THE DEVELOPMENT OF PROCESS KINETICS FOR A WASTE TREATMENT SYSTEM UTILIZING FILAMENTOUS MICROORGANISMS

by

H. Randall Edwards

Thesis submitted to the Graduate Faculty of the

Virginia Polytechnic Institute

in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

in

Civil Engineering

APPROVED:

Dr. C. W. Randall, Chairman

Dr. P. H. King

Dr. R. E. Benoit

Dr. J. M. Wiggert

Dr. H. M. Morris

April, 1970

Blacksburg, Virginia

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ACKNOWLEDGEMENTS

The author is deeply indebted for the assistance and advice from his research advisor, Dr. Clifford W. Randall; to Dr. Paul H. King for his encouragement offered during the months of study; to the rest of the staff of the Sanitary Engineering Department for their interest; to Mr. E. G. Willard for his aid in equipment and material procurement; to Mr. Jerry Harmon and Mr. Bob Armstrong for their untiring assistance in the laboratory; to my colleagues who found time to lend a helping hand and encouragement; to Wytheville Community College for the use of laboratory space and leave time for research; to Dr. Arnold Wirtala for his patience in waiting for the author to move to his new position; and to Mrs. Julia Grubb whose efforts make this publication possible.

A debt of gratitude is also extended to my wonderful wife for her willing sacrifice and generous encouragement.

CHAPTER I

INTRODUCTION

For several years, sanitary engineers and microbiologists have recognized that filamentous microorganisms exhibit certain traits which potentially make them more efficient than typical activated sludge microorganisms for the treatment of soluble organic wastes (2, 12, 24, 27, 35). As an example, filamentous microorganisms have a greater surface area per unit mass than the conventional activated sludge flocs and therefore can remove organics from the solution more efficiently (2). This higher degree of efficiency can potentially reduce the required volume for the treatment of high strength BOD wastes. It is well known that filamentous organisms require smaller quantities of nutrients than the predominant activated sludge organisms, especially with respect to nitrogen (28). Therefore, filamentous organisms are capable of treating wastes that would normally be classified as nutrient deficient. Recent studies have shown that filamentous organisms can effectively stabilize organics over a pH range as low as 2.5 or as high as 9.5 (4, 13, 25, 35). Consequently, a process using these organisms should be able to treat high or low pH wastes with little or no neutralization. Further, it has been observed that filamentous organisms will typically produce a higher quality supernatant

from the treatment of some wastes than can be obtained under non-bulking conditions (4, 12, 25, 35).

The potential advantages of using a filamentous system for a treatment process seem readily apparent when it is recalled that a major cause of operational difficulty with the activated sludge process is sludge bulking. In most instances, sludge bulking occurs when environmental conditions favor the growth of filaments and they outgrow the normal organisms (24, 27, 33, 38). Activated sludge bulks when the nitrogen concentration is low, when the BOD loading is too high, when there is a drastic change in pH, etc. (2, 4, 13, 24, 25, 28, 33, 35, 38, 39). Therefore, a system using filamentous microorganisms would be less subject to upsets from such environmental changes.

Even though it has been noted that the use of filamentous organisms for the treatment of wastewaters potentially has several advantages over the use of activated sludge, they have not been utilized to any extent for waste treatment. A conventional process using filamentous organisms has one great disadvantage, it is extremely difficult to remove the biological solids from the treated effluent after stabilization of the organic content of the waste has been accomplished. Therefore, it is virtually impossible to obtain a clarified effluent or to thicken bulked sludge for solids return (24, 35).

The objective of this research project is to study and define the capabilities of a biological waste treatment system specifically designed to use attached or filamentous microorganisms for the stabilization of organic wastes. The main unit of this system consists of a rectangular shaped plexiglass tank in which vertical plastic screens have been suspended perpendicular to the waste flow. The microorganisms, using the screen as a support media, attach themselves to the screens. The flow of the waste is such that the flow passes through, as well as under the screens. Treatment occurs on the screens as well as in the media where attached filamentous organisms extend. Unattached organisms are washed from the system. No recirculation of organisms is required because reinoculation occurs from the biological mass on the screens. Air, which is supplied by diffusors along the bottom of the unit, adds the needed mixing as well as the required oxygen. To produce a clarified effluent, it is necessary to settle or otherwise remove those sludge clumps that slough off the screens.

CHAPTER II

LITERATURE REVIEW

The literature concerning the properties and use of filamentous or attached microorganisms in the treatment of organic wastes is very limited. The following literature review is an attempt to present a review of the most pertinent articles concerning attached or filamentous microorganisms found in polluted waters and the occurrence of these organisms in biological waste treatment processes.

Filamentous Microorganisms of Polluted Water

A few species of microorganisms form massive colonies in organically polluted water. These can be seen with the naked eye and are referred to in the literature as "sewage fungus." Only some of the organisms involved are, strictly speaking, fungi; some are colonial bacteria and others are animals (23).

Hynes (23) stated that the most important filamentous organism is the sheath-bacterium Sphaerotilus natans, in which the individual cylindrical bacterial cells are embedded end to end in a slimy gelatinous filament. The growths have various forms, the filaments adhering to one

another in different ways or the cells differing slightly in appearance. One form appears to branch and has received the name Cladothrix dichotoma. The common iron bacterium Leptothrix ochracea is also a member of the family of S. natans. In the commonest form the filaments are free and unbranched; aggregations of filaments occur rather rarely, and only when conditions for growth are not good do branched forms appear. Then the cells may become rather indistinct. Increase in length of the filaments is by simple division, and new growths are produced by cells which break out of the sheath and acquire flagella. The cells then swim away and settle down to form new filaments. The species grows satisfactorily only in running water, probably because there it is kept free of other bacteria which tend to smother it in still water. Otherwise it is very tolerant and grows over a wide range of pH and temperature. S. natans is definitely aerobic, but will grow in very low oxygen-concentrations even though its growth is somewhat retarded. It is sensitive to salt concentrations of more than about 300 mg./l, and therefore, is unable to grow in brackish water. It is a heterotroph with a preference for carbohydrates. Nitrogen can be obtained from both organic and inorganic sources. Thus, it thrives where there are amino-acids resulting from protein breakdown, especially where these are mixed with carbohydrates as occurs below sugar factories, breweries, and dairies. S. natans grows most rapidly at fairly high temperatures

(25 - 30°C.), but can also grow well at temperatures as low as 6°C. Thus it tends to be more moticable in winter when it not only extends further downstream because of the slower decay of the organic matter, but is also largely free of competition for foodstuffs from other bacteria, which are more affected by the cold (18, 23).

Where conditions for growth are particularly good, as, for instance, in sewage filters or near to an effluent outfall, another growth-form in which the cells are distributed at random in a branched mass of jelly, from which ordinary filaments sometimes project is produced (18, 23, 33, 38, 39). This is known as Zoogloea ramigera, and it often occurs together with massive Zoogloea colonies in a cumulus cloud-like form. The latter type of growth is the one usually found in sewage filters, where it is the most abundant microorganism (7). This variation in growth-form indicates that the name Zoogloea probably embraces a complex of different types of bacteria (8).

Beggiatoa alba, one of the sulphur bacteria, has been identified as another constituent of sewage fungus (23). It forms a brittle white film on deposits of sludge and is common in the lower regions of bacterial filters (18). The colonies consist of unbranched filaments of cells in which highly refractive deposits of sulphur can often be seen, particularly in older specimens. It occurs where hydrogen sulphide and oxygen are both present and oxidize the former to the element sulphur. It is

therefore found only where reduction of sulfite to hydrogen sulfide occurs near a source of oxygen, that is, for instance, on the mud surfaces which are the boundaries between reduced and oxygenated conditions. Unlike Sphaerotilus it is tolerant of salt, and it is more common in brackish water than in fresh water, probably because sea water is very rich in sulphates (23).

Apodya lactea, often also known as Leptomitis lacteus, a true fungus and a member of the Phycomycetes, is occasionally an important constituent of sewage fungus. Its growths resemble those of Sphaerotilus, but they are not slimy and are more like cotton-wool (18, 23). Under the microscope the filaments, or hyphae, can be seen to be much wider than those of Sphaerotilus and they are constricted at intervals. Apodya occurs where there is an ample supply of oxygen, calcium and nitrogenous organic matter of high molecular weight. It is therefore commonest where there is a very great dilution with hard water (18, 23). Below wood-pulping factories it often largely replaces Sphaerotilus and, like Sphaerotilus, it is more obvious in winter (23). This is possibly because when the water is cold the rate of decay of the organic matter is slower and the oxygen content higher. Conditions are therefore more favorable for Apodya, and it is able to compete successfully with Sphaerotilus (23).

Fusarium (Nectria) aqueductum, an Ascomycete, may be found in sewage fungus but it is much less common than Sphaerotilus (23). Its hyphae are divided into cells by transverse walls, and they sometimes

end in crescent-shaped spores. Macroscopically the colonies resemble the other species, but they are brick-red rather than white or greyish. This species seems to favor rather acid waters, and it grows only where there is a good supply of oxygen; its occurrence in sewage-polluted water is therefore rare, and it could be loosely described as an "industrial sewage-fungus" (18, 23).

A few other species of true fungi also occur in organically polluted water, including species of Mucor, Geotrichum, and Penicillium, but they do not often form massive growths as do the species described previously (18, 23, 24).

The last important constituents of sewage fungus are animals, colonial bell-animalcules of the genus Carchesium and occasionally also Epistylis (23). In organically polluted water, particularly where there is a large population of bacteria together with a good supply of oxygen, these little Protozoa form large white colonies, which may be 2-3 mm. long, resembling the tufts of Sphaerotilus, with which they are often mixed. They cannot withstand total de-oxygenation, and they are usually most abundant at the lower end of the sewage-fungus zone (23).

None of these organisms are confined to polluted water; they all occur elsewhere under quite natural conditions, but the presence of organic pollution affords them opportunities for massive development. Given the right conditions for growth they appear with great rapidity (23).

Occurrence of Filamentous Microorganisms in Biological Waste Treatment Processes

Bulking of sludge has always been a problem with the activated sludge process and research efforts to gain an understanding of the problem began soon after the system came into general use (28, 33, 39). From the early studies it was soon established that large numbers of filamentous organisms, particularly Sphaerotilus natans, were present when bulking occurred and that the condition could be induced by using high carbohydrate wastes (33, 39). Lackey and Wattie (28) showed that the mineral requirements of Sphaerotilus, i.e., the amount of nitrogen and phosphorus needed per unit BOD assimilated, are low compared to typical flocculating bacteria and this enables them to outgrow the other organisms when the ratio of carbohydrates to nutrients is high. More recently, research investigators have implicated filamentous growths of a fungal type such as Geotrichum, and Jones (24) is of the opinion that Geotrichum rather than Sphaerotilus is responsible for sludge bulking under all conditions. The predominant opinion, however, is that expressed by Bhatla (2). He feels that the situation is more complex and that carbohydrate bulking is caused by bacterial filaments such as Sphaerotilus which are retarded by low oxygen tension, whereas noncarbohydrate bulking is associated with fungal filaments such as Geotrichum that are more efficient than normal activated sludge under oxygen deficient conditions. In a recent study, Pasveer (34) proved that E. coli developed filamentous growth in an

oxidation ditch. He concluded that the conditions required for thread formation of E. coli were those in which wastewater is supplied continuously to activated sludge in a complete-mixing situation, and sufficient oxygen is supplied for the simultaneous oxidation of the carbon and nitrogen compounds. He also implied that filamentous growths of E. coli have sometimes been mistaken for growths of Sphaerotilus in activated sludge under bulking conditions.

Although the early investigators defined the properties of filamentous microorganisms associated with waste treatment, all research aims were toward the prevention of the growth of filaments in activated sludge, a trend that still predominates the literature (24, 34, 35). Only in recent years has the utilization of such organisms for waste treatment been seriously considered and the actual studies have been few. Pipes and Jones (35) studied the ability of filamentous cultures, particularly Sphaerotilus species, to decompose organic wastes. Significantly, they found that Geotrichum candidum produced approximately equivalent growths at pH values ranging from 3 to 9. Brower and Gaddis (4) studied the stabilization of wastes by filamentous systems at low pH and they found that effective treatment was accomplished at a pH value as low as 2.5. Bhatla (2), while studying the treatment of papermill wastes, reported that filamentous sludge produced a better quality supernatant than that obtained with non-bulking sludge. He attributed the improved removal performance to the ability of filamentous organisms to expose greater surface area to the liquid medium and, thus, extract food

more efficiently. However, the authors mentioned above made no attempt to develop a practical system utilizing filamentous microorganisms. In fact, in the latter two cases, while the authors were impressed by the potential of the filaments for waste treatment purposes, they reported that they knew no practical way to utilize them because of solids separation difficulties.

Several researchers (18, 23) have studied bacteria found in trickling filters, and have reported the presence of zöogleal bacteria closely related to those found in activated sludge. Beside zöogleal bacteria, filamentous forms such as Sphaerotilus and Beggiatoa were also present.

Fungi are of a more common occurrence in trickling filters than in activated sludge (18, 23). With domestic sewage, bacteria usually predominate, but with introduction of industrial wastes, fungi become dominate, especially in the upper part of the filter (18). Numerous fungi have been isolated from trickling filters but the following are probably the most important, Fusarium aqueductum, Oospora (Geotrichum), Sepedonium, Ascoidea rubescens, Subbaromyces splenders, Sporotrichum, and Penicillium (18, 23). Those with tenacious holdfasts such as Fusarium and Geotrichum were found to be the first to colonize the stones and form a basis for the subsequent establishment of such forms as Sepedonium and Ascoideo (18).

Probably the initial attempt to extensively use filamentous organisms in the treatment of organic wastes was the contact aeration process (45). In this process settled sewage passed between stationary contact media of cement-asbestos plates on which a biological film developed. An aerobic condition was maintained by blowing air through the contact section from perforated pipe mounted below the plates. Submerged aerobic biological life utilized and stabilized the sewage. Since the treatment was quite sensitive to loading changes and operating conditions, proper operation of settling tanks and digesters was essential (45). Treatment action was comparable to the trickling filter where biological life forms on the stones instead of plates. Like the activated-sludge process, biological life was also suspended in the liquor which was kept aerobic by air blown in or mechanically entrained at the tank surface. Dissolved oxygen had to be kept above a minimum value of 1.0 part per million in the primary aerator and above 3.0 parts per million in the secondary aerator effluent (45). Organisms in a properly operated contact aerator were quite similar to those found in trickling filter film and identical with those of activated sludge (45, 49). Improperly functioning units contained many organisms commonly found in digesters or other anaerobic processes (45). Many more sulfur bacteria were usually present in the contact aerator than in most other secondary treatment units. When the dissolved oxygen disappeared and an anaerobic condition developed, the aerators produced a

considerable amount of hydrogen sulfide. Experience in some installations showed that contact aeration plants usually functioned satisfactorily, without recirculation, at 60 percent or less of the design loading, although hydrogen sulfide odors occasionally persisted even below this loading. Recirculation from the effluent channel of the second stage aerator of the inlet of the first stage aerator usually eliminated odors and produced a high degree of treatment at full loading (45, 49).

The most significant and most successful study using filamentous microorganisms that has been conducted was performed by Kato and Sekikawa (25) in Yokohama, Japan. When attempts to treat a nitrogen deficient, alkaline, soft drink waste with activated sludge failed because of bulking, they devised a practical system that provided successful treatment. Besides being nitrogen deficient to the extent that it could not be treated by activated sludge, the waste had a pH of 9.5. Nevertheless, treatment comparable to that normally obtained by an activated sludge plant was accomplished by their process, which they termed "fixed activated sludge (FAS)," without nutrient addition or neutralization. Further studies showed that the FAS process was capable of stabilizing other types of wastes such as octanol plant waste, isobutanol and octanol plant waste, and domestic sewage. The latter studies were designed solely to demonstrate the amenability of various organic compounds to FAS treatment and therefore the wastes were neutralized and nutrients were added before

treatment began. The dissolved oxygen in the growth chamber was kept above 1 ppm. The total air requirement was calculated to be 40 m^3/Kg - BOD for removal for a submerged depth of 3 meters. This total air requirement corresponded favorably with that in the usual activated sludge process and was less than that in the contact aeration process. While Kato and Sekikawa showed that the FAS process was capable of stabilizing various types of wastes and of treating a nitrogen deficient waste, they did not fully define the system in terms of operational capabilities such as BOD loading, pH range of effective treatment, etc. Superficially their system appears to be similar to the contact aeration process, but, in actuality, the use of flow-through screens instead of asbestos cement panels has the advantages of the contact aeration process without its disadvantages, and the microbial environment is considerably different. The microorganisms of Kato and Sekikawa's system are aerobic while the contact aeration process had many anaerobic organisms present near the plates which caused odors to develop.

In summation, the literature shows that filamentous microorganisms are capable of treating organic wastes and that filaments have several properties that potentially give them an advantage over flocculating bacteria for several types of treatment conditions. While the properties have been known for a long time, utilization of filaments has been hampered by the lack of a practical treatment system. A potentially practical system has been devised by Kato and Sekikawa (25). and it should be studied and evaluated.

CHAPTER III

KINETICS OF BIOLOGICAL TREATMENT

When organic matter is removed from solution by microorganisms, two basic phenomena occur, oxygen is consumed for energy, and new cell mass is synthesized. The organisms also undergo progressive autoxidation of their cellular mass. These reactions can be illustrated by the following general equations:

$$\text{Organic matter} + O_2 + NH_3 \xrightarrow{\text{cells}} \text{new cells} + CO_2 + H_2O \qquad (1)$$

and

$$\text{Cells} + O_2 \longrightarrow CO_2 + H_2O + NH_3 \qquad (2)$$

Sludge Synthesis and Oxidation

For each specific steady state condition, a constant mass of biological cells will be synthesized per unit weight of organic matter removed (expressed on a total oxygen demand basis such as COD). One-third of this ultimate oxygen demand (COD) is used for energy and two-thirds is used for synthesis (29). Thus, if an oxygen-to-cellular volatile solids conversion of 0.7 g VSS/g O_2 is used, 0.47 g VSS (volatile suspended solids) will be synthesized for each gram of COD removed (6). In actual

experimentation, Busch (3) showed that total synthesis from glucose is 0.44 grams of cells per gram of COD removed. Similarly, Porges, et al., (37) found that synthesis from lactose is 0.43 g VSS per g COD removed. Sawyer (42) and Gellman and Heukelekian (16) used sewage and several industrial wastes as substrates and reported that 0.5 g VSS is synthesized per gram of BOD_5 removed. Variations in this value on a BOD_5 basis have generally been attributed to endogenous respiration effects. Such variations may also be caused by changes in the growth rate of the culture resulting from changes in the dilution rate. (See section on Theory of Continuous Culture of Microorganisms).

Recently, Servizi and Bogan (44) showed that synthesis is proportional to the change in free energy of oxidation. They reasoned that since the free energy for most organic compounds is the same (-3160 cal/g COD to -3587 cal/g COD), it follows that synthesis would be proportional to the COD of the substrate. Data for a wide variety of substrates (44, 46) yielded a relationship: g VSS = 0.39 g COD removed. When these data were recalculated to a BOD_5 basis where it was assumed that K = 0.1, the resulting synthesis was 0.57 g VSS/g BOD_5 removed (6). Using this relationship, cell synthesis from glucose can be expressed by the equation

$$6\,C_6\,H_{12}\,O_6 + 17O_2 + 4NH_3 = 4C_5\,H_7\,NO_2 + 17CO_2 + 30H_2O \qquad (3)$$

Sawyer (42) has shown that $\underline{a}$ (ratio of sludge synthesis to BOD_5 removed) is in the range of 0.5 to 0.6. Similarly, Gellman and Heukelekian (16)

obtained a yield of 0.5 pounds of volatile solids per pound of BOD_5 fed to the system. Hoover, et al., (22) in dairy waste oxidation studies, reported a 52 to 58 percent biological sludge yield from the oxidation process whereas Eckenfelder and Moore (7) showed that 76 percent of the 5-day BOD removed in a pulp and paper waste treatment process was synthesized to new sludge. Results reported by Symons and McKinney (48) indicate that $\underline{a}$ will increase in the presence of insufficient nitrogen. In addition, Heukelekian et al. (21) and Wuhrman (50) have reported $\underline{a}$ values of 0.49 and 0.64 respectively for domestic sewage.

As discussed by Eckenfelder and O'Conner (9) the rate of endogenous sludge oxidation at any time may be related to the sludge age or the mean length of time the sludge has been undergoing aeration. It may be anticipated that the rate of sludge oxidation will vary inversely with sludge age. Sludge oxidation rates will also vary with temperature and the nature of the waste being treated. Therefore, it is expected that higher net sludge accumulation will result under winter conditions.

Various values of auto-oxidation rates have been reported in the literature. When sludge from an activated sludge process treating semi-chemical and pulping waste was aerated, 7 percent of the volatile solids were destroyed per day by oxidation (9). The oxidation rate of activated sludge from a pharmaceutical waste treatment process was such that a 55 to 60 percent reduction in sludge solids could be obtained in a 5 to 6 day aeration period (9). Hoover,

et al. (22), studied the oxidation of dairy waste sludges and reported a sludge oxidation rate of 24 percent per day at 20° Centigrade.

Sludge will accumulate in the activated sludge process because of the synthesis of new cells and the accumulation of suspended solids present in the influent waste (6, 9). For a soluble waste, the accumulation of biological solids can be computed from the relationship

$$\text{lb } VSS_p/\text{day} = \underline{a} \text{ lb } BOD_r/\text{day} - \underline{b} \text{ lb MLVSS} \quad (4)$$

where $\underline{a}$ represents the fraction of BOD converted to new cells. The coefficient $\underline{b}$ is the fraction per day of the total aeration volatile solids oxidized. When volatile suspended solids are present in the influent waste, Equation 4 is modified to the form

$$\text{lb } VSS_p/\text{day} = (f \text{ lb VSS/day} + a \text{ lb } BOD_r/\text{day}) - b \text{ lb MLVSS} \quad (5)$$

where the coefficient f is that fraction of the volatile solids present in the influent waste which are not biodegraded during the aeration process.

Oxygen Requirement and Utilization

Total oxygen requirements in a system are related to the oxygen consumed to supply energy for synthesis and the oxygen for endogenous respiration. The total oxygen required for the activated sludge process is estimated from the relationship

$$\text{lb } O_2/\text{day} = a' \text{ lb } BOD_5/\text{day} + b' \text{ lb MLVSS (mixed liquor volatile suspended solids)} \quad (6)$$

Eckenfelder (6) in a review of the literature states that on a COD or

oxygen basis a = 0.39 x 1.42 = 0.55 in accordance with the data of Servizi and Bogan (44). (1.42 is the conversion multiple used here since only 0.7 g VSS were produced per gram of oxygen consumed in Servizi, et al. experiments.) Since $a + a' = 1$, a' is estimated as 0.45 on a COD basis or 0.65 on a BOD_5 basis where K = 0.1.

In a variety of industrial waste oxidation systems the coefficient a' has been found to vary from 0.35 to 0.55 (9). Eckenfelder, et al. (9) have reported that a' is equal to 0.52 for domestic sewage. However, Wuhrman (51) discovered that the availability of nutrients will influence a'. He stated that only 16 percent of glucose was respired by washed activated sludge in the absence of nitrogen while 50 percent was respired when nitrogen was present. Symons and McKinney (48) confirmed Wuhrman's observations. They found that oxidation in the presence of low nitrogen resulted in a large accumulation of sludge of high polysaccharide content, and correspondingly, a lower fraction oxidized.

The endogenous respiration rate of domestic sewage activated sludge has been reported to vary from 1.9 to 9.8 mg O_2/hr g of sludge undergoing aeration for 24 hours with no nutrient addition (40). In an activated sludge plant treating semi-chemical and pulping wastes, the endogenous respiration rate varied from 3 to 7 mg O_2/hr-gram of sludge (volatile solids basis) over a 10 hour aeration period at 32°C. Gehm (15) studied the oxidation of sludge obtained from kraft mill wastes and reported a mean endogenous

respiration rate of 2.1 mg O_2/hr-g volatile suspended solids.

Oxygen consumption values may be measured (a) by the direct absorption of gaseous or dissolved oxygen or (b) by the indirect method of measuring the drop in the oxygen demand of a sludge (9). In the direct method a sludge is respired in a closed oxygen or air atmosphere at constant temperature. The sample is agitated and the oxygen utilized is measured with respect to time by noting the decrease in gas volume or pressure. The Warburg (9) and Hach manometric BOD apparatus (Appendix I) are typical examples. The weight of sludge and BOD (or COD) present before and after a run are determined. From these values oxygen consumption values can be calculated.

Theory of the Continuous Culture of Microorganisms

A more theoretical approach to microbial growth has been developed by Herbert, et al. (1956) based on the classical treatment of Monod (1942) for growth of microorganisms in continuous cultures, and the equations of Bartlett (1958) for continuous growth of microorganisms in multiple stage chemostats.

Consider microorganisms growing in a completely mixed continuous culture vessel, with the inflowing medium containing a single limiting organic substrate at a concentration S_1. Since the other substrates are present in excess, and assuming that the chemostat is sufficiently aerated

to provide an excess of oxygen at all times, the supply of limited organic substrate will be the only growth limiting factor. The variables within the immediate control of the researcher are substrate concentration, pH, temperature, and the flow of substrate (dilution rate) into the chemostat. A complete theory should explain how these variables effect the growth rate of the microorganisms and substrate concentration in the chemostat.

When no organisms are being supplied from external sources, the organisms are growing in the continuous culture growth vessel at a rate described by Equation 7 and being simultaneously washed away at a rate determined by Equation 8.

$$\frac{dx}{dt} = k_1 x \tag{7}$$

$$-\frac{dx}{dt} = Dx \tag{8}$$

x = concentration of organisms in the growth vessel (dry weight of organisms per volume at a time t)

k_1 = the specific growth rate

D = the dilution rate (flow rate entering the system divided by the volume of the growth vessel).

The net increase in concentration of organisms is given by:

Increase = growth - output

$$\frac{dx}{dt} = k_1 x - Dx \tag{9}$$

therefore if $k_1 > D$ the value $\frac{dx}{dt}$ will be positive and there will be a continuous increase in organism concentration until a shortage in the critical nutrient reduces k_1. If $D > k_1$, $\frac{dx}{dt}$ is negative and the organism concentration will decrease, eventually to zero, therefore the culture will be washed from the growth vessel. When $k_1 = D$, $\frac{dx}{dt} = 0$ and x is constant; therefore a steady-state is reached. This equation has been experimentally verified for various microbial systems. To understand completely what dilution rate gives a steady-state, the effect of dilution rate upon substrate concentration must also be examined.

In the culture vessel, substrate is entering at a concentration S_1, being consumed by the organisms and flowing out at a concentration S. The net rate of change of substrate concentration is obtained by Equation 10.

$$\text{Increase} = \text{input} - \text{output} - \text{consumption} \tag{10}$$

Schulze (1964) shows that the yield factor, Y, is not constant. The variance of Y, which is the ratio of weight of bacterial cells produced to the weight of substrate consumed per unit time, is conveniently established at a series of varying growth rates k_1 by the continuous flow technique. The amount of substrate consumed is given by $(S_1 - S)$ times the feed per hour, and the amount of cells produced is given by the concentration x times the feed volume per hour. The flow rates cancel out and

$$Y = \frac{x}{S_1 - S} \tag{11}$$

where S_1 = substrate concentration in feed, mg/l

S = substrate concentration in growth vessel or in growth vessel effluent, mg/l

In the Schulze experiment it appears that at high growth rates substrate is more effectively converted to cell material.

The rate of substrate consumption per gram cell weight per hour at various specific growth rates can easily be calculated from continuous flow data:

$$k_s = \frac{fS_1 - fS}{V_x} = \frac{DS_1 - DS}{x} \tag{12}$$

where k_s = specific rate of glucose uptake in mg per gram cell weight per hour,

and V = volume of growth vessel

A plot of k_s versus $D = k_1$ as given by Schulze demonstrates that k_s increases in direct proportion to the growth rate following the equation

$$k_s = n + hk_1 \tag{13}$$

where n = y - intercept, representing grams of glucose uptake per gram cell weight per hour, $k_1 = 0$.

h = constant, representing grams glucose consumed per gram cell weight formed

The constant h is the inverse of the corrected yield constant Y'. The specific rate of substrate removal can therefore be expressed as

$$k_s = n + k_1 Y' \tag{14}$$

and the growth rate can be related to the rate of substrate removal by

$$k_1 = Y' (k_s - n) \tag{15}$$

In the development of continuous flow equations the growth rate is usually assumed to be a constant fraction of the substrate removal rate:

$$dx/dt = -Y\, ds/dt \tag{16}$$

and inversely the rate of substrate consumption is given by:

$$\frac{ds}{dt} = -\frac{dx}{Y\,dt} = k_1 x/Y \tag{17}$$

Now it appears that Equation 16 has to be changed because of a small amount of substrate consumption which is evident even if no growth occurs so that

$$\frac{ds}{dt} = (nx + k_1 x/Y') \tag{18}$$

and

$$\frac{ds}{x dt} = k_s = -(n + k_1/Y') \tag{19}$$

Equation 19 makes it possible to compute the net yield Y at various levels of k. By definition

$$Y = \frac{k_1}{k_s} \tag{20}$$

and therefore

$$Y = \frac{k_1}{n + (k_1/Y')} \tag{21}$$

This demonstrates that the actual yield factor will decrease with decreasing growth rates.

A balanced equation for the net rate of change of substrate concentration in the growth vessel can be written as:

$$\frac{ds}{dt} = DS_1 - DS - x(n + k_1/Y') \tag{22}$$

Herbert, et al. (1956) formulated the critical value of dilution rate, D_c, above which complete "wash-out" occurs. From Equation 9 it can be seen that this critical value is reached at the highest possible value of k_1. This is theoretically attained when $S_1 = S$. The critical value is given by

$$D_c = k_m \left(\frac{S_1}{k_s + S_1}\right) \tag{23}$$

Some researchers have noted that under certain conditions the theory for the continuous culture of microorganisms does not truly explain all of the growth reactions that occur in a continuous flow system. Gilley, et al. (1965) have shown that yeast in continuous culture show oscillations in growth rate reduced by changes in dilution rate, medium concentration, or temperature. Abrupt changes in dilution rate do not always give smooth transitions from one steady state to another. Instead, decaying oscillations in population seem to occur. The magnitude of the oscillations was shown to be a function of medium concentration and magnitude of dilution rate change. Finn and Wilson (1954) noticed in their work and in the work of others that continuous growth of a yeast culture induced a cycling of the population in contrast to the usual stable steady-state situation. They accounted for this phenomena by assuming that some of the initial disturbance, such as a reduction in flow rate, caused the

number of cells to increase. As each cell manufactured acid, the pH fell, causing the growth rate to decrease. Before long there would be a net wash-out of cells and decrease in population, a sequence of events which would result in steady oscillation if there was a time lag in the response to pH. Yazuda and Matelea (1964) have demonstrated with E. coli in continuous culture that there is a sudden jump in growth rate following an increase in dilution rate and that the growth rate drifts slightly above the new steady state value before completing its adjustment. Edwards (1966) found that attached microorganisms in a continuous culture system produced steady-state populations at various dilution rates. He also recorded that larger total population counts occurred at higher dilution rates less than D_c than at some lower dilution rates. Edwards also found for a filamentous culture that there was no sharp drop in population level at the critical dilution rate but, instead, gradual reductions in total population occurred over a range of dilution rates as the dilution rate was increased above the calculated critical rate.

Growth Rate Constant and Removal Rate Determinations for the Activated Sludge Process

From an engineering viewpoint it has been found in the past by certain researchers that for the design of activated sludge processes it is more convenient to determine growth rates and removal rates in accordance with the

Michaelis-Menten relationship. In a summary of the literature O'Conner and Eckenfelder (9) state that the various phases of sludge growth and BOD removed may be considered to consist of a dynamic relationship between the mass transfer of essential foods into the cell structure and the assimilation and utilization of these foods for energy and growth. At high concentration of organic matter, the rate of assimilation and the growth rate are independent of the external concentration of organic matter. At low food levels in mixed culture systems the rate of growth, and hence the BOD removal rate, are frequently observed to be concentration dependent.

Let a be the fraction of BOD removed, L_r, which is synthesized to sludge at any time for a specific growth rate. Then the sludge production, ΔS can be determined by the following relationship

$$\Delta S = aL_r \tag{23}$$

The total sludge solids, S, present at any time can be determined by the following relationship

$$\Delta S = S_o + \quad S_o + aL_r \tag{24}$$

where S_o is the amount of solids present initially.

They also state that for the log growth phase, during which regular and maximum multiplication of the sludge cells is taking place, can be expressed mathematically

$$\frac{ds}{dt} = K_1 S \tag{25}$$

which in its integrated form is

$$\ln \frac{S_o + S}{S_o} = K_1 t \tag{26}$$

In terms of BOD removal (L_r), this equation becomes

$$\ln \frac{S_o + aL_r}{S_o} = K_1 t = \ln \left(1 + \frac{aL_r}{S_o}\right) \tag{27}$$

A plot of $\ln \left(1 + \frac{aL_r}{S_o}\right)$ against time is a straight line function for the log growth phase. The slope of this line defines K_1, the constant logarithmic growth rate.

From studies with glucose and peptone with mixed cultures, Garrett and Sawyer (12) established maximum reaction rates of 0.08 per hour at 10°C, 0.20 per hour at 20°C, and 0.30 per hour at 30°C. Maximum growth rates varying from 0.05 per hour to 0.13 per hour have been found for various industrial wastes treated with mixed cultures (9).

Eckenfelder (6) states that within any portion of the log growth phase where the percent increase in sludge mass is not greater than 100 percent, relatively little error is introduced by letting $S = S_a$, the average sludge concentration over the range under consideration. The equation for K_1 then becomes

$$K_1 = \frac{aL_r}{S_a t} \tag{28}$$

This equation indicates that the removal rate, expressed in milligrams of BOD removed per hour per gram will be approximately constant (6, 9). The average removal rates for pharmaceutical, brewery, refinery, and spent

liquor were found to be 200, 100, 131, and 107 milligrams of BOD removed per hour per gram of sludge respectively. Wuhrman (51) has shown that the rate of removal of simple specific compounds is usually linear with time, and sludge solids concentration to very low substrate levels (less than one part per million).

Effect of Substrate Concentration on Enzyme Action

The effect of substrate concentration on enzyme action is an important consideration when discussing a biological system. All sludge synthesis and substrate removal is controlled by enzyme action.

Meyers and Free (30) demonstrated that when all variables can be controlled and substrate concentration is low, the velocity of an enzyme action parallels the substrate concentration and that above a certain concentration the rate of enzyme action ceases to increase. Michaelis and Menten (31) showed, in addition to this, that with sufficiently high concentrations the rate of enzyme action is actually slowed.

Nutrient Requirements

Several mineral elements are essential for the metabolism of organic matter by microorganisms. All but nitrogen and phosphorous are usually present in sufficient quantity in the carrier water. Sewage provides a balanced microbial diet, but many industrial wastes do not contain sufficient nitrogen and phosphorous and require their addition as a supplement.

In a review of the literature, Eckenfelder (6) states that when insufficient nitrogen is present, the amount of cellular material synthesized per unit of organic matter removed increases as an accumulation of polysaccharide. He also states that at some point nitrogen-limiting conditions restrict the rate of BOD removal. In the treatment of pulp-and-paper-mill wastes, he notes that the BOD removal efficiency was decreased when the nitrogen content was reduced below 4.4 lb N/100 lb BOD removal. Helmers, et al. (19) revealed that the maximum nitrogen requirement was 5 to 6 pounds of nitrogen per 100 pounds of BOD removed and the critical requirement to be 3 to 4 pounds of nitrogen per 100 pounds of BOD removed. They also showed that the maximum phosphorous requirement was 1.0 pound of phosphorous per 100 pounds of BOD removed. This is approxir ately equivalent to a BOD:N:P ratio of 150:5:1 (6). A BOD:N:P ratio of 100:5:1 in a waste will usually insure adequate nutrition (9). Eckenfelder (6) states that the nitrogen requirements for the activated sludge process can be readily estimated from the sludge synthesized from the BOD removed less the endogenous respiration loss.

Effect of pH

A relatively narrow effective pH range will exist for most biological oxidation systems. Eckenfelder (6) states that for most processes this covers a range of pH of 5 to 9 with optimum rates occurring over the pH range of 6 to 8. He also notes that this relates to the pH of the mixed liquor in contact with

the biological growths and not the pH of the waste entering the system.

Sawyer (41) indicated the desirability of pH adjustment in those systems where weakly ionized acids are converted to highly ionized acids and where neutral substances are converted to acidic forms by oxidation. He stated that deleterious effects of pH are magnified at low temperature. He reported that the effective pH range for cotton kiering liquor and sulfite waste is pH 5 to 11; for spent yeast broth, slaughterhouse waste and candied fruit waste, pH 5 to 9; for boardmill white water and rag cook liquor waste, pH 6 to 9; and for antibiotics waste, pH 5 to 7.

Keefer and Meisel (26) found the optimum pH range for activated sludge treating domestic sewage to be pH 7.0 to 7.5 with an effective range of 6.0 to 9.0. At a pH of 4.0 they found the process was only 43 percent effective. For a pH of 10.0 they showed the process was only 54 percent effective.

O'Conner, et al. (9) noted that a rapid change in pH may decrease the respiratory activity by as much as 75 percent. They also state that in many systems the optimum zone for biological activity may vary appreciably from neutrality.

CHAPTER IV

METHODS AND MATERIALS

A. Description of Apparatus (Figures 1 and 2)

The experimental apparatus and the method of operation is illustrated by Figures 1 and 2. The apparatus consisted of two sections, a growth chamber and a settling basin. The growth chamber, which had inside dimensions of 9 inches width, 10 inches height, and 15 inches length, was made of 3/8 inch plexiglass. Five growth cells of equal volume were developed in the growth chamber with the insertion of four plastic screen panels.

The screen panels consisted of a plastic net stretched in a plexiglass frame. These panels were held in place by a plexiglass fastener which was attached to the top of the growth chamber. The plastic screen panels were located at equal intervals perpendicular to the flow for the support of the attached organisms.

Aseptic air was supplied to each of the growth cells from the laboratory air supply through a rubber hose connected to a diffusor stone. The diffusor stones in the first and fifth chambers were placed

in the center of the cells, while in the second, third, and fourth chambers the stones were placed at the right side of the chamber. The depth of the liquid in the growth chamber was adjusted by the positioning of a movable sharp crested plexiglass weir.

A settling basin of the same material and dimensions as the growth chamber was connected to the growth chamber to receive its effluent. The effluent from the settling basin, which was discharged to a sink, was controlled by a moveable sharp crested plexiglass weir.

Throughout the experiments a 10 liter glass media reservoir was used to supply media through a pump to a glass "T" connector. A 15 liter plastic bottle was used as a reservoir to provide dilution (tap water) water to the same glass "T" connector where dilution of the concentrated media occurred. The tap water reservoir was left open to the air while the concentrated media was supplied with aseptic air from the atmosphere through a cotton plugged glass tube. The diluted media flowed through the glass "T" connector and was allowed to drop by gravity into the first cell of the growth chamber.

All media piping consisted of plastic, rubber, and glass tubing. Concentrated media was fed to the glass "T" connector with a peristaltic pump using a 2 rpm motor (Sigmamotor Company, Middleport, New York). Tap water was fed to the glass "T" connector using a

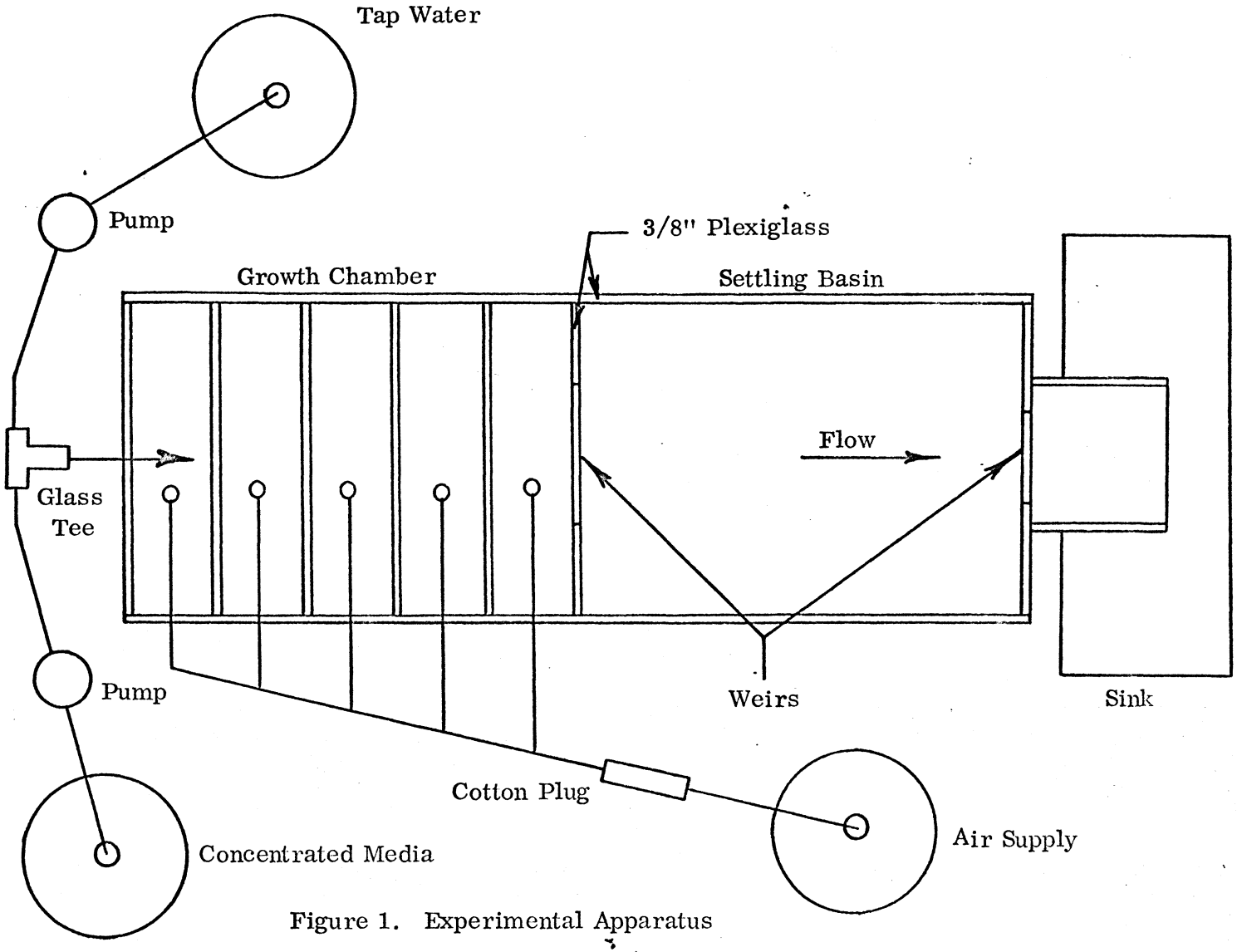

Figure 1. Experimental Apparatus

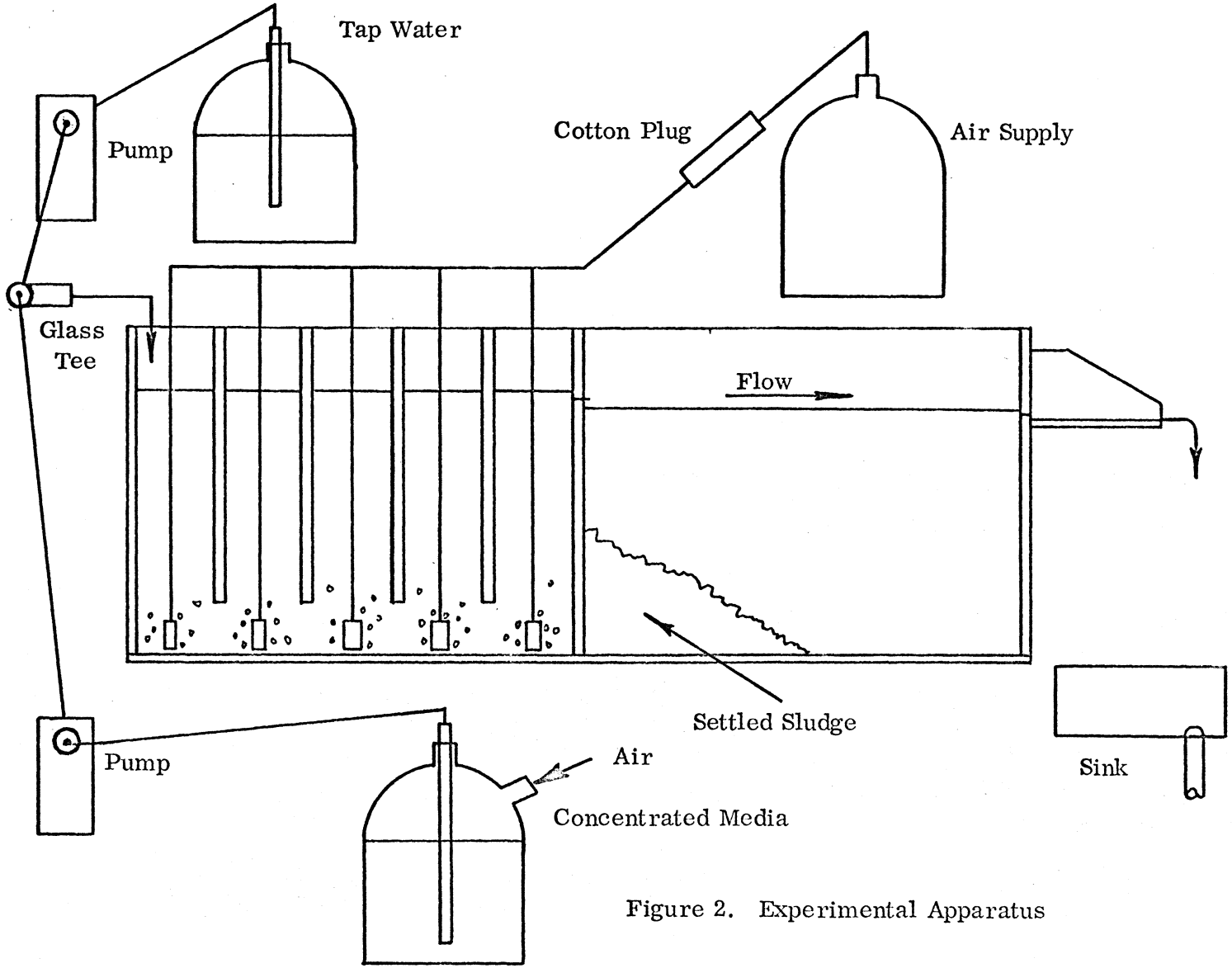

Figure 2. Experimental Apparatus

peristaltic pump (Sigmamotor Company, Middleport, New York) driven by a 1/8 horsepower Westinghouse Electric Motor. The speed of this pump was controlled by a zero-max clutch apparatus.

B. Methods of Sampling and Analysis

To determine the operational characteristics of the described system, the following tests were used in all of the experiments: (1) chemical oxidation demand (COD); (2) dissolved oxygen concentration (DO); (3) suspended solids concentration; (4) pH; (5) biochemical oxidation demand (BOD); (6) flow rate; and (7) total sludge solids production. Temperature checks were also noted at the time of all sampling.

COD test samples were taken from the growth chamber by lowering a clean 50 milliliter beaker into each of cells 2, 3, 4, and 5. Each of the four 50 milliliter samples were then separately filtered through a number 40 Whatman filter and collected in a 250 milliliter Erlenmeyer flask. Twenty milliliter samples were then pipetted from each of the flasks for separate COD determinations. A 20 milliliter sample of the influent, that had been collected in a 500 milliliter graduated cyclinder, was also pipetted as a sample for the COD test. All COD determinations were performed according to Standard Methods, Twelfth Edition, wastewater section.

Dissolved oxygen concentration determinations were made in each of the cells of the growth chamber and in the settling basin at every sampling time with the use of a YSI Model 51 Oxygen Meter and a YSI Model 5104 Oxygen Probe. The probe was extended to mid-depth and quickly moved back and forth across the growth cell until a steady reading was noted on the meter. The dissolved oxygen apparatus was calibrated once every two weeks according to the operation instructions for the YSI Model 51 Oxygen Meter (Yellow Springs Instrument Company, Inc., Yellow Springs, Ohio).

Suspended solids samples were taken from the effluent end of the settling basin using a pipet. Four samples were collected for each suspended solids determination so an average value might be readily obtained. The suspended solids content of the influent were also determined. All suspended solids determinations were performed according to Standard Methods, Twelfth Edition, wastewater section (47), except that Reeve angel grade 934AH glass fiber filters were used as the filter medium.

pH determinations for each of the five cells of the growth chamber, the settling basin and the influent were made using a Corning Model 5 pH meter and a matched pair of Series 500 Corning electrodes. To determine the pH in the five cells of the growth chamber and the settling basin, the electrodes were lowered directly

into each of the five growth chamber cells and settling basin, and gently moved back and forth until a steady reading could be recorded. To determine the pH of the influent, a 200 milliliter sample was collected in a 500 milliliter graduated cylinder and then transferred to a clean 250 milliliter beaker into which the electrodes were immersed. Between each pH reading the electrodes were washed with distilled water. To preserve the electrodes between test runs they were immersed in clean distilled water.

The Hach Model 2173 Manometric BOD apparatus (Hach Chemical Company, Ames, Iowa), placed in a room which had a temperature of $20^{o}C \pm 0.5^{o}C$, was used for 5-day BOD measurements and oxygen consumption studies. BOD tests were performed at least once for each different experiment to correlate the COD data to BOD data. One hundred fifty-seven milliliter samples were taken from the influent and cell number five of the growth chamber for each run. The tests were run according to the procedures listed in the Instruction Manual for the Hach Manometric BOD Apparatus Model 2173. Each time the BOD studies were run, oxygen consumption studies were also run in a similar manner on the following media dilutions: 50:1; 100:1; and 1000:1.

The sludge solids production test was run according to the following procedure:

1. The settling basin was drained and washed with tap water.
2. The settling basin was refilled with tap water.
3. The time was then noted.
4. Sludge was allowed to be expelled from the growth chamber to the settling basin at the wishes of the growth chamber. (The aeration diffusor stone in the fifth cell was placed in front of the sharp crested weir in the center of the cell to expel the excess sludge from the fifth cell into the settling basin).
5. The average influent solids concentration was determined each day by filtering two 20 milliliter samples of the influent through two different 934 AH grade Reeve angel's glass fiber filters. They were then dried for one hour in a 103°C oven. The filters were then cooled in a dessicator jar to room temperature and then weighed. The average increase in weight of the filters was then calculated.
6. The average effluent solids concentration was determined in a similar way for the effluent from the settling basin.
7. After 3 to 5 days the sludge collected in the settling basin was carefully siphoned into a 15 liter galvanized metal container, and the total volume of the sludge was measured.
8. The time was again noted.
9. While the contents of the container were constantly stirred, a

group of eight 20 milliliter samples were pipetted. The average concentration of the samples were then determined in a similar manner as noted in listing 5 above.

10. The total sludge production was then calculated by using the following formula:

$$\Delta X = (\Delta X_S)\ (Vol) + (\Delta X_E - \Delta X_I)\ (t)\ (Q)$$

ΔX = total sludge production calculated for system in grams.

t = length of time between sludge withdraws from settling tank in hours.

Vol = Volume of sludge withdrawn from the settling tank in milliliters.

ΔX_S = average sludge concentration taken from the settling basin in grams per milliliter.

ΔX_E = average concentration of solids in the effluent in grams per milliliter.

ΔX_I = average concentration of solids in the influent in grams per milliliter.

Q = flow rate of influent in milliliters per hour

The flow rate of the influent was determined by collecting the influent in a clean 500 milliliter cylinder for a period of 6 minutes. The flow rate was then converted to the dimensions of milliliters per hour.

Temperature was measured for all cells of the growth chamber, the settling basin, and the influent by an Ertco A 88050 centigrade thermometer whenever samples were drawn for analysis.

C. Description of the Study

This research was a study of a biological wastewater treatment system specifically designed to use filamentous microorganisms for the stabilization of soluble organics. The basic objective was to define the usefulness and some of the capabilities of the proposed treatment systems.

1. Experiment to Determine Some of the Effects of Flow Rate, Screen Area, and COD Loading on Plant Efficiency.

In this initial study, as well as all that followed, the previously described apparatus was used. Sampling and analysis procedures were as listed in Section B.

Initially the screens of the apparatus were positioned with bottoms of the screens making contact with the bottom of the growth chamber. Each of the five cells of the growth unit were then innoculated with a total of 30 milliliters of activated sludge obtained from the Corning Glass Work's sewage treatment plant in Blacksburg, Virginia. The growth chamber was then filled with tap water. The concentrated medium and tap water pumps

were started and the diffused air supply was added.

The system was allowed to stabilize for one week. Large attached growth tenacles, varying in color from brown to white to orange, developed on the screens. Similar large free floating flocs were also noted in all of the five cells.

Analysis was begun on the eight day. The screens were left on the bottom until sufficient data was obtained for a flow rate in the range of 500-600 ml/hr. The screens were then lifted 3/4 inches off the bottom to obtain additional data at this flow rate. From data analysis, it was then decided to run the remainder of the experiments with the screens 3/4 inches off the bottom of the growth chamber so that sludge production data could be obtained.

The cell liquid depth was held at eight inches which made the volume of the contents in the growth chamber equal to 17.79 liters. The concentrated medium pump operated constantly at a rate of two revolutions per minute which provided from 50 to 60 milliliters of medium per hour to the glass "T" connector. The speed of the tap water pump was controlled to provide the desired total flow rate to the growth chamber. The concentrated medium used in this experiment was that listed in Table 1. The pH of the influent to the system was controlled in the range of

5.6 to 7.0.

Additional flow rate ranges studied in this experiment were as follows:

a. 1,300 to 1,400 milliliters per hour

b. 1,600 to 1,900 milliliters per hour

c. 2,000 to 2,500 milliliters per hour

d. 2,501 to 3,000 milliliters per hour

e. 3,001 to 3,800 milliliters per hour

2. Experiment to Determine the Effects of Low pH Conditions on Plant Efficiency

The apparatus and methods of sampling and analysis used in this study were the same as those discussed earlier. The flow rate range used in this experiment was the 2,000 to 2,600 milliliter per hour range. The volume of the growth chamber was kept constant at 17.79 liter. The culture used in this experiment was that developed in the growth chamber in the preceding experiment.

The medium used in this experiment was that shown in Table 1 except that the phosphate buffer was omitted. The pH of the influent was adjusted with appropriate additions of concentrated sulfuric acid to the concentrated medium vessel. The pH was gradually lowered from a pH of 5.6 down to a pH of 2.4.

Appropriate lengths of time were allowed at each new pH level for acclimation and analysis.

3. Experiment to Determine the Effect of BOD:Nitrogen Ratio on Plant Efficiency

In this study the apparatus and sampling and analysis methods discussed earlier were used. The flow rate range used was the 2,000 to 2,600 milliliter per hour range and the culture used was that developed in earlier experiments. The medium used in this experiment was that shown in Table 2.

The initial phase of this study was run using the pH range of 2.65 to 2.85. One week of time was allowed for acclimation each time the BOD:N ratio was changed. The final phase of this experiment was run using the pH range of 6.8 to 7.4. Approximately one week of time was again allowed for acclimation each time the BOD:N ratio was changed.

4. Experiment to Determine the Effect of High COD Loadings on Plant Efficiency

For this study the previously discussed apparatus and sampling and analysis methods were used. The flow rate was held within the 2,000 to 2,600 milliliter per hour range and the culture used was that developed in earlier work. The

medium used was similar to that shown in Table 2 except the amount of dextrose was increased and appropriate adjustments were made in gravimeter additions of other constituents to keep the ratio of constitutes equal to what they are in Table 2 but provide a higher COD level. pH was held in the 6.5 to 7.2 range. The BOD:N ratio was approximately 30:1.

The initial phase of this experiment was run at a COD concentration of approximately 700 ppm. Other phases of this experiment used COD concentrations of approximately 1000 ppm, 1400 ppm, and 1700 ppm. Approximately one week of time was allowed for the acclimation of the system before analysis began.

D. Composition and Preparation of Medium

Medium of the composition shown in Table 1 was used in the initial experiments. Medium of the composition shown in Table 2 was used in the BOD:N ratio experiments and the high COD loading experiments. All gravimetric determinations of quantities used in the medium were made using an Ainsworth Type 10N balance. All liquid measurements were made using appropriate sized graduate cylinders.

After each new medium had been prepared and placed in the medium vessel, it was then autoclaved for 15 minutes at 15 p.s.i.

After autoclaving the vessel containing the medium was allowed to stand until cooled to room temperature. The medium vessel was then connected to the glass "T" by sterilized plastic and glass tubing (autoclaved at the same time as the medium). After the connection procedure was completed, the pumps were again started to supply food to the microorganisms in the growth chamber.

COMPOSITION OF MEDIUM

TABLE I

Medium 1

Component	Quantity
Yeast extract	4 g
Bacto peptone	30 g
Dextrose ($C_6H_{12}O_6 \cdot H_2O$)	126 g
*Phosphate buffer	20 ml
**Distilled water	variable

*Composition of Phosphate buffer

Sodium phosphate, monobasic ($NaH_2PO_4 \cdot H_2O$)	5.6 g
Sodium phosphate, diabasic ($Na_2HPO_4 \cdot 7H_2O$)	36.9 g
Sodium phosphate, diabasic (Na_2HPO_4)	33.4 g
Ammonium chloride (NH_4Cl)	1.7 g
Distilled water	1 liter

**The distilled water was added in a manner to give the desired strength of the medium.

COMPOSITION OF MEDIUM

TABLE II

Medium 2

Component	Quantity
Dextrose ($C_6H_{12}O_6 \cdot H_2O$)	134 g
$MgSO_4 \cdot 7H_2O$	13.4 g
$Fe\ Cl_3 \cdot 6H_2O$	0.0766 g
$Mn\ SO_4 \cdot H_2O$	1.34 g
$CaCl_2$	1.034 g
*$(NH_4)_2\ SO_4$	variable
**Phosphate buffer solution	30 ml
Distilled water	10 l

*The quantity of $(NH_4)_2\ SO_4$ was so adjusted to give the appropriate quantity nitrogen needed for a particular BOD:N ratio.

** Phosphate buffer solution composition

$K\ H_2PO_4$	34.0 g
Distilled water	1 l

In the neutral pH study in which this medium was used, the pH of this phosphate buffer was adjusted to 7.2 by the addition of 1N NaOH.

CHAPTER V

EXPERIMENTAL RESULTS

The results of the previously described study are presented below, primarily in graphical form. The results are grouped and presented in the following manner: effects of flow rate, profile of percent COD remaining with relation to support media area (Screen Area), effects of organic loading, solids production, oxygen utilization, effects of pH, effects of BOD:N ratio, microorganisms present at neutral and acid pH conditions, and a summary of typical operating conditions.

Effects of Flow Rate

Figure 3 shows the substrate removal efficiency of the experimental unit as related to flow rate. The percent COD remaining stayed relatively constant at approximately 4.5 for flow rates up to 1.31 liters per hour. This corresponds to an aeration period of 13.16 hours. For flow rates greater than 1.31 liters per hour, the percent COD remaining increased linearly to greater than 10 at an average flow rate of 3.41 liters per hour (5.21 hours aeration).

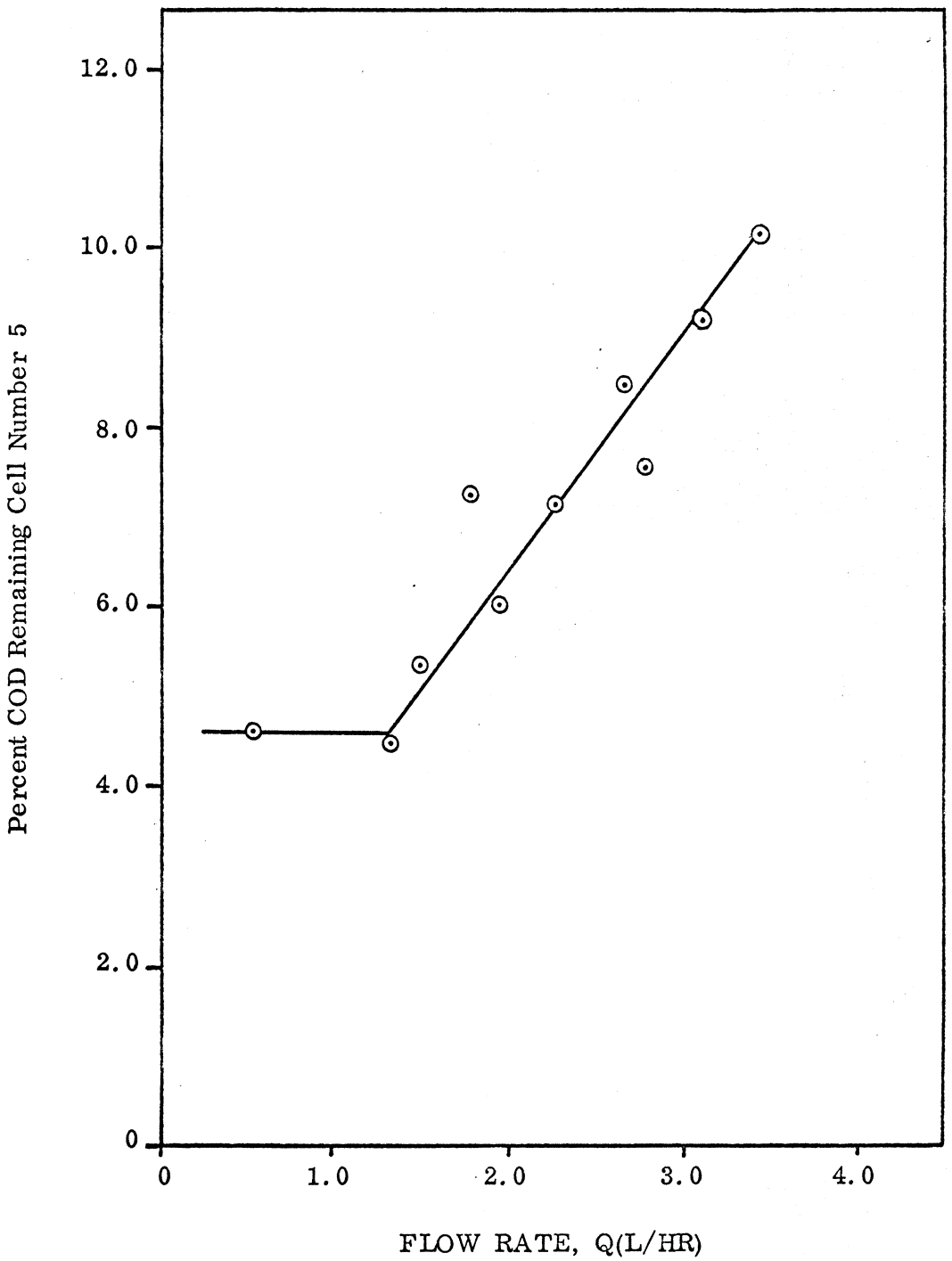

Figure 3. Percent COD Remaining in Cell Number 5 versus Flow Rate [pH_I = (5.6 - 6.9)]

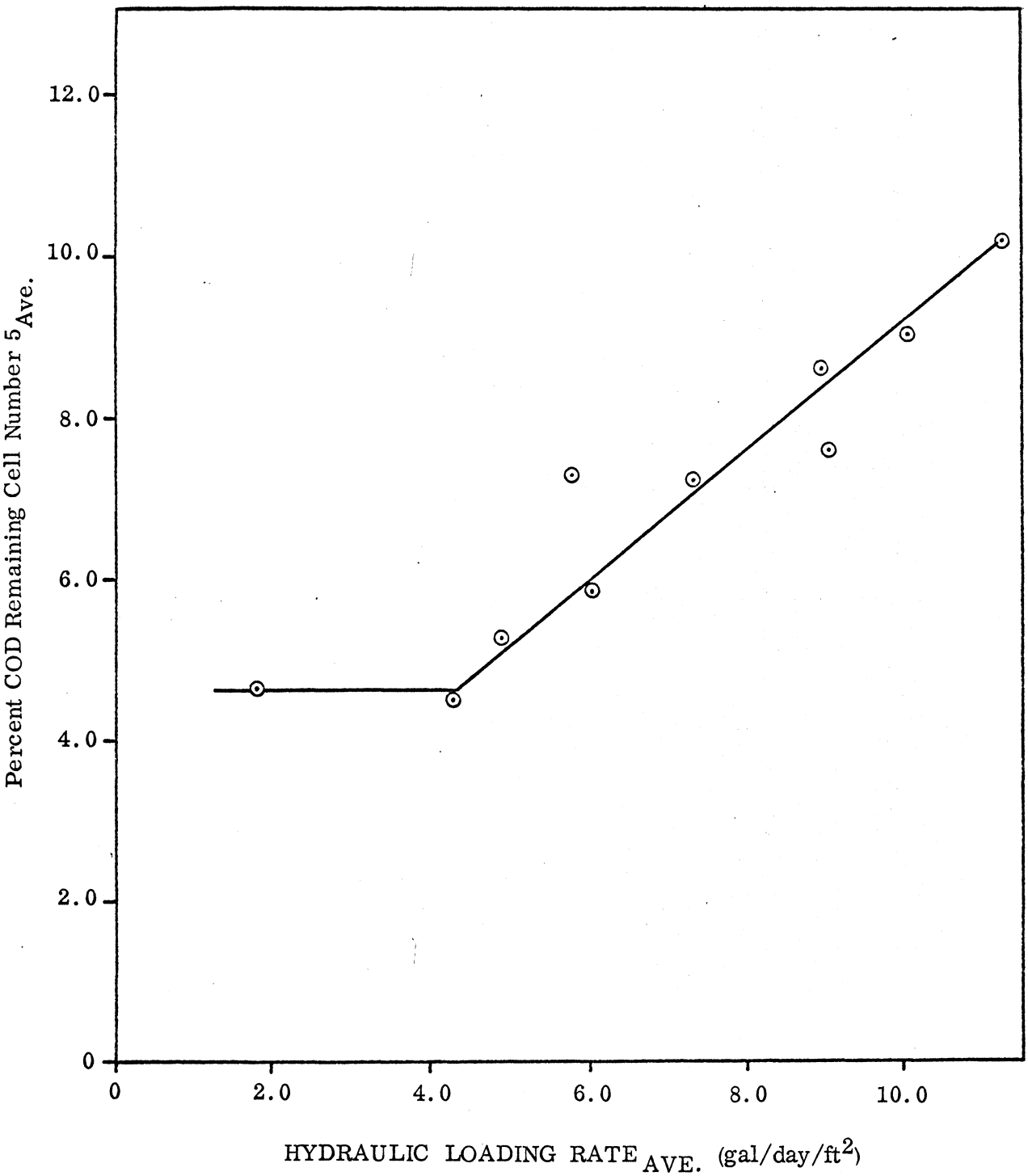

Figure 4. Percent COD Remaining versus Hydraulic Loading Rate [pH_I = (5.6 - 6.9), COD Loading Rate = (3.70 - 15.00) $\frac{\text{grams}}{\text{day - ft}^2}$]

The same effect can be seen in a plot of COD remaining versus hydraulic loading rate (Figure 4). The percent remaining in cell number five remained approximately constant at 4.5 until the hydraulic loading rate exceeded 4.29 gal/day/ft^2 of screen area. From this loading rate to a loading rate of 11.16 gal/day/ft^2, the percent COD remaining increased constantly up to a value of 10.1.

In Figures 5 through 10, the removal rate, that is, the influent COD concentration minus the final COD concentration divided by the detention time of the growth chamber, is plotted versus the influent COD concentration for various average flow rates. These figures show that removal is directly related to the organic loading. A summary of the plots (Figure 11) shows that the rate constant K, which is determined from the slopes of these graphs, increased constantly from 0.38×10^{-1} hrs.$^{-1}$ for an average dilution rate of 0.031 hrs.$^{-1}$ to a value of 1.75×10^{-1} hrs.$^{-1}$ for a dilution rate of 0.192 hrs.$^{-1}$. Dilution rate is the flow rate divided by tank volume.

In Figure 12 the suspended solids concentration of the effluent is plotted against the flow rate. As flow rate increases from zero to a value equal to 2.76 liters per hour, the suspended solids concentration increases constantly from zero to approximately 11.5 milligrams per liter. At a flow rate of 2.76 liters per hour there is an apparent break in the curve and a more rapid increase in suspended solids concentration in the effluent is illustrated between the flow rates of 2.76 liters per hour and 3.41 liters per hour.

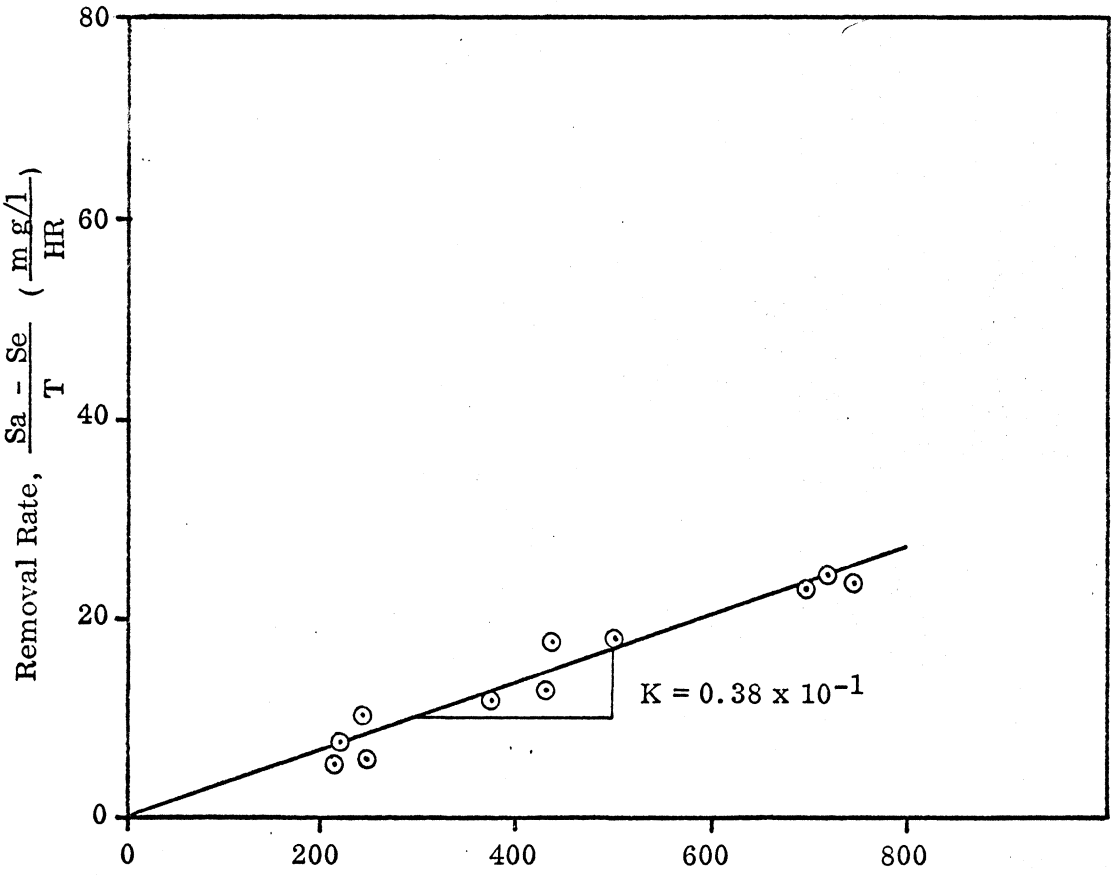

Figure 5. Removal Rate versus Influent COD Concentration

[Volume = 17.25 liters, pH_I = (5.6 - 6.9), $Q_{Ave.}$ = 0.54 L/HR]

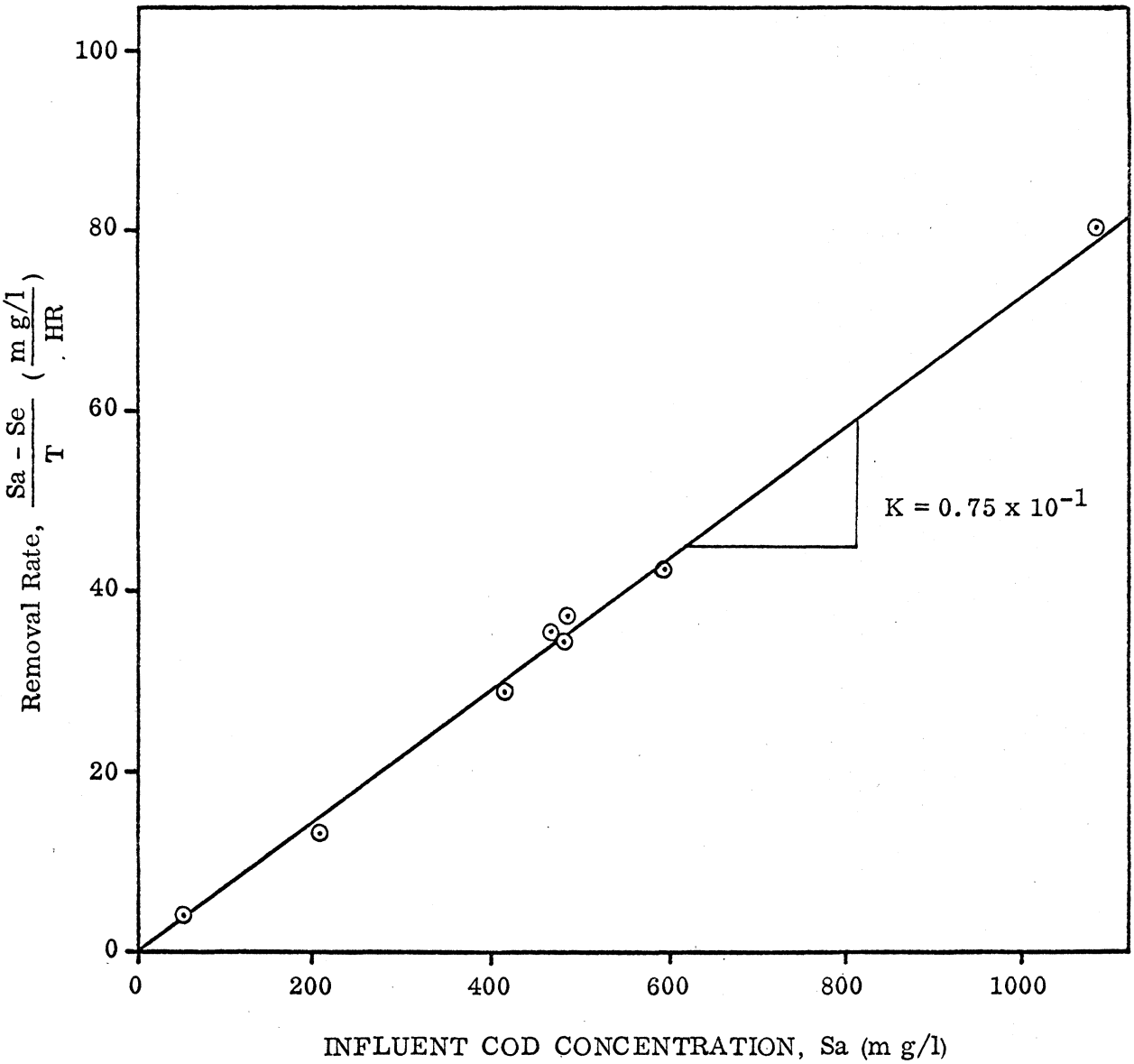

Figure 6. Removal Rate versus Influent COD Concentration
[Volume = 17.25 liters, pH_I = (5.6 - 6.9), $Q_{Ave.}$ = 1.31 L/HR]

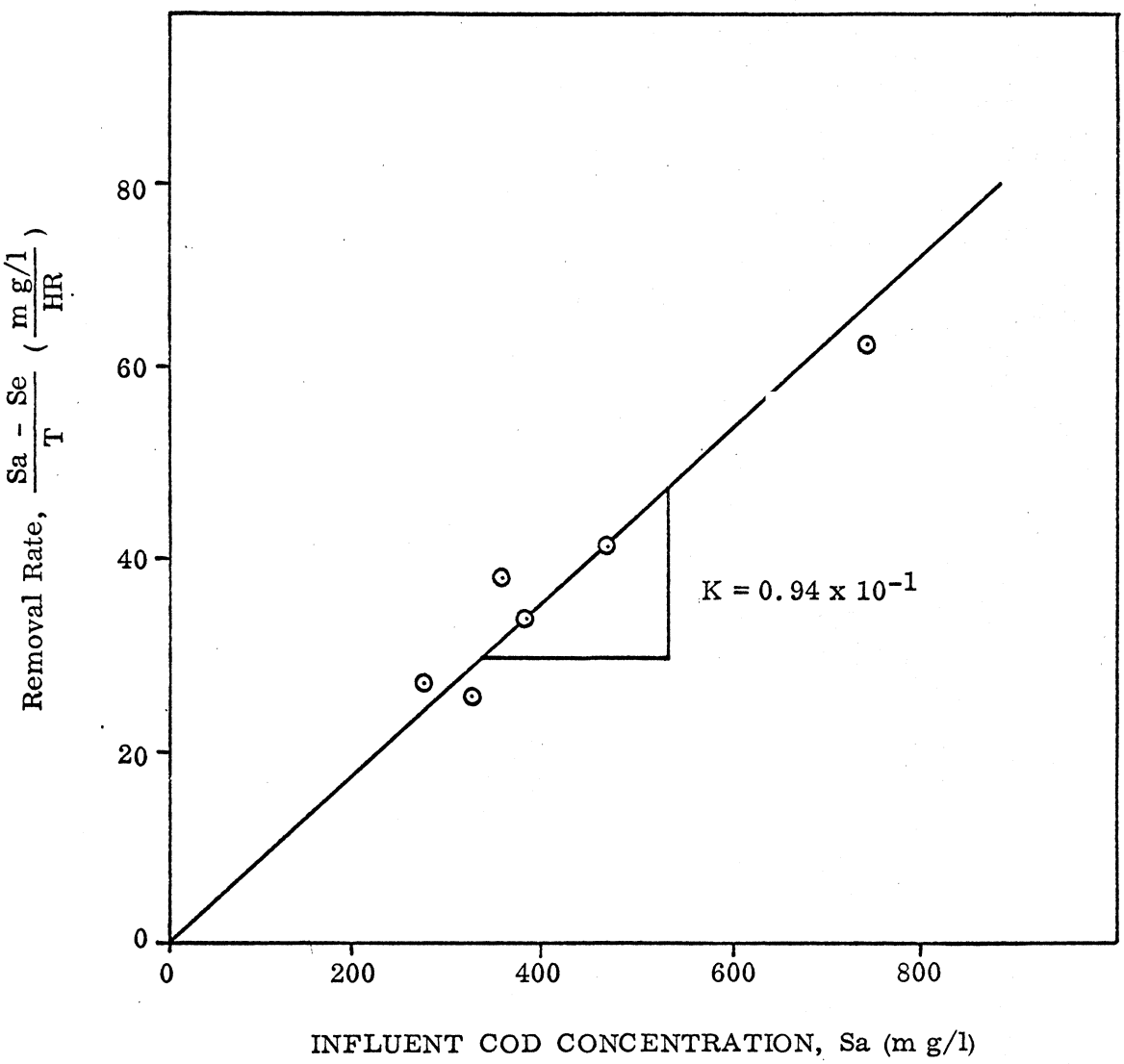

Figure 7. Removal Rate versus Influent COD Concentration [Volume = 17.79 liters, pH_I = (5.6 - 6.9), $Q_{Ave.}$ = 1.75 L/HR]

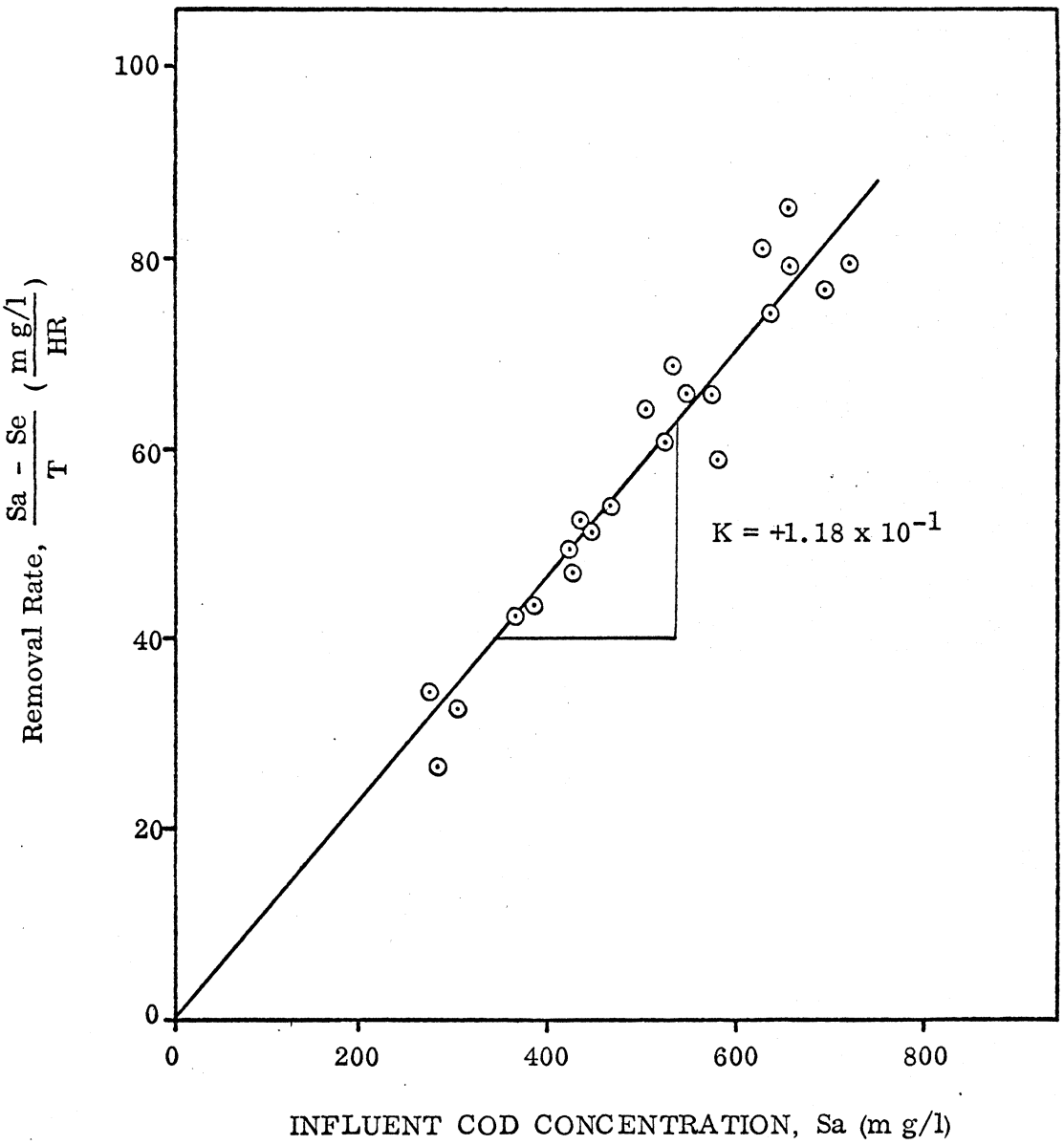

Figure 8. Removal Rate versus Influent COD Concentration

[Volume = 17.79 liters, pH_I = (5.6 - 6.9), $Q_{Ave.}$ = 2.23 L/HR

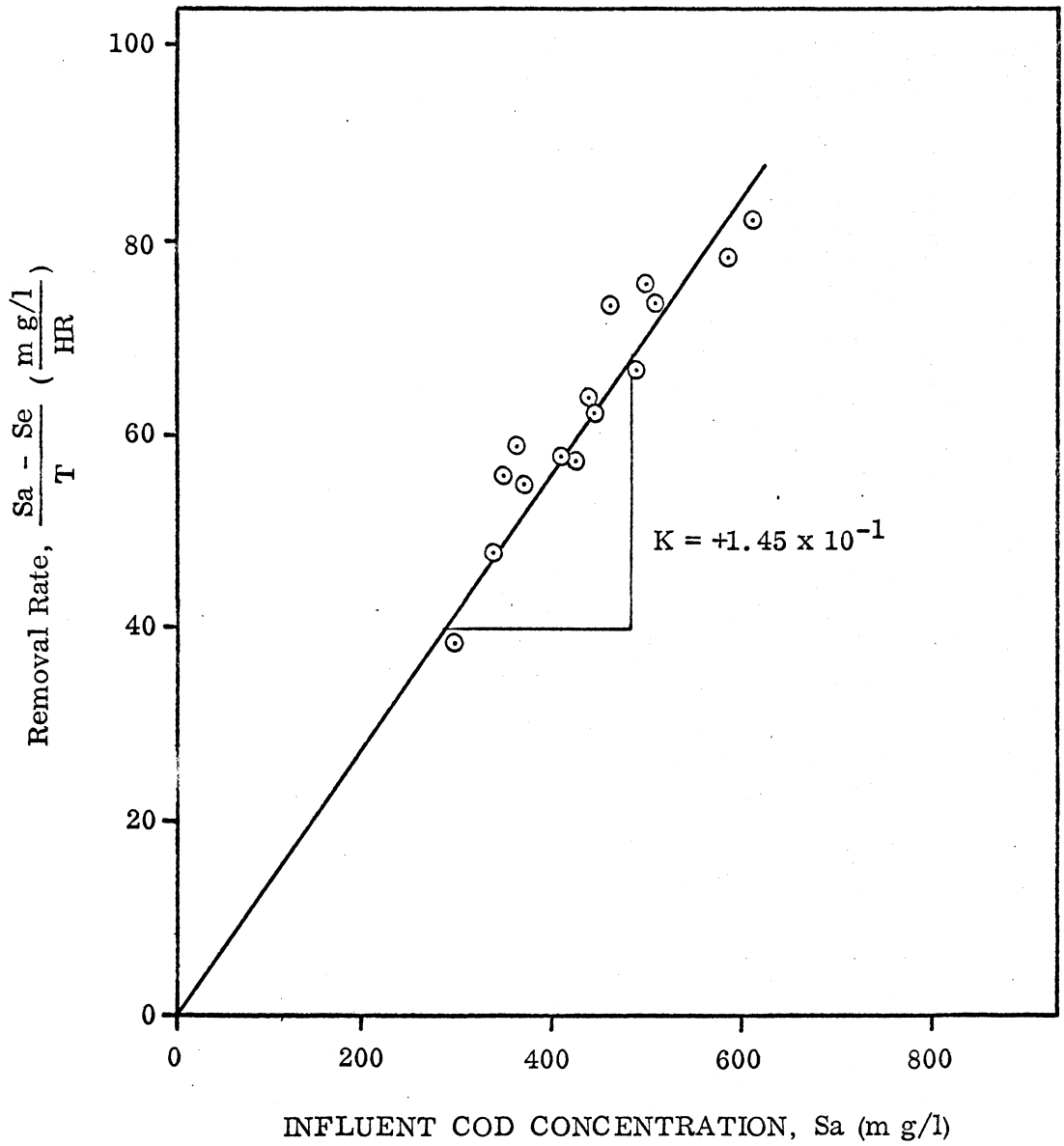

Figure 9. Removal Rate versus Influent COD Concentration

[Volume = 17.79 liters, pH_I = (5.6 - 6.9), $Q_{Ave.}$ = 2.76 L/HR]

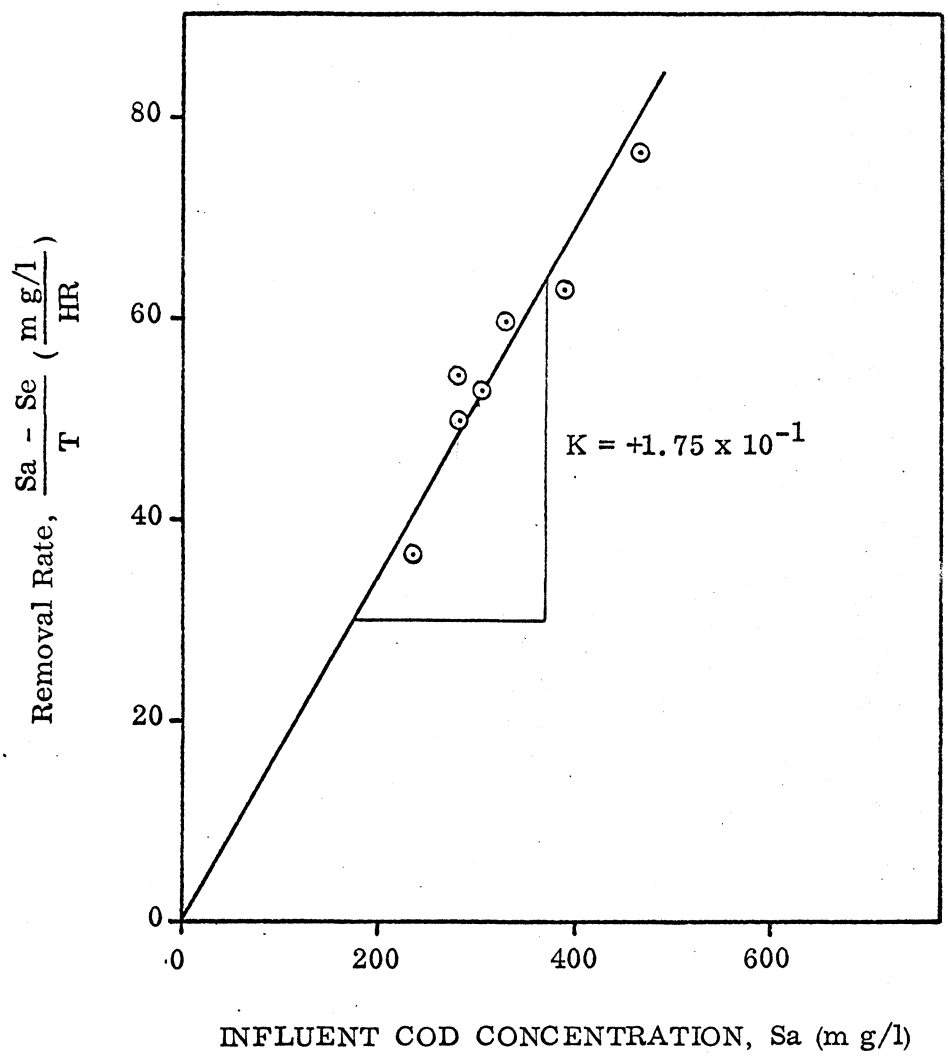

Figure 10. Removal Rate versus Influent COD Concentration

[Volume = 17.79 liters, pH_I = (5.6 - 6.9), $Q_{Ave.}$ = 3.41 L/HR]

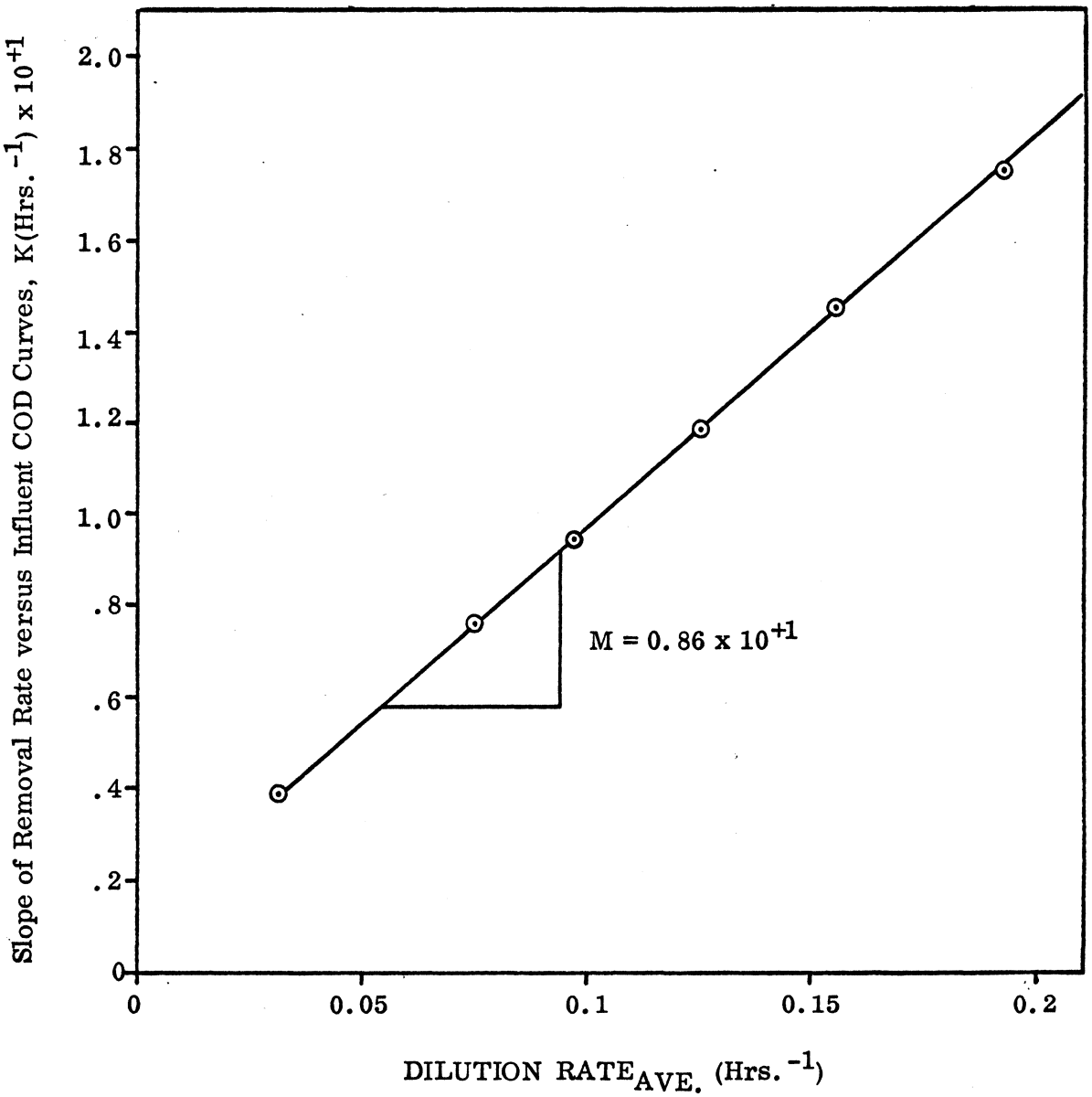

Figure 11. Slope of Removal Rate Curve versus Dilution Rate [pH = (5.6 - 6.9)]

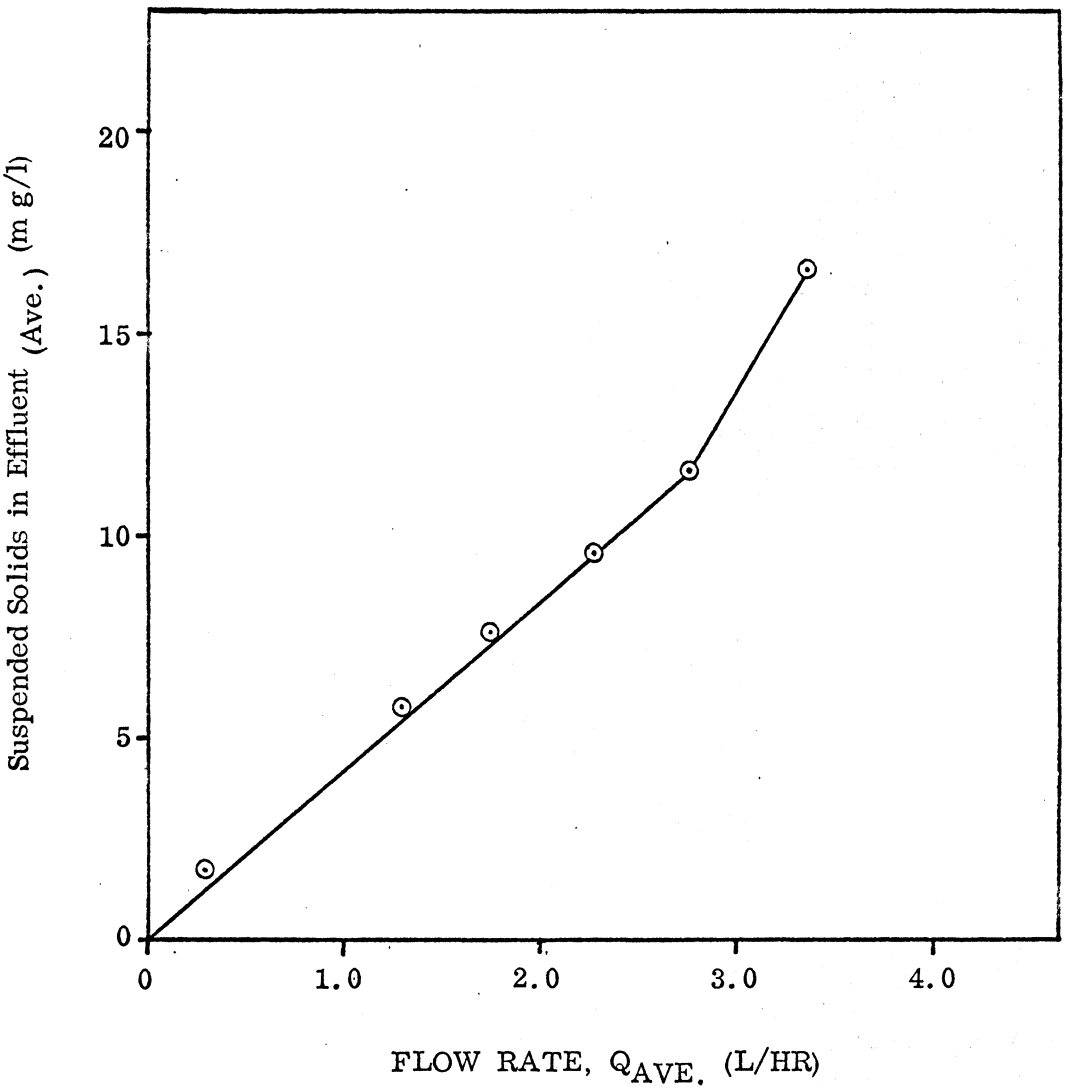

Figure 12. Suspended Solids versus Flow Rate
[pH = (2.75 - 7.45)]

Profile of Log Percent COD Remaining With Relation to Screen Area

Figures 13 through 18 illustrate the relationship between log percent COD remaining and screen area for average flow rates of 0.54 liters per hour, 1.31 liters per hour, 1.75 liters per hour, 2.23 liters per hour, 2.76 liters per hour, and 3.41 liters per hour, respectively. All these figures show some COD removal without any screen area. Figures 13 through 16 display a constant decrease in log percent COD remaining with an increase in screen area. At an average flow rate of 2.76 liters per hour, a break in efficiency is noticeable (Figure 17). The plot shows a constant decrease in log percent COD remaining with an increase in screen area over a screen area range of approximately 70 square inches up to approximately 210 square inches, a slightly lower constant reduction in log percent COD remaining is illustrated. Correspondingly, for an average flow rate of 3.41 liters per hour (Figure 18), the decrease in log percent COD remaining with increase in screen area is far from constant, yet, even at this flow rate approximately ninety percent COD removal is accomplished through the growth chamber.

Effect of Organic Loading

The relationship between percent COD remaining in cell number five and COD loading rate in grams/day/ft^2 for a pH range of 5.6 to 7.2 and a flow range of 0.54 to 3.41 liters per hour is illustrated by Figure 19. The COD loading rate is defined as the quotient of the influent COD (grams/day)

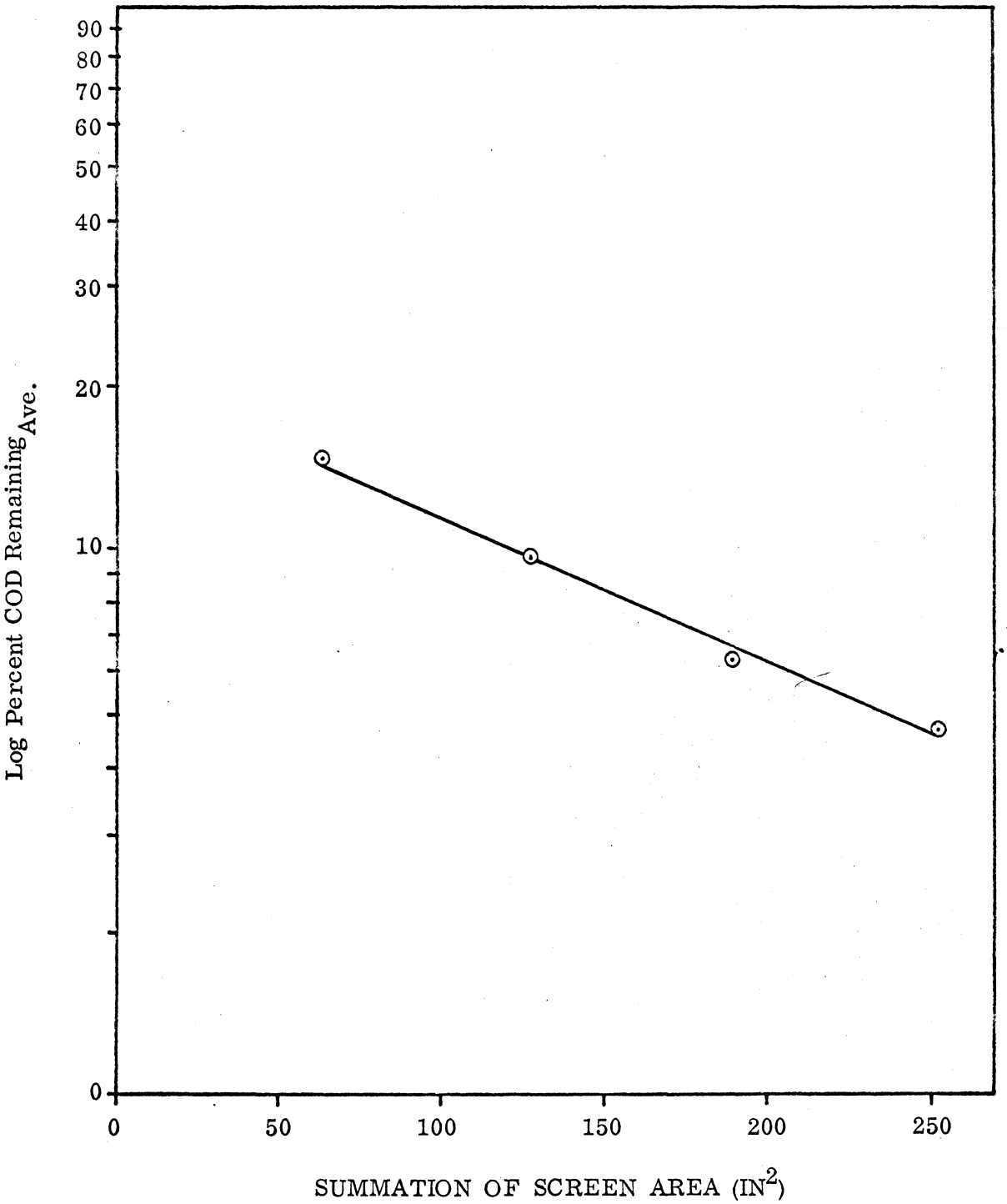

Figure 13. Log Percent COD Remaining$_{Ave.}$ versus Summation of Screen Area

[Volume = 17.25 liters, pH_I = (5.6 - 6.9), $Q_{Ave.}$ = 0.54 L/HR]

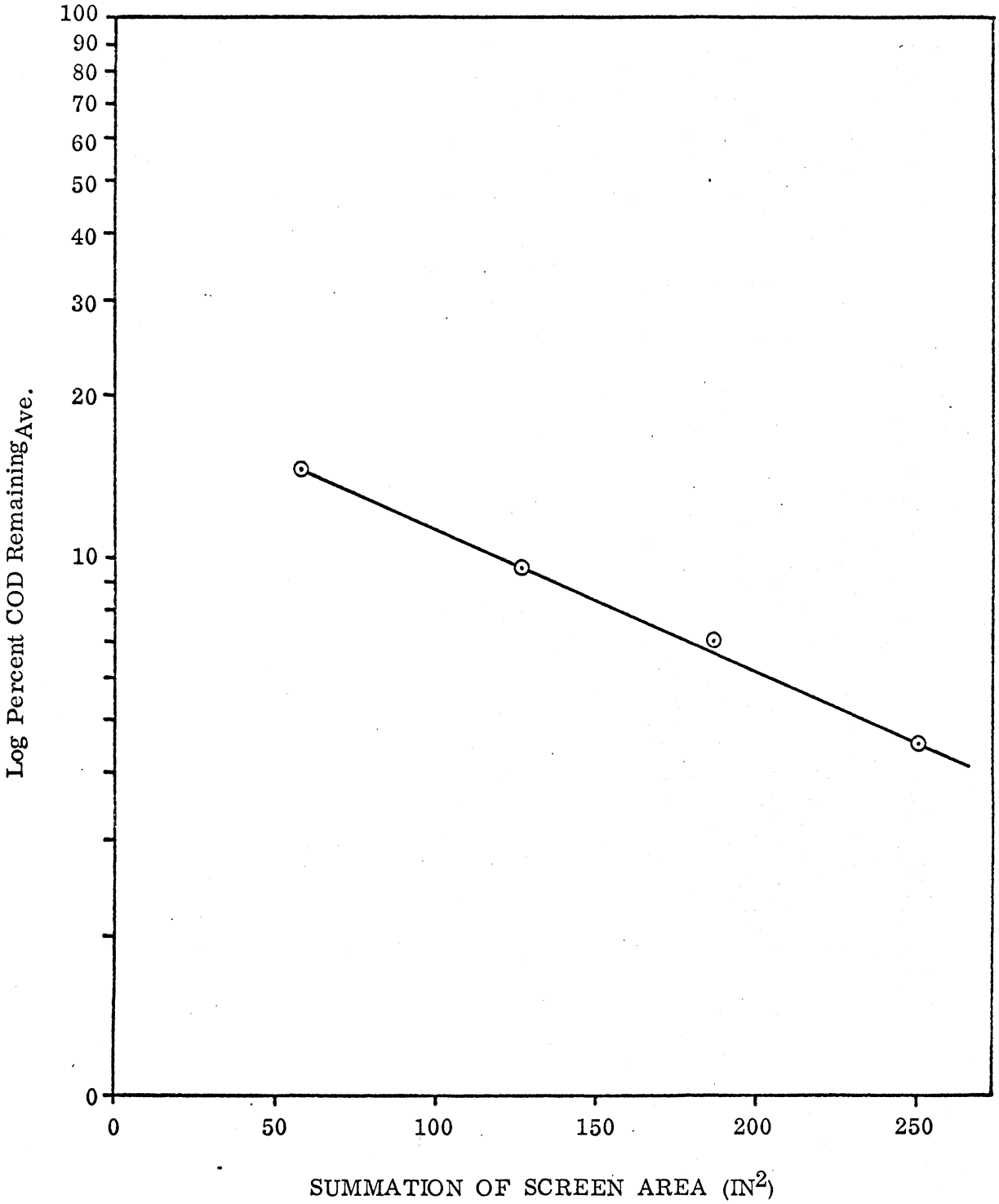

Figure 14. Log Percent COD Remaining$_{Ave.}$ versus Summation of Screen Area

[Volume = 17.25 liters, pH_I = (5.6 - 6.9), $Q_{Ave.}$ = 1.31 L/HR]

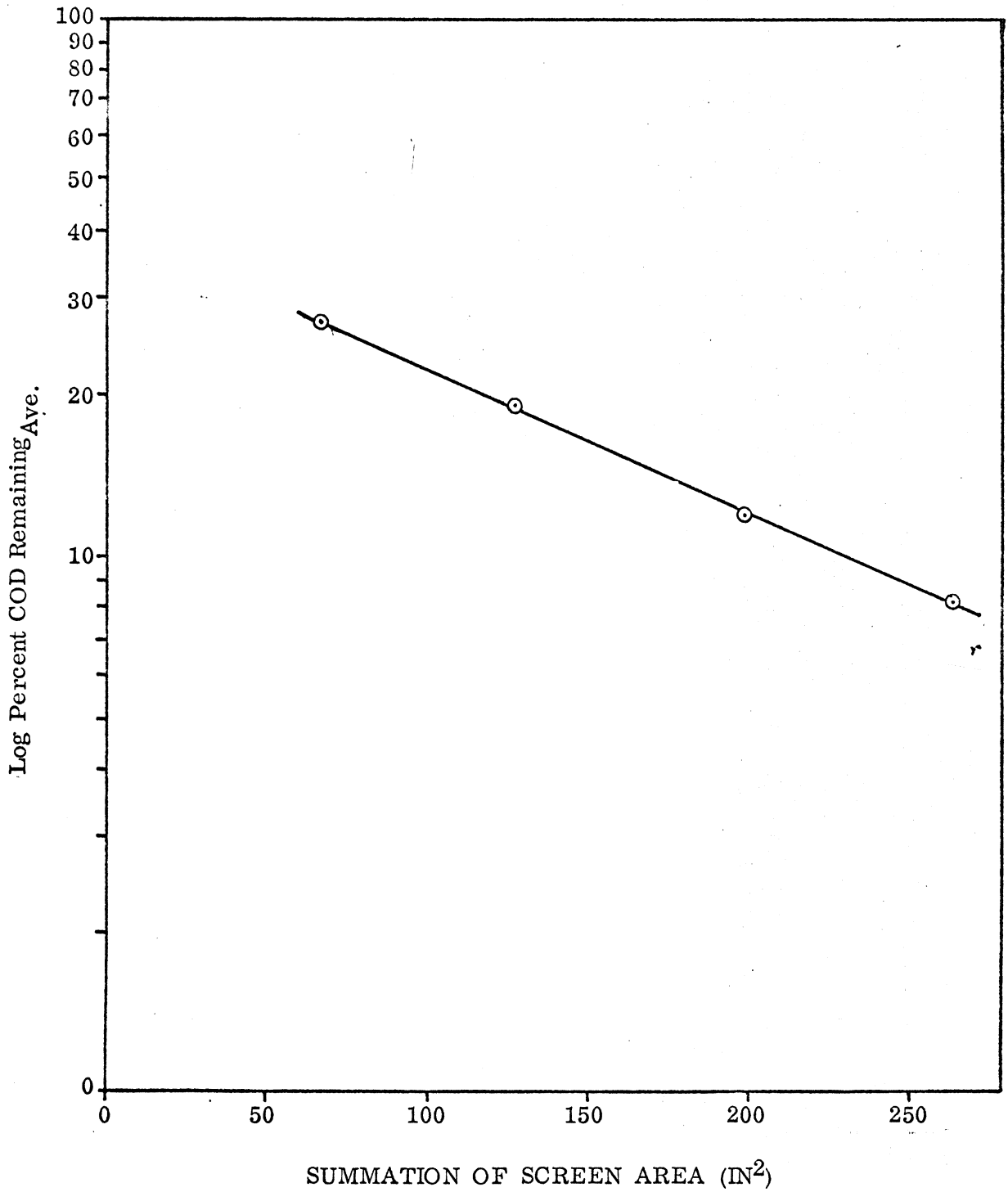

Figure 15. Log Percent COD Remaining$_{Ave.}$ versus Summation of Screen Area

[Volume = 17.79 liters, pH_I = (5.6 - 6.9), $Q_{Ave.}$ = 1.75 L/HR]

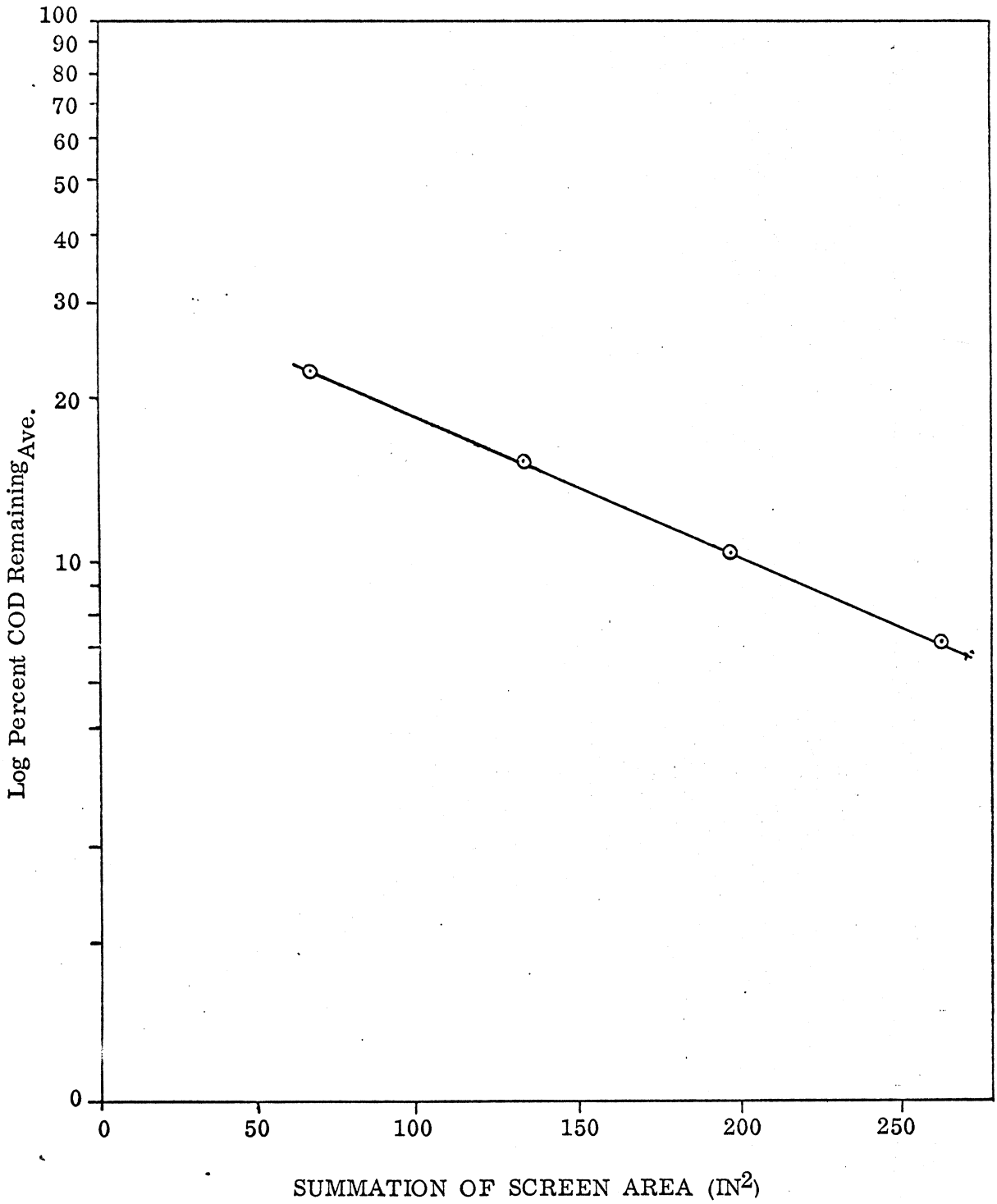

Figure 16. Log Percent COD Remaining$_{Ave.}$ versus Summation of Screen Area

[Volume = 17.79 liters, pH_I = (5.6 - 6.9), $Q_{Ave.}$ = 2.23 L/HR]

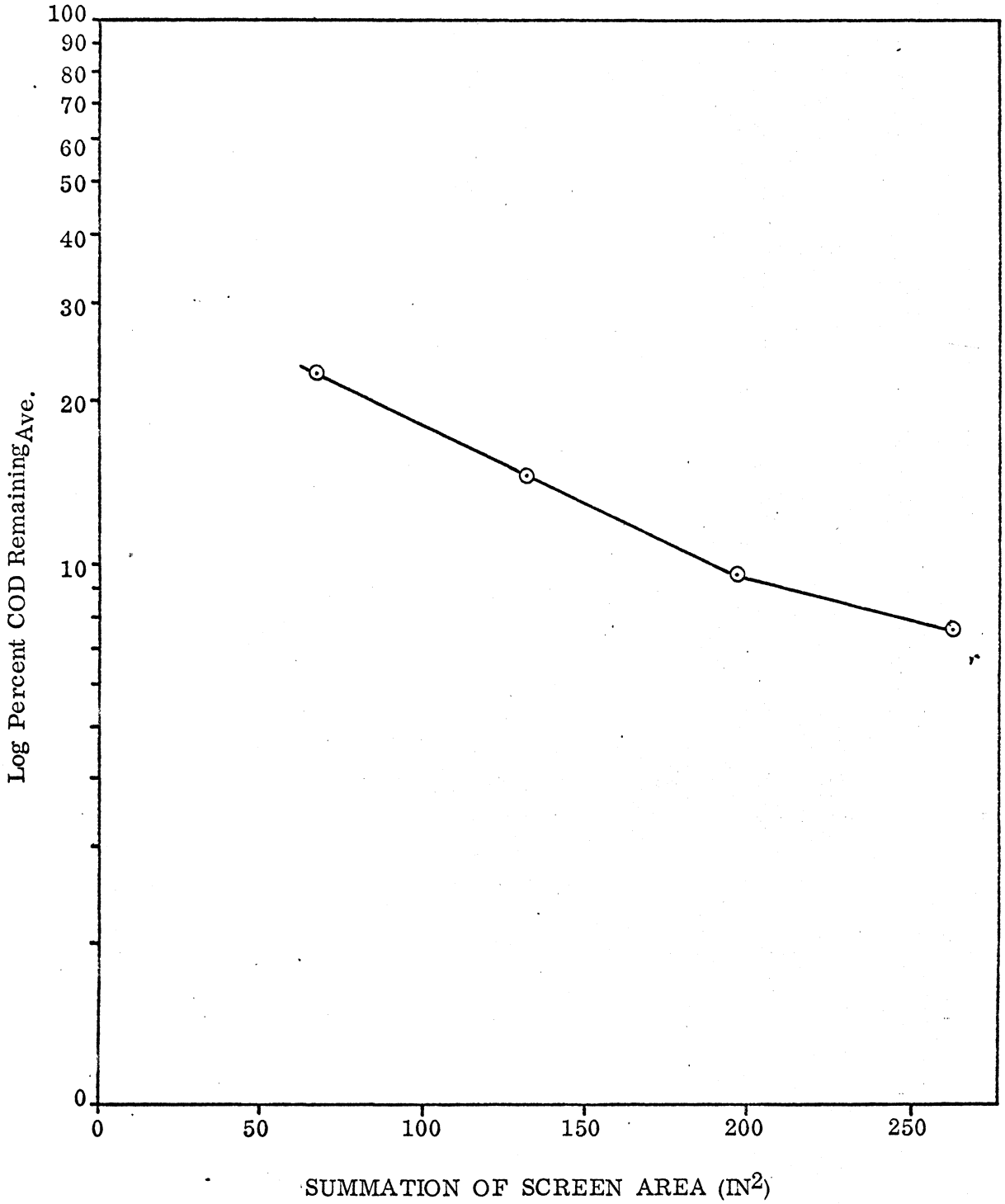

Figure 17. Log Percent COD Remaining$_{Ave.}$ versus Summation of Screen Area

[Volume = 17.79 liters, pH_I = (5.6 - 6.9), $Q_{Ave.}$ = 2.76 L/HR]

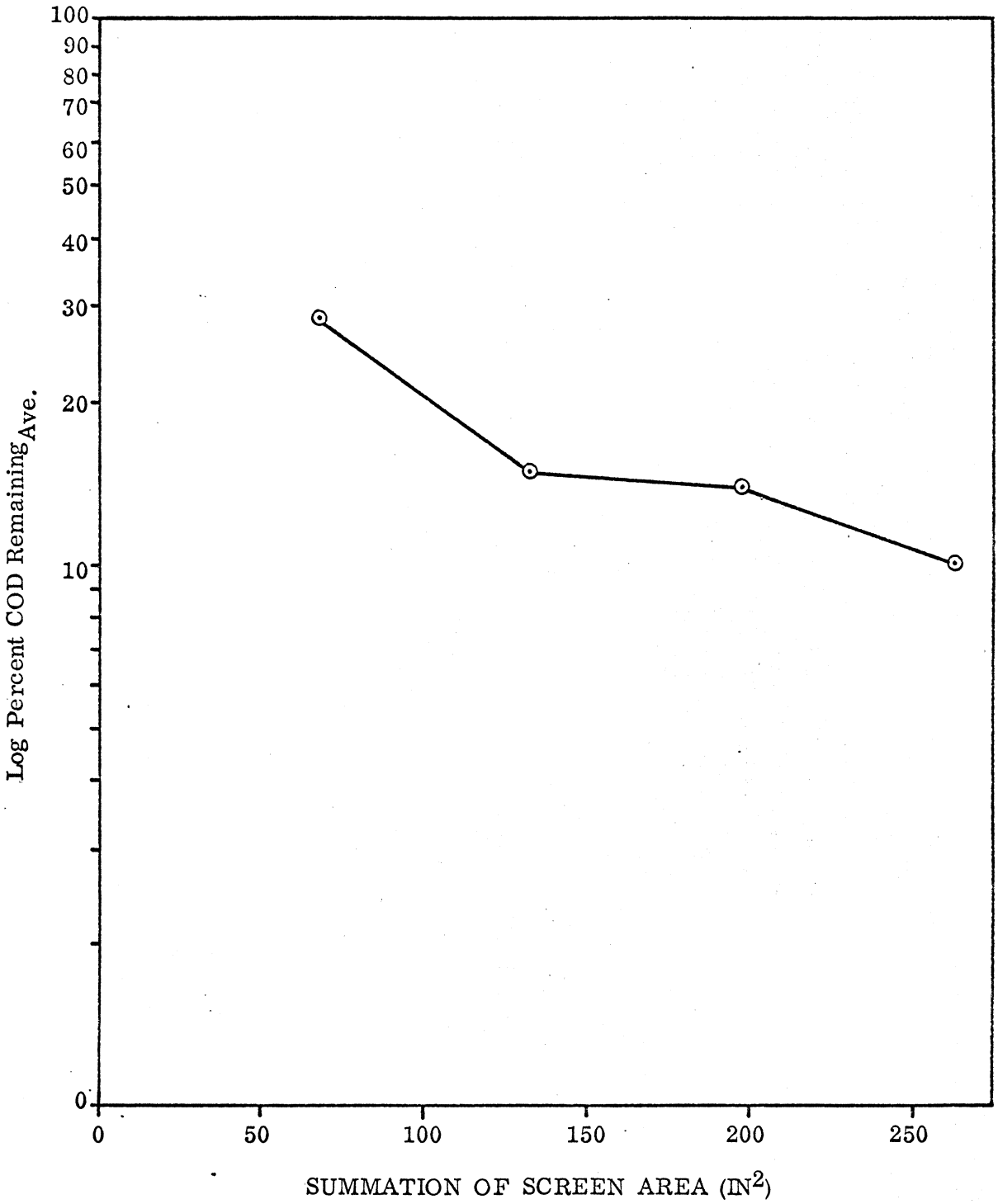

Figure 18. Log Percent COD Remaining$_{Ave.}$ versus Summation of Screen Area

[Volume = 17.79 liters, pH_I = (5.6 - 6.9), $Q_{Ave.}$ = 3.41 L/HR]

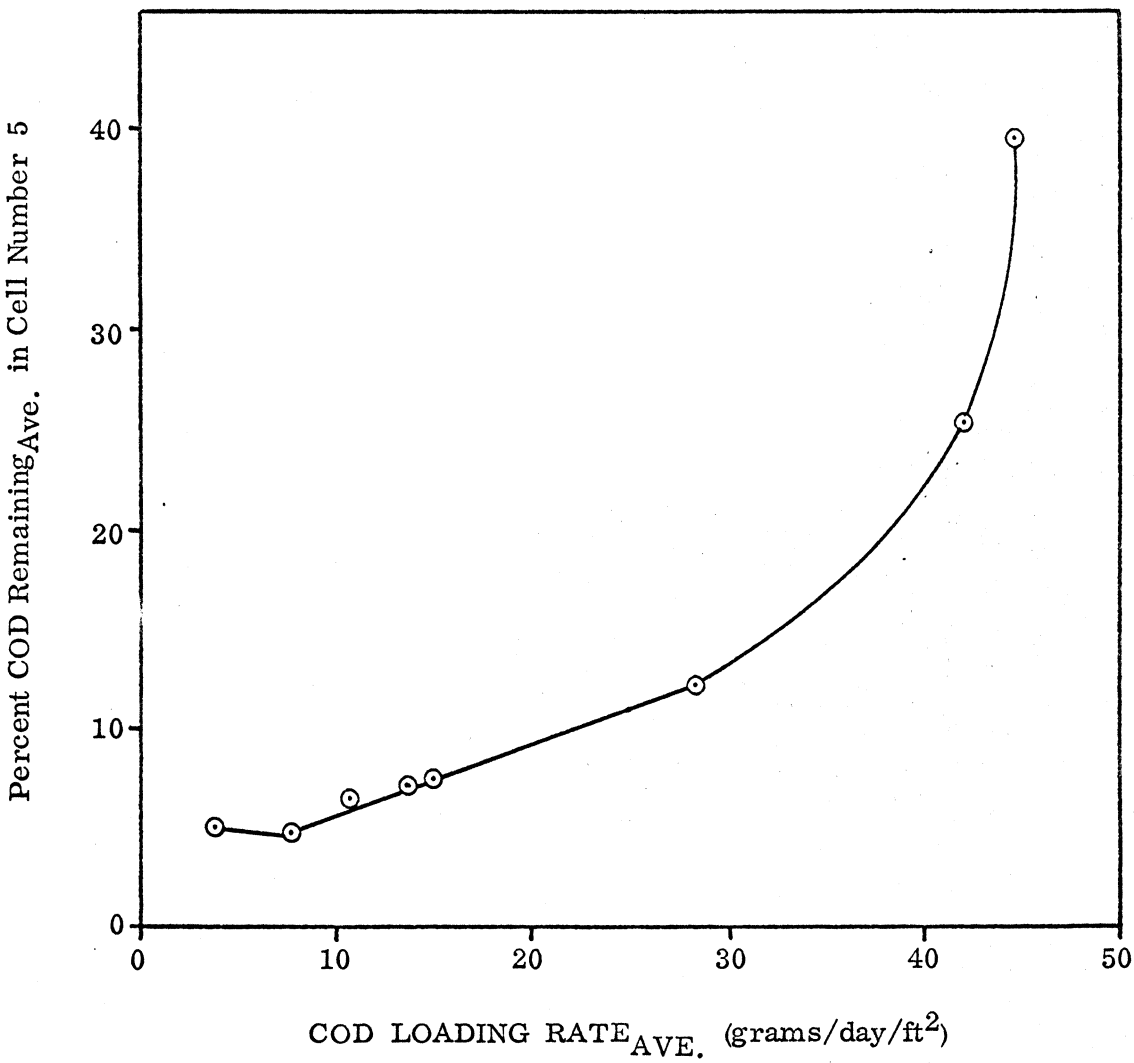

Figure 19. Percent Remaining versus COD Loading Rate [pH = (5.6 - 7.2), $Q_{Ave.}$ = (0.54 - 3.41) L/HR

and the sum of screen area in the growth chamber (square feet). The percent COD remaining stayed relatively constant at 4.5 for COD loading rate values from 3.25 grams per day per square foot to 7.5 grams per day per square foot, then increased linearly from 4.5 to 12.0 when the COD loading rate was increased from 7.5 grams per day per square foot to 28.0 grams per day per square foot. When the COD loading rate was increased above 28.0 grams per day per square foot there was a much more rapid increase in percent COD remaining. In terms of influent COD concentration, the efficiency breakpoint occurred above the concentration of 1040 mg/l when the detention time was 8 hours (Figure 20). The percent remaining in cell number five increased constantly from a value of 4.5 for an influent COD concentration of approximately 201 milligrams per liter, to a value of 12.1 for an influent COD concentration of 1040 milligrams per liter. For influent COD concentrations greater than 1040 milligrams per liter, the percent COD remaining in cell number five increased sharply.

Change in removal rate with influent COD concentration for an average flow rate of 2.23 liters per hour and a pH range of 5.6 to 7.0, is shown in Figure 21. Removal rate increased at a constant rate as the influent COD concentration increased up to an influent COD value of 1200 milligrams per liter. Above this influent COD concentration, there was a reduction in the rate of increase of the removal rate with increase in influent COD concentration to an influent COD concentration value of approximately 1500 milli-

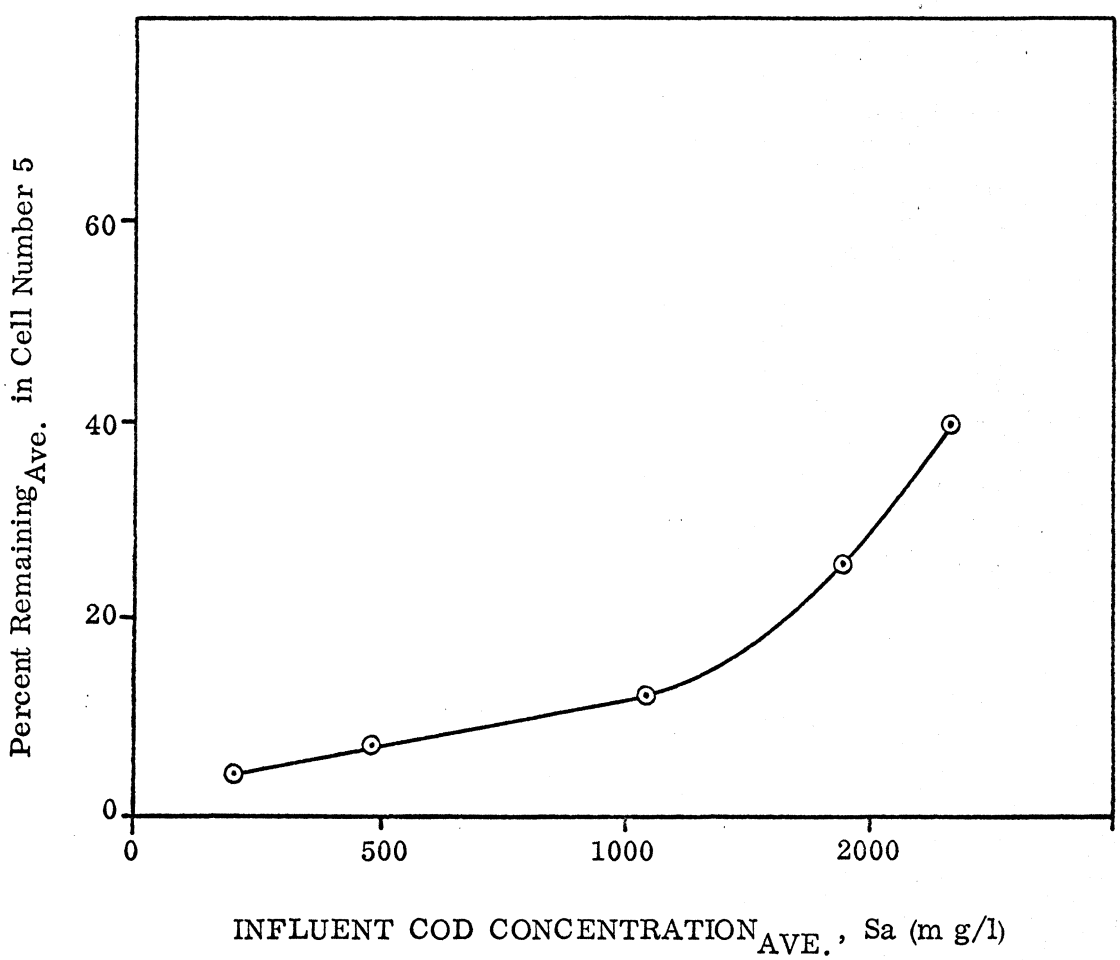

Figure 20. Percent COD Remaining in Cell Number 5 versus Influent COD Concentration

[Volume = 17.79 liters, pH = (5.6 - 7.45), $Q_{Ave.}$ = 2.23 L/HR]
Detention Time = 7.98 Hrs.

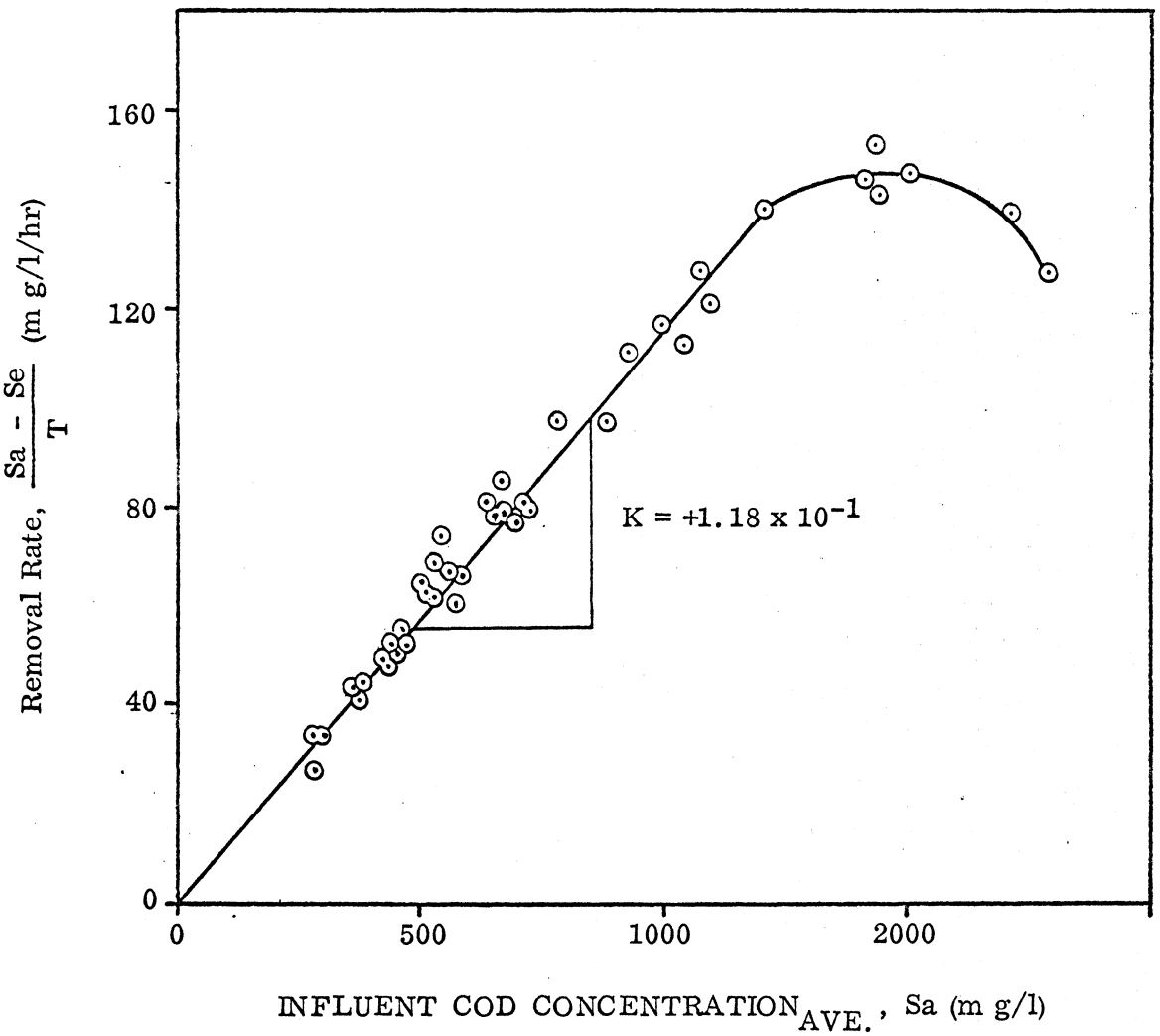

Figure 21. Removal Rate versus Influent COD Concentration

[Volume = 17.79 liters, pH = (5.6 - 7.0), $Q_{Ave.}$ = 2.23 L/HR]

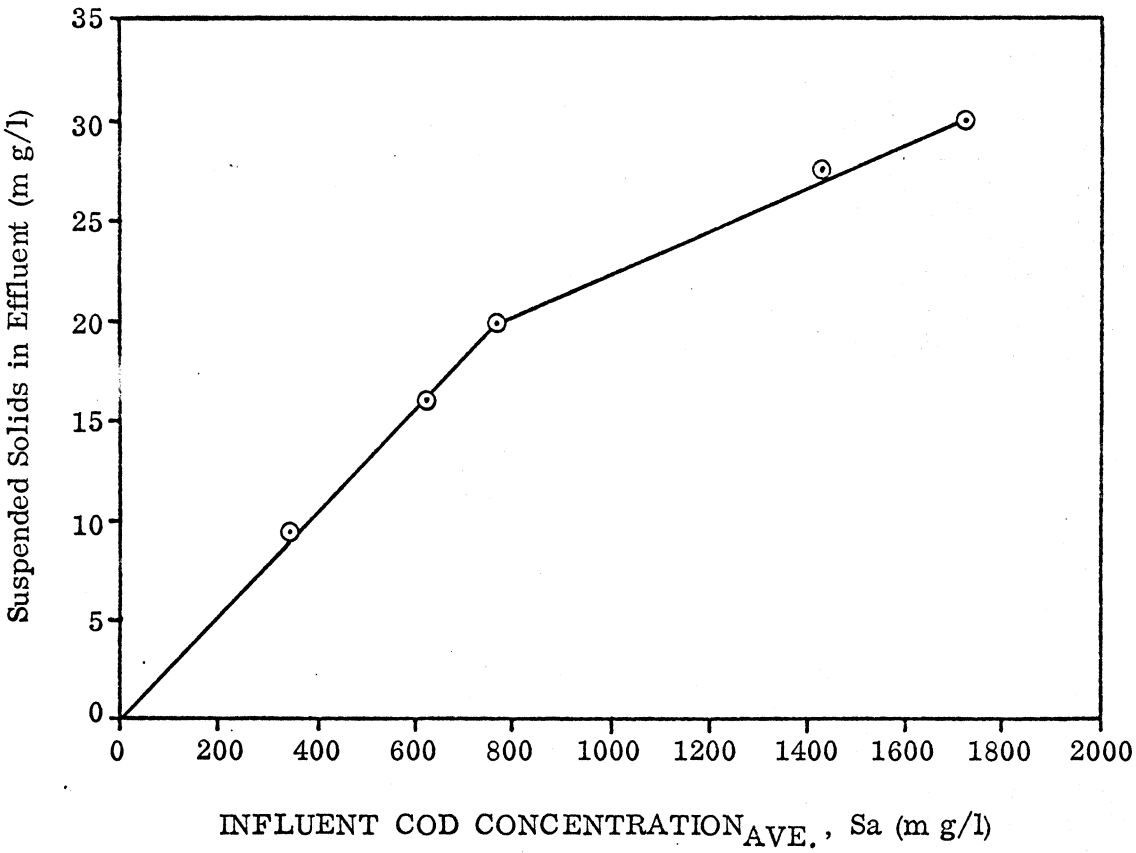

Figure 22. Suspended Solids of Effluent versus Influent COD Concentration

[pH = (6.7 - 7.2), $Q_{Ave.}$ = 2.23 L/HR]

grams per liter. Above 1500 milligrams per liter, the removal rate decreased. Variation in the suspended solids concentration in the effluent from the settling tank with influent COD concentration is illustrated by Figure 22. A constant increase in suspended solids concentration with influent COD concentration occurred up to an influent COD concentration of approximately 760 milligrams per liter. Above this influent COD concentration, the rate of increase in effluent suspended solids concentration per unit influent COD increase, was somewhat less.

Solids Production and Oxygen Utilization

Data presented in Table 3 summarize the effect of flow rate and organic loading rate on "a" and "b" for the pH range of 5.6 - 6.9. The constants "a" and "b" are as defined below:

1. "a" is defined as the quotient of total sludge production and total COD removed during a specific time interval where both values are measured in grams.
2. "b" is defined as the quotient of sludge solids reduction in a sample during endogenous respiration and total solids present in the same sample before endogenous respiration.

Average values for "a" and "b" remained relatively constant at approximately 0.48 and 0.07 respectively on a total solids basis. The BOD_5 removed

TABLE III

Summary of "a" and "b" Data

Q_{Ave} (L/HR)	pH_I Range	Organic Loading $Rate_{Ave}$ $(\frac{Grams}{Day\text{-}Ft^2})$	b_{Ave} $(\frac{mg\ Reduction}{Day})$	a_{Ave} (COD Basis)
0.54	5.6 - 6.9	3.70	0.067	0.48
1.31	5.6 - 6.9	7.84	0.071	0.48
1.75	5.6 - 6.9	10.65	0.069	0.48
2.23	5.6 - 6.9	13.52	0.065	0.48
2.76	5.6 - 6.9	14.95	0.071	0.48
3.41	5.6 - 6.9	13.52	0.069	0.48

was found to average 0.79 of the COD removed. Therefore, "a" on a BOD_5 basis was equal to 0.61. For the range of the experiments, the average volatile solids was found to equal 0.70 of the total solids. Therefore, "a" on a COD and volatile solids basis was equal to 0.34 and, on a BOD_5 and volatile solids basis, was equal to 0.43.

The relationship between oxygen consumed and COD removed is shown in Figure 23. The slope of the plot, a', is 0.54 while the ordinate intercept, b', is 0.05. a' is defined as the fraction of the total oxygen requirement needed for synthesis, and b' is the fraction of the total oxygen requirement needed for endogenous respiration. Figure 24 illustrates the relationship between oxygen consumed and five day BOD removed. The slope of the plot, a', is 0.70 and the ordinate intercept, b', is 0.05. Both Figures 23 and 24 are plotted on a total solids basis. Using the value of the volatile solids equal to 0.70 of the total solids, a' remains equal to the previously noted values, but b' in both cases becomes 0.071.

Effect of pH

The relationship between organic substrate removal efficiency and the pH of the influent for an average flow rate of 2.23 liters per hour and an average influent COD concentration of 490 milligrams per liter, is illustrated in Figure 8. For pH values of 5.25 to 6.8 the percent COD remaining in cell number five was approximately equal to 7.2. At a pH value of 3.94 the percent

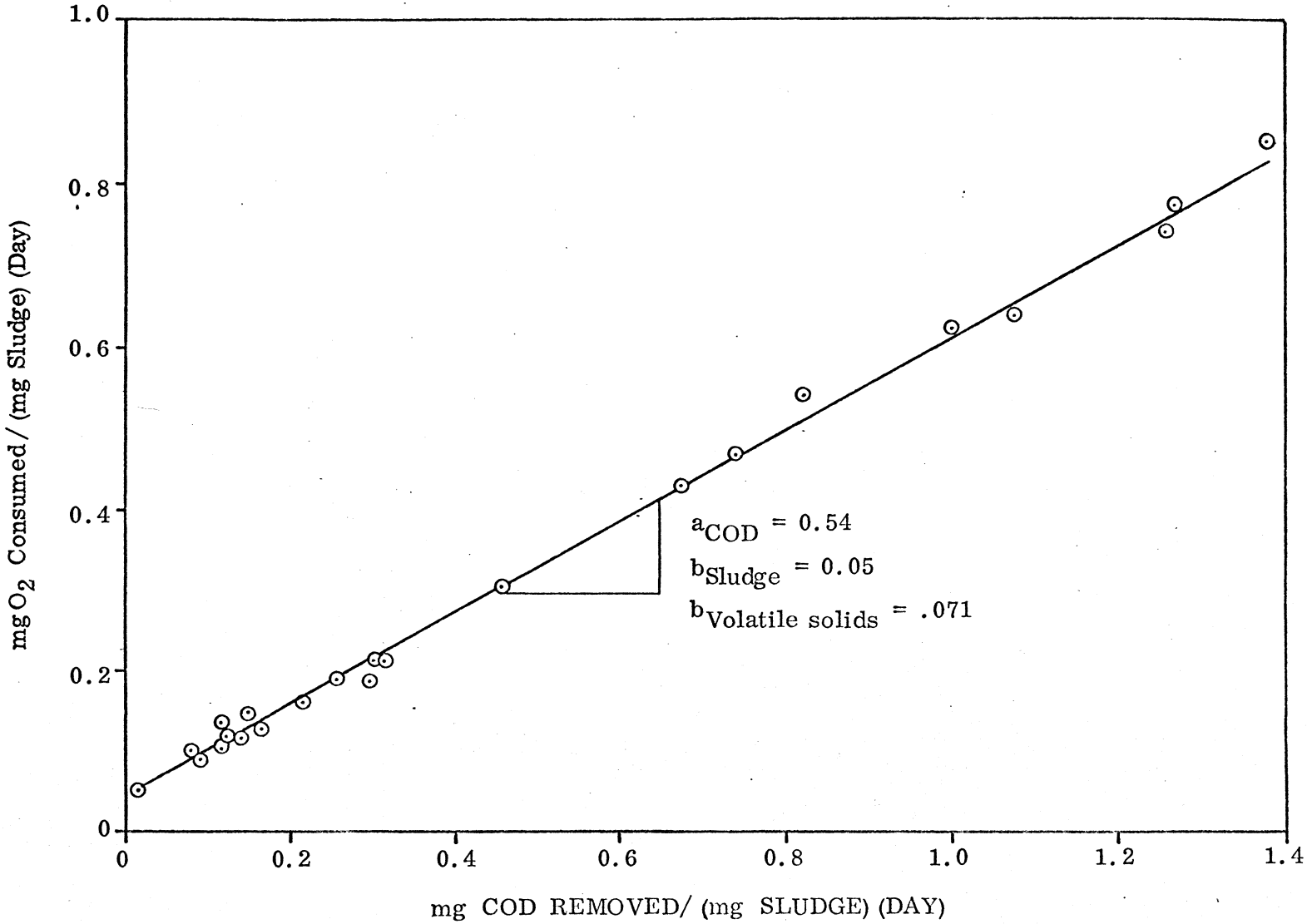

Figure 23. Milligrams Oxygen Consumed per (Milligrams Sludge) (Day) versus Milligrams Removed per (Milligram Sludge) (Day) [pH = 2.65 - 7.2]

remaining in cell number five increased to 9.5. There was a further small increase in percent COD remaining with reduction in pH to a value of 2.65 where the percent COD remaining was approximately 11.6. Then when the pH was lowered from 2.65 to 2.60, the percent COD remaining increased abruptly from 11.6 to 32.7. Further reduction in pH from 2.60 to 2.50 resulted in an increase in percent COD remaining in cell number five from 32.7 to approximately 47.0.

The effect of pH on the rate of organic substrate removal at an average flow of 2.23 liters per hour is shown in Figures 26 through 28 and Figure 8. These figures illustrate an abrupt change in removal rate between the pH values of 2.65 and 2.60. A summary of the values of the removal rate constant, K, with the pH of the influent is shown in Figure 29. A slight reduction in K from 1.18×10^{-1} hrs^{-1} for a pH of 6.8 (Figure 8) down to a value of 1.12×10^{-1} hrs^{-1} for a pH of 2.65 is illustrated. Then a sharp decrease in K from 1.12×10^{-1} hrs^{-1} down to 0.80×10^{-1} hrs^{-1} occurred when the pH was reduced from 2.65 to 2.60. A constant value of 0.80×10^{-1} hrs^{-1} was recorded for K when the pH was equal to 2.60, 2.50, and 2.40.

Figure 30 is a plot of suspended solids concentration versus pH of the influent for an average flow rate of 2.23 liters per hour and an average influent solids concentration of 490 milligrams per liter. This figure shows that the suspended solids concentration of the effluent remained relatively constant at a value of 9.5 milligrams per liter over a pH range of 2.75 to

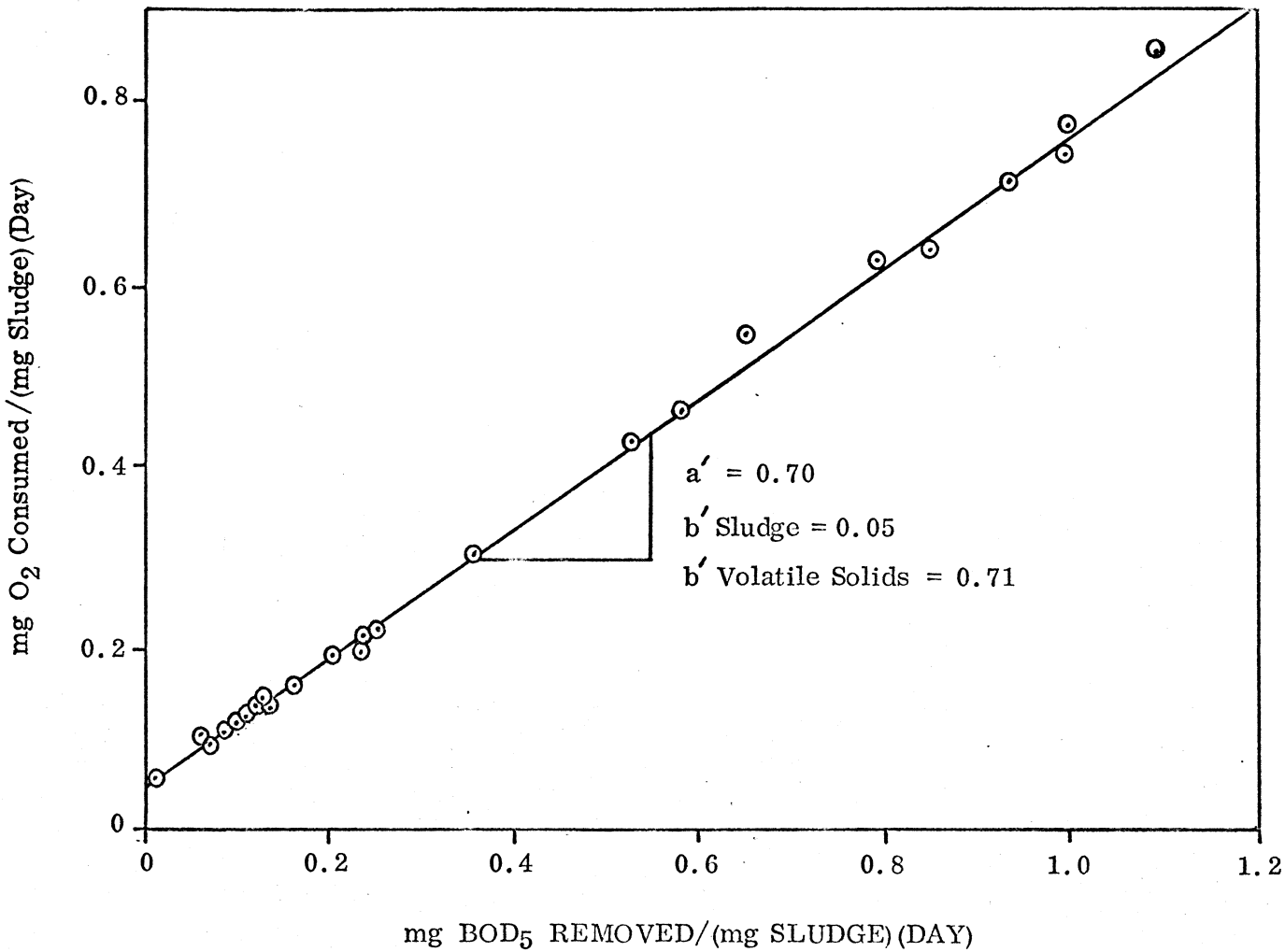

Figure 24. Milligrams Oxygen Consumed per (Milligram Sludge)(Day) versus Milligram BOD_5 per (Milligram Sludge)(Day)

[pH = (2.65 - 7.2)]

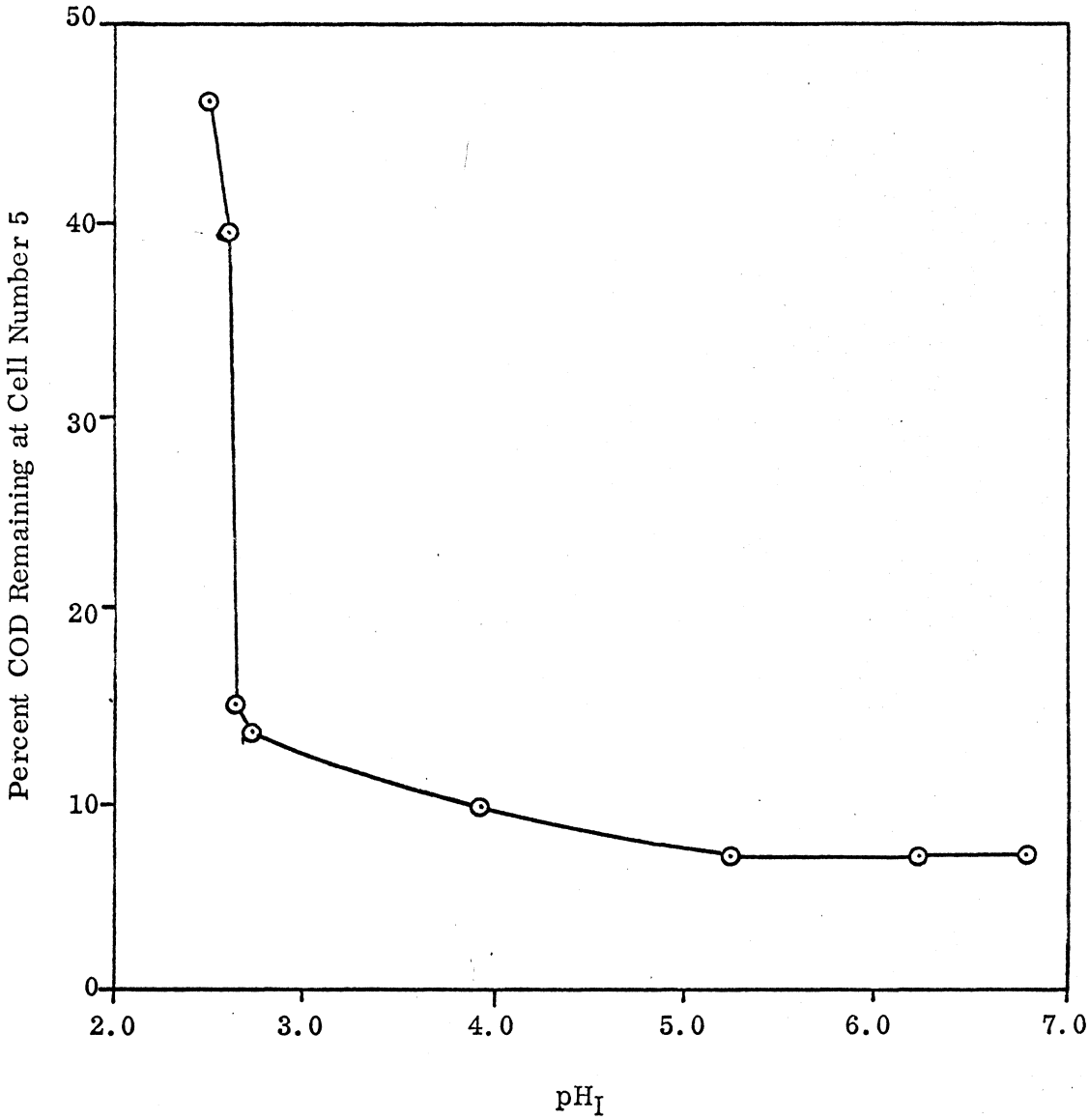

Figure 25. Percent COD Remaining at Cell Number 5 versus pH_I

[Volume = 17.79 liters, Sa_{Ave} = 490.11 mg/l, Q_{Ave} = 2.23 L/HR]

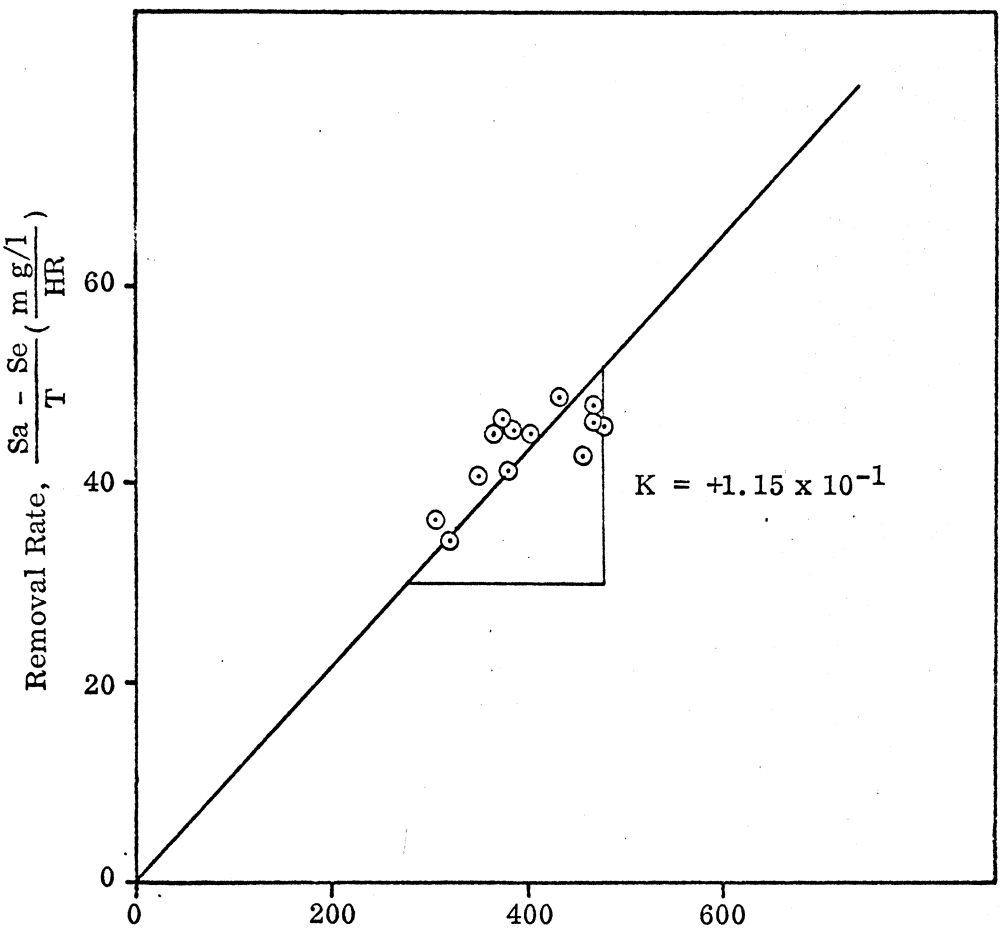

Figure 26. Removal Rate versus Influent COD Concentration

[Volume = 17.79 liters, pH = (3.0 - 5.0), Q_{Ave} = 2.23 L/HR]

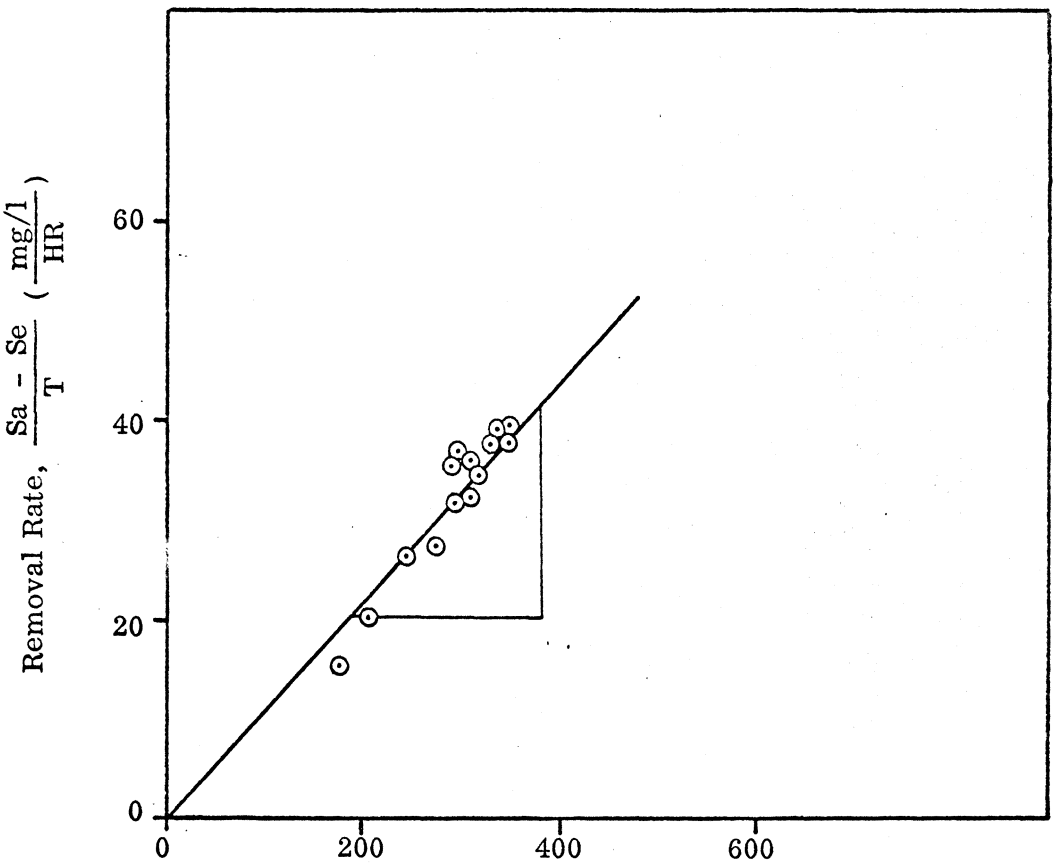

Figure 27. Removal Rate versus Influent COD Concentration

[Volume = 17.79 liters, pH = (2.65 - 2.99), Q_{Ave} = 2.23 L/HR

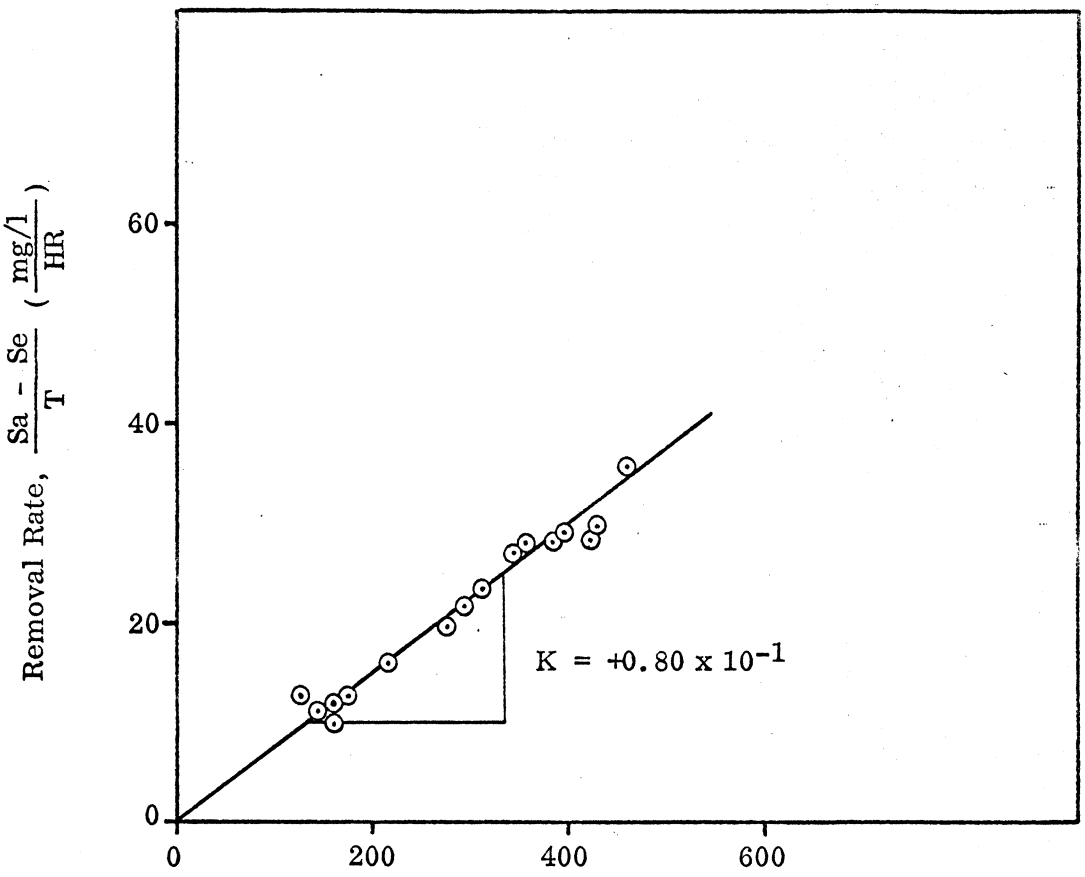

Figure 28. Removal Rate versus Influent COD Concentration

[Volume = 17.79 liters, pH = (2.40 - 2.60), Q_{Ave} = 2.23 L/HR]

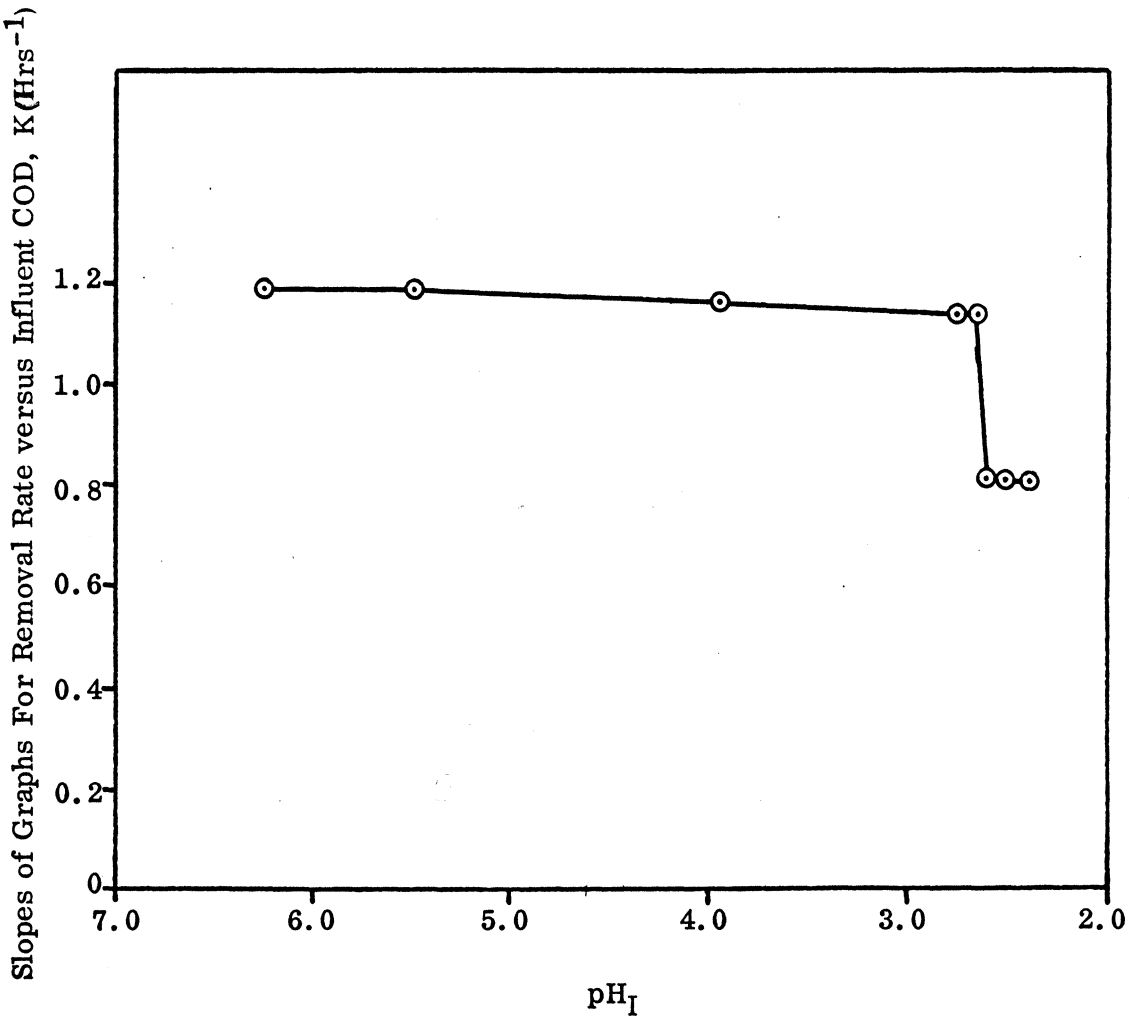

Figure 29. Slopes of Graphs For Removal Rate versus Influent COD versus pH of Influent

[Volume = 17.79 liters, Sa_{Ave} = 490.11 mg/l, Q_{Ave} = 2.23 L/HR]

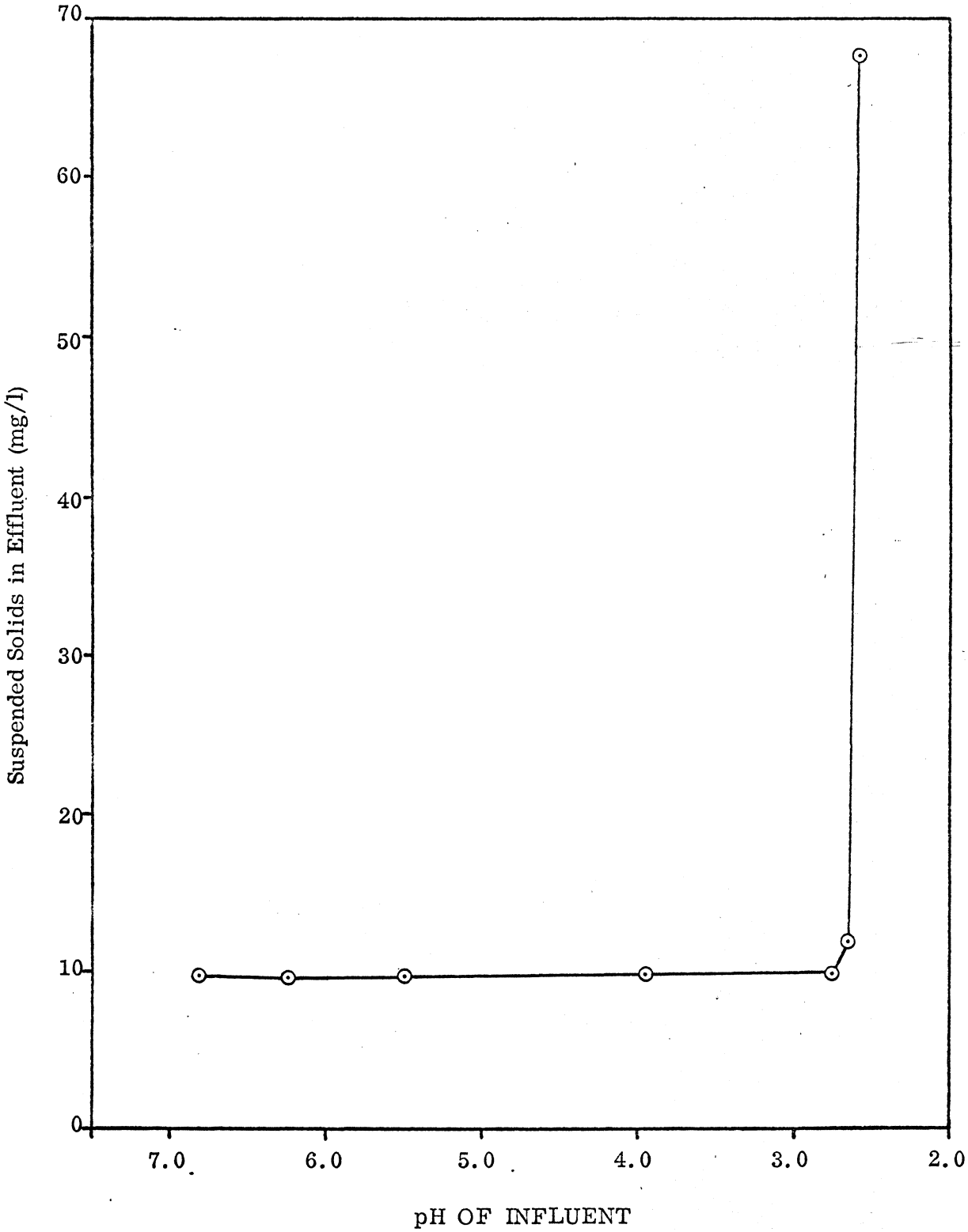

Figure 30. Suspended Solids versus pH of Influent

[Volume = 17.79 liters, Sa_{Ave} = 490.11 mg/l, Q_{Ave} = 2.23 L/HR]

TABLE IV

Summary of Effects of pH on "a" and "b"

Q_{Ave} (L/HR)	pH_I Range	Organic Loading $Rate_{Ave}$ $(\frac{Grams}{Day\text{-}Ft^2})$	b_{Ave} $(\frac{mg\ Reduction}{Day})$	a_{Ave} (COD Basis)
2.23	6.80	13.52	0.066	0.48
2.23	6.25	13.52	0.067	0.48
2.23	5.25	13.52	0.071	0.48
2.23	3.94	13.52	0.073	0.48
2.23	2.75	13.52	0.072	0.48
2.23	2.65	13.52	0.075	0.48
2.23	2.60	13.52	0.083	0.18

6.8, then increased slightly from 9.5 to 11.6 milligrams per liter when the pH was decreased from 2.75 to 2.65. Further lowering of the pH from 2.65 to 2.60, caused a sharp increase in the suspended solids concentration in the effluent from 11.6 to 67.4 milligrams per liter.

Table 4 is a summary of the effects of pH on "a" and "b" for an average flow rate of 2.23 liters per hour. Both "a" and "b" remained relatively constant at 0.48 and 0.07 on a total solids and COD basis for a pH range of 6.80 to 2.65. At a pH of 2.60 "a" and "b" were calculated to be 0.18 and 0.083 respectively.

Effect of BOD to Nitrogen Ratio

The relationship between percent COD remaining and the BOD:N ratio at low pH for an average flow rate of 2.23 liters per hour and an average influent COD concentration of 490 milligrams per liter is shown by Figure 31. For BOD to nitrogen ratios from approximately 8:1 to 45:1, the percent COD remaining in cell number five was relatively constant at approximately 11.0. Above this ratio the percent COD remaining increased to a value of 53.4 for a BOD:N ratio of 90:1. At normal pH levels (6.6 to 7.45), the percent remaining in cell number five stayed relatively constant at approximately 10.5 for BOD:N ratios of 16:1 to 90:1 (Figure 32). Above the latter BOD:N ratio, the percent COD remaining increased to 66.0 for a BOD:N ratio of 135.1.

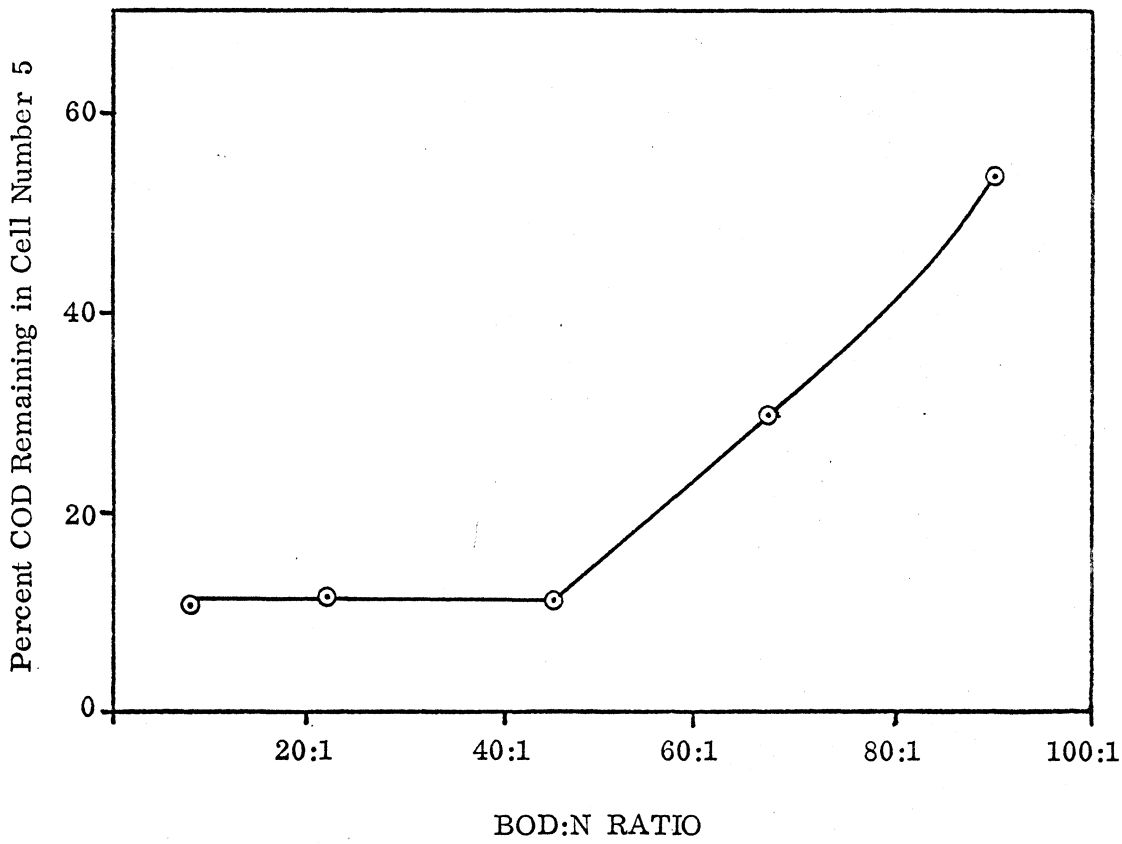

Figure 31. Percent COD Remaining in Cell Number 5 versus BOD:N Ratio at Low pH

[Volume = 17.79 liters, pH = (2.65 - 2.85) Sa_{Ave} = 490.11 mg/l, Q_{Ave} = 2.23 L/HR]

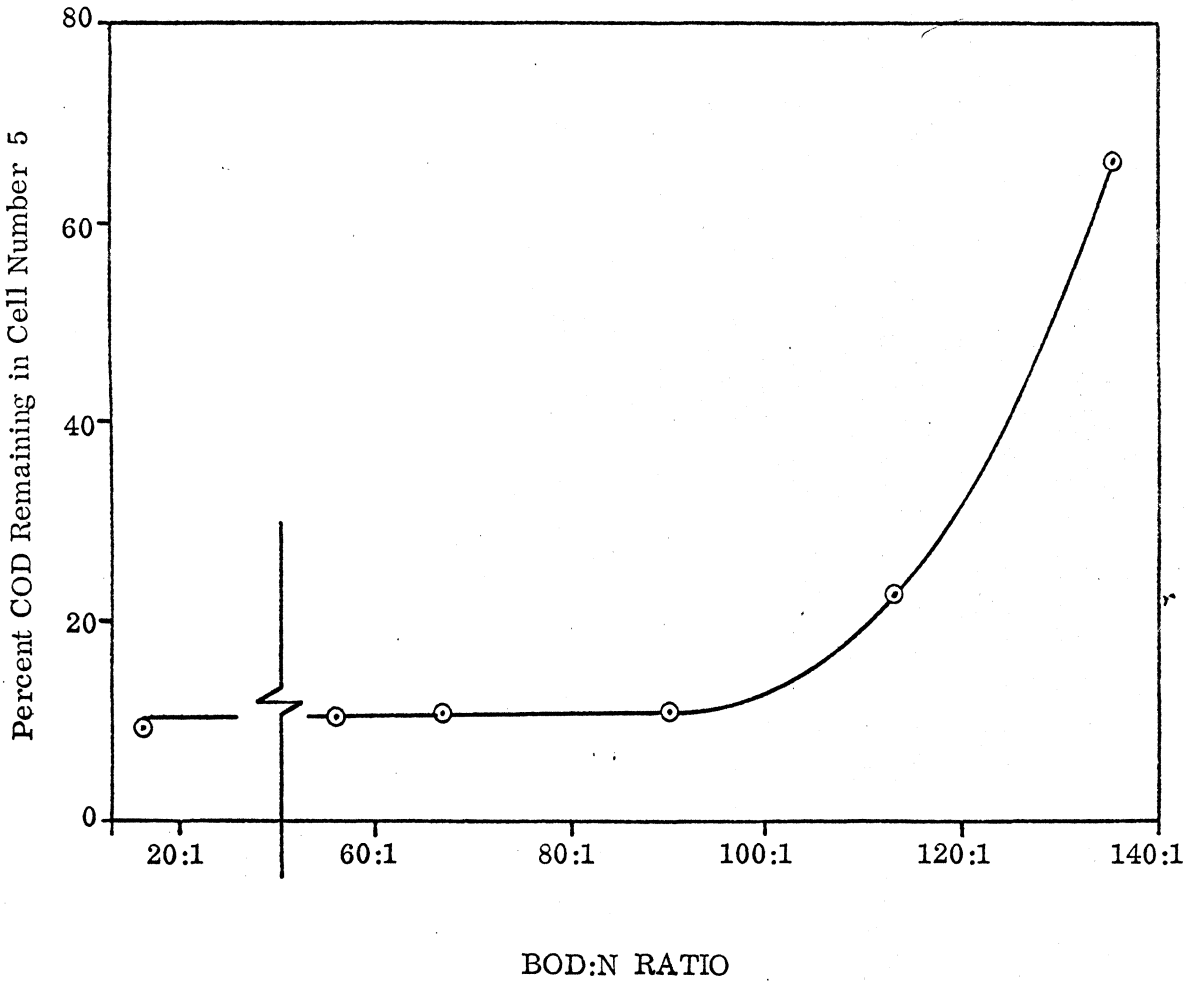

Figure 32. Percent COD Remaining in Cell Number 5 versus BOD:N Ratio

[Volume = 17.79 liters, pH = (6.6 - 7.45), Sa_{Ave} = 490.11 mg/l, Q_{Ave} = 2.23 L/HR]

TABLE V

Summary of Effects of BOD:N Ratio on "a" and "b"

Q_{Ave} L/HR	pH_I Range	Loading $Rate_{Ave}$ $\frac{Grams}{Day\text{-}Ft^2}$	BOD:N Ratio	a_{COD} Based on Sludge Solids	b $(\frac{mg\ Reduction}{Day})$
2.23	2.65 - 2.85	13.52	8:1	0.48	.075
2.23	2.65 - 2.85	13.52	23:1	0.48	.074
2.23	2.65 - 2.85	13.52	45:1	0.48	.074
2.23	2.65 - 2.85	13.52	68:1	0.51	.069
2.23	2.65 - 2.85	13.52	90:1	0.60	.069
2.23	6.6 - 7.45	13.52	16:1	0.48	.065
2.23	6.6 - 7.45	13.52	23:1	0.48	.066
2.23	6.6 - 7.45	13.52	45:1	0.48	.065
2.23	6.6 - 7.45	13.52	68:1	0.48	.066
2.23	6.6 - 7.45	13.52	90:1	0.48	.067
2.23	6.6 - 7.45	13.52	113:1	0.52	.068
2.23	6.6 - 7.45	13.52	135:1	0.64	.068

Data presented in Table 5 summarize the effect of the BOD:N ratio on "a" and "b" for pH ranges of 2.65 to 2.85 and 6.6 to 7.45. For the pH range of 2.65 - 2.85, the average values for "a" and "b" were calculated to be constant at 0.48 and 0.07, respectively, over a BOD:N range of 8:1 to 50:1. Both "a" and "b" increased for BOD:N ratio larger than 50:1. The data shows that for the normal pH range of 6.6 to 7.45, the average values for "a" and "b" remained constant over a BOD:N range of 16:1 to 90:1. Both "a" and "b" increased for BOD:N ratio larger than 90:1 and at a BOD:N ratio of 135:1, "a" and "b" were 0.64 and 0.068, respectively.

Microorganisms Present at Acid and Neutral Conditions

Macroscopic examination of neutral or near neutral pH revealed the presence of large attached growth tenacles, varying in color from white to brown to orange, on the screens, with similar flocs floating free in all five of the cells of the growth chamber. Microscopic examinations by Dr. Robert Benoit of the Microbiology Department of Virginia Polytechnic Institute showed that the test culture at or near neutral pH consisted of approximately 50 - 60 percent fungi with the rest being composed of Sphaerotilus, free moving bacteria, and Zooglea ramigera. At a low pH of 2.65, large attached growth tenacles that were brick red in color were present on the screens and similar flocs were in all five of the cells of the growth chamber. From microscopic examination, Dr. Robert Benoit reported that approximately 90 percent of

the microorganisms were fungi and yeast with about 10 percent of the organisms being Sphaerotilus.

Summary of Typical Operating Conditions

A summary of typical experimental data for various operating conditions is given in Table 6 and Table 7. These results were determined through a carefully controlled experimentation process and display the versatility of the system for the treatment of organic wastes under a wide variety of experimental conditions.

TABLE VI

Summary of Experimental Data

Q_{Ave} (4HR)	pH_I Range	Organic Loading Rate$_{Ave}$ $(\frac{Grams}{Day\text{-}Ft^2})$	Influent $\frac{BOD}{COD}_{Ave}$	Effluent $\frac{BOD}{COD}_{Ave}$	$K \times 10^1$
0.54	5.6 - 6.9	3.70	0.79	0.36	0.38
1.31	5.6 - 6.9	7.84	0.79	0.36	0.75
1.75	5.6 - 6.9	10.65	0.79	0.36	0.94
2.23	5.6 - 6.9	13.52	0.79	0.36	1.18
2.23	6.80	13.52	0.79	0.36	1.18
2.23	6.25	13.52	0.79	0.36	1.18
2.23	5.25	13.52	0.79	0.36	1.18
2.23	3.94	13.52	0.78	0.36	1.15
2.23	2.75	13.52	0.78	0.36	1.13
2.23	2.65	13.52	0.77	0.37	1.12
2.23	2.60	13.52	0.76	0.38	0.80
2.76	5.6 - 6.9	14.95	0.79	0.36	1.45
3.41	5.6 - 6.9	13.52	0.79	0.36	1.75

TABLE VII

Summary of Experimental Data

Q_{Ave} (4HR)	pH_I Range	b_{Ave} $\frac{\text{mg Reduction}}{\text{Day}}$	a_{Ave} COD and Total Solids Basis	Percent COD Remaining Ave	Effluent Suspended Solids Ave mg/l	a^1 BOD and Total Solids Basis	b^1 BOD and Total Solids Basis
0.54	5.6 - 6.9	0.067	0.48	4.68	1.8	0.70	0.05
1.31	5.6 - 6.9	0.071	0.48	4.50	5.8	0.70	0.05
1.75	5.6 - 6.9	0.069	0.48	7.27	7.5	0.70	0.05
2.23	5.6 - 6.9	0.065	0.48	7.17	9.5	0.70	0.05
2.23	6.80	0.066	0.48	7.17	9.5	0.70	0.05
2.23	6.25	0.067	0.48	7.17	9.5	0.70	0.05
2.23	5.25	0.071	0.48	7.17	9.5	0.70	0.05
2.23	3.94	0.073	0.48	9.6	9.5	0.70	0.05
2.23	2.75	0.072	0.48	13.5	9.5	0.70	0.05
2.23	2.65	0.075	0.48	14.8	11.8	0.70	0.05
2.23	2.60	0.083	0.18	39.3	67.3	*	*
2.76	5.6 - 6.9	0.071	0.48	7.6	11.5	0.70	0.05
3.41	5.6 - 6.9	0.069	0.48	10.1	16.6	0.70	0.05

*Note: No meaningful results could be obtained using the Hach Manometric BOD apparatus becuase a very long lag period occurred in the bottle at this pH.

CHAPTER VI

DISCUSSION OF RESULTS

The objective of this research project was to study and define the capabilities of a biological waste treatment system specificially designed to use attached or filamentous microorganisms for the stabilization of organic wastes. The main unit of this system consisted of a rectangular shaped plexiglass growth chamber in which vertical screens had been suspended perpendicular to the waste flow. The microorganisms used the screens as support media by attaching themselves to the screens. The general flow of the waste was through the screens as well as some underflow. The attached organisms extended from the screens into the media in both directions and treatment occurred along the exposed surface of the organisms. Unattached organisms were washed from the growth chamber and settled in the settling basin which followed the growth chamber in the flow diagram of the system. No recirculation of organisms was necessary to maintain the biological population since the primary biological mass was attached to the screens. Aseptic air, which was supplied by diffusor stones along the bottom of the unit, added the needed mixing as well as the required oxygen.

The literature indicates that filamentous organisms are efficient organisms for the removal of organic wastes, but it also points out that, previously, no process had been developed to use this type of organism to its maximum potential. The data from this research proves that the utilization of filamentous organism for the biological oxidation of wastewaters is both feasible and practical, and that a high degree of treatment of an organic waste can be accomplished without recirculation or sludge return. The results, given in the preceding chapter, define the operational capabilities of the system with relation to various parameters. A discussion of the significant aspects of the results follows:

Effects of Flow Rate

The percent COD remaining in cell number five of the growth chamber for flow rates of 0.54 liters per hour and 1.31 liters per hour was approximately the same, about 4.5. Thus, the average percent COD reduction through the growth chamber at flow rates of 1.31 liters per hour or less was 95.5 percent. When the flow rate was increased from 1.31 liters per hour to 3.41 liters per hour, there was a small corresponding increase in percent COD remaining in cell number five of the growth chamber. However, even at a flow rate of 3.41 liters per hour (detention time approximately 5.21 hours) there was greater than 89 percent reduction of the influent COD through the growth cell which illustrates the high efficiency of the process. The data

shows that flow rate, that is, hydraulic loading rate, does not have an effect on the efficiency of the system used when the detention time is less than 13.6 hours. However, when flow rate was increased above 1.31 liters per hour, efficiency was a function of hydraulic loading.

The results illustrated by Figures 5 through 11 show that the biological removal rate of the system is a linear function of the organic loading rate. K, the removal rate constant, increased from 0.38×10^{-1} hrs.$^{-1}$ for an average dilution rate of 0.031 hours^{-1} to a value of 1.75×10^{-1} hrs.$^{-1}$ for a dilution rate of 0.192 hrs.$^{-1}$. Since the quantity of biological mass fixed on the support media (screens) remained relatively constant, an increase in hydraulic loading or dilution rate actually meant an increase in the food-to-microorganism ratio. Therefore, as the food-to-microorganism ratio was increased, there was a linear increase in the removal rate constant, K. In much of the literature, removal rate is considered to also be a function of the concentration of biological mass present in the system. Thus, classical definition for removal rate is that it is equal to the substrate removal divided by the product of the detention time and the mass of organisms present in the growth chamber. The organic mass present in the experimental system used during this research can be estimated by the support media surface area (screen area) perpendicular to the flow. In these experiments the screen area was held approximately constant at 1.9 square feet. In future studies with this system, the definition of removal rate should probably be altered to

include screen area if comparisons between systems are to be made. Such an alteration would change the definition of removal rate to the following:

> The removal rate is equal to the influent COD concentration minus the final COD concentration divided by the product of the detention time of the growth chamber and the support media area.

Because of the nature of the system, the effluent suspended solids concentration is also a function of hydraulic loading. As flow rate increased from zero to 2.76 liters per hour the suspended solids concentration of the effluent from the settling tank increased constantly from zero to approximately 11.5 milligrams per liter. When the flow was increased above 2.76 liters per hour there was a greater rate of increase in suspended solids than for the previously mentioned flow rate range. However, even at a flow rate of 3.41 liters per hour, the effluent suspended solids concentration was only about 16.5 milligrams per liter. These statements indicate that the suspended solids concentration was a function of flow rate but that over the total flow rate range studied, the flow was not great enough to cause an excessive suspended solids concentration in the effluent. The results imply that, as flow rate is increased, an ever increasing amount of immature attached microorganisms is stripped off the support media. The immature filamentous growths tend to be more voluminous and, therefore, are more difficult to settle in the settling basin.

Relationship Between Screen Area and Percent COD Remaining

As anticipated, the results show that screen area is strongly related to the COD removal that occurs in the system and, therefore, to the percent COD remaining in the effluent. For flow rates in the range of 0.54 to 2.23 liters per hour, there was a constant rate of decrease in log percent COD remaining with increase in screen area. However, when the average flow rate was 2.76 liters per hour, the log percent COD remaining decreased at a constant rate with an increase in screen area until the screen area exceeded 210 square inches. For further increase in screen area up to 280 square inches, the rate of COD reduction increased with increased screen area, but at a considerably lower rate. A similar decrease in COD removal rate was observed for all screen areas when the average flow was 3.41 liters per hour.

The probable explanation for the change in efficiency is that at the higher flow rates, organics that are more difficult to metabolize are only partially removed as compared to the lower flow rates because of the decreased contact time between the organics and the attached microorganisms on the later screens. For this reason a lower percentage reduction was noted in the latter chambers for the higher flow rates than was noted for these chambers at lower flow rates. Even though at the two higher flow rates there was not a single constant decrease in log percent COD remaining with an increase in screen area, there was a high percent

removal (approximately 90 percent) of influent COD by the growth chamber. It was also observed that some removal was achieved before any screen area came into action indicating metabolism by unattached microbes. However, the presence of unattached organisms in the system was possible only because of the screens. The organisms attached themselves to the screens and reinoculation of the growth chamber was achieved from the attached growth.

Effect of Organic Loading

Percent COD remaining in cell number five of the growth chamber remained relatively constant at 4.5 for COD loading rate values of 3.25 to 7.5 grams per day per square foot of screen area (Figure 19). When the COD loading rate value was increased from 7.5 to 28.0 grams per day per square foot there was a gradual increase in percent COD remaining in cell number five from 4.5 to 12.0. When the COD loading rate value was increased above 28.0 grams per day per square foot the percent COD remaining increased rapidly. The results indicate that this system, as used for experimental purposes, has a critical COD loading rate of 28.0 grams per day per square foot of screen area. Thus, treatment is efficient only at lower loading rates.

Further effects of organic loading were observed by noting that when the flow rate was equal to 2.23 liters per hour (waste retention time of 8 hours), the percent COD remaining in cell number five increased at a constant rate with influent COD concentration over a concentration range of

201 to 1040 milligrams per liter (Figure 20). On the other hand, when the influent COD concentration was increased above 1040 milligrams per liter, COD removal efficiency decreased rapidly. The decreased metabolism of the microbial population was probably caused by substrate concentration inhibition.

For the same flow rate (2.23 liters per hour) the removal rate increased at a constant rate with influent COD concentration increase up to an influent COD concentration value of 1200 milligrams per liter. Then a breakpoint was observed. This breakpoint was higher than the breakpoint for COD remaining (1040 milligrams per liter). No reason for this difference is apparent at this time. Above the removal rate breakpoint, the removal rate increased slowly with increase in influent COD concentration up to an influent COD concentration of approximately 1500 milligrams per liter. Then, for influent COD concentration above 1500 milligrams per liter, the removal rate decreased. Thus, it appears that the removal rate was a function of influent COD concentration up to a value of approximately 1200 milligrams per liter. Above this COD concentration other environmental factors become important. This observation is similar to the findings of Michaelis and Menten (31) in their work with enzyme action. They found that when substrate concentrations are sufficiently high, the rate of enzyme action is actually slowed because of substrate concentration inhibition.

The suspended solids concentration in the effluent from the settling tank increased constantly with influent COD concentration up to an influent COD concentration of approximately 760 milligrams per liter. For concentrations above 760 mg/l the rate of increase was somewhat less. This indicates that the optimum organic loading condition, as far as suspended solids concentration in the effluent is concerned, may be above an influent COD concentration of 760 milligrams per liter when the detention time of the growth chamber and settling basin is approximately eight hours.

Solids Production and Oxygen Utilization

For a pH range of 5.6 - 6.9, the average ratio of total solids production to total COD removed, "a", remained relatively constant at 0.48. Although the average value for "a" at all flow rates was noted to be constant, there was some fluctuation in "a" for each specific operating condition. This fluctuation indicated that sludge would build up on the screen for a period of time and then would be released. Even though this condition did seem to exist, the data did not show a definite cycling pattern. "b", the rate of sludge solids reduction per day during endogenous respiration, was approximately seven percent reduction per day for all flow rates studied. This value is identical to that found by Eckenfelder (9) for an activated sludge process treating pulping and semi-chemical waste.

"a" on a BOD_5 and total solids basis was found to equal 0.61 and on a BOD_5 and volatile solids basis was calculated to be 0.43. The average value of this ratio on a COD and volatile solids basis was noted to be 0.34. The value of "a" equal to 0.48 on atotal solids and COD basis is somewhat higher than that found by Busch (3) for activated sludge (a = 0.44). However, the "a" value of 0.34 on a COD and volatile solids basis is somewhat lower than the 0.39 value determined by Servizi and Bogan (43) for activated sludge. It is apparent that "a" for this process is relatively close to that of conventional biological treatment processes.

In studying the relationship between oxygen consumed and COD removed, a', the fraction of total oxygen requirement needed for synthesis, was found to equal 0.54 on a COD basis and 0.70 on a BOD basis. b', the fraction of the total oxygen requirement needed for endogenous respiration, was equal to 0.05 grams O_2 per gram of sludge per day on a BOD and COD basis where total solids values were used. b', was determined to be 0.071 grams O_2 per gram of sludge per day on both a BOD and a COD basis where volatile solids values were used. The value for a' of 0.54 fits near the top of the range of a''s that Eckenfelder (9) found for a variety of industrial waste oxidation systems and is only slightly higher than the a' value of 0.52 he reported for domestic sewage. The b' value of 0.05 grams O_2 per gram of sludge per day falls near the lower end of the range for endogenous respiration as stated in the literature (b' = 0.046 - 0.24 grams of O_2 per gram of sludge

per day). From these statements it can be seen that oxygen requirements for synthesis and endogenous respiration for the system are close to those for other biological waste treatment systems.

Effect of pH

The pH studies were run at an average flow rate of 2.23 liters per hour and an average influent COD concentration of 490 milligrams per liter. For pH values of 5.25 to 6.8, the percent COD remaining in cell number five of the growth chamber was relatively constant at 7.2. There was a general increase in percent COD remaining in cell number five as the pH was lowered toward a pH of 2.65. A very large increase in percent COD remaining (11.6 to 32.7) was noted when the pH was lowered from a value of 2.65 to 2.60. A further reduction in pH from 2.60 to 2.50 gave an increase in percent COD remaining from 32.7 to 47.0. The reduction in the efficiency of the system at the lower pH values occurred because the dominant microorganisms being unable to operate efficiently at these pH values. The removal rate constant, K, changed only slightly from 1.18×10^{-1} hrs^{-1} down to 1.12×10^{-1} hrs^{-1} when the pH was lowered from 6.8 to 2.65. However, it decreased sharply from 1.12×10^{-1} hrs^{-1} to 0.80×10^{-1} hrs^{-1} when the pH was 2.50 and 2.40. The reduction in K as the pH was lowered from a pH value of 2.65 to 2.60 was most likely caused by a change in the composition of the microbial population.

There was no significant change in the suspended solids concentration of the settling tank effluent over a pH range of 2.75 to 6.8. It was relatively

constant at 9.5 mg/l. When the pH was lowered from 2.65 to 2.60, however, the suspended solids in the effluent increased from 11.6 to 67.4 mg/l, a six-fold increase. Since the given figures are equilibrium values, this is further evidence of a change of predominant biological forms in the process when the pH drops below 2.65.

A summary of the effects of pH on sludge production is given in Table 4. Average values for "a" and "b" on a total solids and COD basis remained relatively constant at 0.48 and 0.07, respectively, over a pH range of 6.80 to 2.65. When the pH was lowered from 2.65 to 2.60, the average values for "a" and "b" changed to 0.18 and 0.083, respectively. The "a" value illustrates that the dominant organisms are very inefficient at pH values below 2.65. The change in "a" and "b" values as the pH is lowered from 2.65 to 2.60 indicate a change in population composition.

All of these statements concerning the effect of pH indicate that with an appropriate amount of time for acclimation, an organic waste with an acid pH of 2.65 or above can be treated very efficiently without the occurrence of excessive suspended solids in the effluent from the settling basin.

Nitrogen Requirements

The BOD:N studies showed that the experimental process has a much lower nitrogen requirement than activated sludge. For the influent pH range of 2.65 to 2.85, there was no change in COD removal efficiency for BOD:N ratio until, at a BOD:N ratio of 45:1, the percent COD remaining in the last

cell of the growth chamber was 53.4 for a flow rate of 2.23 liters per hour. By contrast, for the influent pH range of 6.6 to 7.45 the percent COD removal efficiency was relatively constant for BOD:N ratios as high as 90:1. Above 90:1, the percent COD remaining in the effluent increased gradually up to 66.0 for a BOD:N ratio of 135:1.

The results of the BOD:N studies establish that the critical BOD:N ratio of the process over an influent pH range of 2.65 to 2.85 was greater than 45:1, and for an influent pH range of 6.6 to 7.45, it was greater than 90:1. The change in the critical value of BOD:N ratio for the two different influent pH ranges studied was probably caused by the differences in the predominate microorganism population present at the difference pH conditions. The test culture used in this experiment was that developed in the low pH studies. Certain organisms that were present in the original culture used in the original neutral pH studies were probably eliminated by pH toxicity before reacclimation to neutral pH. This possibly accounted for the fact that the percent COD remaining for an average flow rate of 2.23 liters per hour, when the BOD:N ratio was below the critical ratio and the pH range was 6.6 to 7.45 was somewhat higher after pH acclimation than before (10.5 as compared with 7.2).

Both "a" and "b" average values were constant for BOD:N ratio below the critical BOD:N ratio for both the low pH and neutral pH studies. These values were the same as those found in the rest of the study. Above the critical BOD:N ratio, "a" increased significantly for both pH ranges. This is

in agreement with the literature which states that more sludge is produced per unit COD removed when a system is operating above the critical BOD:N ratio. "b", for both pH cases, showed only minor increases when the BOD:N ratios were increased above the critical ratio.

Microorganisms Present at Acid and Neutral Conditions

A macroscopic examination at neutral or near neutral pH's revealed that the screen growths were large attached tenacles, varying in color from white to brown to orange, with similar free floating flocs occurring in all five of the cells of the growth chamber. Microscopic examination by Dr. Robert Benoit of the Microbiology Department of Virginia Polytechnic Institute revealed that the test culture at or near neutral pH consisted of approximately 50-60 percent fungi while the rest of the culture was composed of freemoving bacteria, Sphaerotilus, and Zooglea ramigera. At a pH of 2.65, large attached growth tenacles that were brick red in color appeared on the screens and in all five of the growth chambers. Further microscopic examination by Dr. Robert Benoit showed that approximately 90 percent of these microorganisms were fungi and yeast with about 10 percent of the organisms being Sphaerotilus:

For the various environmental conditions studied, the types of microorgansims found were reasonably consistent with the literature, the exception being that the percentage content of Sphaerotilus was lower than expected.

CHAPTER VII

CONCLUSIONS

The experimental results show that the process studied is an efficient and effective method for treating organic wastewaters under a wide variety of environmental conditions. Based on an analysis and evaluation of the collected data, the following conclusions were made:

1. The efficiency of this system is dependent on flow rate, i.e., hydraulic loading rate.
2. The removal rate is a function of COD loading and, since COD loading is a function of flow rate, the removal rate constant, K, varies directly with the flow rate.
3. An increase in flow rate or organic loading causes an increase in suspended solids in the effluent from the settling tank.
4. The system can effectively treat an organic waste over a pH range of 2.65 to 7.45.
5. A decrease in pH of the influent below 2.65 disrupts

the system and causes a tremendous increase in the concentration of COD and suspended solids in the effluent from the settling basin. There is a corresponding reduction in the removal rate and the removal rate constant, K.

6. An increase in organic loading or concentration causes an increase in the percent COD remaining in the final growth cell.
7. The system can treat an organic waste having a COD loading rate as high as 22 grams per day per square foot of screen area with at least ninety percent removal of COD through the growth chamber.
8. Using an average flow rate of 2.23 liters per hour (detention time equal to eight hours), the removal rate is a linear function of influent COD concentration from an influent COD concentrations of approximately zero up to 1200 milligrams per liter.
9. Solids production and oxygen requirements or utilization for the experimental system are similar to those of other biological systems presently used to treat organic waste.

10. This system can effectively treat a nitrogen deficient organic waste at both acid and neutral pH's.
11. Recirculation of biological solids is not necessary to keep a sufficient amount of culture in the system for effective treatment.
12. Fungi were the dominant microorganisms present in the system at both acid and neutral pH.

CHAPTER VIII

SUMMARY

The objective of this investigation was to study and define the capabilities of a biological treatment system specifically designed to use attached or filamentous microorganisms for the stabilization of organic wastes. Various chemical and physical tests such as pH, suspended solids, Chemical Oxygen Demand, Biochemical Oxygen Demand, sludge production, and oxygen utilization were run at various operating conditions.

The investigation showed that the removal rate constant, K, is a function of the organic loading rate or food-to-microorganism ratio. Correspondingly, the organic loading rate is a function of flow rate or hydraulic loading rate in addition to the organic concentration of the waste, because of the fixed nature of the microbial mass. Therefore an increase in hydraulic loading caused an increase in the removal rate constant, suspended solids concentration in the effluent, and percent COD remaining in the final cell of the growth chamber.

This study also showed that an organic waste could be effectively treated by the attached filamentous system over an influent pH range of

2.65 to 7.45 with an oxygen requirement similar to that found in other biological treatment processes. The decrease in influent pH below 2.65 caused a tremendous decrease in COD removal efficiency in the growth chamber and a similar increase in the suspended solids concentration of the effluent with a corresponding reduction in the organic removal rate and the removal rate constant, K.

The results of this experimental project further showed that the growth chamber as designed can accomplish a minimum of 90 percent COD removal from organic waste when the COD loading rate is as large as 22 grams per day per square foot of screen area. Generally, an increase in the organic loading or the concentration of the influent COD caused an increase in the percent COD remaining in the fifth cell of the growth chamber and the suspended solids of the effluent. For an average flow rate of 2.23 liters per hour (approximately eight hours detention), the removal rate was found to be directly proportional to the influent COD concentration from a concentration of approximately zero to a concentration equal to 1200 milligrams per liter.

The investigation also showed that this system could treat a nitrogen deficient waste at both neutral and acid pH's. The critical BOD:N ratios were found to be greater than 45:1 for a waste with a pH in the range of 2.65 - 2.85 and greater than 90:1 for a waste with a pH from 6.6 - 7.45.

Under the operating conditions studied, recirculation of biological solids was found to be unnecessary to keep sufficient culture in the growth chamber for effective treatment. The average sludge production per unit of COD removed, "a", was found to be relatively constant at 0.48 on a total solids basis for most of the operating conditions of this study. "a" decreased significantly to 0.18 when the pH of the influent was at a pH of 2.60 and increased significantly when the BOD:N ratio was above the critical ratio. The waste solids which sloughed from the screens during treatment settled readily and were easily concentrated.

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APPENDIX I

Hach Manometric BOD Apparatus - Model 2173

Principle of Operation

A measured sample of sewage or wastewater is placed in one of the bottles on the apparatus, and the bottle is connected to a closed end mercury manometer. Above the sewage or water sample is a quantity of air which contains 21 percent oxygen. Over a period of time bacteria in the sewage utilize the oxygen to oxidize the organic matter present in the sample, and thus dissolved oxygen is consumed. The air in the closed sample bottle replenishes the utilized oxygen which results in a drop in air pressure in the sample bottle. This pressure drop is registered on the mercury manometer and is read directly as ppm BOD. During the test period (usually five days) the sample is continually agitated by a magnetic stirring bar which is rotated by a pulley system connected to a motor. Carbon dioxide is produced by the oxidation of organic matter and must be removed from the system to prevent development of positive gas pressure which would result in an error. This is accomplished by the addition of several drops of potassium hydroxide solution to a wick assembly which is placed in each sample bottle.

APPENDIX II

Cleaning of Glassware

Because minute quantities of organic and inorganic matter may cause serious errors in COD and gravimetric analysis, it was necessary to take great care in the cleaning of glassware used. The glassware was first soaked in 1:100 solution 7X detergent, (Limbro Chemical Company, New Haven, Connecticut) for several hours and then scrubbed and rinsed eight times in tap water and four times in distilled water. The glassware was then shaken by hand and inverted upon paper towels and left in the air to dry.

The pipettes were soaked in 1:100 7X solution for more than 24 hours and then rinsed with an automatic continuous device for at least one hour in cold tap water. These pipettes were then drained and air dried.

APPENDIX III

YSI Model 51 Oxygen Meter

Operation Principle

The YSI Oxygen Probe is a polarographic system.

The cathode is a gold ring imbedded in a lucite block; the anode is a silver coil recessed in the central well. The interior is filled with an aqueous solution of potassium chloride (KCl). A thin Teflon membrane stretched across the end of the sensor isolates the sensor elements from their environment. The membrane is permeable to gases and allows them to enter the interior of the sensor. When a suitable polarizing voltage is applied across the cell, oxygen will react at the cathode causing a current to flow through the cell. The amount of current which flows is proportional to the amount of oxygen to which the membrane is exposed. The sensor actually measures the oxygen pressure. Since oxygen is consumed at the cathode, it can be assumed that the oxygen pressure inside the membrane is zero (this is nearly so at the gold cathode). Hence, it can be seen that the force causing oxygen to diffuse through the membrane is proportional to the absolute pressure of oxygen outside the membrane. If the oxygen pressure increases, more oxygen diffuses through the

membrane and more current flows through the cell. A lower pressure results in less current. The membrane diffusion is directly proportional to pressure and the oxygen-cell current relationship obeys stoichiometric laws, thus a linear relationship exists between external oxygen pressure and cell current.

Temperature control is important because the membrane permeability varies with temperature and changes at a rate of about 4 percent per ^{o}C depending on the membrane material.

APPENDIX IV

Maintenance of the Experimental Apparatus

There was very little maintenance necessary to keep the system operational. Diffusor stones were cleaned once every week to make sure that their full potential for supplying air and mixing was being achieved. To clean the diffusors, the pumice stones were removed from the liquid and scraped with a metal spatula.

Whenever the first cell of the growth chamber became clogged with excess growth, the first screen was raised three inches off the bottom of the growth chamber and additional diffused air was added to the cell to help flush some of the clumps from the system. This cleaning process was accomplished with a minimum of effort.

Other maintenance necessary was the occasional replacement of a hose and the oiling of the pumps and pump motor.

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THE DEVELOPMENT OF PROCESS KINETICS FOR A WASTE TREATMENT SYSTEM UTILIZING FILAMENTOUS MICROORGANISMS

by

H. Randall Edwards

ABSTRACT

The capabilities of a biological waste treatment system designed to utilize filamentous microorganisms were defined in this study. Various chemical and physical tests such as pH, suspended solids, COD, BOD, sludge production, and oxygen utilization were used to determine the effects of parameters like flow rate, support media area, organic loading, pH, and the BOD-to-nitrogen ratio.

Findings of the study indicated that the removal rate, suspended solids concentration in the effluent, and percent COD remaining in the final growth cell of the growth chamber increase as the dilution rate or flow rate increase.

The study also showed that an organic waste can be effectively treated by a filamentous system over an influent pH range of 2.65 to

7.45 with an oxygen requirement similar to that found in other biological treatment systems. An increase in organic loading caused an increase in the suspended solids of the effluent with a decrease in COD removal efficiency. The removal rate was found to be directly proportional in influent COD concentration from a concentration of approximately zero to a concentration equal to 1200 milligrams per liter.

A nitrogen deficient waste at both neutral and acid pH's was treated effectively by this system. For a pH range of 2.65 to 2.85, the critical BOD:N ratio was found to be greater than 45:1. A BOD:N ratio in excess of 90:1 was found to be critical for a waste with a pH range of 6.6 to 7.45.

Recirculation of biological solids was not needed to retain sufficient culture in the growth chamber for efficient treatment of the waste. Sludge production values were found to be close to values for the activated sludge process treating organic wastes. The average value for "a" on a COD and total solids basis was found to equal 0.48 for most of the conditions studied. However, a tendency for sludge to build up for a period of time and then slough off was noted. The average value for "a" decreased significantly to 0.18 when the pH was lowered to a pH of 2.60 and increased significantly for BOD:N ratios above the critical ratios.