hour detention time.

The effluent COD increased as the detention time was decreased. The effluents from the units operated at the 12 and 8-hour detention times were found to have COD's of 81 and 127 mg/1, respectively, which amount to 90 per cent and 88 per cent biochemical purification efficiency. It is significant to note that rather good COD removal was observed at the 8-hour aeration period. A COD removal of 85 per cent can be obtained at this nitrogen level if a

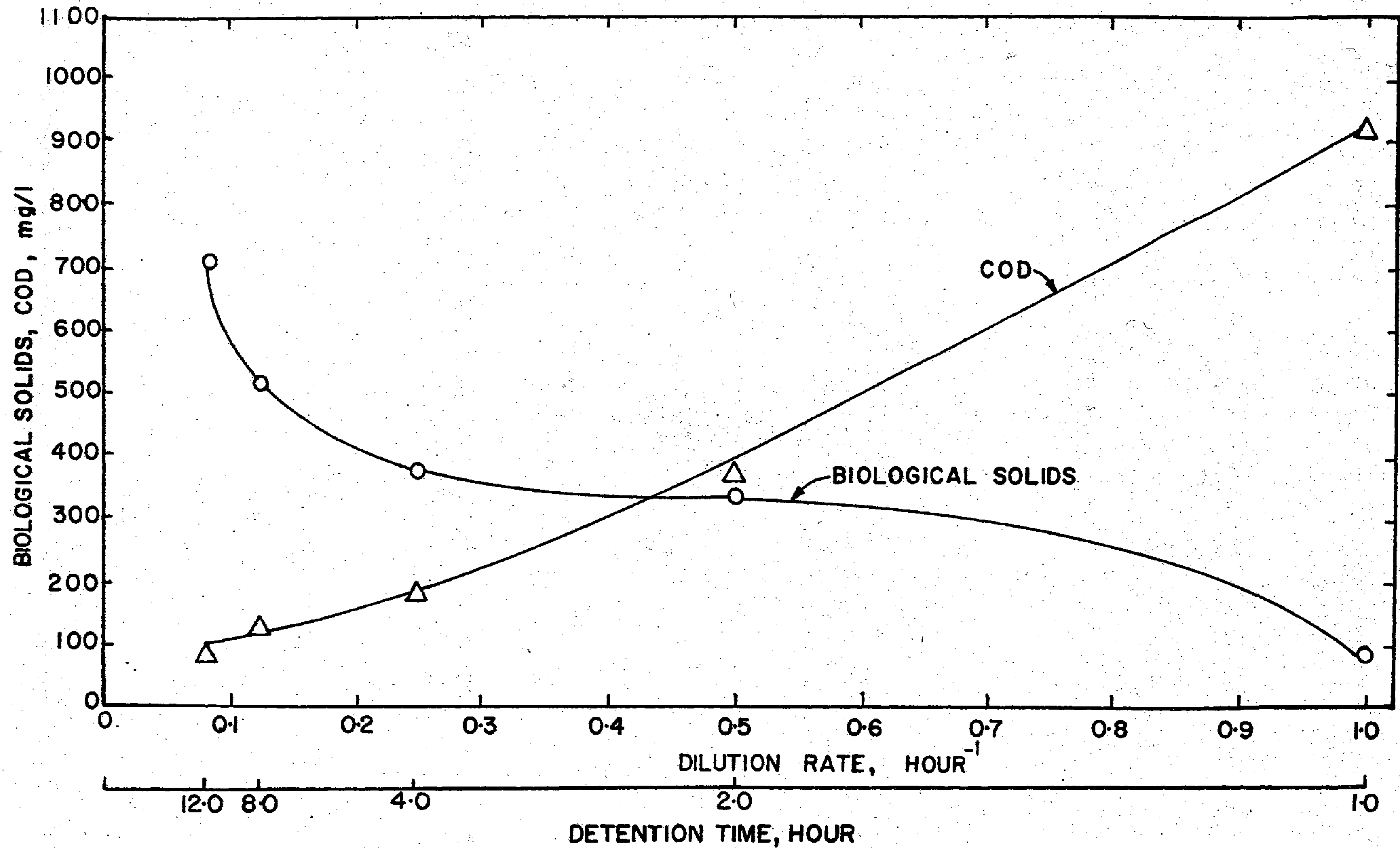


Figure 52. Dilute-out curves for biological solids and substrate with S_i = 1000 mg/l glucose and COD:N = 40:1.

detention time of at least 4.25 hours is provided. It may be recalled that at a COD:N ratio of 70:1 a satisfactory removal was obtained only at the 12-hour aeration time. The results indicate that an increase in nitrogen supplementation from COD:N of 70:1 to 40:1 allows the same degree of purification in a shorter aeration period.

The sludge yield was found to vary between 0.43 and 0.73. No definite relationship was observed between the sludge yield and the detention time. These values are, however, in the range of sludge yields usually observed. Gaudy and Gaudy (105) have stated that although wide ranges of yields have been reported, the yield constants generally accepted are approximately 0.5 and 0.67 expressed as fractions of BOD and COD loading, respectively. It is noted that the yields at a COD:N ratio of 40:1 are lower than those observed at COD:N equal to 70:1 at all detention times.

The analyses for sludge composition show that at this particular nitrogen supplement, i.e., COD:N = 40:1, the cells contained a higher proportion of carbohydrate at the lower detention times. The carbohydrate content varied from 31 to 36 per cent of the dry cell mass. These values are lower than those observed at a COD:N ratio of 70:1 for the same detention times. However, it is important to note that the total amount of carbohydrate synthesized from the carbon source increased as detention time was increased. The cells were found to be very slimy and sticky at low nitrogen levels. It thus appears that most of the carbohydrate

synthesis is due to the production of extracellular slime, especially at the highest COD:N ratio (70:1). It also appears that as the detention time increases, the sludge accumulates considerable amounts of compounds other than carbohydrate or protein. It is likely that lipids may be present at high detention times.

As observed in the case of systems with a COD:N ratio of 70:1, the total amount of protein in all of the systems was found to be relatively constant (between 125 and 140 mg/l). However, this level of protein synthesis is considerably higher than that observed for systems operated at a COD:N ratio of 70:1. Thus, as could be expected, the supplementation with a greater amount of nitrogen resulted in greater amounts of protein in the system.

At the low nitrogen concentrations in the medium (high COD:N ratios) the cells exhibited low protein content (as low as 11.5 per cent of dry weight of the solids). This may be taken as an indication that there were not as many viable cells present to utilize the substrate as there were at the higher nitrogen level. Therefore it follows that this lesser number of feeders would require a longer time to effect the same degree of purification than would be required by the greater number of viable cells which one would expect to be present in systems exhibiting a higher protein content. The cells at low nitrogen levels were slimy, and the mixed liquor was very viscous, indicating that the higher carbohydrate content was due to a large

amount of capsular material. Thus, at the low nitrogen levels, the cell mass consisted of a lower number of viable cells, but each cell possessed a high amount of extracellular capsular material. This synthesis was accomplished at the expense of the substrate in the synthetic waste.

The analysis of ammonia nitrogen in the final effluent shows that 90 per cent or more of the nitrogen in the medium (26 mg/l) was incorporated in the cells. The maximum utilization was observed to be 93 per cent. The concentration of ammonia nitrogen in the final effluent ranged between 1.9 and 2.7 mg/l. Helmers, et al. (56) had advocated that the nitrogen content in the effluent should be at least 2 mg/l for efficient purification. The results of this study show that systems containing 2 mg/l ammonia nitrogen in the effluent may or may not be efficient from the point of view of organic removal; e.g., the system operated at the 4-hour detention time with a COD:N ratio of 40:1 did have 2 mg/l ammonia nitrogen in the effluent, but its biochemical purification efficiency was only 83 per cent. Similarly, the effluent from the system operated at a 2-hour detention time contained 2.2 mg/l nitrogen, yet its biochemical purification efficiency was only 65 per cent, which is, in general, an unsatisfactory level of purification.

3. Effects of Detention Time on Steady State Parameters (COD:N = 25:1)

The "dilute out" curve in Figure 53 shows the effects

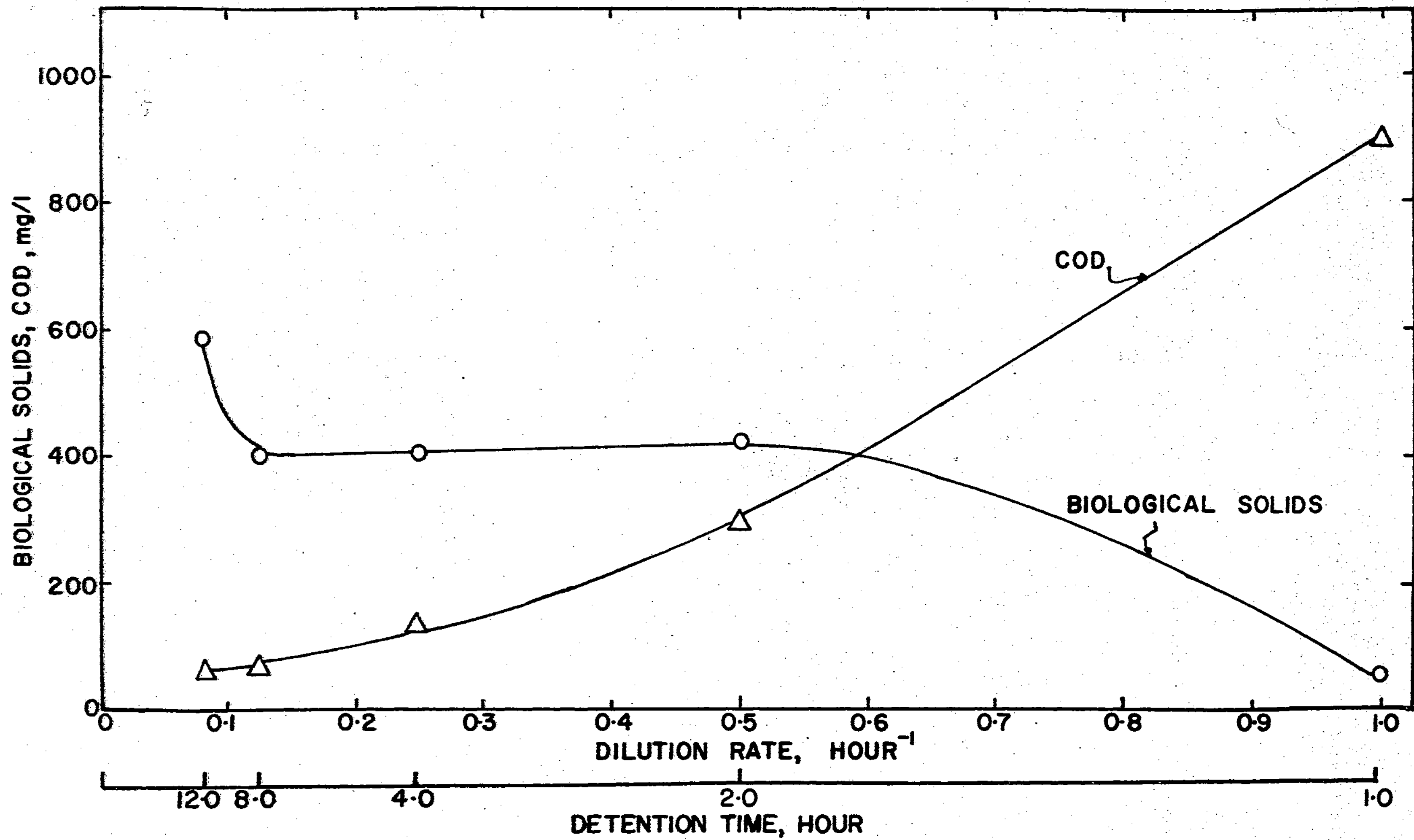


Figure 53. Dilute-out curves for biological solids and substrate with S_i = 1000 mg/l glucose and COD:N = 25:1.

of detention time (or dilution rate) on COD removal and biological solids concentration. As in the systems at a COD:N ratio of 40:1, the concentration of biological solids was not the same at eight and twelve hours, and so it is not possible to predict the maximum biological solids concentration that the system can carry at this nitrogen level. It is possible that the biological solids at detention times in excess of twelve hours may be higher than that observed in the system with a 12-hour aeration time. However, detention times in excess of twelve hours are rarely used (except for total oxidation systems), and thus may be of little practical significance. Complete wash-out of the cells was not observed even at the 1-hour detention time.

The COD data (Figure 53) indicate that 85 per cent COD removal, which corresponds to an effluent COD of 160 mg/l, can be obtained at this nitrogen level by providing a detention time of 3.125 hours or more. It will be recalled that the detention time required for 85 per cent COD removal for the systems operated at COD:N ratios of 70:1 and 40:1 were 12 and 4.25 hours. Thus, for obtaining the same biochemical purification efficiency, a shorter aeration time coupled with higher nitrogen supplementation seems to be satisfactory.

The sludge yield was found to fluctuate between 42 per cent and 62 per cent. As observed at other nitrogen levels, no specific trend was observed in the variation of sludge

yield with detention time.

The carbohydrate content of the sludge varied between 25 per cent and 30 percent, the higher fraction being observed at the lowest detention time. At this COD:N ratio it appears that the carbohydrate fraction in the dry weight of biological solids is higher at shorter aeration periods, but the total amount of carbohydrate was less variable than at the lower nitrogen concentrations.

The nitrogen analysis shows that the amount of nitrogen utilized varied between 80 per cent and 94 per cent, being lower at higher detention times. If one assumes that the presence of at least 2.7 mg/l of ammonia nitrogen in the effluent indicates that these systems were not nitrogen-limited, then it would appear that protein synthesis was not restricted due to the nitrogen concentration. One may also then conclude that a COD:N ratio of 40:1 does not seem to be nitrogen-limited.

The protein content of the sludge was found to be approximately 50 per cent in systems operated at detention times of 2, 4, and 8 hours, whereas it dropped to 30 per cent in the system operated at a detention time of twelve hours. These results appear to show that the hydraulic loading controls protein synthesis through forcing the cells to grow at a rate equal to the dilution rate. The amount of protein actually synthesized may be controlled by the protein requirement for the particular species present. It is also possible that the RNA content of the sludge

increased with increasing detention time. The efficiency of nitrogen utilization decreased with increasing detention time. However, Ramanathan (79) observed that the protein content in carbon-limited systems was higher at higher detention times. The nature of the two systems is very different. The medium used by Ramanathan was designed for a carbon-limited system and necessarily contained excess nitrogen (2.5 times that employed in the present studies). His operational conditions might be considered as leading to "force feeding" of nitrogen. Under such conditions it seems possible that the longer detention times might provide for excess protein synthesis.

Table VII shows the effects of COD:N ratios and detention times on the amount of solids produced per unit of nitrogen taken up by the cells. It is seen that at a particular COD:N ratio in the feed, as the detention time increases, more solids are produced for the same nitrogen utilization. The table also shows that at a particular detention time, as the nitrogen supplementation is increased, the amount of solids produced per unit of nitrogen incorporated into the cells decreases.

Table VIII shows the effects of COD:N rations and detention times on the amount of COD removal per unit of nitrogen incorporated in the cells. It appears that at particular COD:N ratios, as the detention time is increased, more COD is removed for the same nitrogen utilization.

TABLE VII

RELATION BETWEEN AMOUNT OF SOLIDS PRODUCED PER UNIT OF NITROGEN TAKEN UP BY THE CELLS AT DIFFERENT DETENTION TIMES AND COD:N RATIOS IN THE FEED

Detention Time Hours	Milligrams Biological Solids per Milligram Nitrogen used		
	COD:N = 70:1	COD:N = 40:1	COD:N = 25:1
2	14.7	13.8	10.6
4	19.0	15.1	10.3
8	46.0	21.0	10.8
12	46.3	29.8	17.2

TABLE VIII

RELATION BETWEEN AMOUNT OF COD REMOVAL PER UNIT OF NITROGEN TAKEN UP BY THE CELLS AT DIFFERENT DETENTION TIMES AND COD:N RATIOS IN IN THE FEED

Detention Time Hours	Milligrams Biological Solids per Milligram Nitrogen used		
	COD:N = 70:1	COD:N = 40:1	COD:N = 25:1
2	15.9	28.3	19.3
4	32.0	35.6	24.0
8	54.7	38.4	27.5
12	60.7	41.0	28.9

4. Effects of Different Nitrogen Levels on the Performance of the Activated Sludge Process

The interrelationship between detention time, the nitrogen supplementation, and effluent COD is shown in Figure 54. It is seen that to obtain 85 per cent COD removal, i.e., an effluent COD of 160 mg/l, the nitrogen requirements at 12, 8, and 4-hour detention times correspond to COD:N ratios of 70:1, 45.5:1, and 35:1, respectively. By interpolation, the nitrogen requirement for a 6-hour detention system is found to correspond to a COD:N ratio of 39:1. In order to compare the results of the present study with those of past workers, it was necessary to express the nitrogen supplementation used in terms of BOD:N. The BOD of glucose was, therefore, determined. The ratio of BOD and COD was found to be 0.667 for glucose. Therefore, the nitrogen supplenetation for 85 per cent COD removal corresponds to BOD:N ratios of 47:1, 30:1, 26:1, and 23:1 for 12, 8, 6, and 4-hour detention times. Since the nitrogen requirement is found to be dependent upon the detention time employed, it does not seem advisable to specify one ideal BOD:N or COD:N ratio for all detention times. Sawyer (12) had recommended a BOD:N ratio of 32:1 when stabilization using the minimum amount of nutrient is desired. He apparently intended that the ratio could be applied to any detention time. Moreover, his conclusions were based on batch experiments. The results of the present study show that a BOD:N ratio of 32:1 is satisfactory

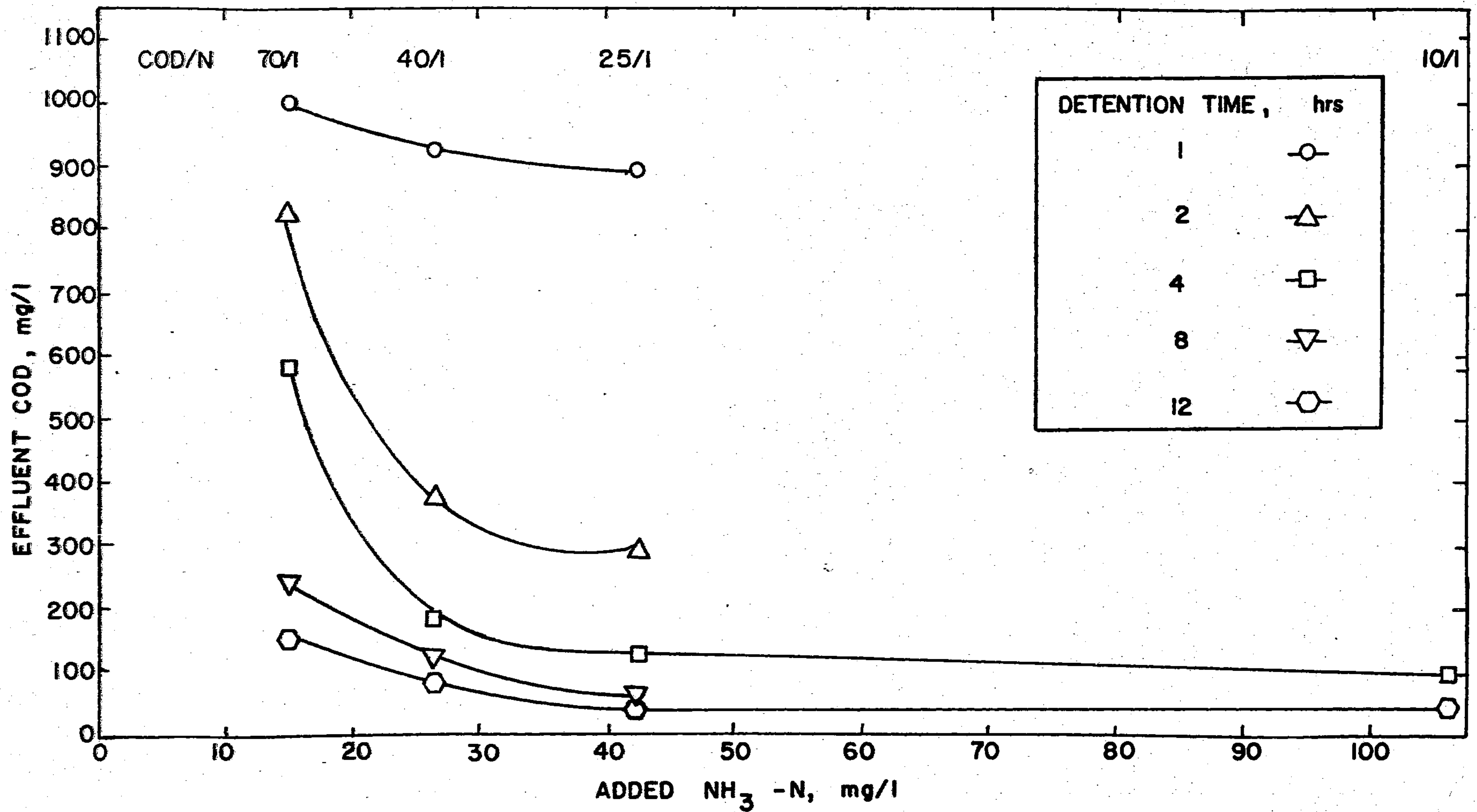


Figure 54. Relationships between detention time, supplemental nitrogen and effluent COD.

for a completely-mixed continuous flow system when the detention time in the aerator is slightly more than eight hours, but is insufficient for systems with lower detention times.

On the other hand, Eckenfelder and O'Conner (14) suggested a ratio of BOD:N equal to 20:1 for satisfactory organic removal. The results of the present study show that the nitrogen requirement is really lower than that given by these authors for a system operated at a detention time of four hours. Hattingh (61) advocated slightly higher nitrogen requirements (BOD:N = 19:1) than that suggested by Eckenfelder and O'Conner (14).

The results shown in Figure 54 also reveal that a 4-hour detention time is close to the minimum retention which could be recommended for treatment of nitrogen-deficient wastes without depending upon sludge recirculation to effect higher removals irrespective of the amount of nitrogen supplementation.

The relationship between the amount of ammonia nitrogen in the effluent and the amount of nitrogen in the feed is shown in Figure 55. It is seen that all systems operated at a COD:N ratio of 40:1 yielded approximately 1.8 to 2.8 mg/l ammonia nitrogen in the effluent, whereas the amount of nitrogen in the effluent at a nitrogen level corresponding to a COD:N ratio of 25:1 was found to be quite different at various detention times. The 12-hour system was the most wasteful system with respect to nitrogen,

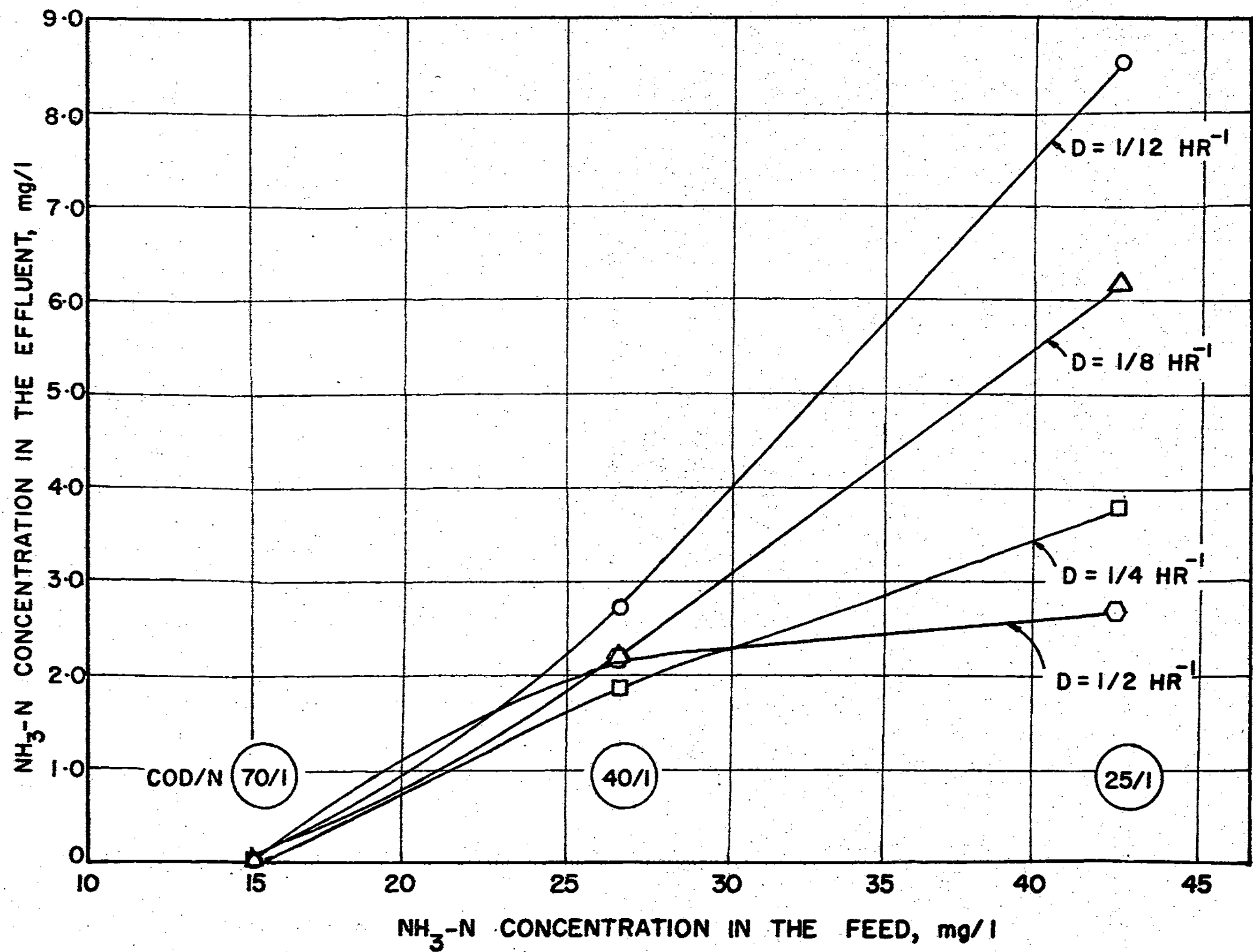


Figure 55. Relationship between the amount of supplemental nitrogen and nitrogen in the effluent at different detention times.

whereas the 2-hour system utilized nitrogen to the greatest extent. It is apparent from this graph that nitrogen levels in excess of a COD:N ratio of 40:1 are not desirable because they lead to more wastage of supplemental nitrogen. In fact, nitrogen supplementation could be reduced to a lower level by adoption of a higher aeration period.

Thus, in cases where escape of nitrogen in the stream is a critical factor, longer detention times and lower nitrogen supplementation are to be preferred. Based on the data given in Figure 55, it appears that in systems without cell recycle nitrogen starts leaking into the effluent at some COD:N ratio between 70:1 and 40:1 at all detention times. However, the exact COD:N ratio at which nitrogen starts appearing in the effluent cannot be determined from the data presented. It is possible that cell recycle could affect the amount of nitrogen in the effluent. The possibility of lower NH_3-N in the effluent in systems employing cell recycle is not ruled out.

For cases when some nitrogen in the effluent may be tolerated, the exact amount of nitrogen supplementation will depend upon the detention time. Although longer aeration time reduces the cost of supplemental nitrogen, it increases construction and operating cost of the plant. Since engineering economy requires optimizing the cost to benefit relationship, no definite detention time and COD:N ratio can be recommended without stipulating the requirement and condition for designing the plant. However, as

pointed out earlier, a 4-hour aeration period is the minimum detention time which this author would recommend. The need for pilot plant studies on the waste to be treated is thus apparent for optimizing the cost to benefit relationship. There is no one COD:N or BOD:N ratio that can be recommended for all cases. Considering the complexity of the wastes being treated by activated sludge processes, there is every reason to believe that pilot plant studies will be advocated increasingly before attempting to design a treatment facility. This is a healthy sign, because the era of using rule of thumb design criteria is hopefully now coming to a close.

It is felt that the present study has helped to define the range of COD:N ratios which should be studied when making pilot plant studies to obtain design and operational criteria for particular wastes.

The relationship between aerator biological solids concentration, amount of supplemental nitrogen and detention time in the aerator is shown in Figure 56. It is seen that an increase in supplemental nitrogen increases the concentration of biological solids at low detention times. This is expected, because low detention times (up to four hours) were found to be inadequate for satisfactory COD removal. Thus, a supply of additional nitrogen increased the purification efficiency in these otherwise inefficient systems to some extent, though not to a satisfactory level.

On the other hand, in the systems with 8 and 12-hour

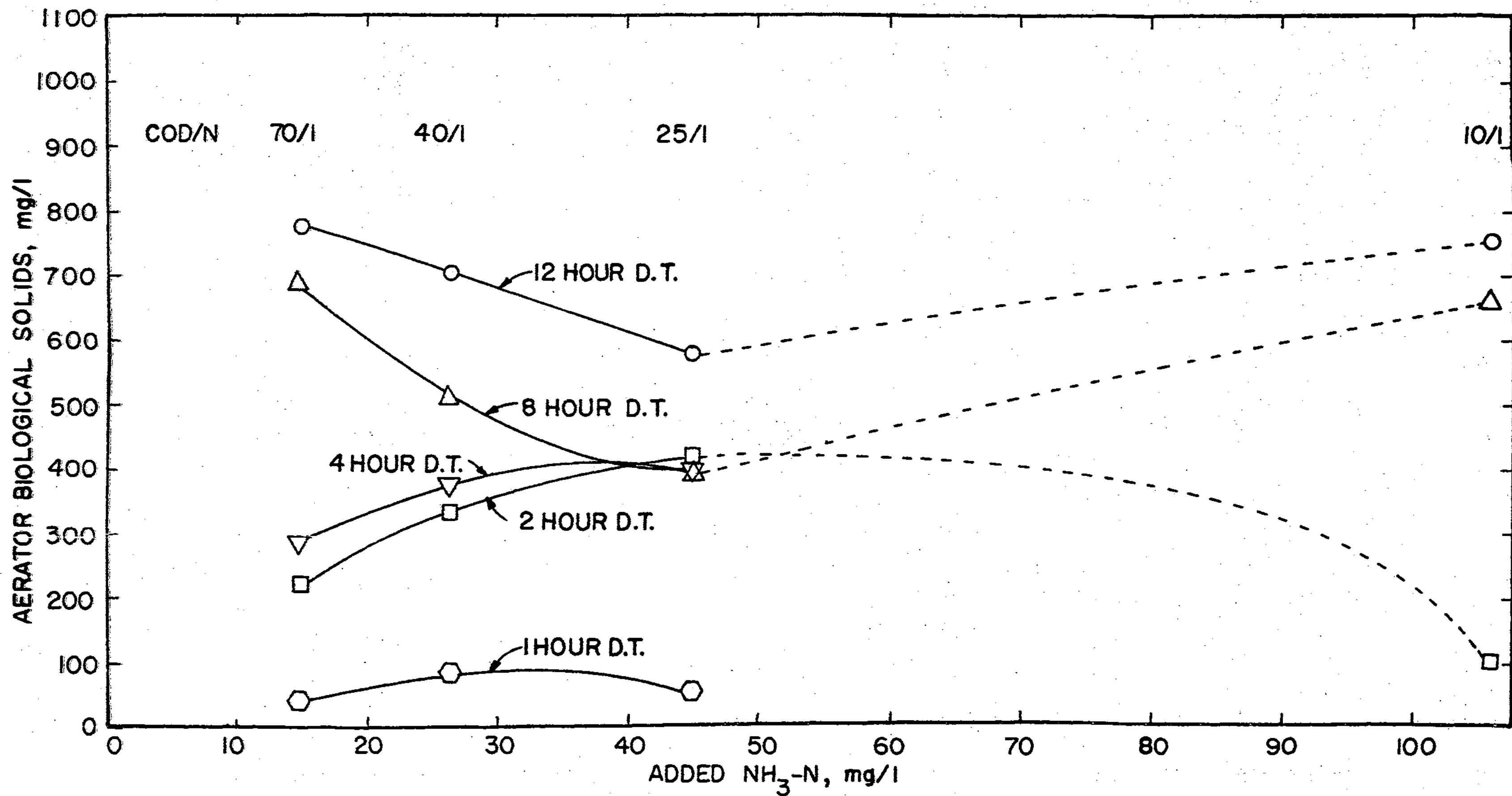


Figure 56. Relationship between supplemental nitrogen and mean biological solids concentration at different detention times.

detention times, the concentration of biological solids was higher at lower nitrogen levels. This is so because both of these systems were able to remove a large amount of organic matter by synthesizing carbohydrate, i.e., growth at low nitrogen levels was not balanced and consisted largely of carbohydrate. As pointed out earlier, glycogen synthesis is a mechanism for a "cheap" synthesis, i.e., cheap in the sense of energy utilization. At very low nitrogen levels (i.e., COD:N = 70:1) it is apparent that the carbohydrate was extracellular material and not an internal metabolic store of carbohydrate. Growth at higher nitrogen levels was more balanced and, therefore, more energy was required for sludge production. Utilization of a greater amount of energy for balanced growth thus reduced the total growth (weight basis) in these systems. Higher biological solids concentrations at lower nitrogen levels were also observed by Symons and McKinney (60) and Wilkinson (83).

From Table VII it is seen that there was no direct relationship between the biological solids concentration and the percentage of nitrogen utilization. It may be noted that the nitrogen escape in systems where the COD:N ratio was maintained at 25:1 ranged between 6.5 and 14.6 per cent of the added nitrogen as the detention time was increased from two to eight hours. However, the solids concentration remained essentially the same for these detention times. At the 12-hour aeration period the system

carried more biological solids, yet the nitrogen utilization was lower. It would seem logical that in a system in which the cells were growing slower, the need for nitrogen would be less. It is not known whether the extent of nitrogen utilization is different in systems with and without sludge recycle; i.e., it is not known whether the return of sludge, thereby maintaining solids levels in excess of those attained by growth, would allow for a greater nitrogen incorporation in the sludge mass. The effect of concentration of biological solids on the nitrogen requirements in systems where sludge recirculation is employed is not known, but would seem to be a logical subject of future investigation.

5. Release of Metabolic Intermediates and/or End Products

Larger amounts of metabolic intermediates and/or end products were present in the effluent at lower nitrogen levels. Also, the detention time seemed to have a direct effect on the release of metabolic intermediates; they were present in lower concentration at the higher detention times. It appears that very low detention times, e.g., two hours, do not provide adequate time for complete utilization of the carbon source in the waste; i.e., the microorganisms are not able to complete the process of full degradation of the substrate, and only partial oxidation takes place. Release of such metabolic intermediates and/or end products has been reported by several workers using pure cultures (73, 74) and heterogeneous populations (72). Krishnan (71) observed that volatile acids were released

under nitrogen-deficient shock loading conditions (predominantly acetic acid) and the results of the present study also support his finding. However, Krishnan (71) had observed small quantities of isobutyric, valeric, and hexanoic acids and had not found any ethanol. In the present study ethanol was also detected at 2-hour detention time at a COD:N ratio of 70:1. At higher nitrogen levels ethanol was not observed. Unlike the results of Krishnan (71), isobutyric, valeric, and hexanoic acis were not found in any sample.

The results of this study indicate that the metabolic intermediates and/or end products, other than acetic and propionic acids and ethanol, were also present because the total amount of intermediates, i.e., total COD minus anthrone COD, was found to be much in excess of the sum of compounds detected by gas chromatography using the poly pak column.

On the basis of paper chromatographic analysis, Bechir and Symons (68) indicated the possibility of release of glutarate in nitrogen-deficient systems in continuous flow. As only volatile acids were detectable in the column used in the gas-liquid chromatographs, no definite statement can be made regarding the presence or absence of glutarate in the systems studied in the present investigations.

6. Growth Parameters under Nitrogen-Limiting Conditions

a. Growth Rate

The data presented in Figures 20 through 22 and Table IV

showed that the concentration of nitrogen source affects the rate of growth. However, this effect was observed only at very low nitrogen concentrations. Nitrogen requirements for attainment of maximum growth rate, so far as ammonia nitrogen is concerned, seem to lie between 10 and 20 mg/l. Thus, provision of 20 mg/l NH_4^+ would ensure attainment of maximum growth rate insofar as nitrogen source is concerned. At nitrogen levels lower than this value, the rate of growth in some cases was retarded due to nitrogen limitation.

b. Total Biological Growth

The maximum biological solids production was seen to be close to 275 mg/l in all flasks (Figure 19) containing at least 20 mg/l NH_4^+, whereas it was found to be 22, 38, and 105 mg/l in flasks which contained intiaial nitrogen concentrations of 1, 2, and 10 mg/l. It thus appears that provision of 20 mg/l NH_4^+ for every 500 mg/l glucose ensured maximum biological growth. This corresponds to a COD:N ratio of 32:1. The presence of lower nitrogen concentrations than this caused restriction of total growth. This should not be confused with the critical nitrogen requirements from the point of view of removal of organic matter. It has been shown by Helmers, et al. (57) that satisfactory organic removal may be observed even when the nitrogen source concentration is lower than that needed for balanced growth. Also, Komolrit, Goel, and Gaudy (16) have shown that substrate removal can be effected without nitrogen being present in the medium.

c. <u>Removal of Organic Matter</u>

It can be seen from Table IV that the presence of 20 mg/l NH_4^+ for every 500 mg/l glucose ensured satisfactory purification efficiency in all studies. It is seen that the purification efficiency at 10 mg/l NH_4^+ was distinctly lower than that at 20 mg/l NH_4^+. The source of the seed did not seem to have any direct effect on the purification efficiency, since the purification efficiency was observed to be lower at 10 mg/l NH_4^+ as compared to 20 mg/l in all cases.

It is strange that in some cases the maximum organic removal was found to be 80 per cent or lower, even when the nitrogen source was present in excess. These experiments were terminated when the biological growth reached a steady value or started decreasing. It is possible that in these studies some oxidation of substrate took place after the cessation of growth.

d. <u>Utilization of Supplemental Nitrogen</u>

The ammonia analyses on the mixed liquor filtrates after the cessation of growth shows that almost all of the supplemental nitrogen was utilized when its initial concentration was 20 mg/l or below. It is seen from Table IV that at an NH_4^+ concentration of 30 mg/l, at least 5 mg/l NH_3-N remained after the cessation of growth. Therefore it seems logical to assume that growth in flasks containing at least 30 mg/l NH_4^+ for every 500 mg/l glucose was not nitrogen-limited. This nitrogen level corresponds to a COD:N ratio of 21:1. On the other hand, growth at lower nitrogen

concentrations may or may not be nitrogen-limited.

A survey of the literature (87)(85) reveals that the term "nitrogen-limiting" has been used without ever defining it. Herbert (87) used a medium in which the COD:N ratio amounted to 30:1, whereas Ecker and Lockhart (85) used a medium containing COD:N equal to 15:1. Both of these media were classified by the authors as nitrogen-limiting. It appears to be more logical to use the term "nitrogen-limiting" where the nitrogen source is exhausted before the carbon source. Such a definition would, however, require analysis of nitrogen and carbon sources at different times to see which one is eliminated first. This definition also fits with the results of Ecker and Lockhart (85). They found that at a COD:N ratio of 15:1 in the medium, the nitrogen source was exhausted from the medium before the carbon source. This medium would thus be classified as "nitrogen-limiting." It may be pointed out that when these authors used a medium in which nitrogen supplentation corresponded to a COD:N ratio equal to 5:1, part of the nitrogen remained in the medium even after all of the carbon source was exhausted. This medium would thus be classified as "carbon-limiting." In the present study almost complete removal of ammonia nitrogen was observed when its initial concentration was 10 mg/l or less; whereas in flasks containing an intitial concentration of 20 mg/l NH_4^+, ammonia nitrogen remained in the medium after the carbon source was exhausted. It would thus appear that media containing 10

and 20 mg/l NH_4^+ for every 500 mg/l glucose were nitrogen-limiting and carbon-limiting, respectively. This is substantiated by the fact that the biological growth at a nitrogen level of 10 mg/l NH_4^+ was lower than that observed at 20 mg/l NH_4^+.

B. Effect of Nitrogen Levels in Batch Operated Activated Sludge Systems

The results of this study showed that "old cells" acclimated to low nitrogen levels are capable of purifying waste. However, the time required to do so seems to be affected by the nitrogen levels maintained. To lower the COD from approximately 2000 mg/l to 150 mg/l, the 10:1, 25:1, 40:1, 55:1, and 70:1 systems required 1.7, 1.7, 7.0, 13.2, and 15.0 hours, respectively. However, use of aeration periods as long as 13 hours or more is not very common in water pollution control plants. A 6-hour detention time is more commonly used. Figure 23 shows that these systems contained COD's of 125, 95, 265, 620, and 850 mg/l at the end of six hours' aeration, yielding biochemical purification efficiencies of 94, 95, 87, 69, and 57 per cent, respectively. These results show that satisfactory COD removal can be obtained in a 6-hour aeration period using reasonably low initial solids levels if the medium contains a nitrogen source corresponding to a COD:N ratio of 40:1.

As observed in the continuous flow experiments, the amount of nitrogen supplementation can be reduced by

increasing the aeration period. It is possible, therefore, that the nitrogen requirements, i.e., COD:N ratio, for satisfactory COD removal in extended aeration systems may be lower than 70:1. This remark, based on the results of this study, thus seems to support the suggestion of Simpson (63), who advocated a COD:N ratio of 90:1 in extended aeration systems for satisfactory organic removal. Symons and McKinney (60) had also observed satisfactory COD removal in systems with a COD:N ratio of 85:1, when long aeration periods and high biological solids concentrations were employed.

Phase II

A Modification of the Activated Sludge Process for Nitrogen-Deficient Wastes

A. Batch Experiment

The results of this experiment show that waste consisting of acetic acid (which is a component of various industrial wastes) can be treated biologically by a process involving the modified flow sheet shown in Figure 1.

A comparison of results using glucose, presented elsewhere (16), and acetic acid in the present study, shows that in the feeding phase the yield of biological solids was lower in the acetic acid system. Low sludge yields such as those observed in this experiment are frequently observed for fatty acids (99). The carbohydrate content of cells grown on acetic acids was found to be lower than for

cells grown on glucose. The protein content of cells grown on acetic acid was found to be higher than for cells grown on glucose.

The results observed for the endogenous phase of the process show that the chief storage product is not carbohydrate. It seems possible that poly-β-hydroxybutyrate may have been the chief storage product, which was partially converted to protein when the cells were allowed to metabolize endogenously in the presence of nitrogen. The possibility of such a storage product is indicated in the literature (111)(126)(127)(128).

A comparison between the rate of substrate removal in the initial feeding phase and the nonproliferating refeeding phase shows that the regenerated sludge was approximately 60 per cent as efficient as the original cells. This comparison is based on the assumption that rate removal is directly proportional to the initial biological solids concentration. However, the comparison between systems A and C (Figure 26) shows that, for approximately the same initial biological solids concentration, the regenerated sludge was as efficient as the sludge which received nitrogen in the feeding and endogenous phases. The poor COD removal in system D shows that the addition of nitrogen in the endogenous phase helped greatly to regenerate the sludge which initially underwent a nonproliferating feeding phase.

B. Continuous Flow Experiments

1. Glucose

The results of this study show that the flow sheet proposed earlier by Komolrit, Goel, and Gaudy (16) is operationally feasible for treatment of a synthetic waste containing glucose as the sole carbon source. The system provided excellent purification efficiencies at a COD:N ratio as high as 70:1. It is possible that the system may be successfully operated at even lower nitrogen levels.

Operation at different nitrogen levels revealed several interesting features of the system. The settling characteristics of the sludge became poorer as the nitrogen supplementation was decreased. This observation is substantiated by the fact that the COD contribution of the biological solids which did not settle in the settling tank and escaped in the effluent amounted to 28 mg/l at a COD:N of 10:1, whereas it was 55 mg/l at a COD:N ratio of 70:1. Thus, the optimum COD:N ratio may be determined in part by the settling characteristics of the sludge. This may not be a serious consideration in the present study, because the settling capability of the sludge at the COD:N ratio of 70:1 was fairly good.

The sludge yield was found to decrease from 0.80 to 0.258 as the nitrogen level was reduced. Thus, a greater portion of the substrate was oxidized at lower nitrogen levels in this system. It is not known whether sludge yield would decrease further at still lower nitrogen levels.

A reduction in the sludge yield would seem to be one advantageous aspect of the process, since it reduces the load to the sludge digestor. Sludge wasting amounted to only 17 per cent at the COD:N ratio of 70:1. Thus, as the nitrogen level is reduced, the process moves closer to a total oxidation process.

In the absence of nitrate or nitrite analyses it is not possible to make a rigid nitrogen balance. However, approximate calculations show that, at low nitrogen levels, almost all of the nitrogen was used up in the synthesis of protein and nucleic acids. This possibility of leakage of a part of the nitrogen as nitrate or nitrite is very remote, due to the relatively short detention time employed in the aeration tank.

The protein content of the sludge in the feeding aerator was found to decrease as the amount of nitrogen supplementation was decreased. However, the protein content of the recycled sludge, i.e., after intracellular transformations, was essentially the same at all nitrogen levels.

The carbohydrate content of the sludge in the feeding aerator was found to increase as the nitrogen level in the system was decreased. After reactions in the endogenous aerator, the sludge contained a lower fraction of carbohydrate. The carbohydrate content of the recycled sludge was found to be approximately 20 per cent of the dry weight of the sludge in systems with COD:N equal to 10:1, 30:1, and 40:1, whereas it was approximately 25 per cent in the

systems operated with COD:N ratios equal to 50:1 and 70:1.

The results of the "turnover" experiment indicate that a detention time of nine hours or less is sufficient for the intracellular turnover to protein.

2. Acetic Acid

The high degree of purification obtained at a COD:N ratio of 70:1 using acetic acid as substrate shows that the process affords an excellent method for treatment of non-carbohydrate waste. However, it was seen that the degree of purification obtained was slightly lower (by 3 per cent) than that observed for the glucose system. This may be due to maintenance of lower biological solids in the aerator.

The settling characteristics of the sludge in the glucose and acetic acid systems were comparable. For the acetic acid system, good settling was observed even at a COD:N ratio of 70:1.

The yield of biological solids in the acetic acid systems did not decrease at lower nitrogen levels. Sludge yields are usually found to be lower on acetic acid than on glucose in carbon-limited systems (99). This point is also illustrated in these studies since the sludge yield for carbohydrate wastes was about twice that of the acetic acid system when the system was supplied with nitrogen corresponding to a COD:N ratio of 30:1.

The cell composition data show that the protein content of sludge in the feeding aerator grown on acetic acid was higher than that grown on glucose, whereas the carbohydrate

content was essentially the same in both systems. However, a significant difference between the glucose and acetic acid systems is that the carbohydrate content of the sludge decreased in the endogenous aerator in glucose systems, whereas it remained practically unchanged in the acetic acid systems. This result indicates that the increase in protein content in the endogenous phase in the glucose system is due to a reduction in carbohydrate content, whereas in acetic acid systems it is due to reduction of some other storage compound, i.e., apparently carbohydrate was not the carbon storage product. In the absence of lipid analysis, it cannot be definitely concluded that the increase in protein content was due to a reduction of lipids. However, since lipid would be the only other major class of storage compounds, it would appear that lipid was stored by the cells.

The ammonia analysis shows that both the glucose and acetic acid systems produced final effluents devoid of any ammonia when supplied nitrogen at a COD:N ratio of 70:1. However, at a COD:N ratio of 50:1, some differences in the glucose and acetic acid systems were observed. Where acetic acid was the carbon source, nitrogen was not utilized to the full extent, and considerable amounts of nitrogen were carried over to the feeding aerator along with the recycled sludge. Even the final effluent contained a little ammonia nitrogen.

The results of the "turnover" experiment show that the system could be operated by reducing the aeration period in

the endogenous aerator to approximately nine hours.

No effort was made in these continuous flow studies to optimize the design parameters. Further work is needed to examine the feasibility of reducing the aeration period in the feeding aerator. Some of the factors affecting nitrogen requirements are discussed elsewhere in this dissertation. Similar studies are required to obtain design parameters.

CHAPTER VII

SUMMARY AND CONCLUSIONS

A. Nitrogen Requirements in Completely-Mixed Continuous Flow Activated Sludge Systems

1. The nitrogen requirement in a completely-mixed activated sludge process without sludge recycle is dependent on the detention time employed. To attain the same biochemical purification of wastes, nitrogen supplementation can be reduced by providing longer detention times. Eighty-five per cent COD removal can be obtained by providing nitrogen equivalent to COD:N ratios of 70:1, 45:1, and 35:1 with 12, 8, and 4-hour aeration times, respectively.

2. All supplemental nitrogen was consumed in systems with COD:N equal to 70:1. In 40:1 systems, approximately 2 mg/l of ammonia nitrogen was present in the final effluent. At 25:1, the effluent contained from 2.7 to 8.6 mg/l NH_3-N; this increased as detention time increased.

3. Addition of nitrogen supplementation in excess of a COD:N ratio of 40:1 does not seem warranted. The nitrogen supplementation may actually be reduced to a level lower than 40:1, where leakage of nitrogen in the final effluent is not permissible. In such cases longer

detention times would be required.

4. The amounts of solids produced, as well as the amount of COD removed, per unit of nitrogen utilized were found to be higher at higher detention times at a given nitrogen level in the feed. Also, at a given detention time both biological solids concentration and COD removal increased when the nitrogen supplementation was increased.

5. Cell yield is independent of the detention time but appears to be high in systems with low nitrogen content.

6. At a fixed nitrogen level the carbohydrate content of the sludge was higher at low detention times. At a fixed detention time the carbohydrate content was higher in systems with low nitrogen supplementation than in systems which contained a higher level of nitrogen.

7. At a particular nitrogen level the protein content of the sludge was higher at lower detention times. Also, at a given detention time the protein content was usually higher in systems with higher nitrogen supplementation.

8. In systems containing nitrogen supplementation equivalent to COD:N ratios of 70:1 and 40:1, the concentration of protein in the reactor was the same for all detention times employed.

9. Sludges with a protein content as low as 11.5 per cent of the dry weight were able to purify the wastes satisfactorily.

10. The amount of metabolic intermediates and/or end products was higher in low nitrogen systems. Acetic acid

was the chief volatile acid detected in the mixed liquor filtrates.

B. Effects of Nitrogen Levels on Growth Parameters

1. The concentration of nitrogen source affects not only the total biological growth, but also the rate of growth.

2. For cells harvested from continuous flow units operated with a COD:N of 40:1 in the feed, provision of 20 mg/l NH_4^+ in shaker flasks ensured maximum growth rate insofar as nitrogen source was concerned; whereas for cells harvested from the continuous flow units operated with a COD:N ratio of 25:1, the nitrogen requirement for attainment of μ_{max} corresponds to 10 mg/l NH_4^+. Thus, it is concluded that NH_4^+ concentration of 20 mg/l would ensure attainment of μ_{max}.

3. Provision of nitrogen supplementation corresponding to a COD:N ratio of 32:1 ensures full biological growth of microorganisms and attainment of full substrate removal during growth. Presence of lower nitrogen concentrations (from 32:1 to a lower level) imposed a restriction on growth and substrate removal.

4. All supplemental nitrogen was utilized for growth in flasks containing nitrogen corresponding to a COD:N ratio of 32:1. In flasks with higher nitrogen supplementation, ammonia nitrogen was detected even after cessation of growth.

5. The μ_{max} with respect to nitrogen source seems to be independent of the source of the seed. It did not matter

whether the seed was taken from a continuous flow unit with low or high nitrogen supplementation, or from a unit operated at high or low detention time.

C. Nitrogen Requirements in Batch-Operated Activated Sludge Systems

1. "Old cells" acclimated to low nitrogen levels (even up to a COD:N ratio of 70:1) are able to purify the waste to a reasonably satisfactory level. The time required for purification depended upon the nitrogen supplementation. Systems operated with COD:N ratios of 10:1, 25:1, 40:1, 55:1, and 70:1 required 1.7, 1.7, 7.0, 13.2, and 15.0 hours for 92 per cent COD removal.

2. At the COD and biological solids levels employed in these studies (i.e., approximately a COD/biological solids of 1.5) linear biological growth was observed in all systems. The biological growth accounted for 33-48 per cent of the COD removed.

D. A Modification of the Activated Sludge Process for Nitrogen-Deficient Wastes

1. Based upon batch studies to examine the mechanistic feasibility and continuous flow studies to investigate the operational feasibility, it is concluded that wastes containing acetic acid and glucose can be successfully treated by the activated sludge process modification proposed earlier by Komolrit, Goel, and Gaudy (16) at savings in the cost of nitrogen supplementation and without leakage of any nitrogen in the final effluent. It has also been shown

that this new process can treat actual industrial wastes (131). Thus, the earlier prediction by Komolrit, Goel, and Gaudy (16) has been proven to be true. On the basis of the diversity of wastes employed in various studies, it is believed that this process modification can be employed for all nitrogen-deficient wastes amenable to biological treatment.

2. Lower sludge yield and cell carbohydrate content as well as higher protein content were characteristic of sludges developed on acetic acid as substrate as compared to the sludges developed on glucose.

3. The sludge yield was found to decrease as the nitrogen level was reduced, thus reducing the amount of sludge and possibly the load on sludge disposal facilities.

4. The protein content of the cells in the glucose system decreased as the COD:N ratio was increased. However, the carbohydrate content increased.

5. A detention time of nine hours or less is sufficient for the intracellular turnover of stored products to protein.

CHAPTER VIII

SUGGESTIONS FOR FUTURE WORK

Based on the results of this investigation, the following suggestions are offered for future work:

1. Studies including sludge recirculation employing various COD:N ratios would be valuable in order to determine whether the concentration of biological solids has any effect on the nitrogen requirement for optimum COD removal or attainment of μ_{max}.

2. Further studies at high detention time and low nitrogen levels (e.g., COD:N = 70:1 and D = 1/12) are needed to obtain more precise data on the chemical composition of cells. In the present studies 50 per cent or more of the cell weight consisted of compounds other than carbohydrates and protein.

3. In order to arrive at a more rigid nitrogen balance, future work should include analysis of total nitrogen content of the cells, and analysis of ammonia, nitrite, nitrate and organic nitrogen in the effluent. In view of the short detention times involved in the present studies, the chances for nitrification to take place were remote.

4. Studies to examine the possible relationship

between sludge composition and number of viable cells under conditions of nitrogen deficiency could provide valuable data, since it was seen from the present study that sludge yield and protein content are n o t good criteria upon which to base a judgment as to the substrate removal capability of the microbial population.

5. Studies are needed to determine the nature of other metabolic intermediates and/or end products which may be produced during metabolism at various nitrogen levels. In the present study only volatile acids were determined.

6. In view of the fact that considerable attention is being focused on the discharge of phosphates into receiving streams, it would be desirable to study the phosphorous requirements in heterogeneous cultures under completely-mixed conditions since it may be possible to operate a system successfully at COD:P ratios lower than are presently used in the field.

7. Pilot plant studies on the modified activated sludge process are required to gain knowledge regarding different design parameters, such as detention times in the feeding and endogenous aerators, and biological solids concentration in the recycled sludge.

8. Research is needed on various whole wastes to examine the spectrum of industrial wastes that can be successfully treated by the new process.

9. The feasibility of treatment of wastes at nitrogen levels lower than those provided by a COD:N ratio of

70:1 should be investigated, since the present studies show that the wastes can be treated successfully at a COD:N ratio of 70:1.

10. Studies should be conducted to determine whether the excess sludge from the new process is readily digested.

11. It would be interesting to examine the possibility of a similar mode of operation for phosphorous-deficient wastes. It may be possible that the phosphorous requirement can be reduced using the modified process.

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VITA

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Membership in Professional Societies: Water Pollution Control Federation, American Society of Civil Engineers, and American Water Works Association.

Publications: Author and co-author of the following technical publications concerning water pollution control problems:

1. Komolrit, K., Goel, K. C., and Gaudy, A. F. Jr., "Regulation of Exogenous Nitrogen Supply and Its Possible Applications to the Activated Sludge Process," Journal Water Pollution Control Federation, 39, 251 (February, 1967).

2. Goel, K. C., Gaudy, A. F. Jr, and Komolrit, K., "A New Approach to Treatment of Nitrogen-Deficient Wastes," Oklahoma Academy of Science Meeting, Stillwater, Oklahoma (December, 1965).

3. Gaudy, A. F. Jr., Goel, K. C., and Freedman, A. J., "Activated Sludge Process Modification for Nitrogen-Deficient Wastes," Proceedings, Fourth International Conference on Water Pollution Research, Prague, Czechoslovakia (August, 1968).