

SEQUENTIAL SUBSTRATE REMOVAL IN
ACTIVATED SLUDGE SYSTEMS

by

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ABSTRACT

A synthetic waste composed of glucose, nutrient broth, and sodium oleate was used to study the removal relationships between the three carbon sources when fed to an activated sludge waste treatment process.

Processes consisting of from one to four activated sludge tanks in series and the relative concentrations of the three components of the waste at various stages of removal were investigated.

The results showed that a single tank process removed more of the waste substrate than a multi-tank process when the processes were the same volume and operated at the same detention time. Glucose was found to be the most rapidly metabolized of the three substrates.

CHAPTER 1

INTRODUCTION

Traditionally the design of activated sludge waste treatment systems has been based almost entirely upon numerous loading factors for such systems. Typical loading factors are pounds of BOD applied per day per cubic foot of aerator volume and pounds of BOD applied per day per pound of mixed liquor suspended solids under aeration. Although numerous schemes of mixing, aerating, and introducing the waste influent into the aeration step have been developed, little has been done relating the biochemical characteristics of the waste being "loaded" into the system and its relationship to the size and configuration of the aeration chamber. This is to say, only total loadings such as BOD or COD in pounds per day have been considered in deciding how large to make an activated sludge unit.

This study examines the chemical components of the waste substrate in terms of how one component can influence the removal of another and how this influence relates to total aerator volume.

CHAPTER 2

LITERATURE REVIEW

Monod (1) was the first to note that the presence of a particular substrate would hinder or inhibit the utilization of a second substrate by microorganisms. He found in particular that, in a combination of sugars such as glucose and sorbitol, the microorganisms would utilize the glucose first and then the sorbitol. Sorbitol utilization appeared to be inhibited until the glucose level was substantially reduced. Monod explains this phenomenon by saying that the presence of glucose acts to inhibit the formation of the enzymes necessary for the organisms to break down the sorbitol. It is only after the glucose (inhibitor) level has dropped to a level that does not interfere with the enzyme formation, that the organisms can utilize the sorbitol.

Willettts (2) points out that when a combination of substrates, one of which inhibits the uptake of another substrate, are fed to a pure strain of microorganisms have growth phases separated by a lag period results. Willettts attributes the lag period to the time needed for the microbes to synthesize the necessary enzymes for the utilization of the previously unusable substrate.

Monod (3) in working with combinations of sugars defined this "phasic" growth phenomenon as "diauxie growth," in which "each growth cycle conforms to the exclusive utilization of one of the constituents of the substrate mixture, due to an inhibitory effect of one of the compounds on the formation of the enzymes attacking the other."

Gaudy, Komolrit, and Bhatla (4) concur with Monod, and explain that when two or more specific carbon sources are present in pure bacterial cultures it sometimes happens that "each source is removed sequentially and not concurrently." Also, an acclimation period exists between the sequential removals. Gaudy further explains that in certain combinations of substrates some are not only preferred by the microbes to other carbon sources, but also prevent the uptake of the less preferred substrate until the repressive compound has been eliminated from the medium. Gaudy finds, however, that such a phenomenon is not only characteristic of pure cultures, but heterogeneous cultures as well. Working with a bacterial population developed from sewage seed, Gaudy (5) found that bacteria, acclimated to sorbitol, were unable to utilize sorbitol when fed a glucose-sorbitol mixture until the glucose was exhausted. Gaudy et al. (4) conclude that "the repressive phenomenon was not an effect characteristic of a few species, but was rather general in nature and more characteristic of substrate relationships."

Stumm-Zollinger (6) points out that a condition termed catabolite repression exists when a substrate, or more particularly, a catabolite of the substrate interferes with complete synthesis of an enzyme necessary for the uptake of another substrate by the cells. Inhibition comes about if a catabolite inactivates a catabolic enzyme already formed. At any rate, both conditions lead to sequential substrate utilization similar to that described by Monod and Gaudy.

From their batch studies Gaudy and Komolrit (7) state conclusively that "sequential substrate removal in heterogeneous populations can occur."

Their explanation of the mechanism for such removals is based upon catabolite repression - the cells choose to form only those enzymes required for the utilization of only one carbon source while those required for another carbon source are not synthesised.

Gaudy and Komolrit (8) also found in continuous studies that the uptake of a substrate to which the system had been acclimated could be blocked by the addition of a particular repressive and/or inhibitory substrate.

Wang and Humphrey (9) noted the preferential utilization of glucose when fed continuously with fructose to E. coli in a bacterial reactor.

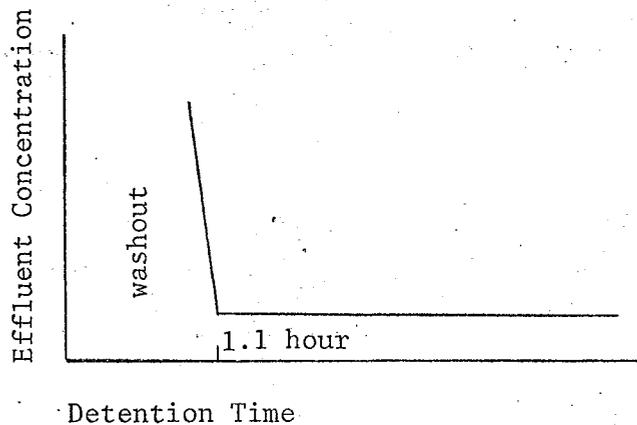


Figure 2.1 Detention time of glucose and fructose when fed separately to microorganisms correlated to effluent concentration

When glucose and fructose are fed simultaneously, however, the result is a preferential uptake of glucose followed by fructose as expressed in figure 2.2.

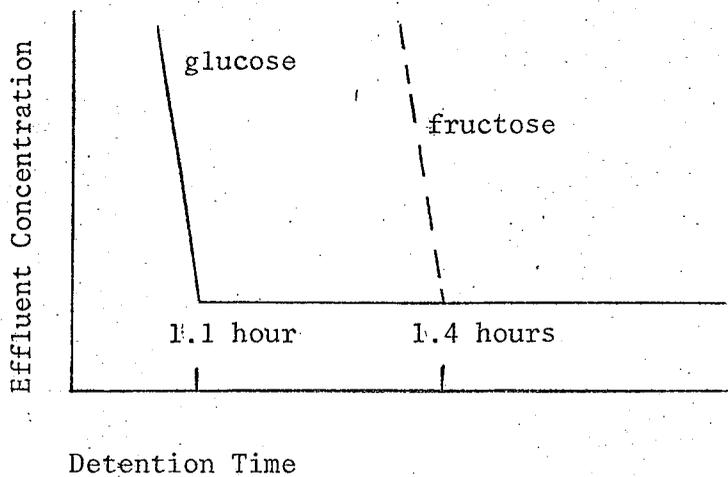


Figure 2.2 Detention time vs. effluent concentration of glucose and fructose when fed concurrently to microorganisms

Wang and Humphrey explain diauxic growth as being the result of catabolite repression in which microorganisms, when faced with a multi-carbon food source, will metabolize the one which they are biochemically most able to utilize first and so forth. The point being that in a multi-carbon waste there undoubtedly exists catabolite repression. Due to this repression, Wang and Humphrey suggest it might be better to design the waste aerators as a multiple-stage operation rather than one tank. For example, in the case of a glucose-fructose combination and a two tank sequence, the glucose could be metabolized at a high rate in the first tank and the fructose removed in the second tank also at a high feed rate. The alternative would be to use a large tank and a low feed rate. As the total volume of the two tanks would be less than one tank (the difference being a function of the rate at which the enzymes

necessary to utilize fructose can be produced) a reduction of aerator volume would result.

Others have reported the advantages of using a multiple aeration system. Webb (10) states that:

1) in a single vessel system it might not be possible to use up enough nutrients in the vessel without slowing down the feed rate unduly and,

2) many biological reactions take place essentially in different stages and optimal conditions may be different for these.

Webb continues to say that in a multi-tank system, the most easily metabolized compounds are consumed in the first tank where the organisms are adapted primarily to the substrate removed the most and the succeeding tanks are of benefit to the other compounds to which the organisms are similarly adapted.

Erickson, Chen, and Fan (11) as well as Milbury, Pipes, and Grieves (12) report superior results when a "tanks-in-series" activated sludge scheme is compared with a single activated sludge tank.

CHAPTER 3

EXPERIMENTAL PROCEDURE

Experimental Design

The first series of experiments were made in an attempt to establish the specific growth rate of activated sludge organisms when fed glucose, nutrient broth, sodium oleate, or a combination of all three. This was attempted by feeding each substrate to an activated sludge reactor at progressively higher flow rates until washout occurred. (See Figure 2.1: for no sludge recycle and a single tank the specific growth rate = the detention time at washout). By comparing the specific growth rate of organisms fed a single substrate to the specific growth rate of organisms fed a combination of all three compounds, one could determine if catabolite repression was taking place such as in the case of glucose and fructose. (See Figure 2.2).

If the composite substrate was of a repressive nature, it was proposed that three activated sludge tanks be combined in series and the systems performance be compared to that of the fourth tank operating as a single-stage. In this way the hypothesis that the multi-tank system would work better than a single-stage system for a multi-carbon waste which exhibited catabolite repression could be appraised. (See Figure 3.1).

The specific growth rates characteristic of the individual substrates were never established as each of these attempts was terminated

Figure 3.1 Phase one flow chart

1. Refrigerator containing 4-20 liter carboys
2. Composit feed, COD = 900 mg/l.
3. Sigma motor pump
4. Sodium oleate feed, COD = 300 mg/l
5. Glucose feed, COD = 300 mg/l
6. Nutrient broth feed, COD = 300 mg/l
7. Air supply line
8. Composit reactor
9. Sodium oleate reactor
10. Glucose reactor
11. Nutrient broth reactor
12. Glass wool air filter
13. Air diffused through distilled water

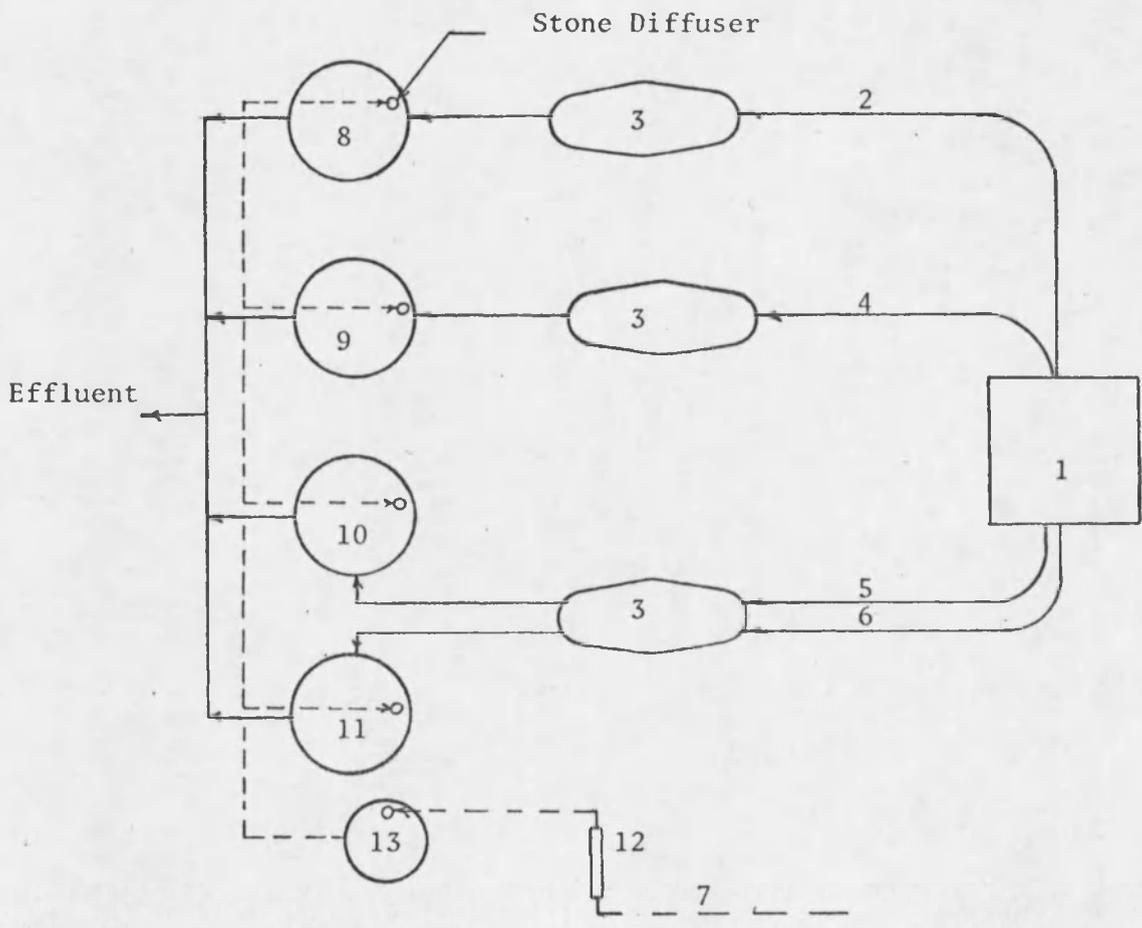


Figure 3.1 Phase one flow chart



Figure 3.2 Phase one activated sludge reactors

Note: The color is due to fungi which is explained in Appendix A.

by a fungi infestation before the maximum feed rate preceding washout (the feed rate which corresponds to the specific growth rate) could be attained. The fungi infestation is discussed in Appendix A.

Adopting an alternative procedure, all tanks were joined and operated as a four-stage process with the combination substrate being fed to the initial tank. (See Figure 3.3) The flow rate was again progressively increased providing for a detention time range from nine to two hours in each tank. By comparing the relative concentrations of each of the individual substrates in each of the tanks, the influence of one substrate on one or both of the others could be ascertained.

Having determined the effect of one of the waste components on the others, a three-stage unit was to be operated in such a way as to minimize the effect. The performance of such a system was then to be compared to a single stage system. (See Figure 3.4).

The substrate was the same in kind as that used by Tenney, Johnson, and Symons (13). The formulation per liter of Tucson tap water consisted of:

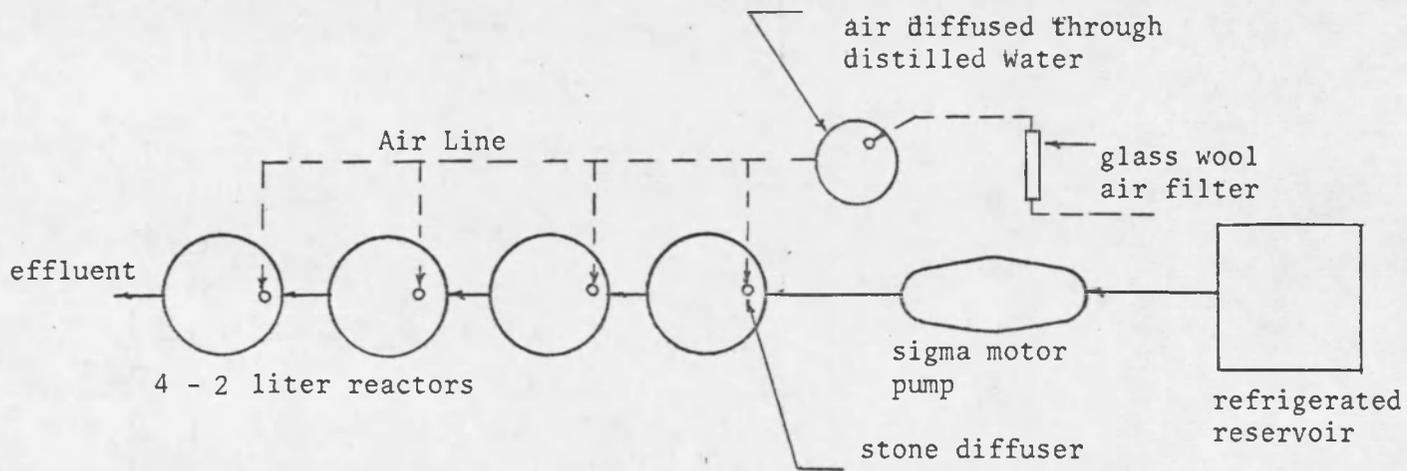
0.210 grams sodium oleate

0.435 grams glucose

0.420 grams nutrient broth

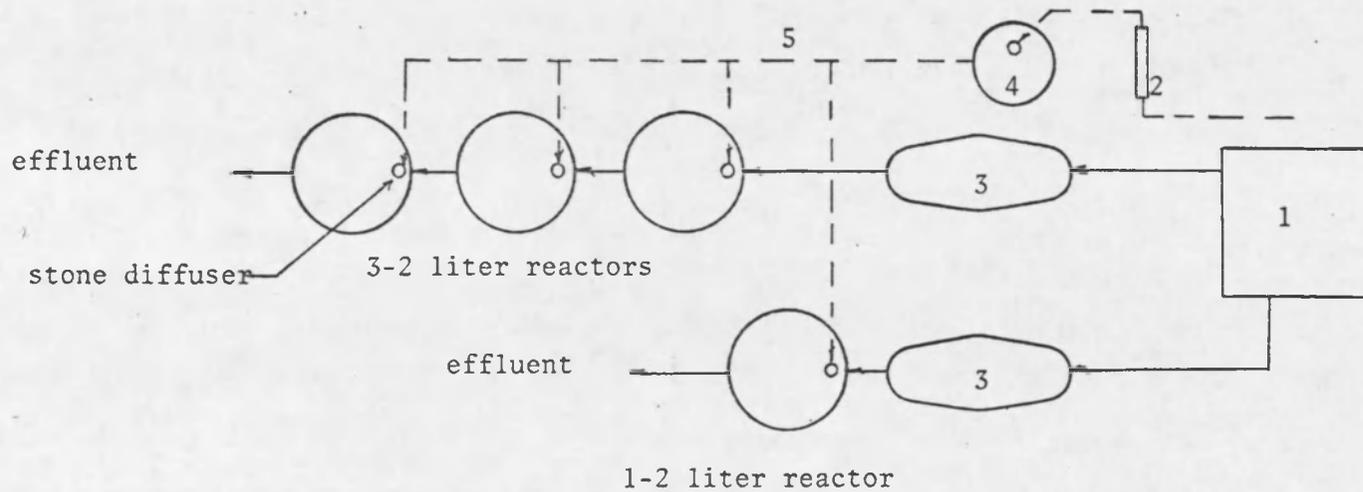
0.4 grams K_2HPO_4 per gram of COD.

The substrate was selected as it provided carbon sources in each of three broad classifications of waste products found in domestic sewage. That is, glucose is a carbohydrate, nutrient broth, is essentially all



Substrate: Composit of all three carbon sources, COD = 900 mg/l.

Figure 3.3 Phase one revised flow chart



1. Refrigerated Reservoir
2. Glass Wool Air Filter
3. Sigma Motor Pump
4. Air Diffused Through Distilled Water
5. Air Line

Figure 3.4 Phase two flow chart



Figure 3.5 Phase two activated sludge reactors

protein, and sodium oleate can be classified as a salt of a fatty acid. Each carbon source yielded a COD of approximately 300 mg per liter. Therefore, when all three were combined into a single substrate the total COD was about 900 mg per liter.

Each reactor was 6" in diameter and had a volume of 2 liters. The stone diffuser supplied the mixing for each tank. It was necessary to refrigerate the substrate to control growth in the influent. The temperature in the refrigerator was 3°C while that of the reactors was 23°C. By diffusing air through water before introducing it into the reactors, the evaporation rate in the reactors was reduced.

General Consideration on Reactor Operation

The activated sludge was initially that of the Tucson sewage treatment plant. When the sludge was obtained, it was screened through a Tyler Standard number 30 sieve to remove any extraneous matter. Sieving was also conducted each day on the reactor contents to reduce any unnaturally large floc.

At the same time that the contents were being sieved, the tanks were thoroughly scrubbed to remove any slime accumulation on the reactor walls and bottom.

To keep the influent COD at a relatively constant level, it was necessary to hold growth in the feed reservoir to a minimum. This was done by washing the reservoirs (20 liter carbons) daily with a strong household bleach solution. All feed lines were acid washed daily. Acid washing was found to be superior to autoclaving or household bleach as the acid wash removed any slime buildup as well as sterilizing the feed lines.

In addition to refrigerating the substrate, it was also found to be advantageous to pre-cool the tap water to about 4°C before mixing in the nutrients.

CHAPTER 4

ANALYTICAL METHODS

General Considerations

Daily measurements were made of the flow rates through the reactors, as well as the pH, temperature, and mixed liquor volatile suspended solids (MLVSS) levels in the reactors. The influent and effluent COD, organic nitrogen, and glucose concentrations were also measured.

The COD contribution of the nutrient broth to the total was determined from the organic nitrogen levels, and the glucose contribution was determined from the glucose concentration. The sodium oleate contribution to the total COD was determined by subtracting COD attributable to the nutrient broth and the glucose from the total.

The correlation between COD and nutrient broth and between COD and glucose was established by running COD analyses and organic nitrogen determinations on several sets of samples of known nutrient broth concentrations. Similar tests were carried out for COD and glucose. The data was fit to a least squares linear regression and the results were:

$$\text{COD} = -5.8 + 10.1(\text{mg nitrogen})$$

$$\text{COD} = 3.5 + 0.84(\text{mg glucose}).$$

The data is plotted in Appendix B.

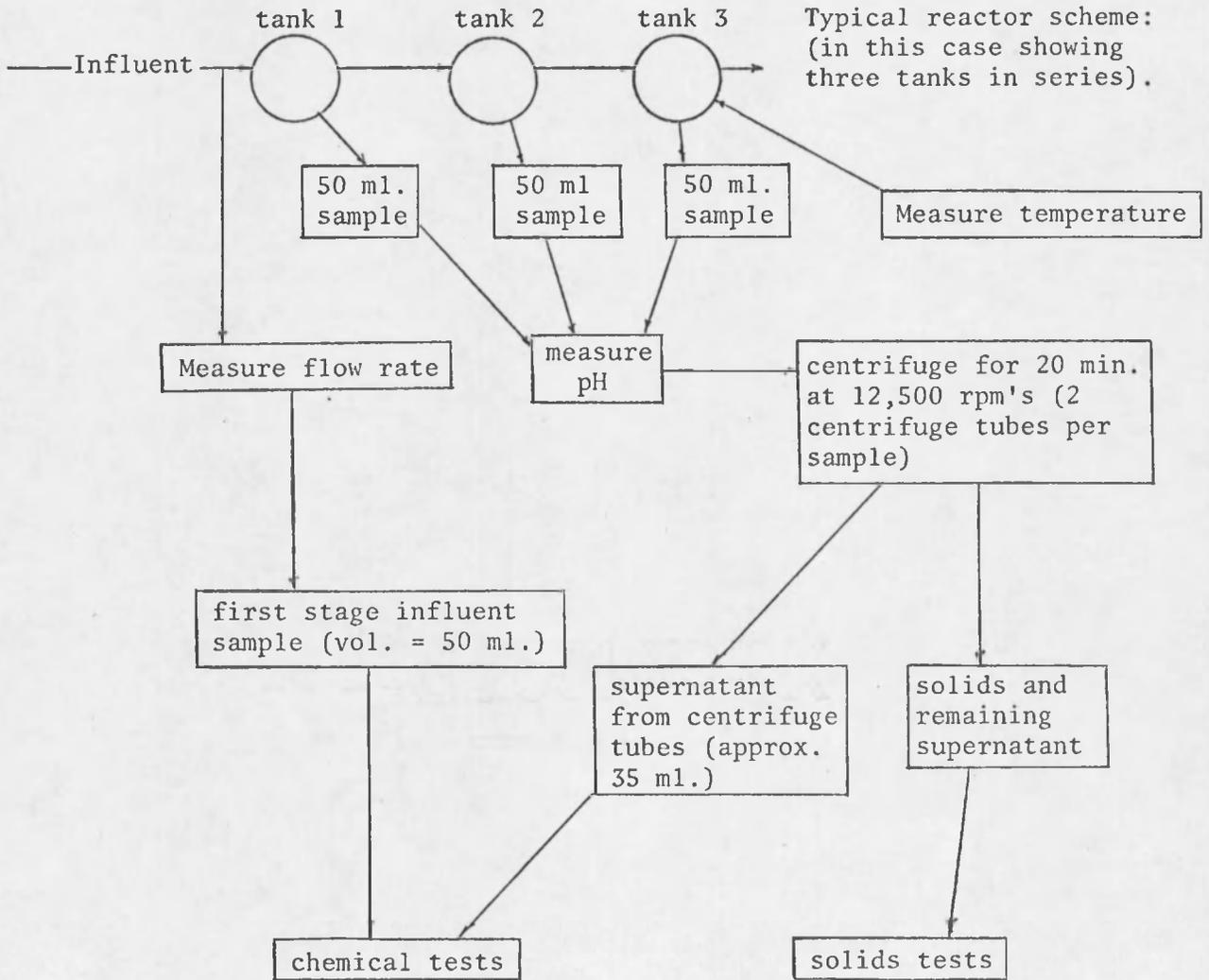


Figure 4.1 Flowchart for analyses of samples taken from reactors

Flowchart Continued

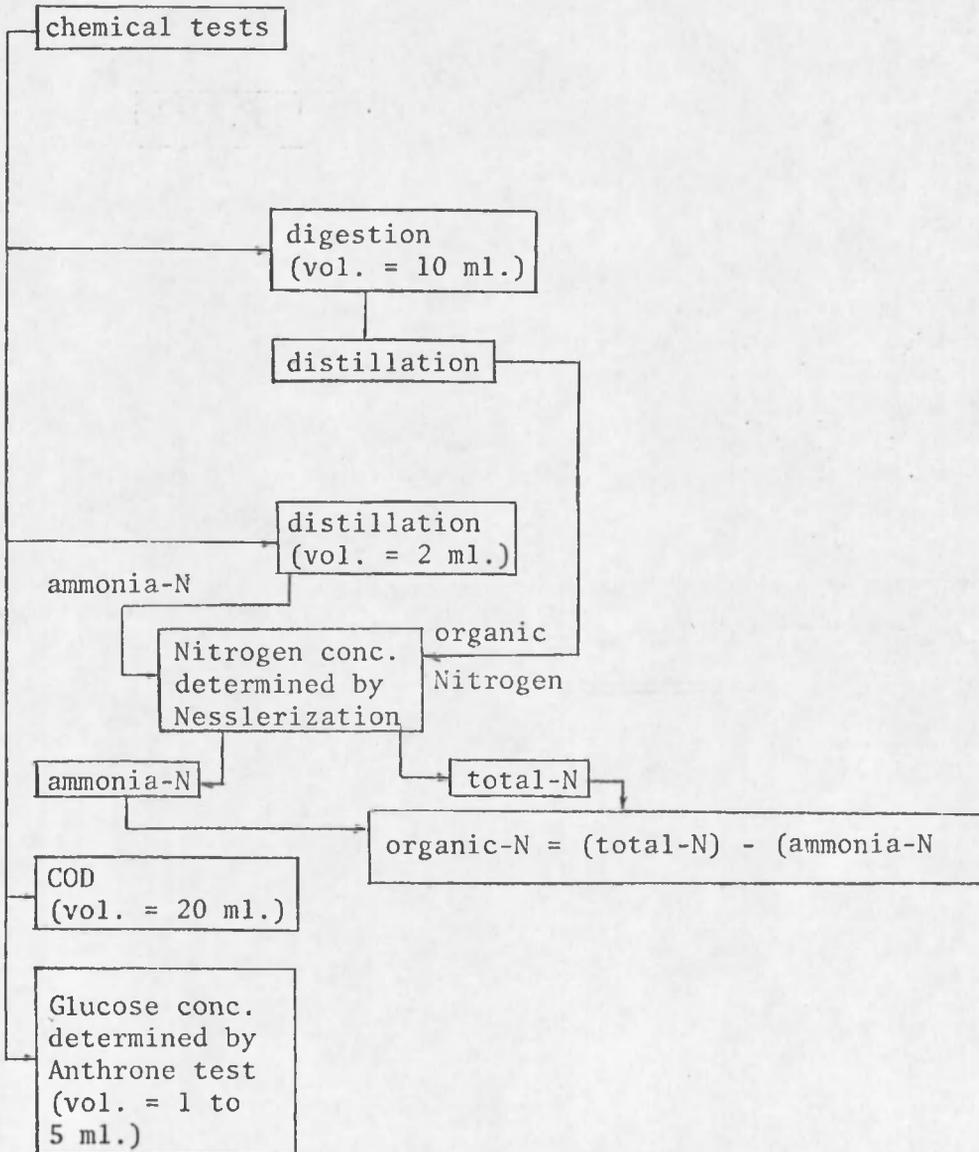


Figure 4.1 Flowchart for analyses of samples taken from reactors
Continued

Flowchart Continued

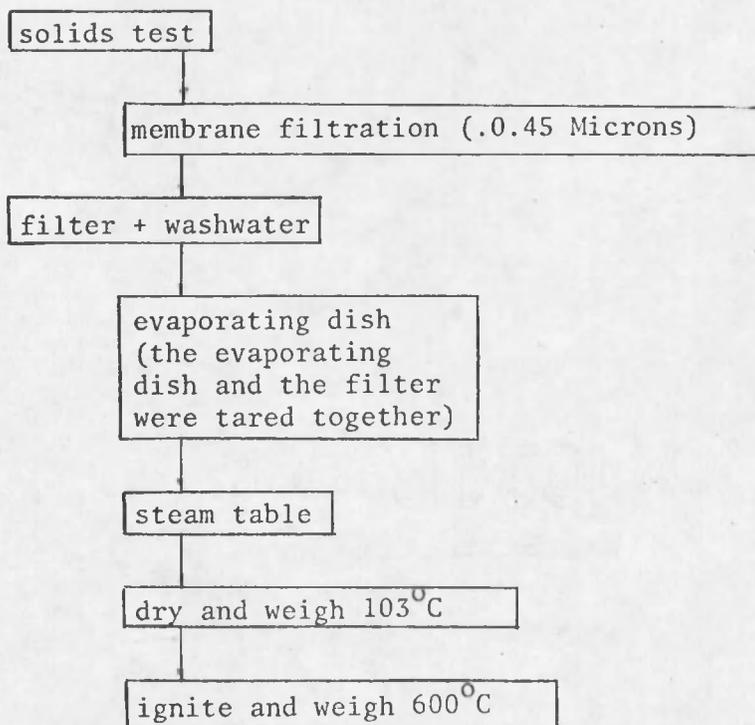


Figure 4.1 Flowchart for analyses of samples taken from reactors
Continued

Explanation of Flow Chart

The first stage influent flow rates were measured using a graduated cylinder and a laboratory timer. The sample volume was determined using a graduated cylinder.

Centrifugation was carried out to separate the solids from the soluble COD and to facilitate the membrane filtration step. The 50 ml sample was divided and placed into two tubes to facilitate the centrifuge operation.

A. Chemical Tests

COD. The COD was measured to determine the concentration of the carbon sources as stated earlier.

The tests were conducted in accordance with Standard Methods (14).

Nitrogen Digestion and Distillation. The nitrogen concentrations were used to indicate the nutrient broth concentrations.

Digestion and distillation were carried out using a modification of the micro-Kjeldahl procedure of McKenzie and Wallace (15). The procedure was modified in that the distillate was not collected in boric acid, but instead no collection agent was used to trap the ammonia. However, to prevent the loss of ammonia in distillation, a second condenser through which ice water was pumped was added to the distillation unit.

Nesslerization. The Nesslerization method of Standard Methods (14) was used to determine the ammonia concentration from the digestion and distillation step. Nesslerization was also used to determine the original ammonia content of the sample. It was found to be advantageous to distill the sample in the manner outlined above before using Nesslerization, however.

Glucose. The glucose concentration was measured using the colorimetric anthrone test developed by Brink, Dubach, and Lynch (16). In concentrated sulfuric acid, anthrone yields a green color when complexed with carbohydrates. Color is measured using a spectrophotometer and is proportional to the glucose concentration.

B. Solids Tests

Mixed Liquor Volatile Suspended Solids. An assessment of a system's performance was made by monitoring the MLVSS level. The procedure used was the membrane filtration test outlined in Standard Methods (14). However, the procedure was modified by the introduction of an ignited and tarred evaporating dish into which the washwater for all glassware used in handling the sample was collected. The dish was also used for convenience in handling the filter from step to step in the analysis. As mentioned above, centrifugation aided filtration as essentially no solids hit the filter until the slug at the bottom of each centrifuge tube was poured out. As the slug was always the last to be poured from the tube, the filter remains unclogged until the last moments of the filtration process. The slug contains essentially all the solids which were originally present in the tube. By reducing filter clogging until virtually all of the liquid portion of the sample had been filtered, it was possible to use larger sample volumes. All glassware was washed with distilled water to ensure the collection of all the solids, and the washwater was collected in an evaporating dish. The filter was placed in the dish and the dish and filter were then dried at 103°C and then cooled and weighed. This was followed by ignition of the

filter and dish and then the dish weighed again. It was found that the filters were virtually ash-free when ignited. This is in accord with the Millipore Corporation's claim that the filters are of negligible ash content (17).

It was assumed that the supernatant used in the chemical tests contained no solids. Therefore, the concentrations were computed based on the original 50 ml sample.

CHAPTER 5

EXPERIMENTAL RESULTS AND DISCUSSION

In order to determine the influence of one substrate on another, the portion of the total COD in a tank attributable to glucose was plotted against the portion of the total COD in a tank attributable to nutrient broth (figure 5.1) and against the COD in a tank for which the sodium oleate was responsible (figure 5.3). The same was also done for nutrient broth and sodium oleate (figure 5.2).

Figure 5.1 suggests that glucose influences nutrient broth removal. When the COD contribution of glucose is in the range of 50 mg per liter or higher, the nutrient broth removal appears to be dependent upon the glucose concentration. As the COD contribution of glucose drops below 50 mg per liter, the nutrient broth reduction appears to be independent of the glucose concentration.

The relationship between glucose and nutrient broth as expressed in figure 5.1 should be linear provided the following:

- a. The presence of one substrate does not effect the removal of the other.
- b. The detention times are greater than the minimum detention times for organisms fed solely nutrient broth or solely glucose.

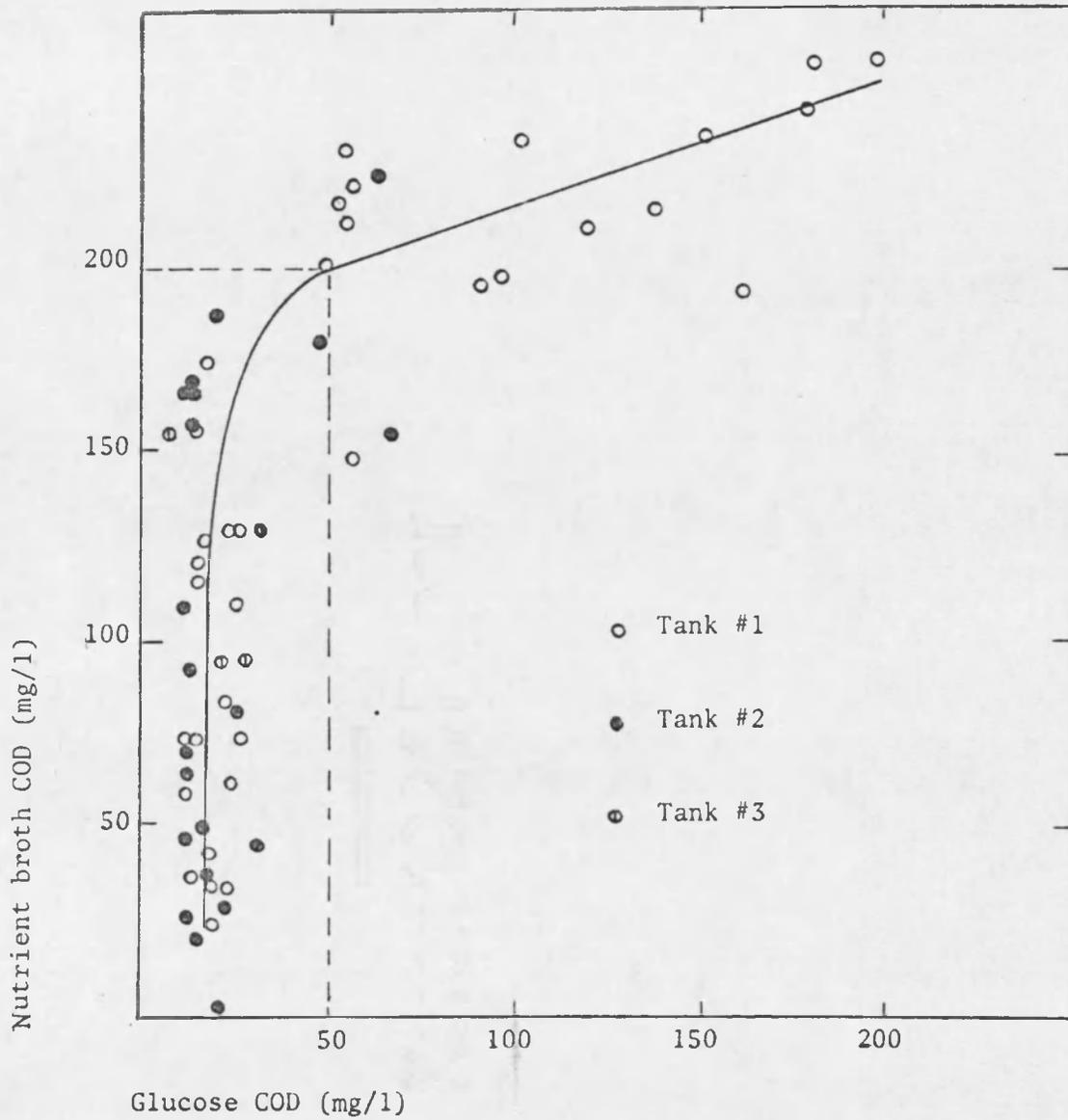


Figure 5.1 Glucose COD vs. nutrient broth COD

If conditions "a" and "b" are met the relationship should appear as below:

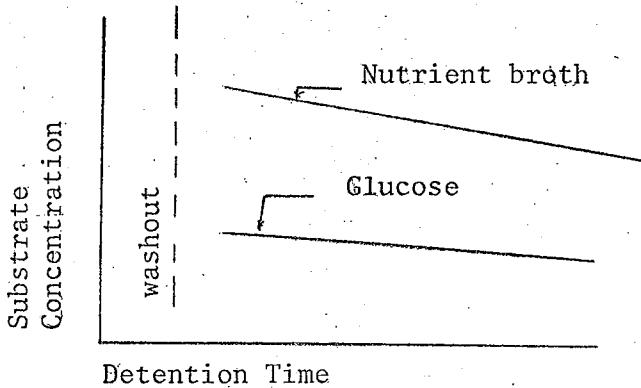


Figure 5.4 Theoretical nutrient broth-glucose detention time relationships

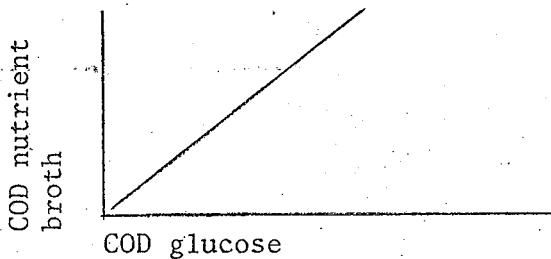


Figure 5.5 Theoretical glucose COD and nutrient broth COD correlation

If the slopes of figure 5.4 were the same (removal rates are equal) the slope of figure 5.5 would be 45° .

With this in mind, figure 5.1 could really imply the existence of either or possibly both of the following phenomenon. Either glucose does have an inhibitory effect on nutrient broth removal and/or organisms when fed glucose have a different minimum detention time than when they are fed nutrient broth. (Condition "b" is not satisfied.) Thus, it is not known at this point whether glucose inhibits nutrient broth

assimilation or glucose is simply utilized at a higher rate than is nutrient broth.

At any rate, glucose is preferred to nutrient broth by the microorganisms, and a substantial nutrient broth reduction will not occur until the glucose concentration is such that its COD contribution is less than about 50 mg per liter.

There is no discernable influential relationship between nutrient broth and sodium oleate (figure 5.2) or between glucose and sodium oleate (figure 5.3). The scatter of data which figures 5.2 and 5.3 (as compared to figure 5.1) reveal, can be attributed to the fact that the COD contribution from the sodium oleate was computed by subtracting the COD values of glucose plus nutrient broth from the total COD value. Thus the COD of sodium oleate reflects the errors inherent in the three substrate analyses.

The above implications were used as the basis for a comparative study of a tanks-in-series operation to a single tank process. The comparison began by operating the first of three equal volume tanks such that the glucose contribution to the total COD in the first tank was about 50 mg per liter. The single tank (designated as #4S) was operated to provide approximately the same detention time as the combined detention time of all three tanks of the series process (designated as #1, #2, and #3). The detention times in all tanks were gradually increased as the comparative operation progressed.

Figures 5.6 through 5.8 are similar to 5.1 through 5.3 and are presented to examine the substrate relationships in the comparative study.

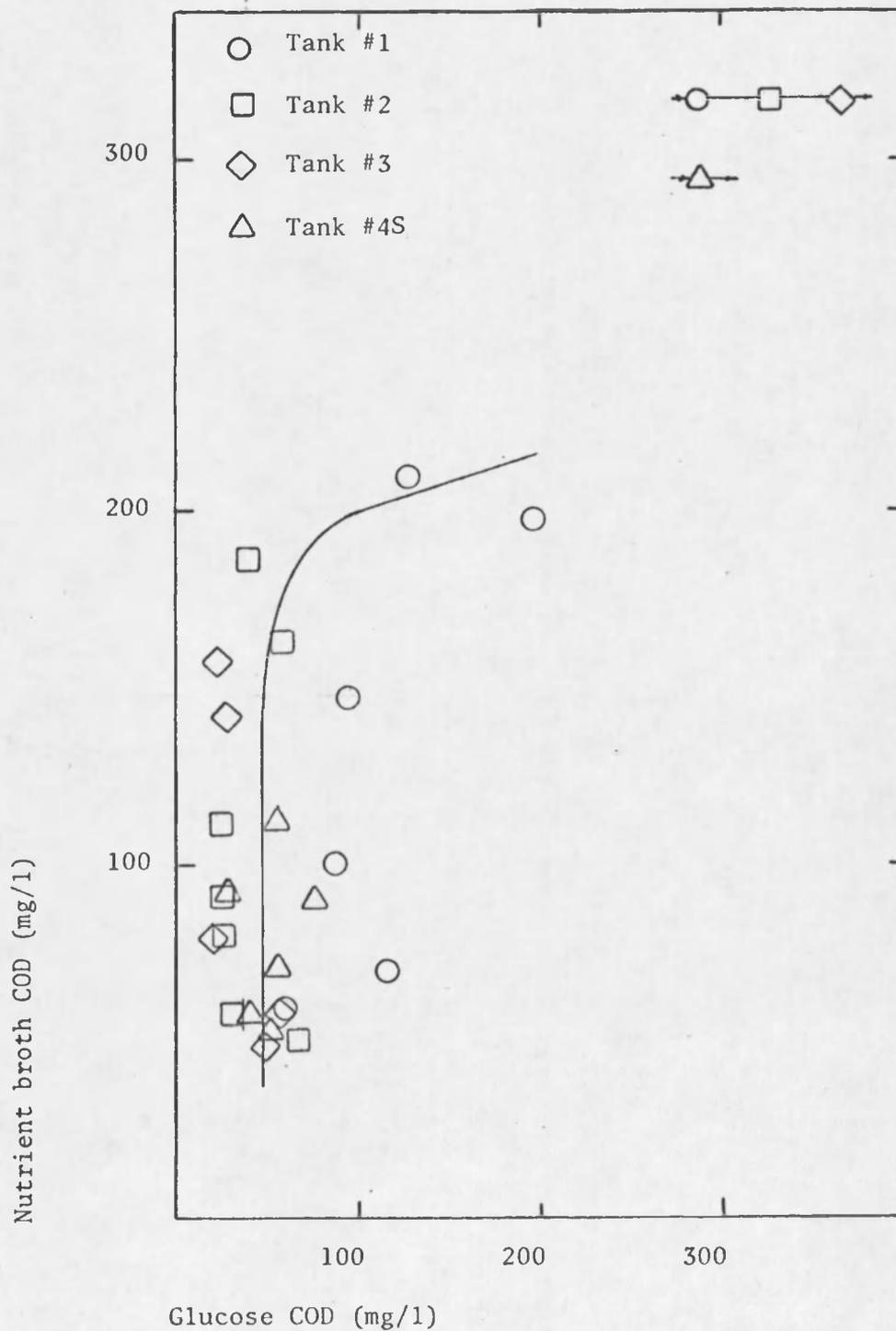
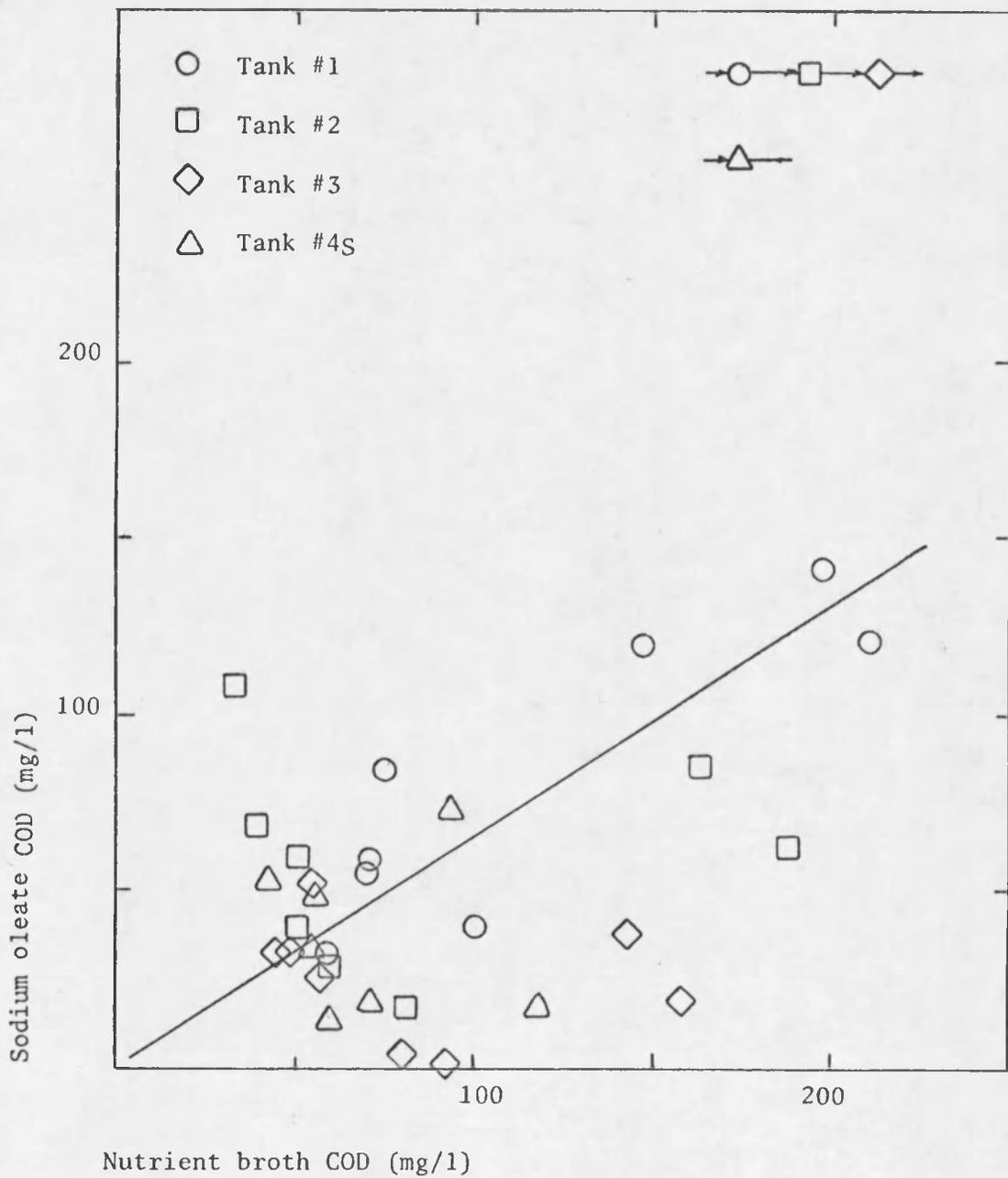


Figure 5.6 Glucose COD vs. nutrient broth COD
Series - single tank comparison



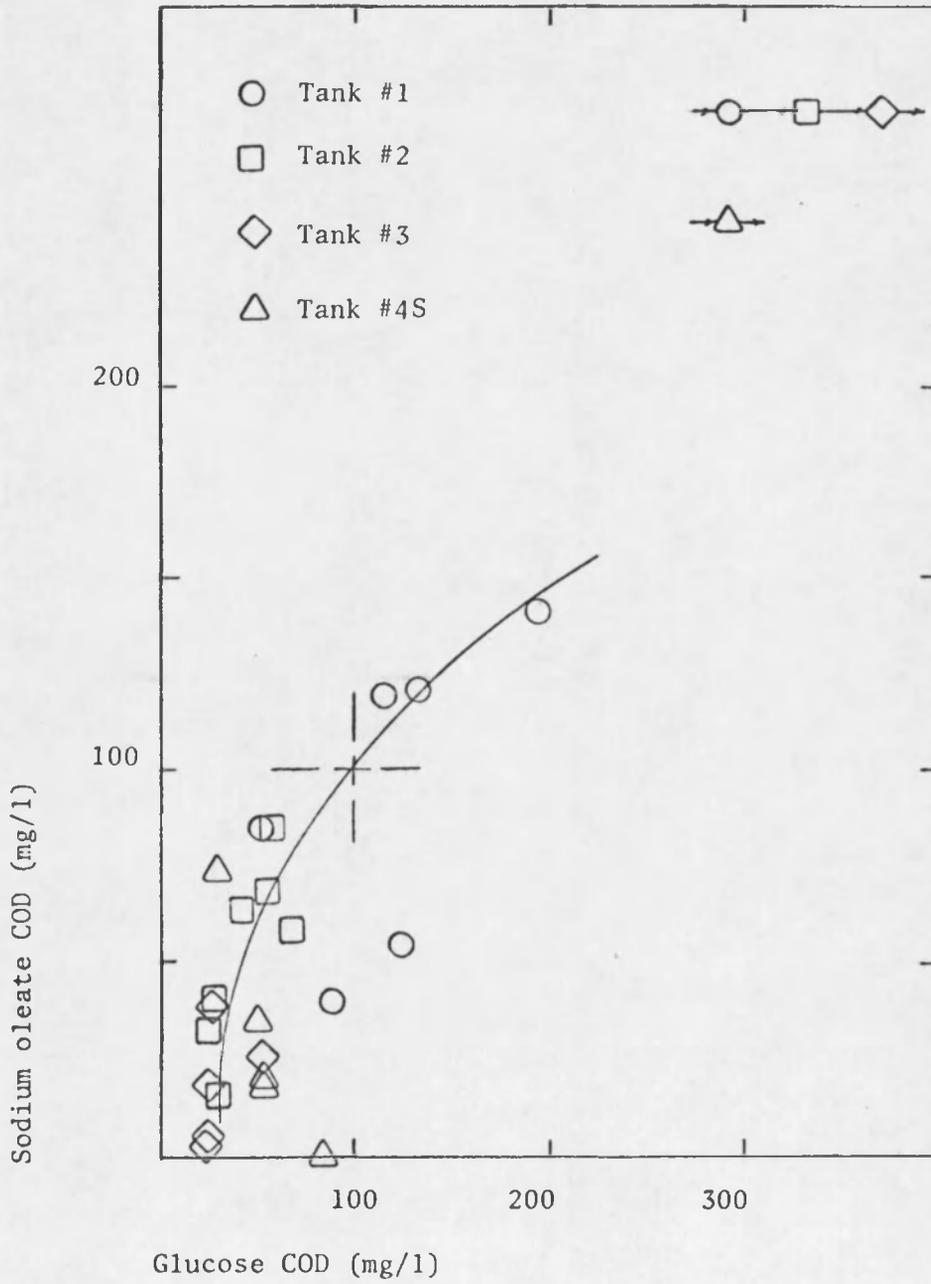


Figure 5.8 Glucose COD vs. sodium oleate COD
Series - single tank comparison

Figure 5.6 reveals that the same glucose-nutrient broth relationship existed in the comparison study as existed in the preliminary investigation. The data of figure 5.7 is not as badly scattered as figure 5.2. If anything, figures 5.2 and 5.7 suggest a linear monotonic association between nutrient broth and sodium oleate. The data of figure 5.8 has far less scatter than figure 5.3. The change of curvature of figure 5.8 is about 50 mg per liter of glucose COD and indeed implies a relationship between sodium oleate and glucose such as that between nutrient broth and glucose. If, however, glucose does influence sodium oleate removal it should not be as substantial an influence as that on nutrient broth as the sodium oleate COD concentration of 50 mg is 100 mg per liter. The nutrient broth COD concentration at the same glucose concentration is 200 mg per liter as seen on figure 5.1.

Figure 5.9 is a plot of the operating characteristics of the three tank system at various hydraulic detention times. The percent COD remaining is based on the total COD entering the first tank and that leaving the tank under consideration.

Figures 5.10 a, b, and c compare the COD performance of a single tank (tank #4S) to the COD performance of the series system of tanks (tanks #1, #2, and #3). The percent COD remaining is based upon the COD concentration entering the first tank and the concentration leaving the tank under consideration. The detention time for a one tank system is the detention time of one tank. For a two tank system, the detention time is the total detention time spent in tank one and two. For a three tank system, the detention time is the time required to pass through all three tanks.

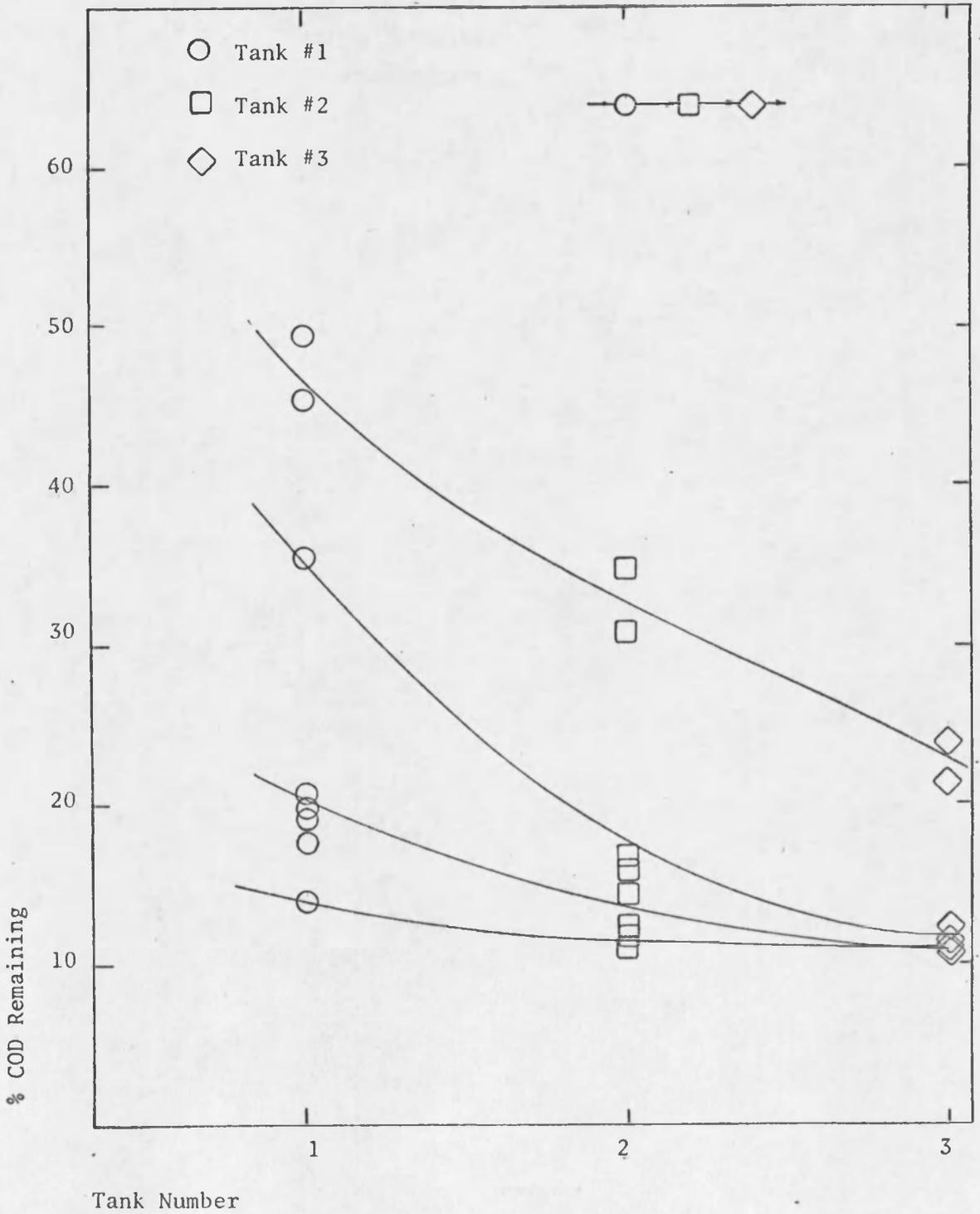


Figure 5.9 COD performance isodetention-times

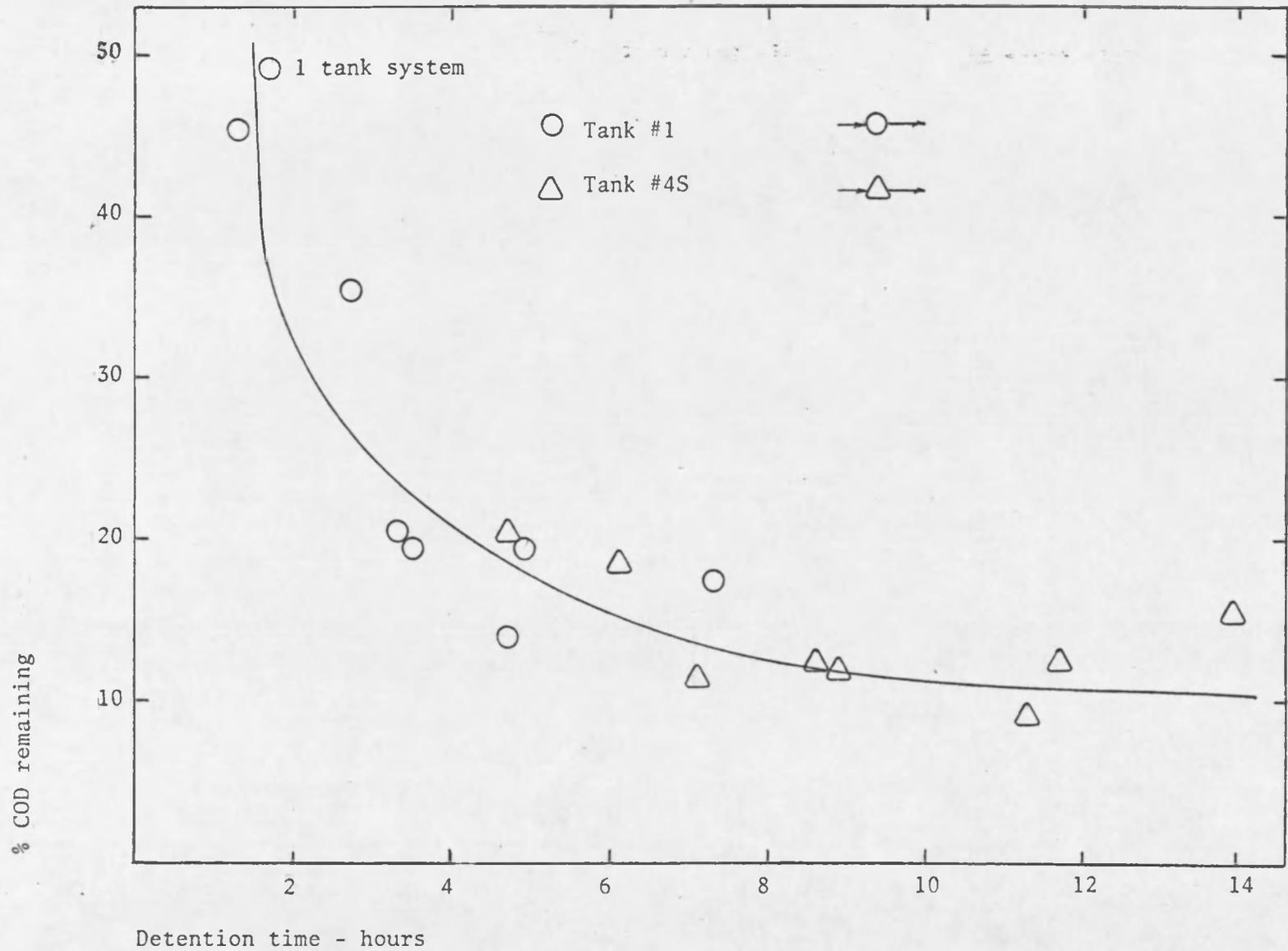


Figure 5.10 % COD remaining vs. detention time: (a) for 1 tank

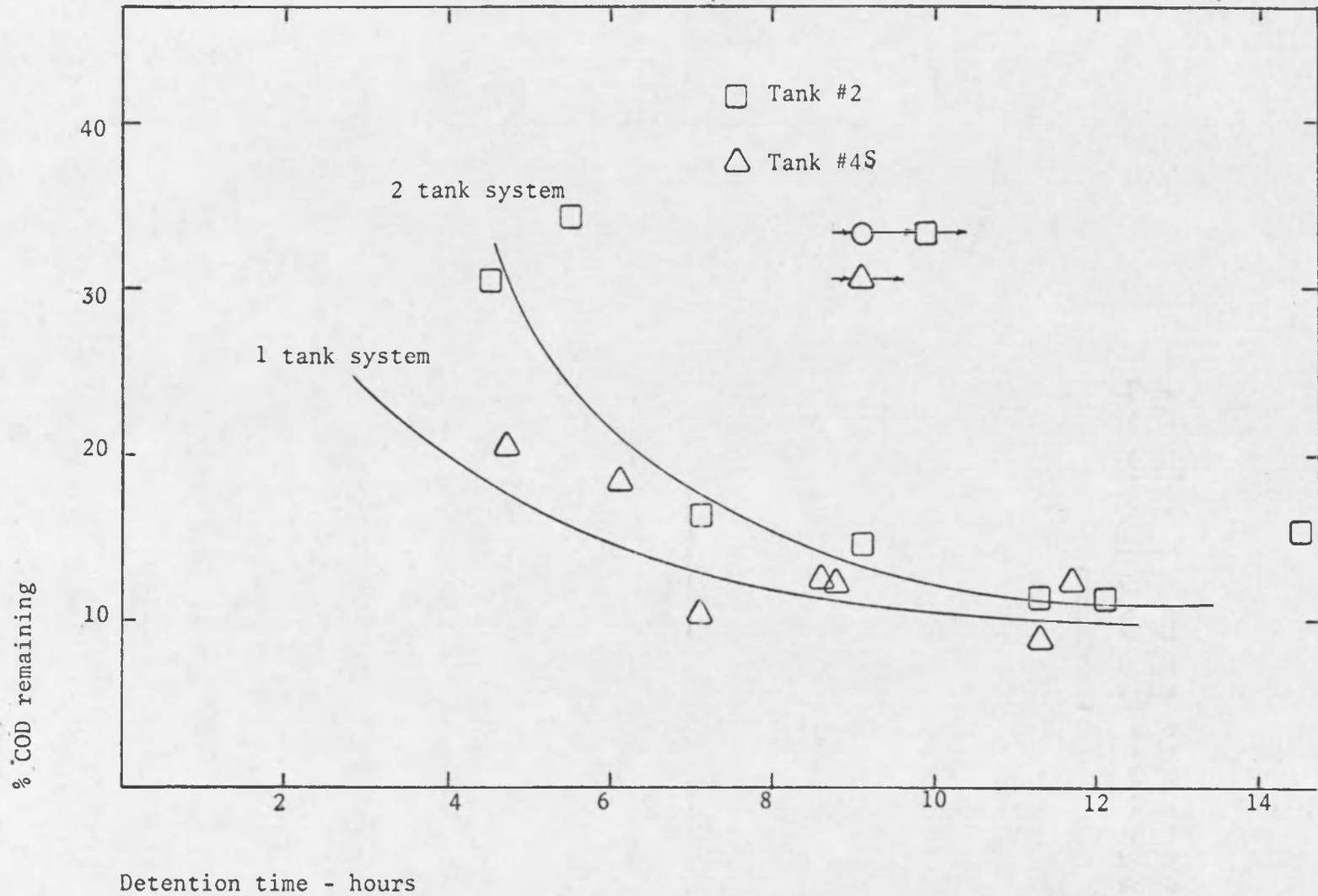


Figure 5.10-continued (b) for 1 tank system compared to a 2 tank system

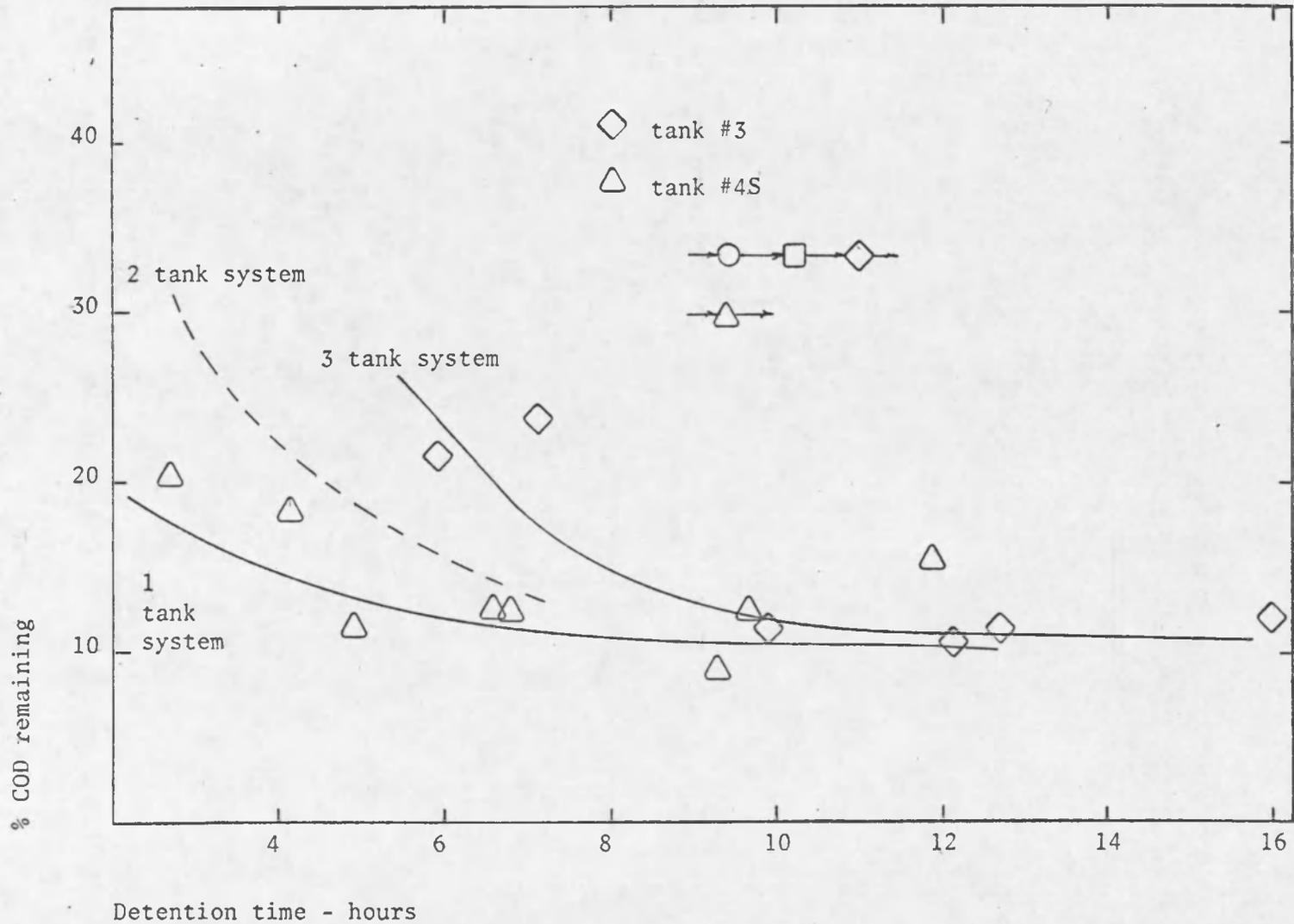


Figure 5.10-continued (c) for a 1 tank system compared to a 3 tank system

Figure 5.10-a shows that the COD performance of the first tank of the series and that of the single tank plot as a continuum. This means that there is no difference in COD performance between tanks #1 and #4S when operated at the same detention time.

In figure 5.10-b the 1 tank system is the same as that of figure 5.10-a. The 1 tank curve and the 2 tank curve of figure 5.10-c are the same as those of figure 5.10-b.

The author concludes from figures 5.10 that at COD reductions approaching 90%, there is no difference between a single tank and a tanks-in-series system. However, at a given detention time which affords less than 90% reduction of COD, the single tank system is superior to the series system.

The percent COD remaining in figure 5.11 is again calculated from the COD concentration fed into the first tank and that leaving the tank of interest. The loading factor is defined as the pounds of COD applied per day per pound of MLVSS under aeration. The loading factor in figure 5.11 is based on the amount of COD fed into the system per day and the amount of MLVSS in the tank under consideration plus the MLVSS found in any preceding tanks. For example, for a three tank system (plotted as \diamond in figure 5.11) the COD applied to the system is the amount of COD fed to the first tank per day. This is then divided by the total weight of MLVSS found in all three tanks to give a loading factor for a three stage operation. One concludes from figure 5.11 that the largest substrate reduction occurs when the ratio of COD applied to MLVSS under aeration is lowest.

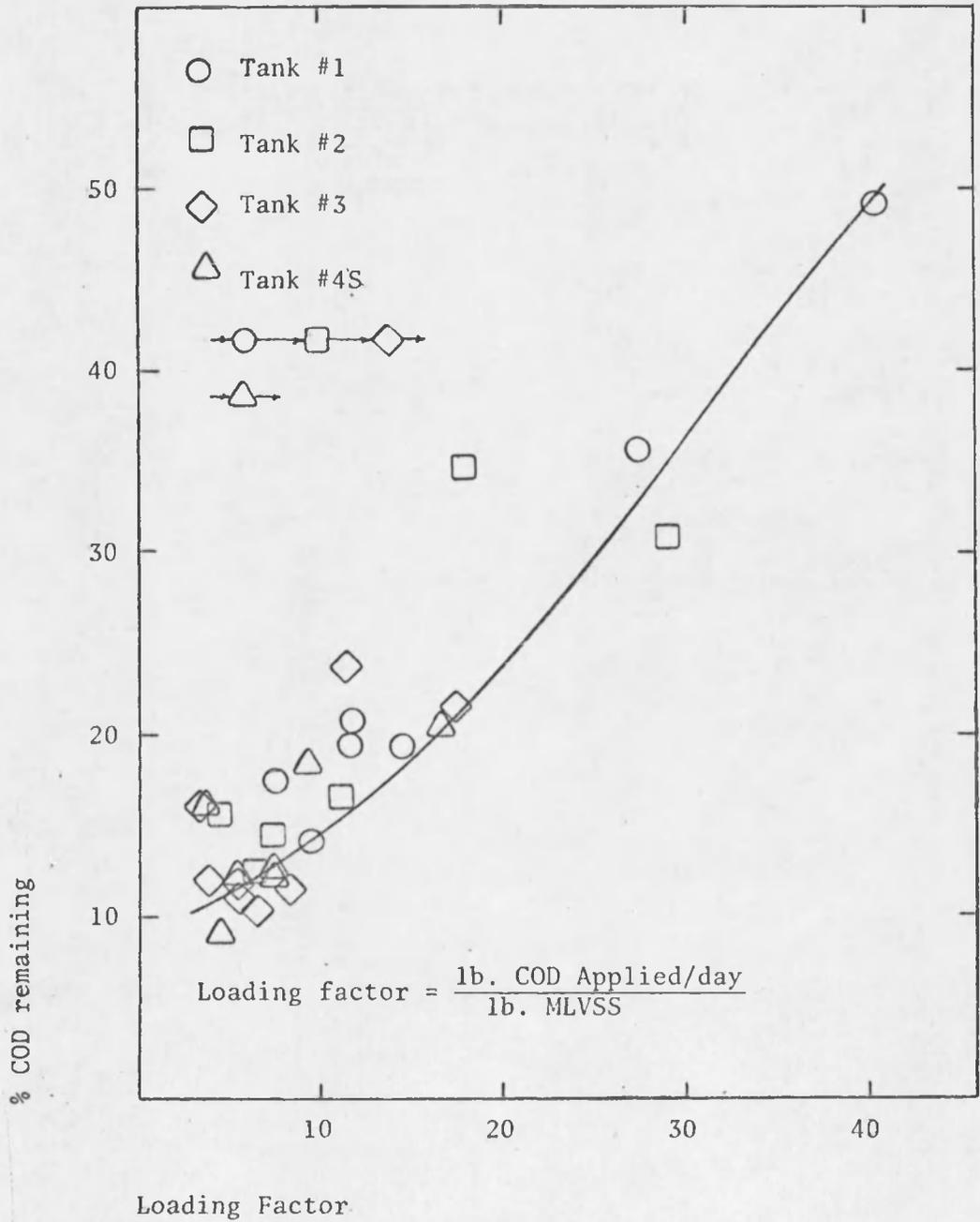


Figure 5.11 Loading factor vs. % COD remaining

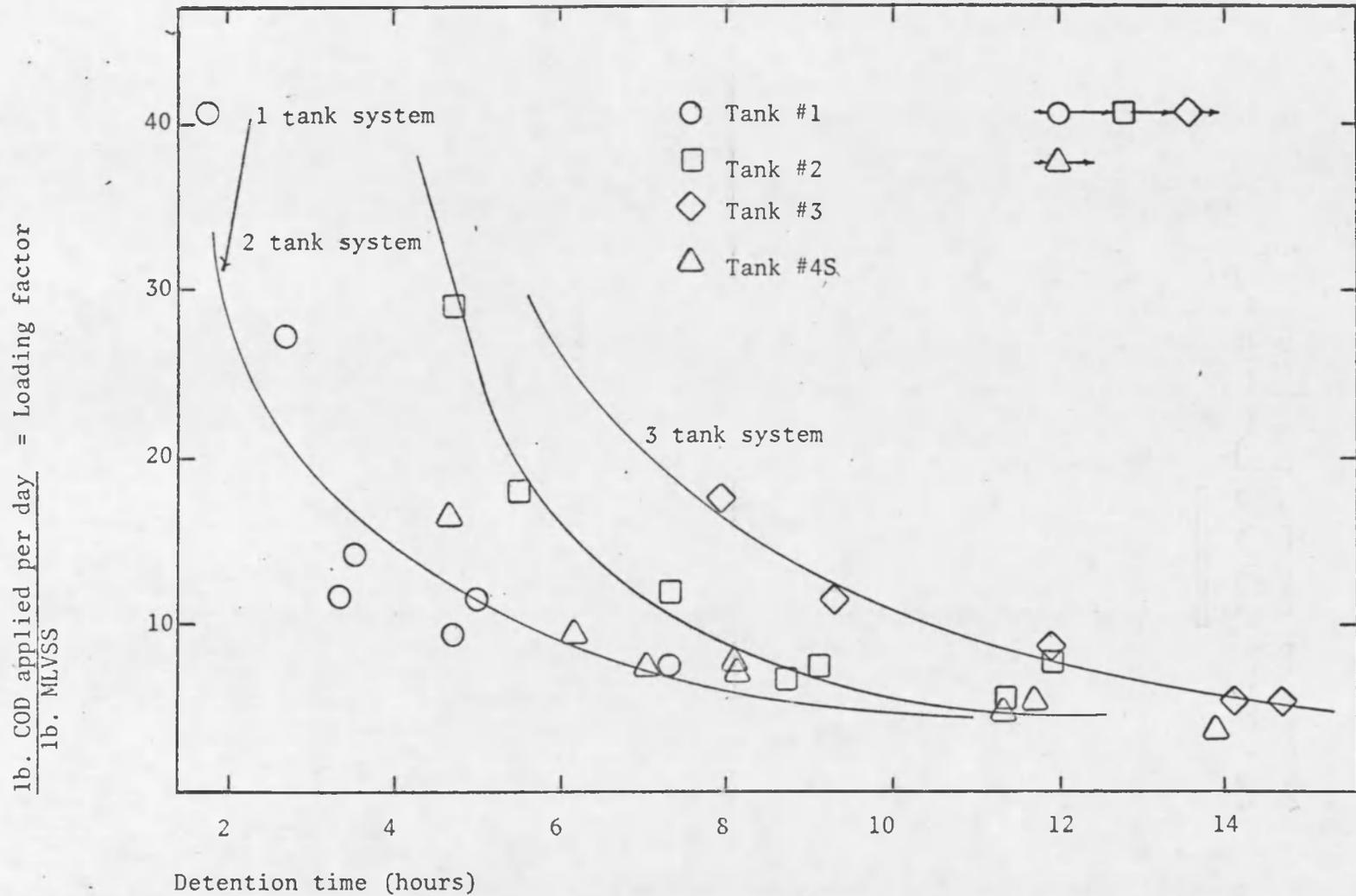


Figure 5.12 Detention time vs. loading factor

Loading factors for figure 5.12 are calculated as they were in figure 5.11. The detention time is again the detention time for the whole system. That is, the detention time is the total volume of the system being considered (1 tank, 2 tanks, or 3 tanks) divided by the flow rate through the system.

In considering figure 5.12, one should note that the one tank system again, as in figure 5.10-a, plots as a continuum. That is, there is no difference in performance between tank #1 and tank #4S when operated at the same detention time. Figure 5.12 further implies that for a given detention time, a one tank system achieves the lowest loading factor, and therefore, the best COD reduction.

On further consideration of the preliminary data (the data on which the comparison study was based) it is interesting to note the maximum specific growth rate as observed in figure 5.13. (The maximum growth rates were that which the author hoped to obtain for pure substrates in the original three trials.) Although the substrate was a mixture of all three carbon sources, one would expect that the minimum detention time for organisms fed only glucose would be about 2.2 hours. This compares to values ranging from 20.4 hours to 2.9 hours as reported by Lawrence and McCarty (18). The minimum for organisms fed solely nutrient broth would appear to be approximately 3.5 hours. Lawrence and McCarty (18) report minimum detention times of about 3.8 hours for a peptone substrate. Although data of figure 5.14 is more scattered than that of figure 5.13, it would appear that the minimum detention time for organisms fed only sodium oleate would be less than 3.5 hours but more than 2.2 hours.

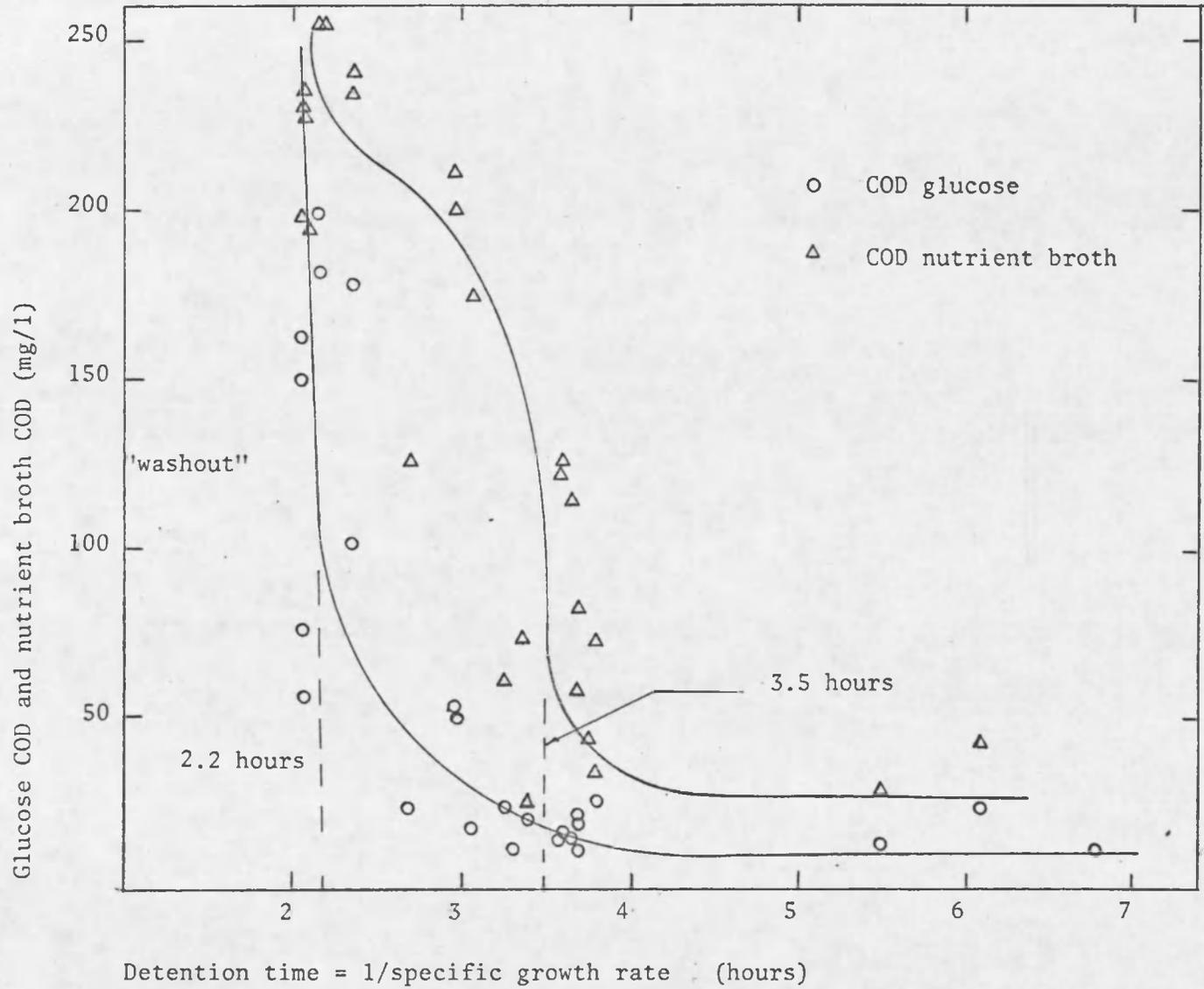


Figure 5.13 Detention time vs. glucose COD and nutrient broth COD.

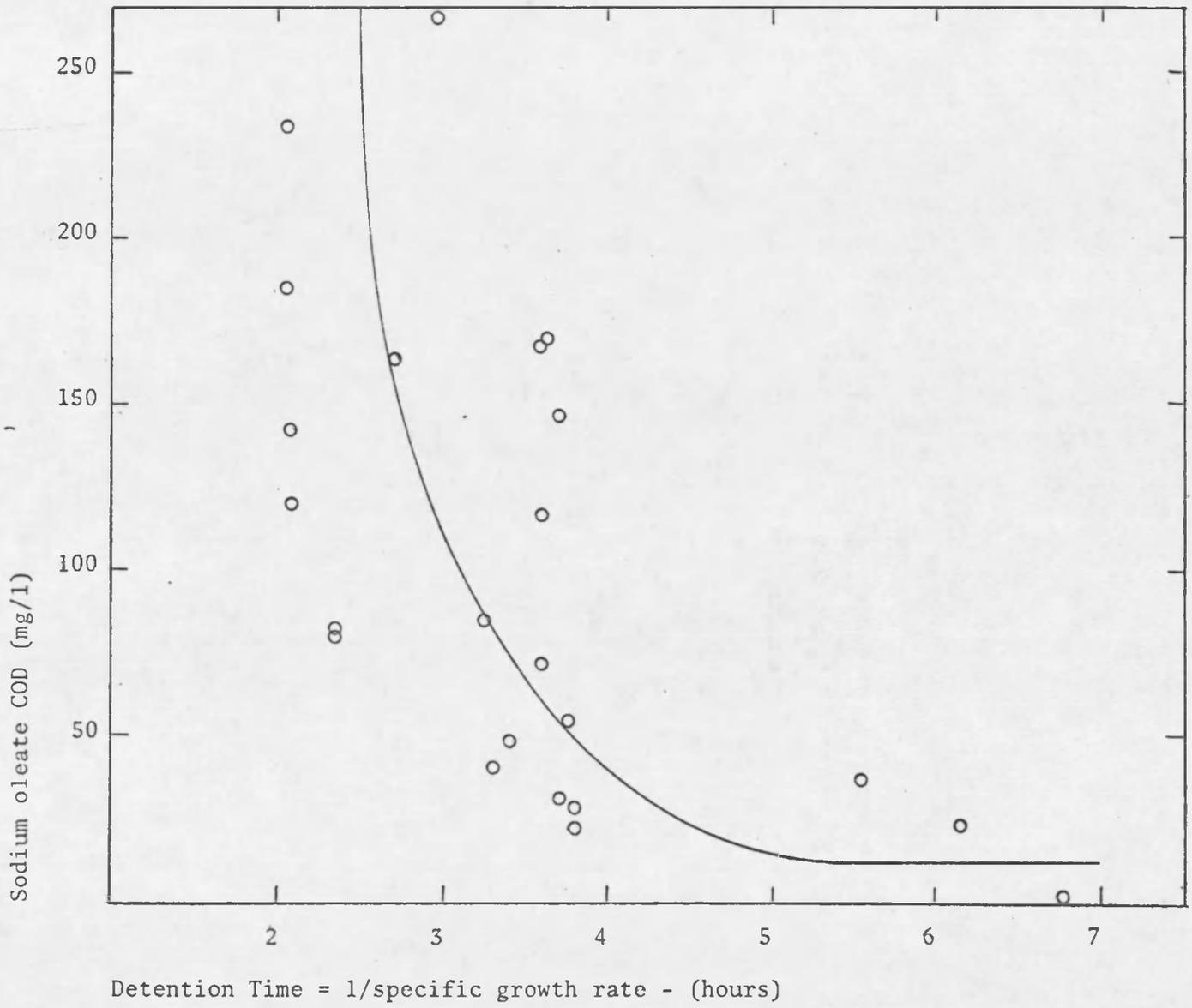


Figure 5.14 Detention time vs. sodium oleate COD

Figure 5.15 shows the effluent COD composition as a function of various COD removals. (See Appendix C for the basis of figure 5.15). At low COD removals nutrient broth seems to be more desirable to the organisms present than sodium oleate. However, at high COD removals, the residual nutrient broth component appears more resistant to degradation than the sodium oleate component. The conclusion one draws from figures 5.13, 5.14, and 5.15 is that glucose is preferred to sodium oleate and nutrient broth by the organisms present in that glucose is the first of the three substrates to be substantially removed.

Again noting figure 5.13, it is presumed that the reason that the COD curves of glucose and nutrient broth become coincident above COD values of 200 mg per liter is that a nitrogen source is needed for further reproduction. In other words, microorganisms cannot live by carbon alone - they must also have nitrogen.

Without having data similar to figures 5.13 and 5.14 for organisms fed pure substrates, that is strictly glucose, sodium oleate, or nutrient broth, it cannot be determined if one carbon source inhibits the uptake of another. If such data were available and inhibition was taking place, one should notice a difference in the minimum detention times between figures 5.13 and 5.14 and those of pure substrates. Such an inhibitory relationship is shown in figures 2.1 and 2.2.

It must also be considered that by providing a readily metabolizable carbon source to the microorganisms, the glucose might actually aid in sodium oleate and nutrient broth removals. This would occur if the detention time was such that it was less than the minimum required

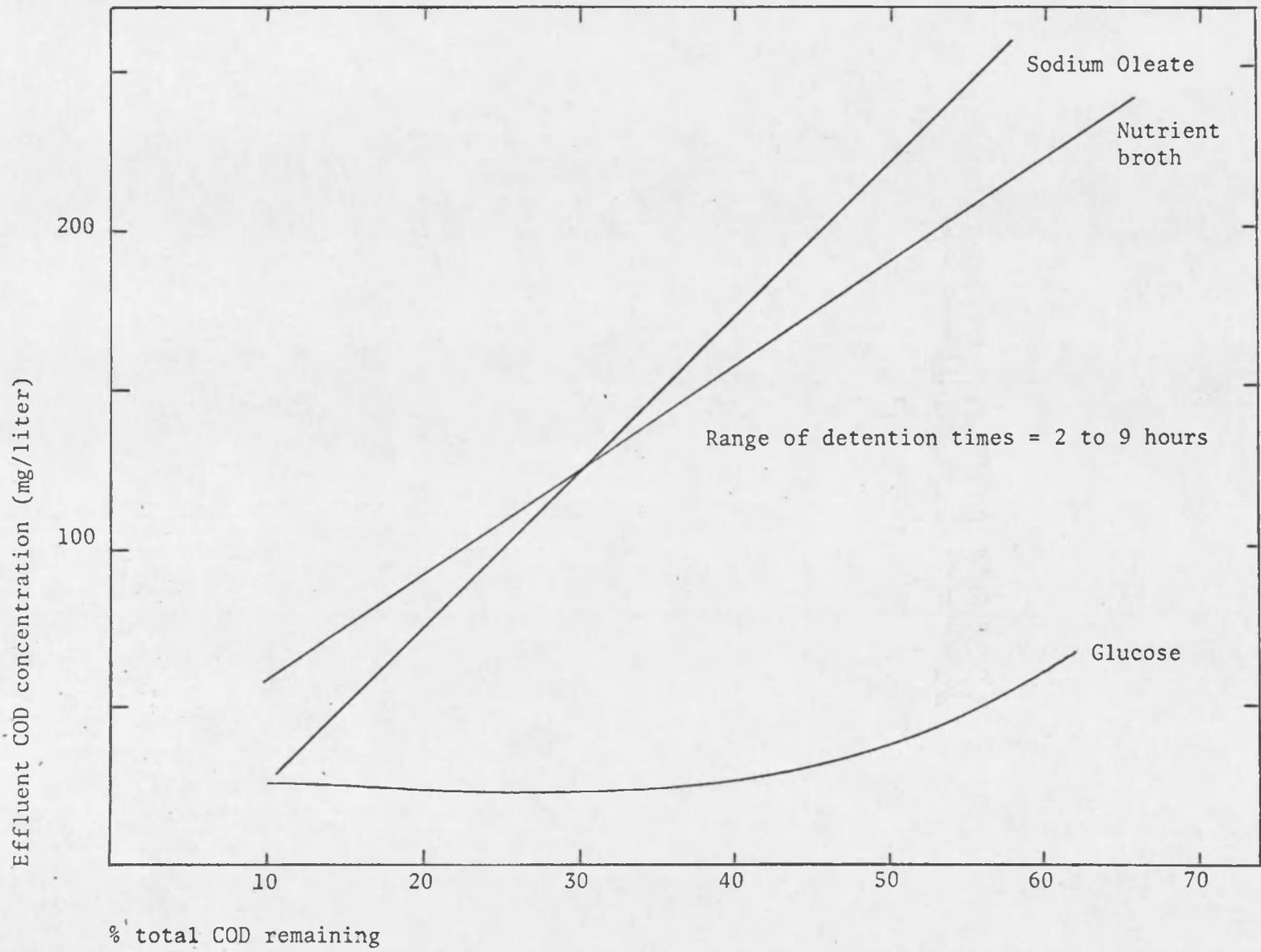


Figure 5.15 % total COD remaining vs. effluent COD concentration.

for cells fed solely nutrient broth and sodium oleate but greater than the minimum for cells fed only glucose. Microorganisms which would be generated quickly from glucose could then in turn metabolize sodium oleate and nutrient broth. Thus if no glucose was present, washout would occur and there would be no organisms present to metabolize anything.

Another consideration is that perhaps the tank volumes of the series operation were not optimum. Erickson et al. (11) propose a scheme for allocating volume to the various tanks in a tanks-in-series process. Erickson and Fan have proposed an optimization procedure based on the kinetic model of Michaelis-Menton in conjunction with hydraulic and economic conditions as a method of allocating volume in an activated sludge system. As the hydraulic and economic objectives are to minimize total aerator volume for a given degree of treatment, the considerations are essentially those of kinetics. Thus the allocation is based upon the Michaelis-Menton constant, K , for a particular waste, where K is equal to the substrate concentration of one half the maximum velocity of reaction (see figure 5.16).

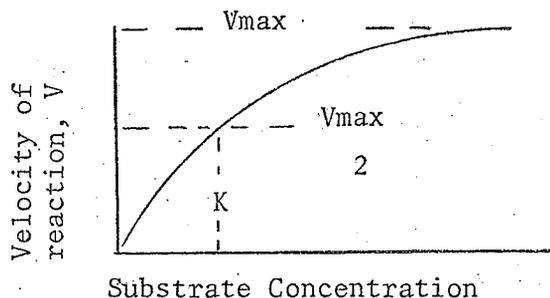


Figure 5.16 Illustration of maximum velocity of reaction

For single tank systems with no sludge recycle, the velocity of reaction is equal to the flow rate through the tank (19).

Defining $K_1 = K/(\text{influent substrate concentration})$, Erickson and Fan propose the following relationship:

From figure 5.17, K is determined to be 200. As 861 is the average influent COD concentration for the data of figure 5.18, $K_1 = 200/861 = 0.232$.

Entering figure 5.18 with $K_1 = 0.232$, the suggested allocation is 65% as compared to a 50% allocation as the author used.

Perhaps the most plausible explanation is offered by Herbert (20). In reference to a chain of biological reactors, Herbert states that such a multi-stage operation is only warranted when a "complex" substrate is fed. The complex substrate consists of several carbon sources such that microbial growth is supported at different rates. In this case, the most easily metabolized carbon source is assimilated in the initial reactor and the remaining substrate in the subsequent reactors. However, Herbert points out that often there is little advantage to a series operation as a single reactor of equal volume is more efficient. The reason for the higher efficiency in the single tank is that "the maximum output of cells obtainable from a multi-stage system is never greater than from a single reactor of the same volume."

Again, referring to figure 5.11, it is seen that the best removals are obtained at the lowest loading factors. Since there was no sludge return, this means that the best removals are obtained when the solids production is highest. This is as Herbert suggests - a single tank generally yields more solids than does a multi-tank system. For the single tank system the yield is 0.32 mg MLVSS per mg of COD removal. For the two tank system, the yield is 0.23 and for the three tank system the

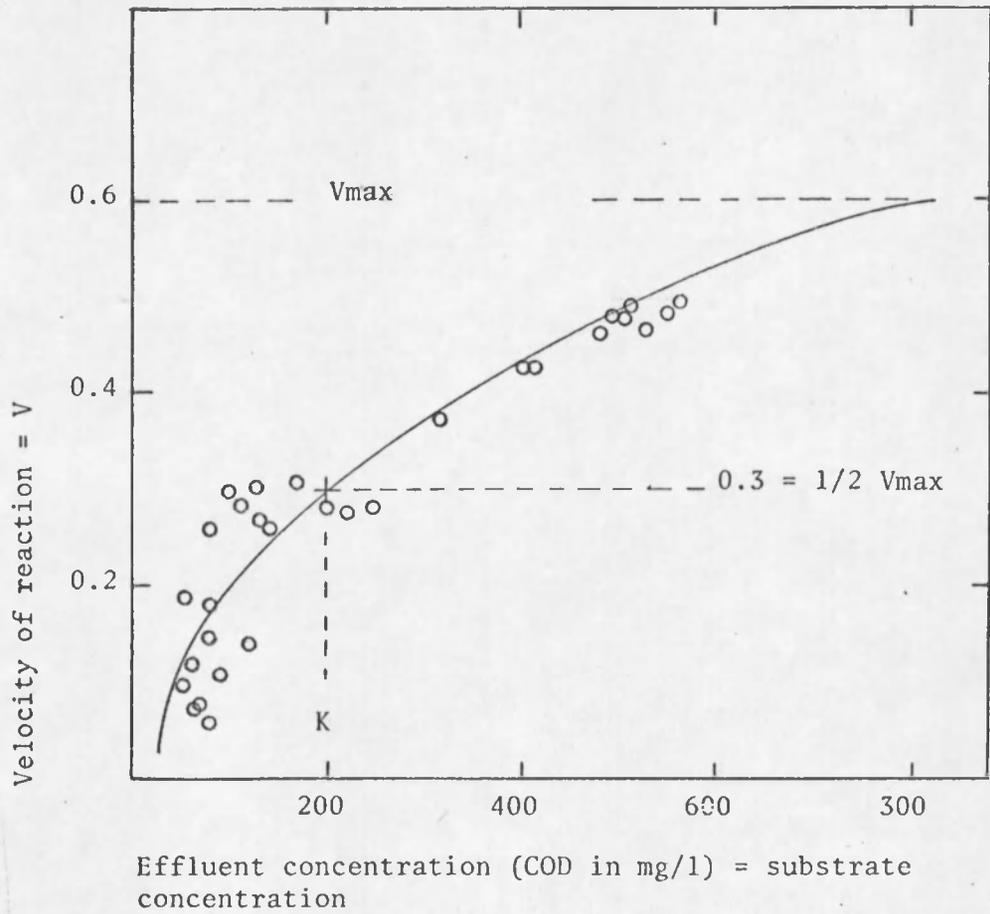


Figure 5.17 Graphical solution for 1/2 the maximum velocity of reaction

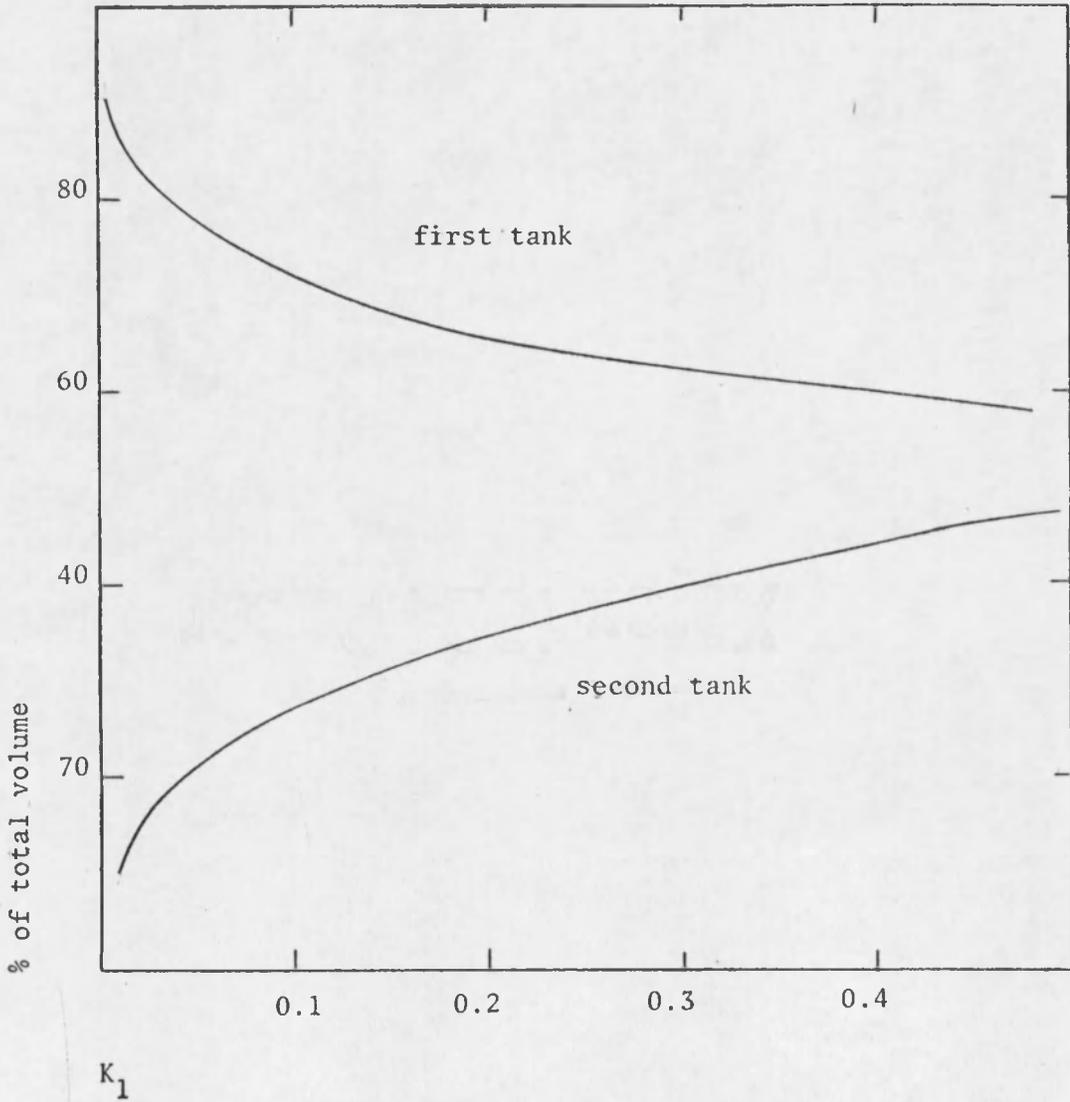


Figure 5.18 Variation of optimal allocation of volume with K_1

Yield is 0.19. Thus for the substrate used in this study the yield does indeed decrease as the number of tanks in series increases. (See Appendix D).

Herbert says that only when the assimilation rates for the different carbon sources are substantially different, is a multi-stage system advisable. Certainly it would not appear that the uptake rates for the carbon sources used in this study are sufficiently different to suggest the use of a multi-stage system as the single tank was superior to the multi-stage system.

CHAPTER 6

CONCLUSIONS

In the substrates studied, glucose was found to be preferred to nutrient broth by the microorganisms as it was the first in the substrate sequence to be substantially reduced in concentration. (See figure 5.15).

It was determined that a single activated sludge tank was superior to a tanks-in-series process in which each of the series tanks was the same volume. The single tank is of course the same volume as the combined tanks-in-series volume. However, as the reduction of substrate approached 90% the difference between systems became negligible.

As the original postulate was that a multi-staged process would degrade more of the substrate at a given detention time than a single-tank process, an explanation as to why it did not is offered below.

It is conceivable that the volume allocation in the multi-staged process was not optimum. The work of Erickson et al. (11) also suggest that it might not have been.

Perhaps, however, the best explanation is that the condition that one substrate be preferred (that is utilized faster) to another is not a sufficient condition on which to base the design of a multi-staged process. It appears that a substrate must not only be preferred to another, but actually prevent the uptake of another carbon source. In the case in point where the single tank was superior to the multiple tank system, it must be assumed that the presence of one substrate did not substantially inhibit the uptake of another. In the absence of such inhibition, the

reproduction efficiency of the microorganisms in a single tank system appears to be higher than where the volume is divided. This is borne out by the fact that lower loading factors are obtainable with a single tank and a given detention time than one can achieve with a multi-staged process and the same detention time. The decrease of sludge yield obtained by increasing the number of tanks is also indicative of a higher sludge production efficiency.

APPENDIX A

FUNGI INFESTATION

The first indication of abnormally great fungi activity was a rapid increase in MLVSS. The MLVSS concentration would double or triple in a matter of two to three days. Microscopic examination over the same period revealed the increase in fungi until fungi were the only microorganisms discernable in the floc. The rapid increase of the fungi population was attributed to the physical nature of the organisms which prevented them from being washed from the activated sludge tanks. Fungi are not a desirable floc component of activated sludge because they are difficult to remove using gravity sedimentation.

McKinney (21) reports that low pH is conducive to fungi development in activated sludge. The author found no low pH values.

The Tucson sewage treatment plant, from which the activated sludge was originally taken, has experienced similar fungi infestations in their activated sludge tanks. When this has occurred, the procedure has been to dump the sludge from the system, reseed the infested tanks and grow new activated sludge (22).

Another indication of extensive fungi growth as observed by the author was an apparent color change in the activated sludge tanks. The color of the sludge would change from brown, to gray and then to blue. The blue color occurred when the fungi became the only discernable species in the floc.

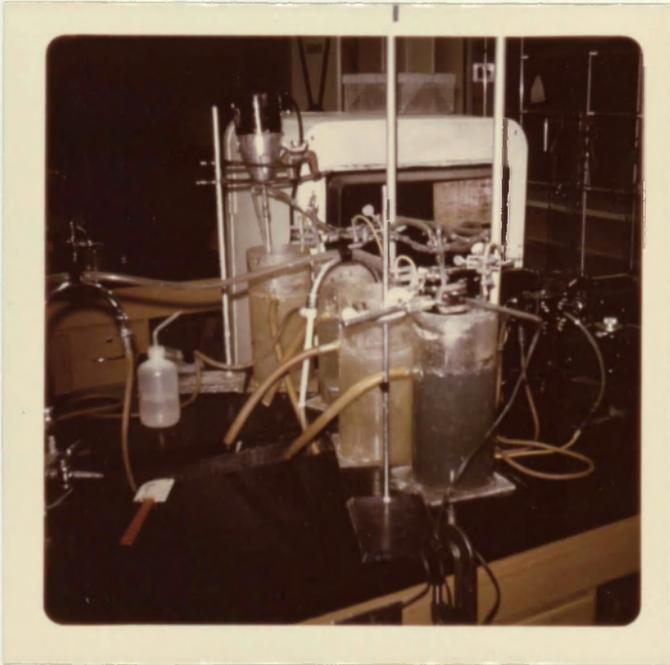
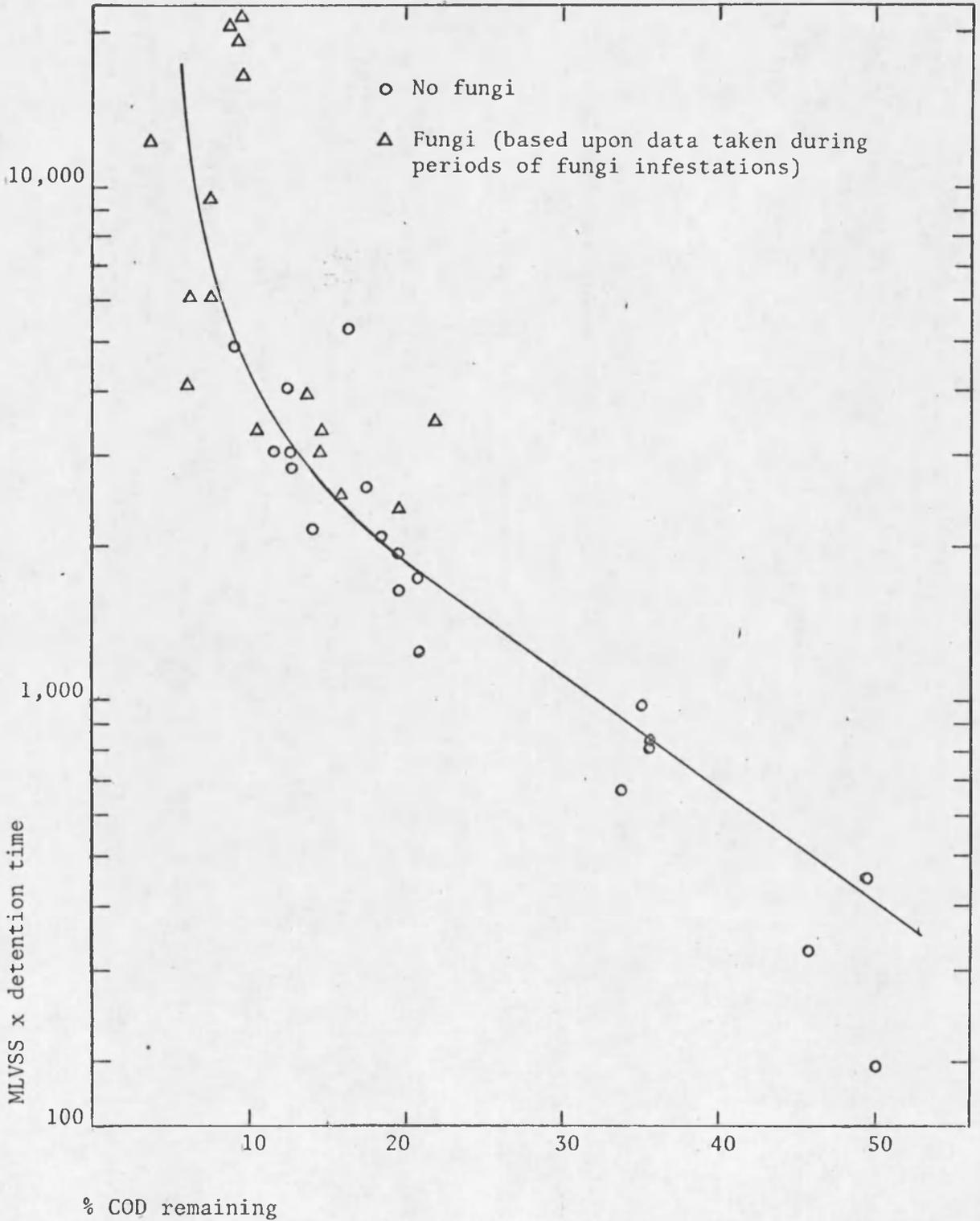


Figure A.1 Reactors showing fungi infestation

As COD removal is a function of detention time and MLVSS concentration, the two were combined into one parameter by multiplying them together and the result was plotted against percent COD remaining in the effluent from a one tank system. The data of figure A-2 is from systems where no fungi was detected and from systems in the early part of the study which contained predominately fungi. It would appear that for the substrate used there is little difference between the COD removing characteristics of the fungi and bacteria developed in the study.



APPENDIX B

COD GLUCOSE AND COD
NUTRIENT BROTH CORRELATIONS

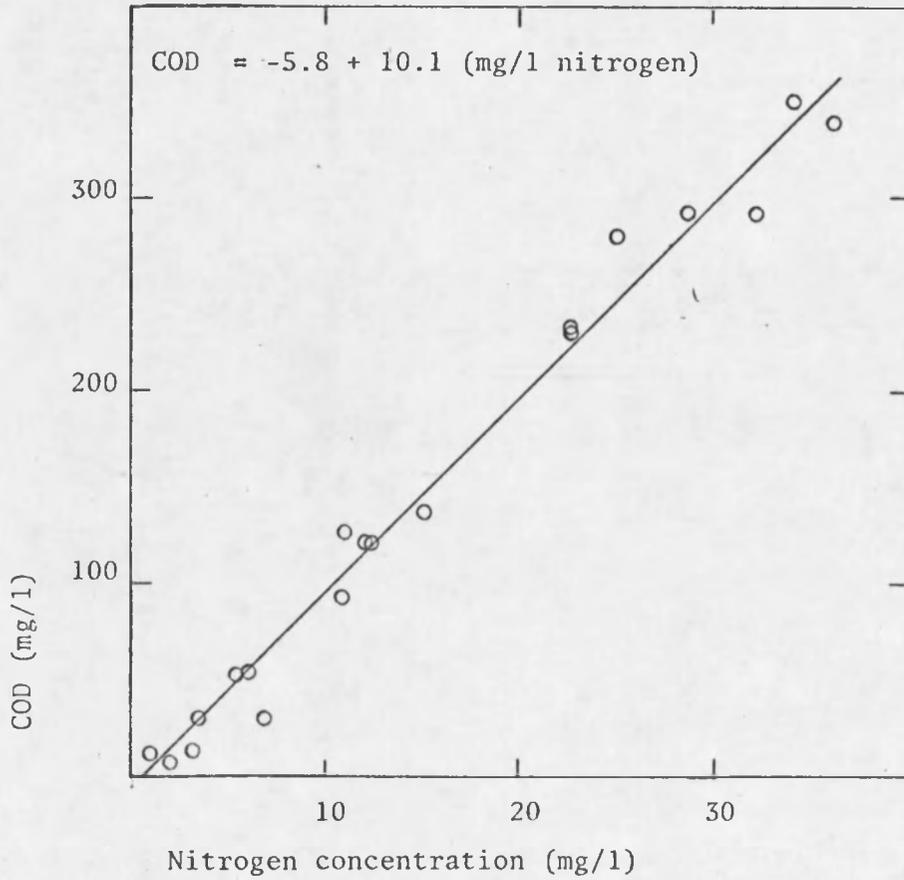


Figure B-1 COD vs. nitrogen concentration

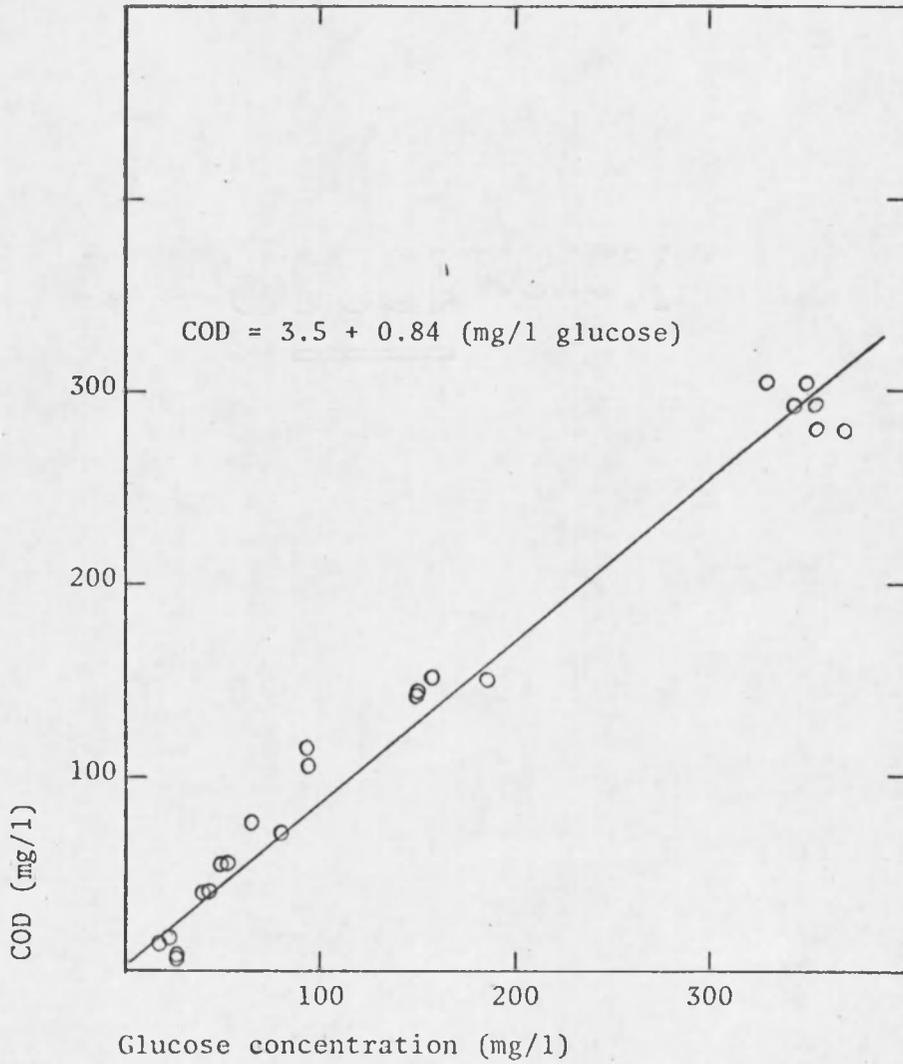


Figure B-2 COD vs. glucose concentration

APPENDIX C

CONSTRUCTION OF FIGURE 5.15

Figures C-1, 2, and 3 are the individual curves which are presented as a composite graph in figure 5.15. The data are from a one tank and two tank system. The substrate COD concentration is that of the system effluent. The percent COD remaining is based on the total COD concentration fed the system and the total COD concentration leaving the system.

A three tank system is not shown as most of its data would plot close to the origin lending little information to the curves.

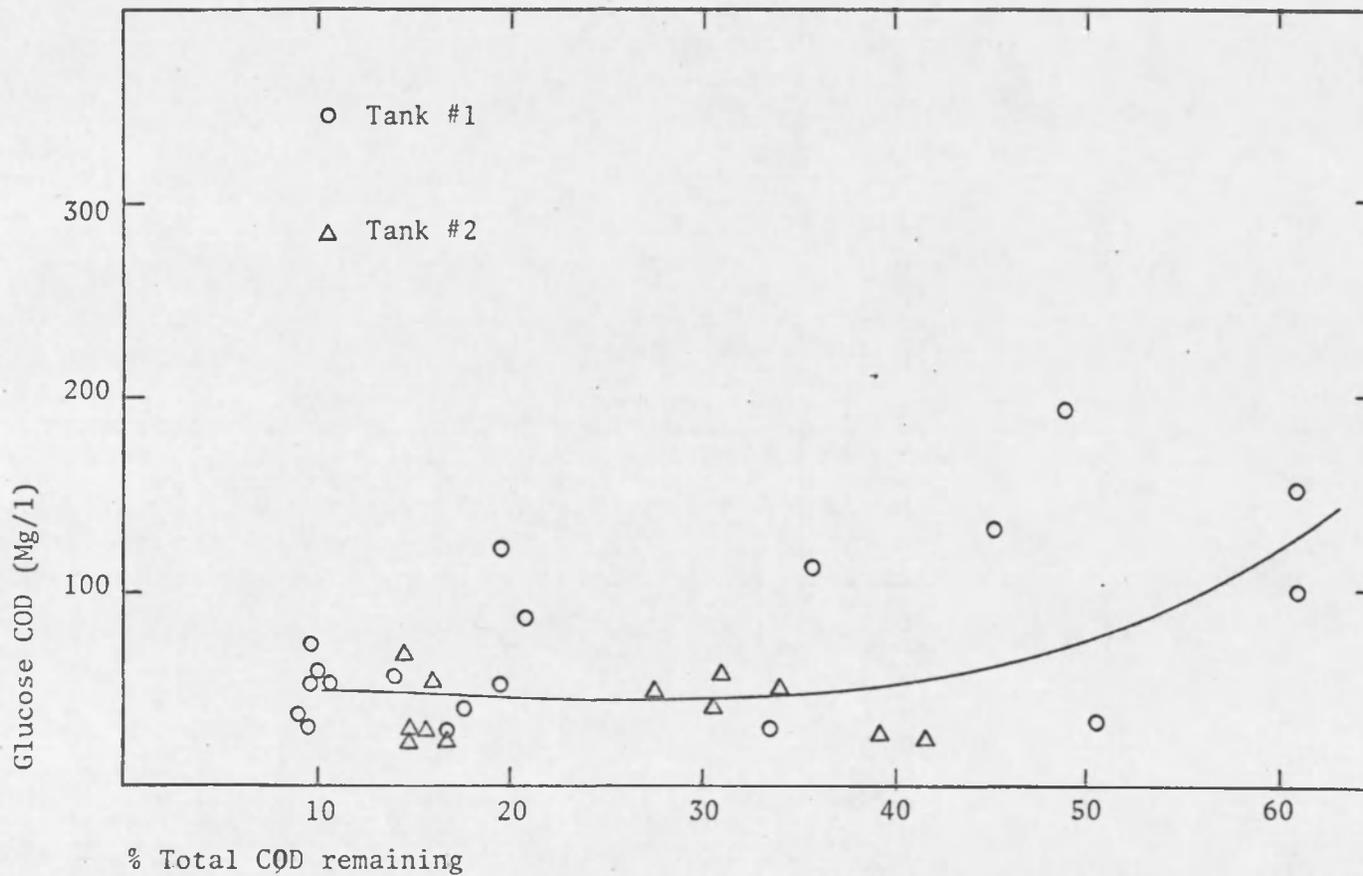


Figure C-1 % total COD remaining vs. glucose COD

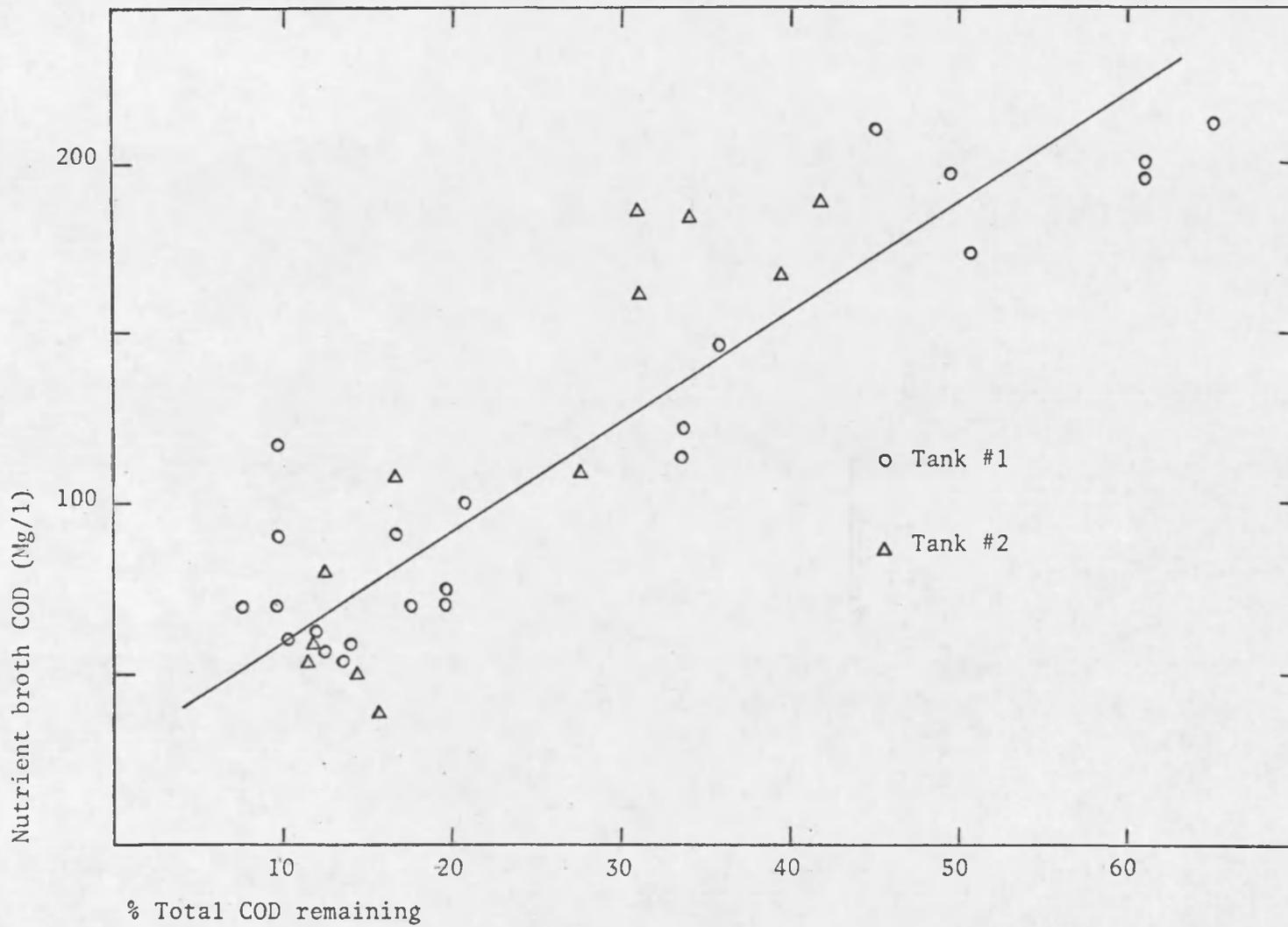


Figure C-2 % total COD remaining vs. nutrient broth COD

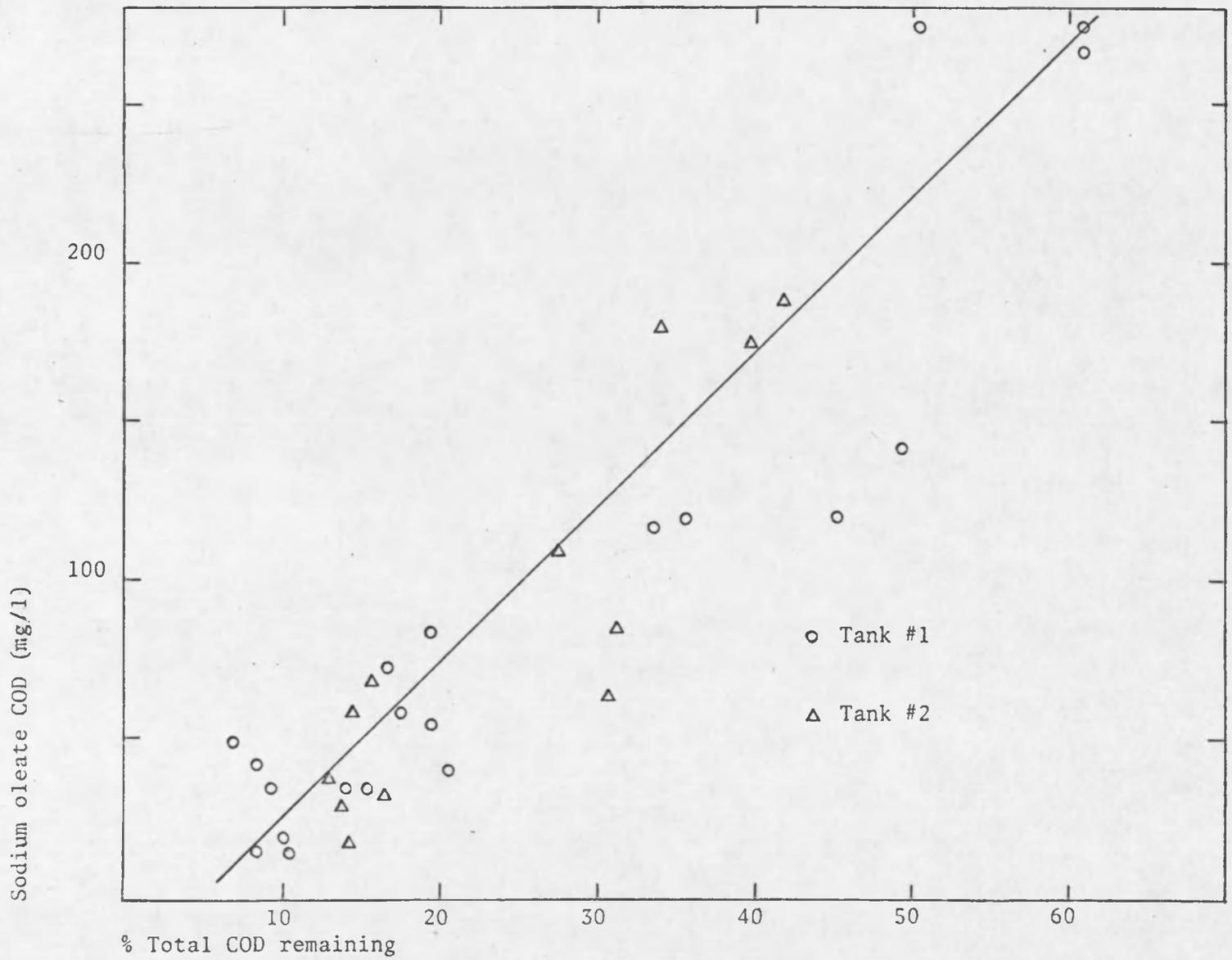


Figure C-3 % total COD remaining vs. sodium oleate COD

APPENDIX D

CALCULATION OF SLUDGE YIELD COEFFICIENTS

The yield of MLVSS per mg of COD removed was calculated in the following manner:

$$\text{Eq. 1} \quad \Delta \text{MLVSS} = a \Delta \text{COD} - b S_a$$

where

$\Delta \text{COD} = \text{COD in} - \text{COD out} = \text{COD removed}$ and expressed in mg/hr.

$a =$ fraction of COD removed that is synthesised to sludge (yield)

$S_a =$ average amount of MLVSS under aeration in mg

$\Delta \text{MLVSS} =$ amount of MLVSS out -

amount of MLVSS in

$=$ amount of MLVSS out (since there is no recycle) and expressed in mg/hr.

$b =$ mean rate of endogenous respiration and expressed as a fraction

dividing eq. 1 by S_a

$$\frac{\Delta \text{MLVSS}}{S_a} = \frac{a \Delta \text{COD}}{S_a} - b$$

$$S_a \quad S_a$$

By plotting $\Delta\text{MLVSS}/S_a$ vs. $\Delta\text{COD}/S_a$, the coefficient, a , and the intercept, b , are obtained as in figure D-1.

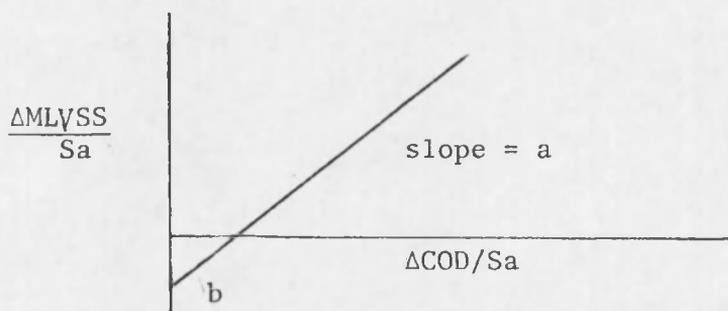
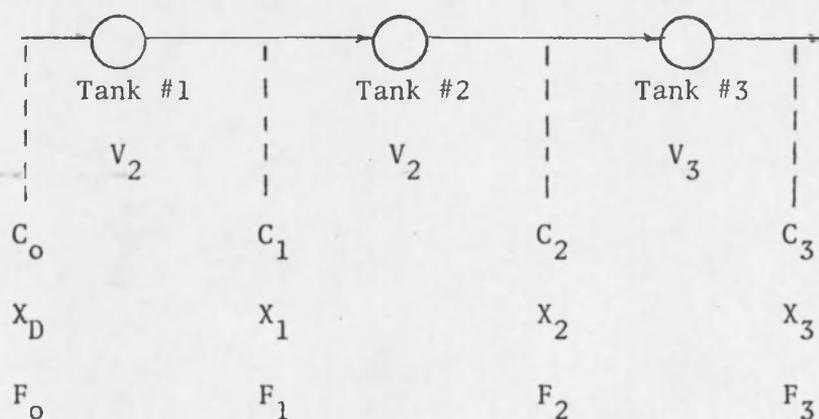


Figure D-1 Illustration of graphical solution for the sludge yield coefficient



C = COD concentration in mg/l

X = concentration of MLVSS in mg/l

F = Hydraulic flow rate in liters/hr.

$$F_0 = F_1 = F_2 = F_3 = F$$

V = Tank volume, $V_1 = V_2 = V_3$

Figure D-2 Illustration for material balance notation

One Tank System

$$\Delta\text{COD} = (C_0 - C_1) F = \text{mg/hr.}$$

$$S_a = (X_0 + X_1) V = X_1 V = \text{mg}$$

$$\Delta\text{MLVSS} = (X_1) F = \text{mg/hr.}$$

The result is plotted as figure D-3.

Two Tank System

$$\Delta\text{COD} = (C_0 - C_2) F$$

$$S_a = (X_1 + X_2) (2V)$$

$$\Delta\text{MLVSS} = (X_2) F$$

The result is plotted as figure D-4.

Three Tank System

$$\Delta\text{COD} = (C_0 - C_3) F$$

$$S_a = (X_1 + X_2 + X_3) (3V)$$

$$\Delta\text{MLVSS} = (X_3) F$$

The result is plotted as figure D-5.

Statistical Analysis

The curves of figures D-3 through D-5 were computed using the techniques of least squares linear regression.

Testing at the 10% significance level shows that the yield for the two tank system is significantly less than the yield for a one tank system. Again at the 10% level, the yield from a three tank system is significantly less than the yield in a one tank system. However, there is no significant difference between the yields of a two tank system and a three tank system.

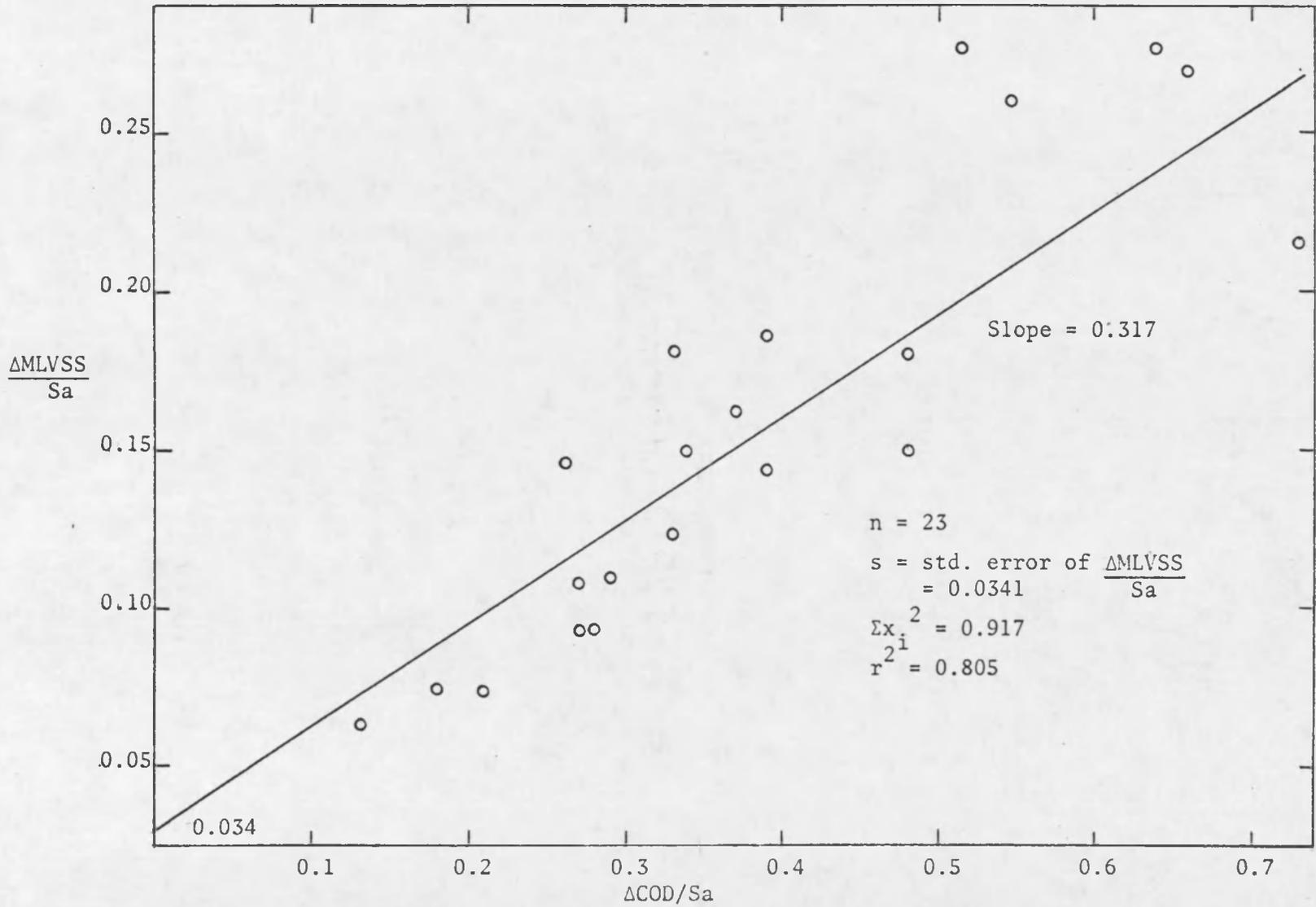


Figure D-3 Graphical solution for the sludge yield coefficient for a 1 tank system

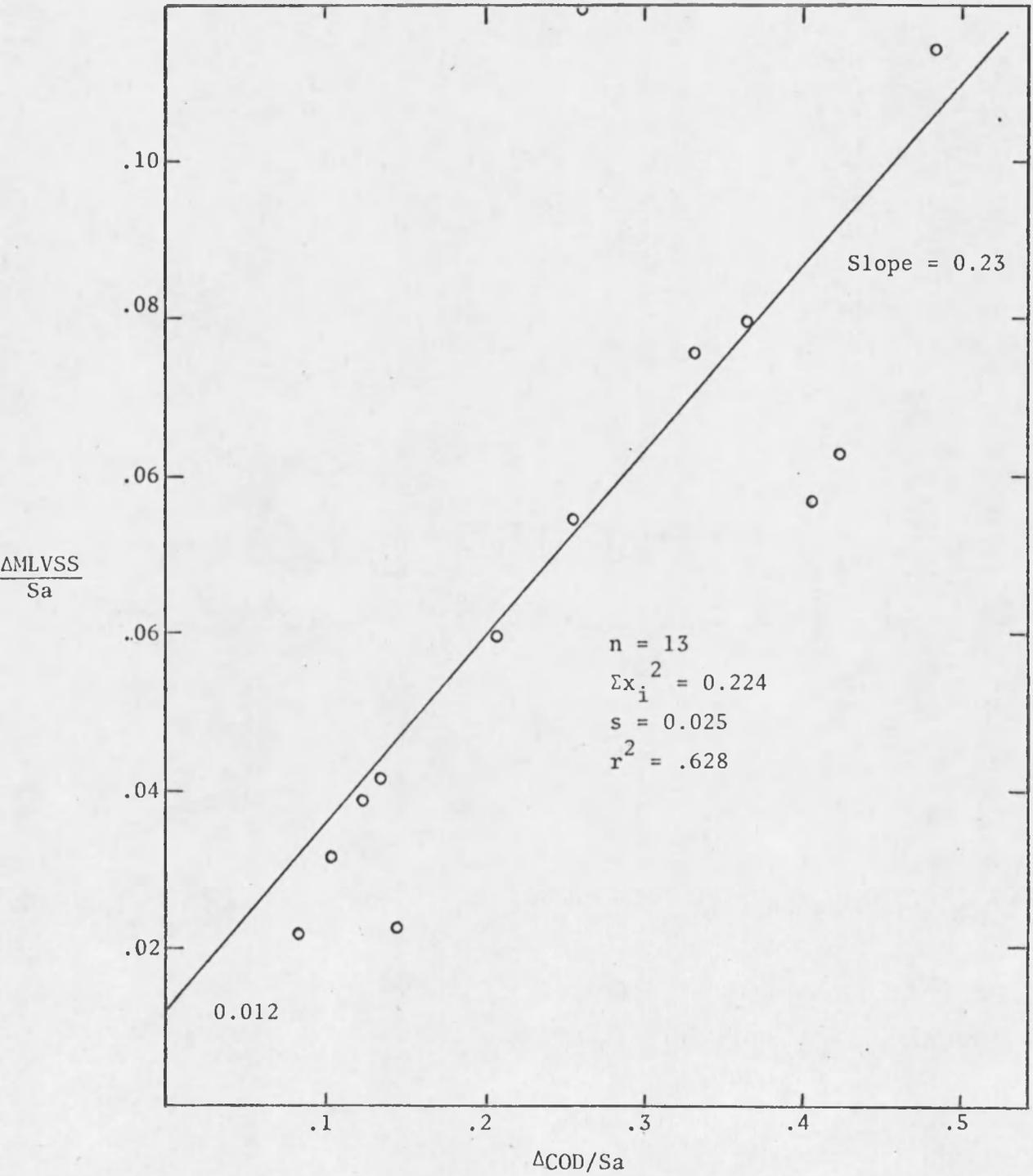


Figure D-4 Graphical solution for the sludge yield coefficient for a 2 tank system

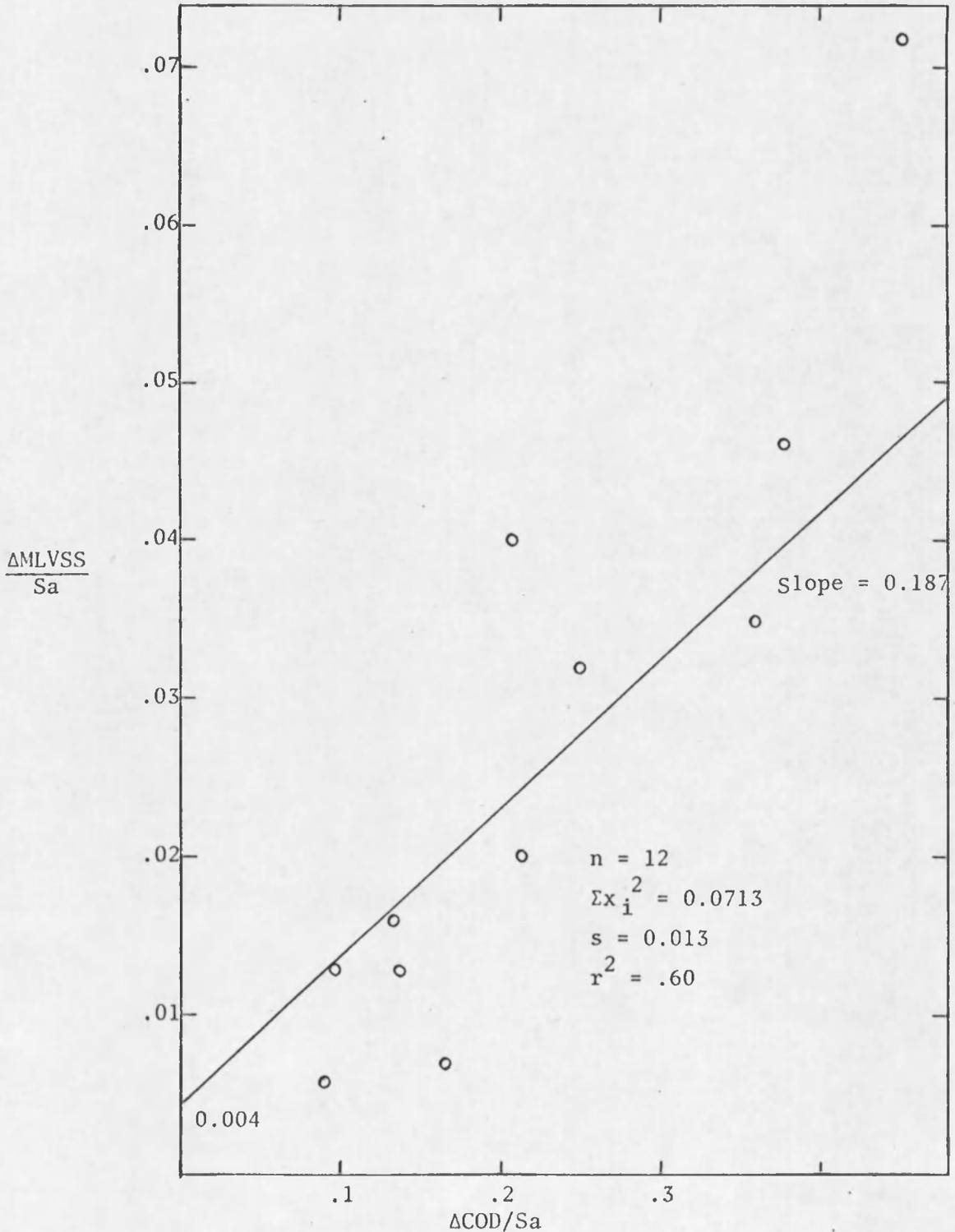


Figure D-5 Graphical solution for the sludge yield coefficient for a 3 tank system

The existence of a positive b in figures D-3 through D-5 imply that no endogenous respiration is taking place. However, it is possible to reject the hypothesis that b is negative (endogenous respiration does take place) for only the single tank system when tested at the 10% level. As it is also not possible to reject the hypothesis that b is positive (no endogenous respiration takes place) for the two and three tanks system when tested at the 10% level, it can only be said that the data is inconclusive as to the existence of endogenous respiration for the two and three tank systems.

LIST OF REFERENCES

1. Monod, J., "The Phenomenon of Enzymatic Adaptation, Growth, no. 11, (1947).
2. Willetts, N. S., "Protein Degredation During Diauxic Growth of E. Coli," Biochemical and Biophysical Research Communication, 20(6), (1965).
3. Monod, J., "The Growth of Bacterial Cultures," Annual Reviews Microbiology, 3, (1949).
4. Gaudy, A. F., Komolrit, K., and Bhatla, M. N., "Sequential Substrate Removal in Heterogeneous Populations," Jour. Water Pollution Control Fed., 35, (1963).
5. Gaudy, A. F., "Studies on Induction and Repression in Activated Sludge Systems," Applied Microbiol, 3, (1962).
6. Stumm-Zollinger, E., "Substrate Utilization in Heterogeneous Bacterial Communities," Jour. Water Pollution Control Fed., May (part 2), (1968).
7. Gaudy, A. F., and Komolrit, K., "Substrate Interaction During Shock Loadings to Biological Treatment Processes," Jour. Water Pollution Control Fed., August (1966).
8. Gaudy, A. F., and Komolrit, K., "Biochemical Response of Continuous Flow Activated Sludge Processes to Qualitative Shock Loadings," Jour. Water Pollution Control Fed., January, (1966).
9. Wang, W. I. C., and Humphrey, A. E., "Biochemical Engineering," Chemical Engineering, December 15, (1969).
10. Webb, F. C., Biochemical Engineering, D. Van Nostrand Company Ltd., London (1964).
11. Erickson, L. E., Chen, G. K. C., and Fan, L. T., "Modeling and Optimizaition of Biological Waste Treatment Systems" Water, American Institute of Chemical Engineers, 90, Vol. 64, (1968).
12. Milbury, W. F., Pipes, W. O., and Grieves, R. B., "Compartmentalization of Aeration Tanks," Jour. Sanitary Engineering Div. Proc. Amer. Soc. Civil Engr. 91, SA3, 45 (1965).

13. Tenney, M. W., Johnson Jr., R. H. Symons, J. M., "Minimal Solids Aeration Complete Mixing Activated Sludge," Journal of the Sanitary Engineering Division, ASCE, Vol. 90, No. SA1, February 1964, pp. 23-42.
14. Standard Methods for the Examination of Water and Wastewater. 12th Edition. American Public Health Association, New York. (1965).
15. McKenzie, H. A., and Wallace, H. S., "The Kjeldahl Determination of Nitrogen: A Critical Study of Digestion Conditions, Temperature, Catalyst, and Oxygen Agent," Australian Jour. Chemistry, 7.55. (1954).
16. Brink, Jr., R. H., Dubach, P., and Lynch, D. L., "Measurement of Carbohydrates in Soil Hydrolyzates with Anthrone," Soil Science 89, (1960).
17. Catalog MC/1, The Millipore Corporation, (1970).
18. Lawrence, A. W., and McCarty, P. L. "A Kentic Approach to Biological Wastewater Treatment Design and Operation," p. 32, Technical Report No. 23, Cornell University Water Resources and Marine Sciences Center, Ithaca, New York, December (1969).
19. Aiba, S., Humphrey, A. E., Millis, N. F., Biochemical Engineering, Academic Press, New York, (1965).
20. Herbert, D., "A Theoretical Analysis of Continuous Culture Systems," Continuous Culture of Microorganisms, 25-53, (Soc. Chem. Ind. Monograph No. 12), (1960).
21. McKinney, R. E., Microbiology for Sanitary Engineers, McGraw-Hill pp. 35-40, (1962).
22. Trueblood, W. J., Sewage Chemist, Tucson Sewage Treatment Plant, personal correspondence.

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