# THE INFLUENCE OF ANAEROBIC CONDITIONS ON ACTIVATED SLUDGE

# THE INFLUENCE OF ANAEROBIC CONDITIONS ON ACTIVATED SLUDGE

By

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SCOPE AND CONTENTS:

A study was made to determine the elimination rate of soluble organic and inorganic compounds during anaerobic storage. Using aerobic storage as a comparison, some insight could then be gained into the metabolic activities of a mixed culture.

The rate of oxygen utilization following anaerobic storage appeared to be dependent on the level of aeration within the reactor prior to storage. There was no liberation of carbon to account for the increased oxygen uptake following storage. Although there was a slight decrease in the solids level, it was independent of the type of storage.

The nitrate ion appeared to act as a hydrogen acceptor during anaerobic storage and, upon reaeration, the nitrate level tended to level off. There was a production of ammonia during both aerobic and anaerobic storage indicating a deamination of cellular material (loss of solids).

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# CHAPTER I

## INTRODUCTION

### The Activated Sludge Process

The conventional activated sludge process has become perhaps the most popular form of sewage treatment on the North American continent since its introduction by Ardern and Lockett (1914). Although the process has been subjected to numerous modifications, it is generally thought of as an aerobic biological waste treatment in which the non-settleable, finely divided, colloidal, and dissolved solids contained in the wastes are converted to a settleable sludge.

The settleable sludge, known as activated sludge, has been defined by the Water Pollution Control Federation Manual of Practice No. 8 (1959) as:

> "Sludge floc produced in a raw or settled sewage by the growth of zoogleal bacteria and other organisms in the presence of dissolved oxygen and accumulated in sufficient concentration by returning floc previously formed."

A flow diagram for the conventional activated sludge process is presented in Figure 1. Raw sewage normally passes through an influent works to the primary sedimentation tanks where a large proportion of the suspended solids is removed. The primary effluent which contains non-settleable and dissolved solids is directed to the aeration section where it is mixed with the activated sludge to form a mixed liquor. After a period of aeration, the mixed liquor is conveyed to a secondary sedimentation tank where the activated sludge settles and a clear supernatant can be decanted. The activated sludge, which has been separated, is returned to the aeration tank although usually some portion of it is wasted.

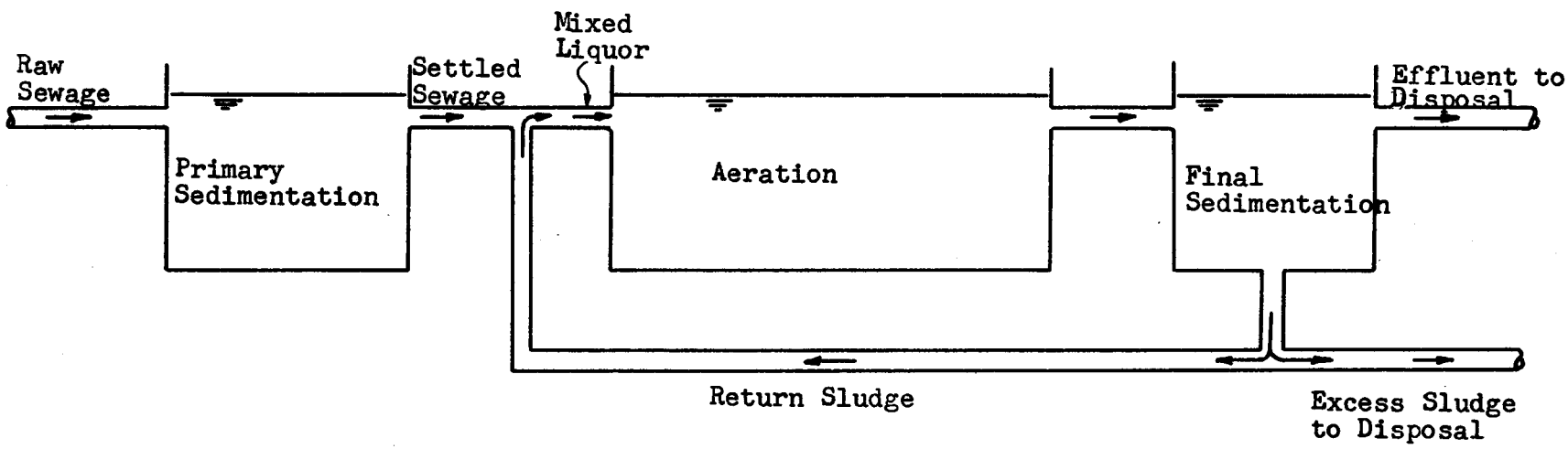



FIG.1 FLOW DIAGRAM FOR THE CONVENTIONAL ACTIVATED SLUDGE PROCESS

In the activated sludge process, when the mixed liquor leaves the aeration basin, the oxygen supply is interrupted. The micro-organisms quickly use up any residual oxygen and then are subjected to "anaerobic conditions," that is, the absence of free molecular oxygen, until they are reintroduced to the aeration section. The anaerobic conditions, sometimes termed anaerobiosis, may last from 2 to 6 hours depending upon the operation and design of the plant.

Purpose of the Investigation

The subjection of activated sludge to prolonged periods of anaerobiosis is generally considered to be harmful to the activated sludge process (WPCF 1959). Work by Wuhrmann (1960), Clesceri (1963) and Westgarth (1963) indicated that periodic anaerobiosis is probably less detrimental to the activated sludge process than has been generally assumed. Hawkes (1963) conceded that the above might be true with soluble wastes but was rather reluctant to accept the view of Wuhrmann, especially if the waste contained suspended or colloidal solids. He suggested that until further evidence was produced on a wider range of wastes, it would seem wise to accept the view that prolonged deoxygenation of the sludge is detrimental, and to provide for the rapid return of sludge to the aeration tanks.

McLellan (1964) attempted to resolve some of the conflicting statements in the literature and made a systematic study of the removal rate of soluble organics and the oxygen utilized by activated sludge previously subjected to anaerobic conditions. He concluded that the removal of organic matter is independent of the type (aerobic or anaerobic) and the length of storage up to 6 hours.

McLellan found that during anaerobic storage the COD (Chemical Oxygen Demand) increased accompanied by a decrease in cellular matter. The

COD which was liberated during anaerobic storage was not sufficient to account for the extra oxygen utilized by the micro-organisms during the subsequent aeration of the anaerobically stored sludge. He also found that the oxygen utilized by endogenously respiring sludge was significantly greater than that utilized by aerobically stored sludge.

This study was designed to confirm or dispute the findings of McLellan and to explain the excess oxygen utilization by anaerobically stored sludge following storage. To this end, a storage period of 6 hours was selected for the endogenously respiring sludge and the progression of solids and organic carbon was studied. Oxygen uptake studies were carried out along with studies on the transformation of the various nitrogenous compounds.

# CHAPTER 2

## LITERATURE REVIEW

### Anaerobiosis of Activated Sludge

The concept that the subjection of return activated sludge to anaerobic conditions is deleterious to the activated sludge process has been accepted by some workers since at least 1933. Spode (1933) reported that failure to maintain aerobic conditions in the activated sludge system caused poor settling sludge, unstable effluent and difficult plant control. Ridenour and Henderson (1934) studied the effects of "stale" return sludge and found that as the sludge became concentrated, it became "staler", thus decreasing the BOD removal in the aeration section. They explained the resultant decrease in efficiency was effected either by failure to provide for the increased oxygen demand of the sludge or by the formation of toxic material during anaerobic conditions. The above attitude is still current as reflected by the WPCF MOP #8 (1959) which stated:

> "The dissolved oxygen in the mixed liquor of the aeration tanks is quickly depleted in the sludge layer of the final tanks, and the rapid return of the sludge is necessary to avoid deterioration of the sludge."

Westgarth (1963) summarized some of the effects attributed to anaerobiosis by other investigators as follows:

(1) floating or rising sludge in the final sedimentation basin, ascribed to denitrification,

(2) high oxygen demand at the influent end of the aeration system, and

(3) a decrease in the removal of organic material, assimilation capacity, by the sludge organisms in the aeration tanks.

Keefer and Meisel (1953) exposed activated sludge to anaerobic conditions for varying periods of time. They found that the rate of oxygen uptake

was greatest with 6 hour anaerobic exposure and least with 96 hour anaerobic exposure. They found that BOD removals were somewhat adversely affected using aged activated sludge although the effectiveness in oxidizing sewage could soon be recovered upon the resumption of aeration.

Recent work by Wuhrmann (1960), Westgarth (1963) and Murphy and McLellan (1965) has indicated that periodic anaerobiosis in the activated sludge process is less detrimental to process efficiency than has generally been assumed. Using washed activated sludge, Wuhrmann (1960) was unable to demonstrate any significant change in the respiration rate following anaerobic storage for sludge respiring endogenously or when fed a glucose substrate following storage. As he had previously demonstrated that the utilization of organic matter was intimately related to respiration rate (Wuhrmann (1955)) he concluded that the purification capacity remained unaffected. Westgarth (1963), working with pilot plants, reported that return sludge stored anaerobically for periods up to 4.5 hours did not have a deleterious effect on the activated sludge process, but under certain conditions, produced less waste sludge more compact in nature. This latter finding could result in the reduction of sludge handling costs.

Murphy and McLellan (1965) supported Westgarth's findings but found that on the basis of their experimental data, oxygen utilization was not strictly proportional to the removal of organic matter. McLellan (1964) suggested that the changes of the various forms of nitrogen during anaerobic storage and the subsequent aerobic period should be investigated as a possible explanation for the unexplained fraction of the extra oxygen utilized. McLellan (1964) used the relationship as postulated by Eckenfelder and O'Connor (1961) to calculate the theoretical oxygen uptake and sludge synthesis.

The general equations are:

$$\frac{dO_2}{dt} = \frac{a'dC}{dt} + B'S$$

$$\frac{dS}{dt} = \frac{adC}{dt} - BS$$

He employed the Chemical Oxygen Demand (COD) test (the Dichromate Silver Sulphate reflux method) as recommended in the 11th Edition of Standard Methods (1960) to determine the amount of soluble organic matter removed or liberated by the sludge under various conditions. A revision of the test procedure in the 12th Edition (1965) recommended the use of sulphamic acid to eliminate the interference of nitrites. Nitrite-nitrogen exerts a COD of 1. 14 milligrams (mg) per mg nitrite-nitrogen present. As a consequence, the COD results obtained by McLellan and his subsequent calculations should be reconsidered in view of the possible nitrite interference.

Inorganic Nitrogen Metabolism

Inorganic nitrogen metabolism has received considerable attention during the past few years by biochemists and microbiologists but relatively little of this work has been utilized by sanitary engineers. Taniguchi et al (1956) found from their investigations a considerable complexity and a multifunctional character of the bacterial reduction of nitrate and nitrite, both from the enzymatic and physiological points of view. Although a general conclusion was difficult to draw, they did state that the so-called "bacterial reduction of nitrate" has at least 2 metabolic aspects; namely, nitrate assimilation and nitrate respiration. Verhoeven (1956) has summarized that at least 3 types of "nitrate reduction" exist:

(1) nitrate assimilation, in which nitrate is reduced only for the building up of cell protein,

(2) incidental dissimilatory nitrate reduction in which nitrate acts as a non-essential hydrogen acceptor, and

(3) true dissimilatory nitrate reduction which, at least under certain conditions, is essential for the well-being of the cell and in which the cell under anaerobiosis accepts nitrate and part of its decomposition products as hydrogen acceptors instead of oxygen.

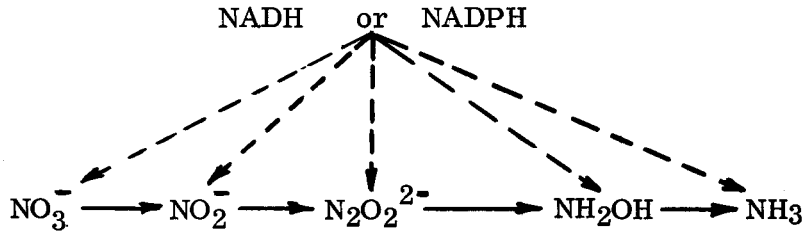
McElroy and Spencer (1956) have agreed with Verhoeven that at least three different types of nitrate reducing systems exist.

Verhoeven reported that certain denitrifiers, cultivated under anaerobic conditions showed an abundant growth and a copious gas production when nitrate was present. The gas consisted of nitrogen and nitrous oxide ($N_2O$) in addition to large amounts of carbon dioxide; there was no formation of ammonia out of the nitrate. However, with another organism Bacillus licheniformis large amounts of ammonia were produced. He considered the above as examples of true dissimilatory nitrate reduction.

Some strains of Escherichia coli have been reported to grow anaerobically only when nitrate is present in the medium. However the accumulation of nitrite is apparently toxic, causing limited growth and indicating incidental dissimilatory nitrate reduction. Taniguchi et al (1956) found that nitrate assimilation and nitrate respiration take place with Escherichia coli.

Conn and Stumpf (1963) also included Bacillus subtilis and Escherichia coli as utilizing nitrate as a terminal electron acceptor instead of oxygen. They suggested that inorganic nitrogen is ultimately transformed to organic nitrogen before it is used by organisms and that this transformation is done in steps, each step involving 2 electrons which are furnished by reduced pyridine nucleotide. The complete sequence including the involved enzymes is shown in

the following scheme:




Conn and Stumpf also suggested that many bacteria (Pseudomonas denitrificans, Denitrobacillus) that carry out respiration produce nitrogen gas instead of ammonia.

Figure 2 is a simplified nitrogen cycle as suggested by Conn and Stumpf and shows the different processes involved.

McElroy and Spencer (1956) suggested the possibility of two broad pathways for the process of nitrate assimilation:

(1) the step-wise reduction of nitrate as indicated above by Conn and Stumpf, and

(2) the incorporation of inorganic nitrogen into an organic molecule at an oxidation level higher than that of ammonia.

This organic nitrogen compound would then be reduced to the amino group level. Both pathways could occur simultaneously.

Although there is general agreement that the immediate product of nitrate reduction is nitrite irrespective of the mechanism involved, there is still some controversy over the character of the immediate product of nitrite reduction. Most workers assume that ammonia is the final compound in the reduction reactions.

Nason (1956) has suggested through his enzymatic studies that a step-wise pathway occurs by way of nitrite and hydroxylamine to ammonia.

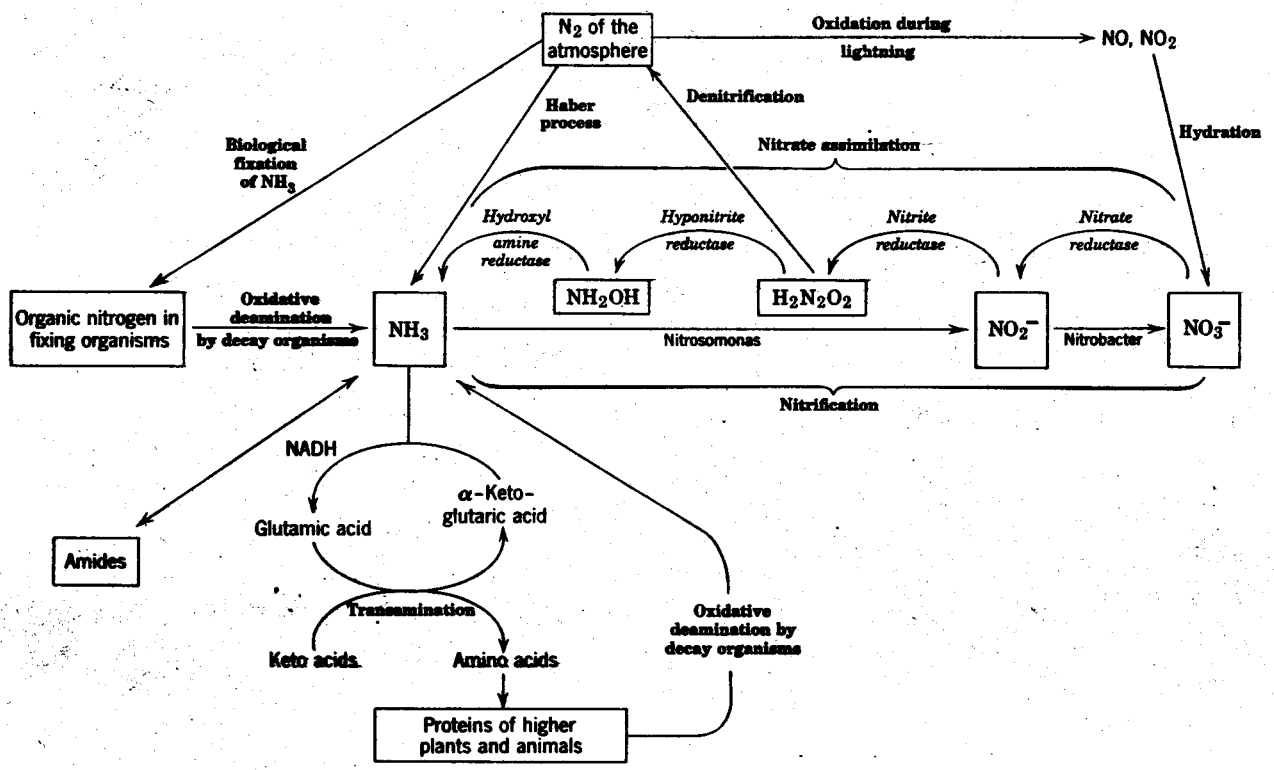
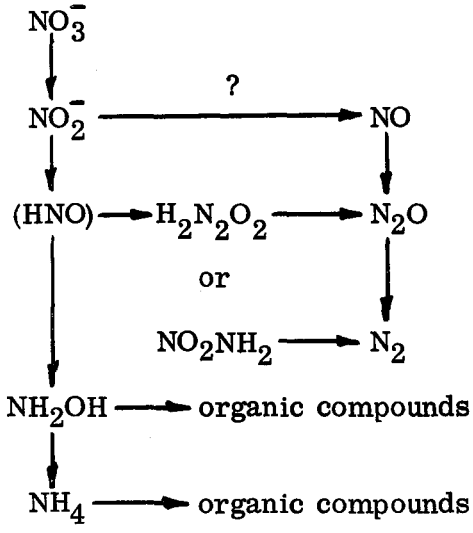



FIG.2 THE NITROGEN CYCLE

Nason postulates that if two electron changes are involved in each step then an undetected intermediate between nitrate and hydroxylamine can be postulated with a nitrogen atom of oxidation number + 1.

Verhoeven (1956) has postulated that a general scheme comprising all aspects of nitrate reduction could be given as:




The first step in nitrite reduction still requires elucidation as it is not yet clearly understood. Nason and Takahashi (1958) summarized the general feeling of investigators by postulating the formation of a hypothetical nitroxyl intermediate on the basis of a presumed 2-electron step-wise change. The presumed spontaneous dimerization of nitroxyl to yield hyponitrite (or nitramide) might be followed by a spontaneous or enzymatic decomposition of hyponitrite involving the splitting off of water thereby yielding nitrous oxide ($N_2O$). Taniguchi et al (1956) have traced the anaerobic reduction of nitrate to ammonia in successive steps. By measuring the accumulation of nitrite, hydroxylamine, ammonia, and ammonia (endogenous) they could recover 80-90% of the reduction products of nitrate. No appreciable denitrification (formation of gaseous products such as nitrogen and nitrous oxide) could be observed manometrically.

Taniguchi et al could not account for the intermediate reduction product between nitrite and hydroxylamine. They could always catalyze the reduction of nitrite by hydroxylamine but the yield of hydroxylamine was always lower than that expected. This finding suggested an unkown intermediate. They found that hyponitrous acid (HON=NOH) was not reduced by a crude extract of a halotolerant bacterium containing all the enzyme systems for the successive reduction of nitrate to ammonia. Verhoeven (1956) mentioned the hypothesis of de la Haba that prior to the incorporation of the nitrogen in a protein molecule, the reduction of a nitrite (or hydroxylamine) molecule proceeds by way of an organo-nitrite compound: $RNO_2$. However, further evidence is still required.

Verhoeven (1956) pointed out that in denitrifying bacteria, there is a unique situation when the processes of nitrate dissimilation and that of respiration are compared. Free oxygen can be replaced by another hydrogen acceptor, in casu nitrate, nitrite, or nitrous oxide, without interfering in the least with the normal metabolic activities of the cells, proliferation included.

Wuhrmann (1960) has in his study on the effect of oxygen tension, carried out nitrogen balances by measuring organic, ammonia, nitrite and nitrate nitrogen. He found that in plants with low oxygen tension, non-nitrifying, a nitrogen balance could be achieved. However with a higher oxygen tension, nearly complete nitrification occurred and an underbalance (-26%) occurred. He suggested that the sludge had taken up nitrate nitrogen for its own future respiration requirements and that by denitrification nitrogen gas was lost to the atmosphere.

Westgarth (1963) in his study of anaerobic storage periods felt that for a nitrogen balance it was necessary to measure nitrate, nitrite, ammonia, protein and total nitrogen. He felt that these tests would show changes in the nitrogen parameters at various points but not the activity at any particular time.

Finally because of the excessive time to do these tests, Westgarth did not consider the use of nitrogen determinations.

Bragstead and Bradney (1937) reported that rising sludge was due to gaseous nitrogen evidently released from nitrites. However, their test of the nitrogen gas was rather elemental in that they collected a gas and bubbled it through O. 1N sodium hydroxide. They deduced that the gas was nitrogen and not carbon dioxide because the alkaline value of the solution was only slightly reduced. Sawyer and Bradney (1945) found that the rising of activated sludge could be correlated to the presence of nitrite and/or nitrate nitrogen. They suggested that the nitrites and nitrates were denitrified to nitrogen gas which, when sufficient quantities were obtained, buoyed the sludge. Although they found that chlorination inhibited the formation of nitrites and nitrates, they advised against pre-chlorination because of the impairment of the purifying ability of the sludge.

Bringmann et al (1959) suggest that nitrogen could be removed from waste water in the form of nitrogen gas in two stages. In the first stage all ammonia nitrogen is converted to nitrite and nitrate by any aerobic biological process.

In the second stage, denitrification of nitrites and nitrates is effected by anaerobiosis. Certain types of bacteria present in the sewage will then make use of the oxygen available from the nitrates and nitrites and thus will liberate nitrogen gas or nitrogen dioxide. Ludzack and Ettinger (1962) in their studies generally agreed with the work of Bringmann et al. They suggested that the form and quantity of nitrogen in the effluent was a good process control parameter, viz:

(a) low ammonia, organic, and oxidized nitrogen indicated good performance,

(b) high ammonia nitrogen indicated a poor air supply and low mixed liquor volatile solids,

(c) high organic nitrogen indicated poor clarification, and

(d) high oxidized nitrogen indicated inadequate denitrification.

# CHAPTER 3

## EXPERIMENTAL TECHNIQUES

### Determination of Sludge Respiration Rates

A parameter of primary importance in the evaluation of the activated sludge process is the quantity of oxygen utilized. Oxygen uptake is usually considered as an indicator of metabolic activity. Wooldridge and Standfast (1936) have established that carbon dioxide is the only gas evolved during the biological oxidation of sewage by sludge. As a consequence, a Warburg Constant Volume Respirometer can be used to measure the uptake of oxygen if the carbon dioxide is absorbed by some chemical. Similarly the Warburg Apparatus can be used to measure the evolution of a gas.

McLellan (1964) used a Warburg respirometer in his studies and found that a shaking rate of 60 oscillations per minute (opm) with a stroke of 4.0 centimeters was adequate to transfer more than 450 microlitres (µl) in a 15 minute period. The same parameters were used in this study and the above transfer rate was greater than any observed during these tests. This would indicate that the uptake of oxygen was independent of the shaking conditions.

Throughout the tests, 20 millilitres of activated sludge were placed into 125 millilitre (ml) reaction flasks. The bath temperature of the Warburg apparatus was held constant at 20°C. Carbon dioxide was absorbed by 1 ml of 15% potassium hydroxide (KOH). The KOH was placed into boats which were subsequently placed into the centre wells of the reaction flasks. The KOH boats (Figure 3) were designed such that the KOH could be removed from the reaction flasks prior to analysis to prevent lysing of the cells as had happened in McLellan's study. The use of boats made duplicate flasks available for both oxygen uptake determinations and chemical tests.

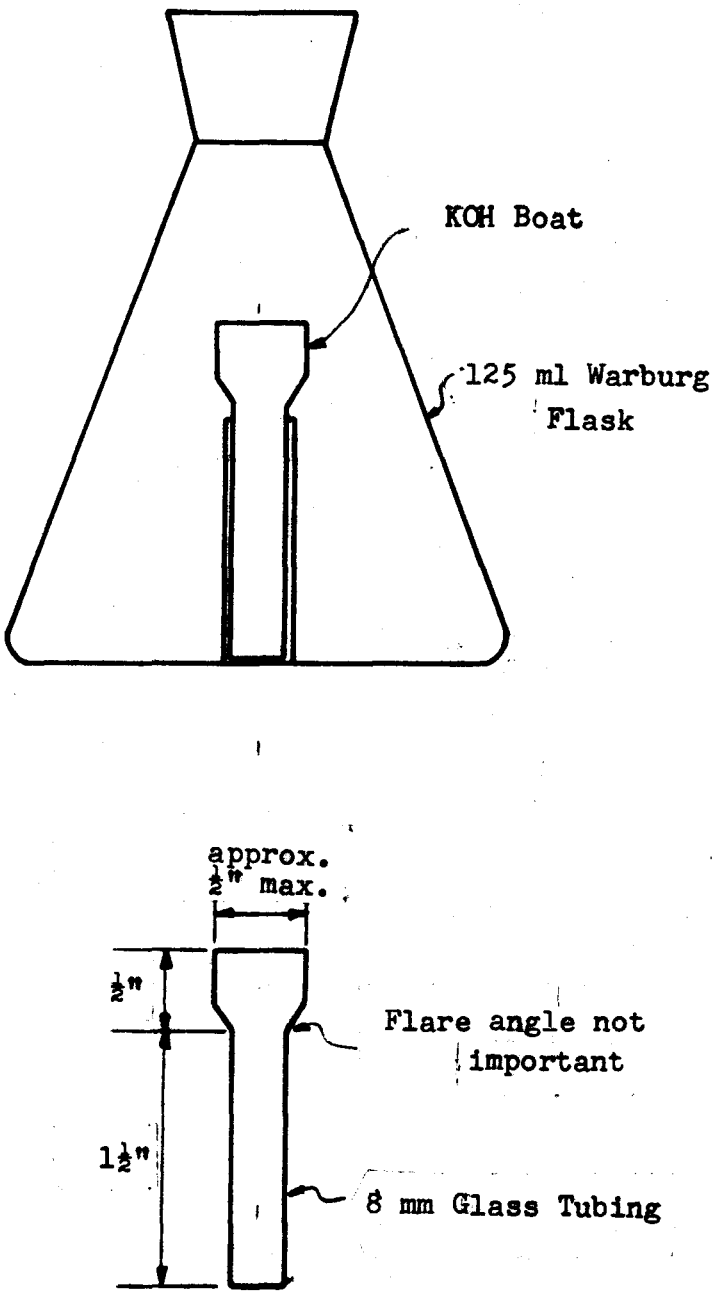



Fig.3 KOH BOATS as Used to Enable Warburg Flask to be Used both for Oxygen Uptake Studies and Chemical Determinations

Standard manometric techniques as described by Umbriet et al (1957) were used. The manometers were shaken in the bath from 15 to 30 minutes with the manometer open to the atmosphere to enable the system to reach a temperature equilibrium. Vessels subjected to anaerobic conditions were flushed with compressed air for 15 minutes and another 15 minutes was allowed for temperature equilibrium prior to the measurement of oxygen uptake.

Gas production during anaerobic storage was measured in a similar manner except that the manometers were closed to the atmosphere during the equilibrium period. The anaerobic periods were considered to start with the introduction of the inert gas (argon or nitrogen) into the reaction flask and gas production measurements were initiated about 30 minutes after.

Determination of Organic Carbon

In present day pollution research, the pollutional strength of a waste is usually defined in terms of oxygen required by the bacteria for the stabilization of decomposable organic matter under aerobic conditions. The major measure of pollution is the 5-day Biochemical Oxygen Demand (BOD) which in the absence of nitrification is considered to be a measure of the carbon present. The major drawbacks for the BOD as a measure are:

(1) it takes 5 days to complete, and

(2) it is rate dependent.

The Chemical Oxygen Demand (COD) has often been substituted as this test can be carried out in a few hours. However, because of the many interfering factors, erroneous conclusions may be drawn from the data obtained.

Relatively recently an instrument has become available which measures, by infra-red absorption, the carbon dioxide produced when carbonaceous matter in solution or suspension is completely oxidized in a stream of oxygen. A Beckman Carbonaceous Analyzer was used in this study to determine the amount of organic

carbon removed or liberated by the sludge under various conditions. The soluble organic carbon which was measured was that present in the filtrate after the cells had been filtered out. Campbell (1966) suggested that, although dissolved (soluble) carbon as a measure of organic pollution has certain weaknesses, on balance it seems to have much in its favour.

The organic carbon determination in this study were made on the filtrate after the mixed liquor had been filtered through a 0.45 micron ($\mu$) membrane filter supported on a Millipore filter holder. The filtrate was collected into 125 ml Erlenmeyer flasks. A portion of the filtrate (usually 4 ml) was diluted with double distilled water at a ratio of 1:1, acidified with a drop of hydrochloric acid (HCl) to a pH in the range from 1 to 2 and stored at $4^{o}C$ for about 12 hours or longer. Krishnan and Gaudy (1966) and Campbell (1966) have indicated that the cooling procedure caused no more loss of organic carbon than that which may have occurred during immediate handling of the sample. It is noted that Krishnan and Gaudy used COD determinations to determine the organic carbon level. The use of 0.45 $\mu$ filters for the removal of micro-organisms would tend to reduce the possibility of any loss in organic carbon during storage.

Prior to the organic carbon determinations, any inorganic carbon (carbon dioxide) was removed by stripping the diluted filtrate with an inert gas (argon or nitrogen) for 20 to 30 minutes. Acidification of the diluted filtrate converted all carbonates to carbon dioxide. A Hamilton 50 microlitre syringe was used to obtain a 20 $\mu$l sample for injection into the carbonaceous analyzer.

Determination of Cell Concentrations

The measurement of the concentration of cells present was necessary because the oxygen utilization and removal of organic matter is dependent upon the quantity of cells present. Although the volatile portion of the suspended

solids is generally taken as a measure of the concentration of cells present, it was assumed for this study that the total suspended solids concentration would be sufficient. The reasons for this assumption were:

(1) only relative values were required, and

(2) the cell source was of a relatively stable nature.

The decision to use vinyl metricell Gelman VM-6 filters came after a series of preliminary studies in which it was found that reproducible measurements could be made with this filter media. It was determined that the least variation in weight of a filter was obtained when the filter was soaked in water for at least 12 hours and then dried in individual desiccators. Heating could not be used prior to filtration as the filters would deform even though the weight was unaltered. The use of glass fiber filters was considered but discarded because the loss of fine fibres could appreciably affect reproducibility. The filters were 47 millimeter diameter with a pore size of 0.45 microns. The filter was supported during filtration on a Pyrex Filter Holder Model XX10 047 00 as manufactured by the Millipore Filter Corporation. A filter bank was constructed of Lucite and each filtration section was equipped with a 3-way valve to permit rapid filtration. The vacuum applied was approximately 28 inches of mercury.

Initially, the solids determinations were carried out by pouring the entire contents of the Warburg flasks into the filter holders. However, when the solids content of the liquor was greater than 40 milligrams, the filters tended to blind, thus increasing the filtration time. As this increased filtration time greatly interfered with the other tests being carried out, a faster filtration period was required. It was found that by using tared centrifuge tubes and filtering only the decanted liquor, an appreciable saving of time was effected. Both the centrifuge tubes and filters were then dried at 103°C for at least 12 hours prior to weighing.

Nitrogen Analyses

Three types of nitrogen analyses were performed:

(1) nitrate nitrogen,

(2) nitrite nitrogen, and

(3) ammonia nitrogen.

Organic nitrogen was not determined because it was assumed that most of the organic nitrogen would be present in the microbial cells rather than in the filtrate. Further the sample size used allowed very little sample for organic nitrogen and there was not enough time to run organic nitrogen determinations while the other tests were being done. In all of the tests double distilled water was used. The double distilled water was found to be free of nitrate and nitrite nitrogen.

1) Nitrate-N Analyses

The method used to determine the concentration of nitrate nitrogen ($NO_3$-N) was the Brucine Method as given in the 12th Edition of Standard Methods. Although the procedure calls for 2 ml of sample, the concentration of nitrate present was such that it was necessary to dilute samples 0.1 to 0.3 ml in size to 2.0 ml. After the Brucine solution was added and the colour developed, the percent transmission of light was measured by the use of pH Coleman Spectrophotometer Model 14 set at 410 mu through a path length of 1.5 cm. A calibration chart was used to determine the amount of nitrate-nitrogen present in the sample.

2) Nitrite-N Analyses

The diazotization method as presented in the 12th Edition of Standard Methods was used to determine the concentration of nitrite-nitrogen in the filtrate. As with the nitrate determination, an excessive amount of nitrite-nitrogen in the filtrate led to a very high dilution ration (0.1 to 0.5 ml di-

luted to approximately 50 ml). The original calibration of the reagents was done in accordance to Standard Methods. It was later decided to use fresh stock nitrite solution to make a calibration curve as samples were in similar range. The spectrophotometer (as above) was set at 520 mu.

(3) Ammonia-N

An Aminco-Koegel Micro-Kjeldahl Apparatus (Steam-Distillation Model) was used to determine the amount of ammonia-nitrogen present in the filtrate. The sample size used in all ammonia-nitrogen determinations was 10 ml. The samples were treated with a drop of sodium hydroxide (250 gm/1) which was enough to turn the phenolphthalein indicator to a pinkish red colour (indicating a pH close to 7.4). The titration method (0.02N $H_2SO_4$) was used to determine the amount of ammonia-nitrogen present. The mixed indicator boric acid solution was prepared in accordance with Standard Methods.

Production of Anaerobic Conditions

Anaerobic conditions were simulated with both high purity argon and nitrogen gas. McLellan found that 15 minutes of flushing was sufficient to establish anaerobic conditions. Flushing was carried out by passing the gas into the closed arm of the manometer, through the flask and out the sidearm of flask for 15 minutes. In order to restore aerobic conditions, compressed air was passed through the flask in the same manner.

Source of Micro-organisms

The micro-organisms used in this study were grown in a batch fed reactor which was seeded with sludge from a conventional activated sludge plant. The reactor used was a Virtis Magnetic Drive Fermenter Model 40B4. An effluent discharge was added to the reactor to facilitate mixed liquor wastage by gravity. The reactor operated for approximately 3 months before the actual study was underway. As with any plant there were operational difficulties dur-

ing start-up that were mainly mechanical. It was found that it was necessary to store feed (see appendix) at 4°C to prevent excessive microbial action which tended to clog up the feed lines. Feeding and sludge wastage were effected by time controlled solenoid valves.

Sludge age has been considered by some workers to be an operating parameter which does have an affect on the purification capabilities of a treatment plant. Wuhrmann (1955) suggests that the term "sludge age" is a rather vague concept and has assumed for his purposes that sludge age is the total detention time of a single sludge floc in the plant. A rough approximation of the time that incoming sludge solids remain in the system is determined by the ratio of the active solids in the system to that of the incoming load;

$$\text{i.e.} \qquad \text{Sludge Age} = \frac{\text{Total solids in reactor}}{\text{Solids produced / day}}$$

In this study the sludge age was held constant at 5 days (see appendix A).

# CHAPTER 4

## EXPERIMENTAL RESULTS

### Oxygen Utilization after Storage

Wuhrmann (1955) considered that the respiration rate of sludge may be related to its ability to remove organic substrates. Accordingly, oxygen utilization studies were performed on endogenously respiring sludge following both aerobic and anaerobic storage. Figure 4 presents the results of oxygen uptake data for run 5 following aerobic and anaerobic storage for 6 hours. Prior to storage of the cells, air was supplied to the 3.0 litre reactor at a rate of 750 ml per minute at room temperature and normal pressure. Assuming a solids level of 3000 mg/l, this is equivalent to an oxygen supply of approximately 1230 mg per gm solids per hour. Following anaerobic storage, the rate of oxygen uptake was 6.3 mg oxygen per gm solids per hour as compared to 4.0 mg oxygen per gm solids per hour for aerobically stored sludge. A similar trend had previously been noted in run 4.

It should be noted that argon had been used to simulate anaerobic conditions in the above runs. As the results appeared to be similar to that of McLellan (1964), nitrogen was used in subsequent runs to simulate anaerobic conditions.

The air supplied to the 3 litre reactor prior to storage then was increased to 975 ml per minute. No difference in respiration rates following storage could be detected (Figure 5). These latter results at higher oxygen input tend to substantiate Wuhrmann's (1960) assertion that no significant change of the endogenous respiration rate occurs when the sludge has been subjected to complete anaerobiosis for several hours.

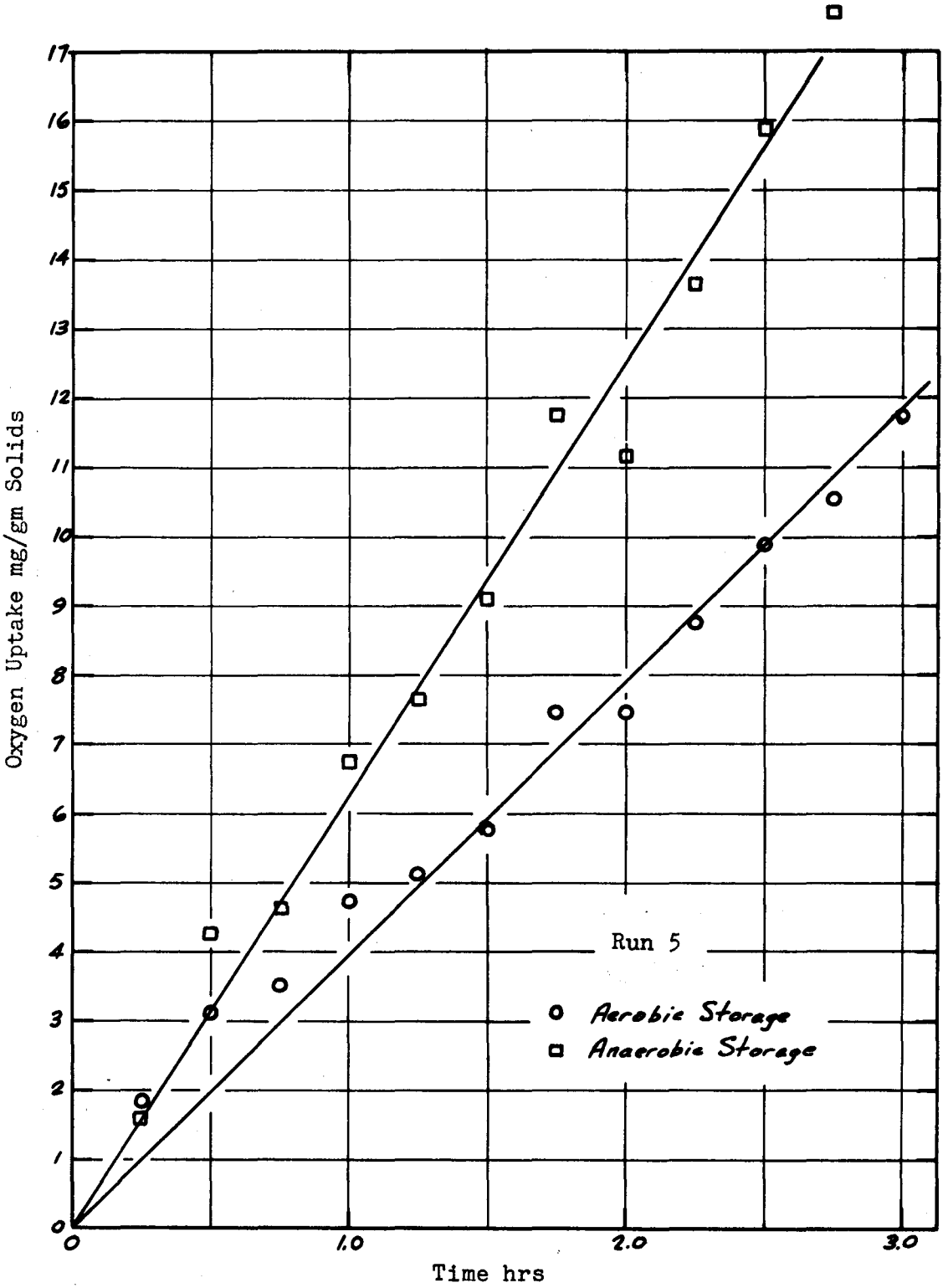



FIG.4 OXYGEN UPTAKE AFTER 6 HOURS STORAGE

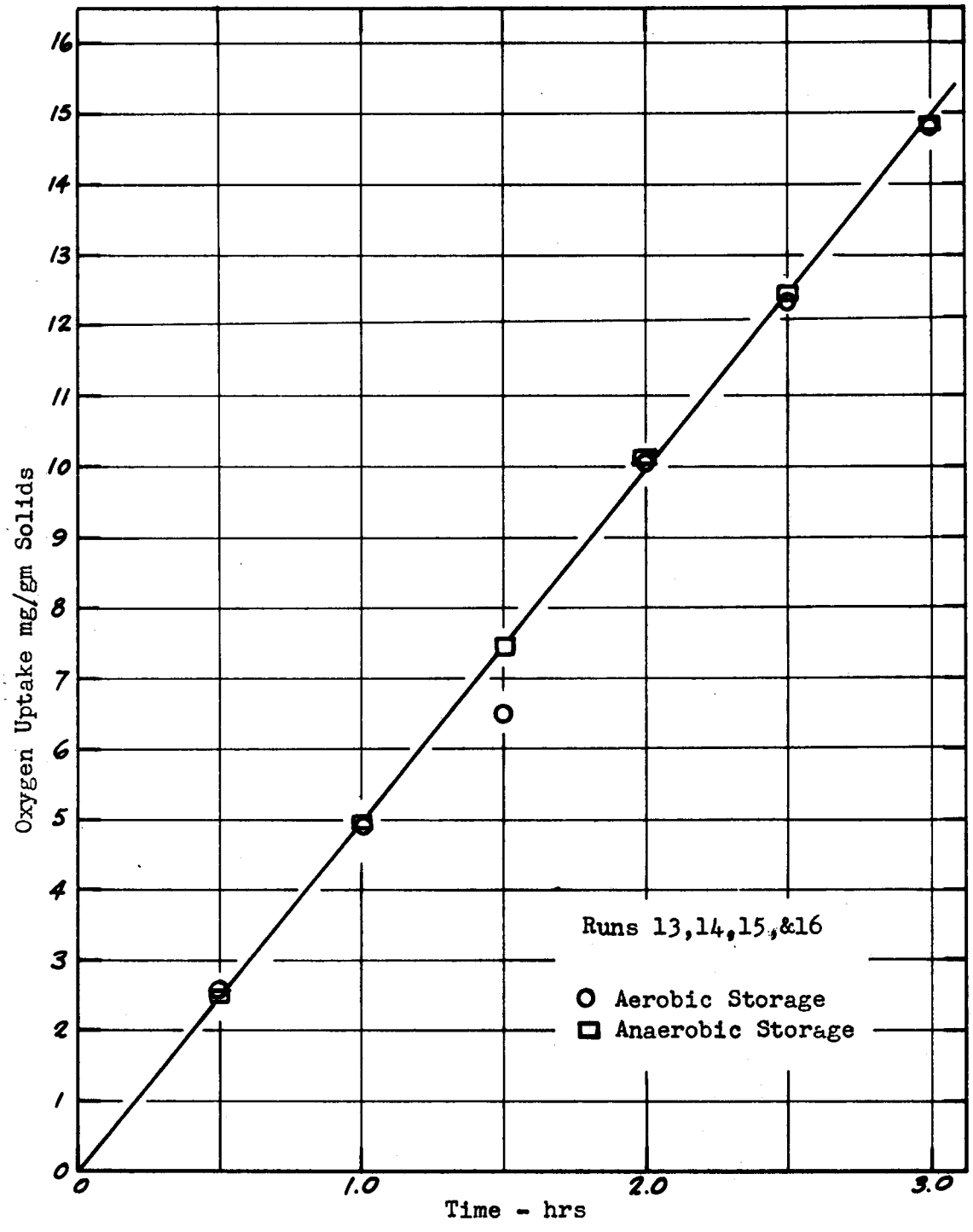



FIG.5 OXYGEN UPTAKE AFTER 6 HOURS STORAGE

Solids and Carbon Progression

McLellan (1964) found that during aerobic storage the concentration of cells remained relatively constant while a slight decrease in COD occurred. During anaerobic storage, a decrease in cell concentration was observed and was accompanied by a corresponding increase in COD which was taken to be indicative of a liberation of soluble organics from cellular decomposition.

Figure 6 presents a typical solids progression for 2 separate runs. It would appear from the data that the type of storage (aerobic or anaerobic) has no effect on the mass of solids measured gravimetrically. It was difficult to determine for individual runs whether the solids level remained constant or whether there was a slight decrease in concentration as time progressed. As the initial solids concentrations varied from run to run the grouped data was analyzed using ratios whereby the solids concentration at any time was compared to the initial concentration. As an analytical aid, a computer program was developed employing statistical techniques. The mean values of the solids ratios are presented in Figure 7. The equation for the line of trend of the solids ratio regression (least squares) for both aerobically and anaerobically stored sludge was:

$$Y = 1.000 - 0.006T$$

An F-test was used to check the significance of the above equation by comparing them to a horizontal line through the mean. The F-test compares the variance or scatter of the measurements about the regression line with the variance of the measurements about the mean. Here the F-test was used to determine whether the error removed by the least square lines was significant as compared to the error removed by a horizontal line through the mean. The 0.01 level of significance was chosen as the criterion for validity. The F-test indicated that the above equation was significant.

In Figure 8 the carbon progression of 2 typical runs is presented. Similar ratios were employed to evaluate the carbon data in a manner similar to that used

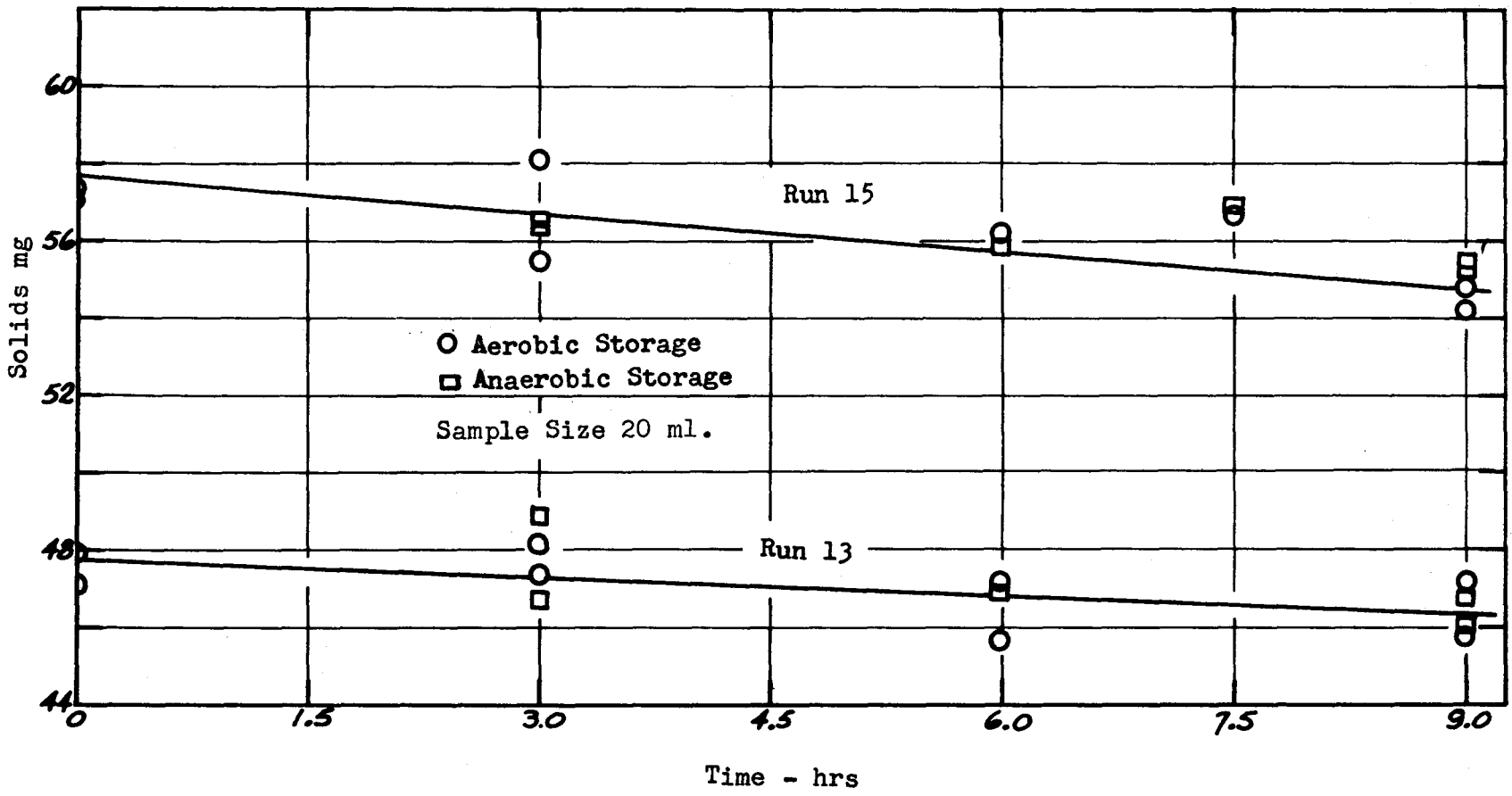



FIG.6 TYPICAL SOLIDS PROGRESSION

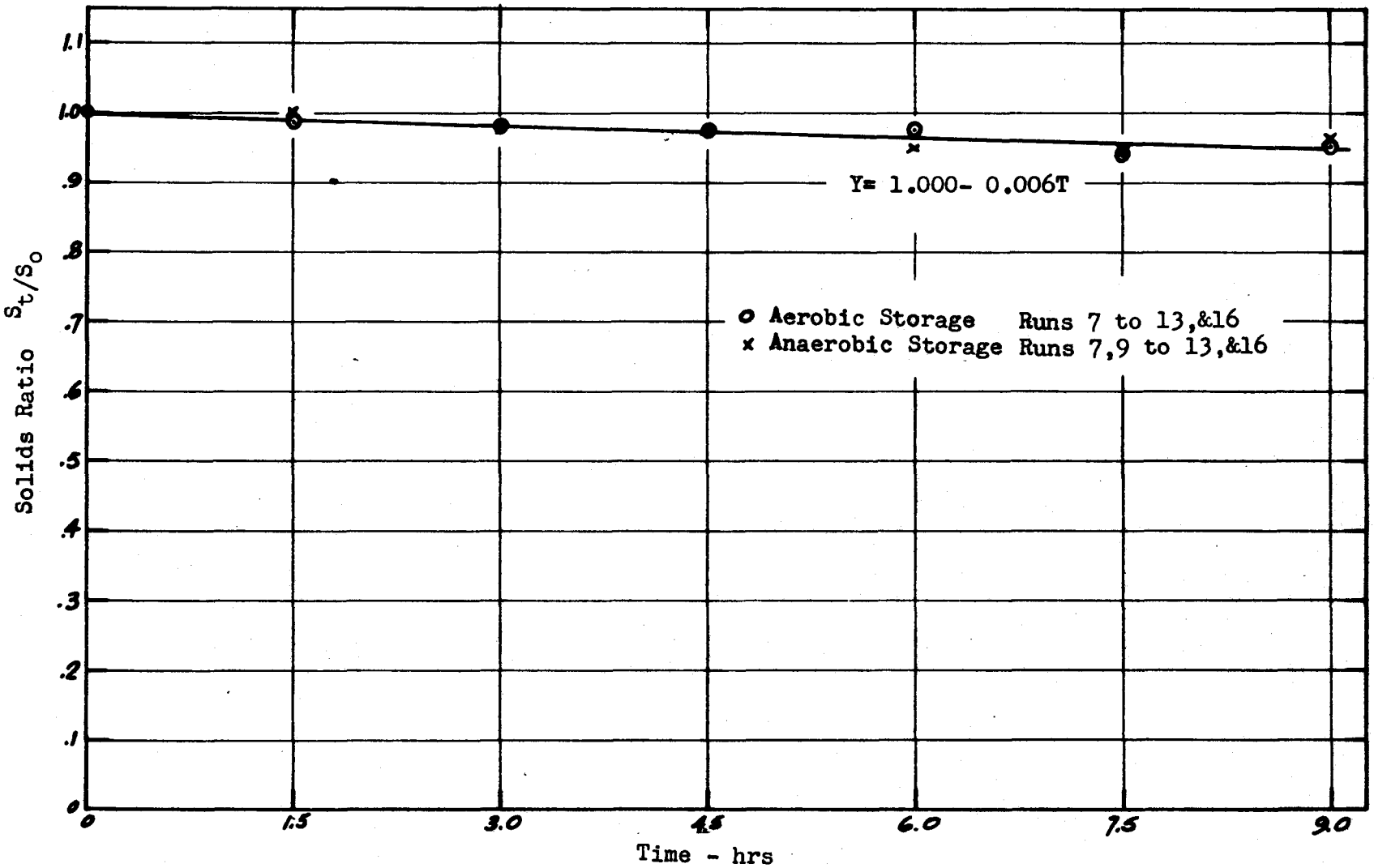



FIG.7 PROGRESSION OF SOLIDS RATIO

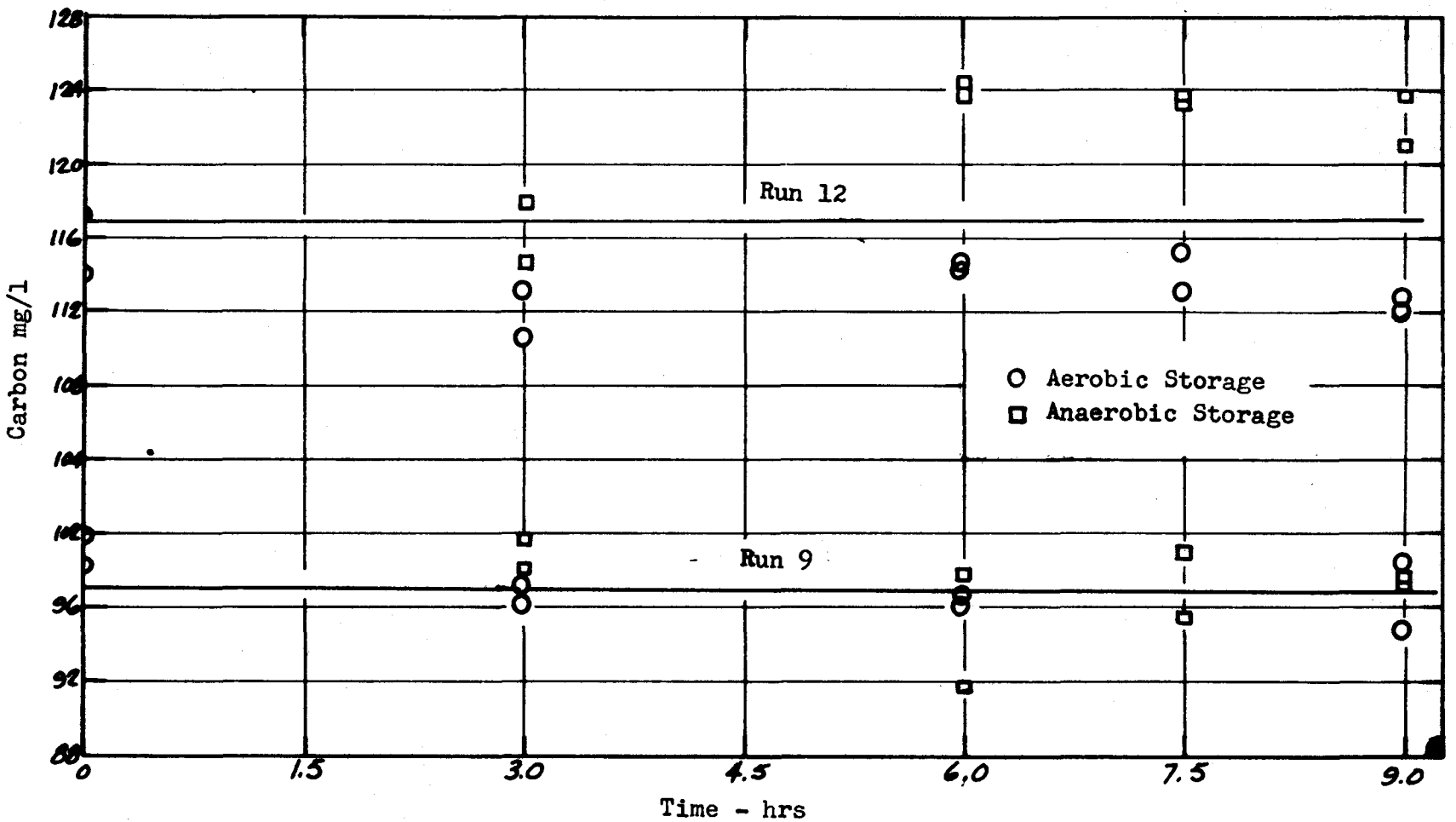



FIG.8 TYPICAL CARBON PROGRESSION

for the solids data and the mean values are presented in Figure 9. The equations for the line of trend of the carbon ratio progression for both aerobically and anaerobically stored sludge was developed. An F-test indicated that the regression line was not significant at the 0.01 level indicating no regression of carbon with time. The equation adopted was:

$$Zanc = 1.018$$

Inorganic Nitrogen Progression

From the literature reviewed it would appear that nitrate and possibly nitrite would be removed from a medium by microbial metabolism during anaerobic storage. This then could cause a quantitative change in the concentration of the nitrogen compounds present. During runs 6 to 11, the activated sludge samples were stored in 125 ml Warburg flasks both aerobically and anaerobically for 6 hours. During runs 12 to 16, the anaerobically stored samples were reaerated for 3 hours following the 6 hour storage period.

The air rate to the reactor from which samples of activated sludge were obtained was increased for runs 13 to 16. This was done to attempt to decrease the concentration of nitrites (Jaworski and Stankey(1961)) as it is difficult to measure accurately high concentrations using the diazotization method. The increase in air rate did not decrease the concentration of nitrites in the medium. Nitrite and nitrate levels during run 13 could not be measured because of experimental difficulties.

Quantitatively, it was observed that during anaerobic storage the amount of nitrate-nitrogen decreased for all runs as compared to the samples stored aerobically. Figure 10 presents a typical example. Once again, ratios were employed to analyze the nitrogen data in a manner similar to that used in the analysis of carbon and solids data. The results of these ratios are plotted as mean values on Figure 11. Using the least squares method a regression line was developed for

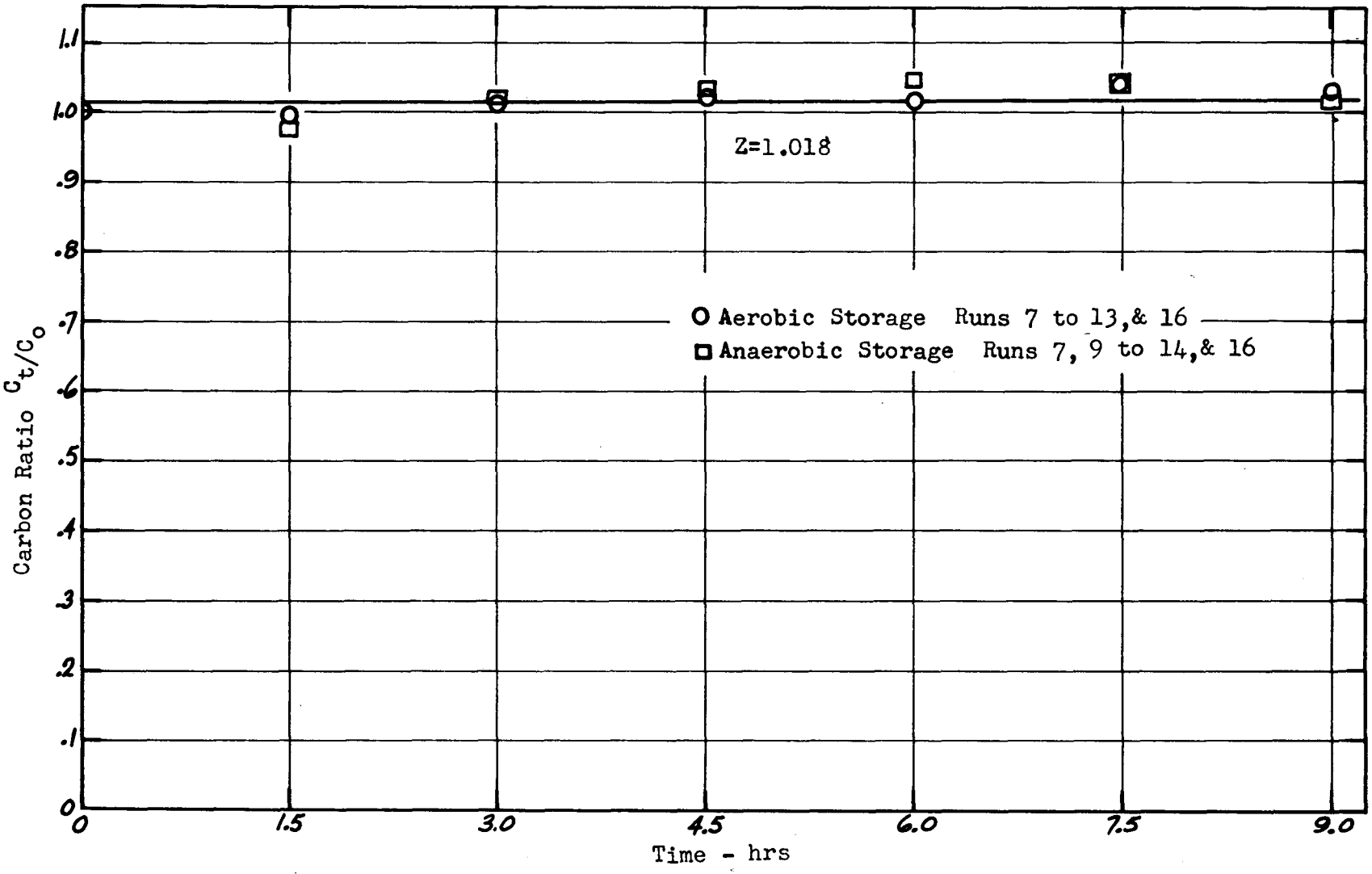



FIG.9 PROGRESSION OF CARBON RATIO

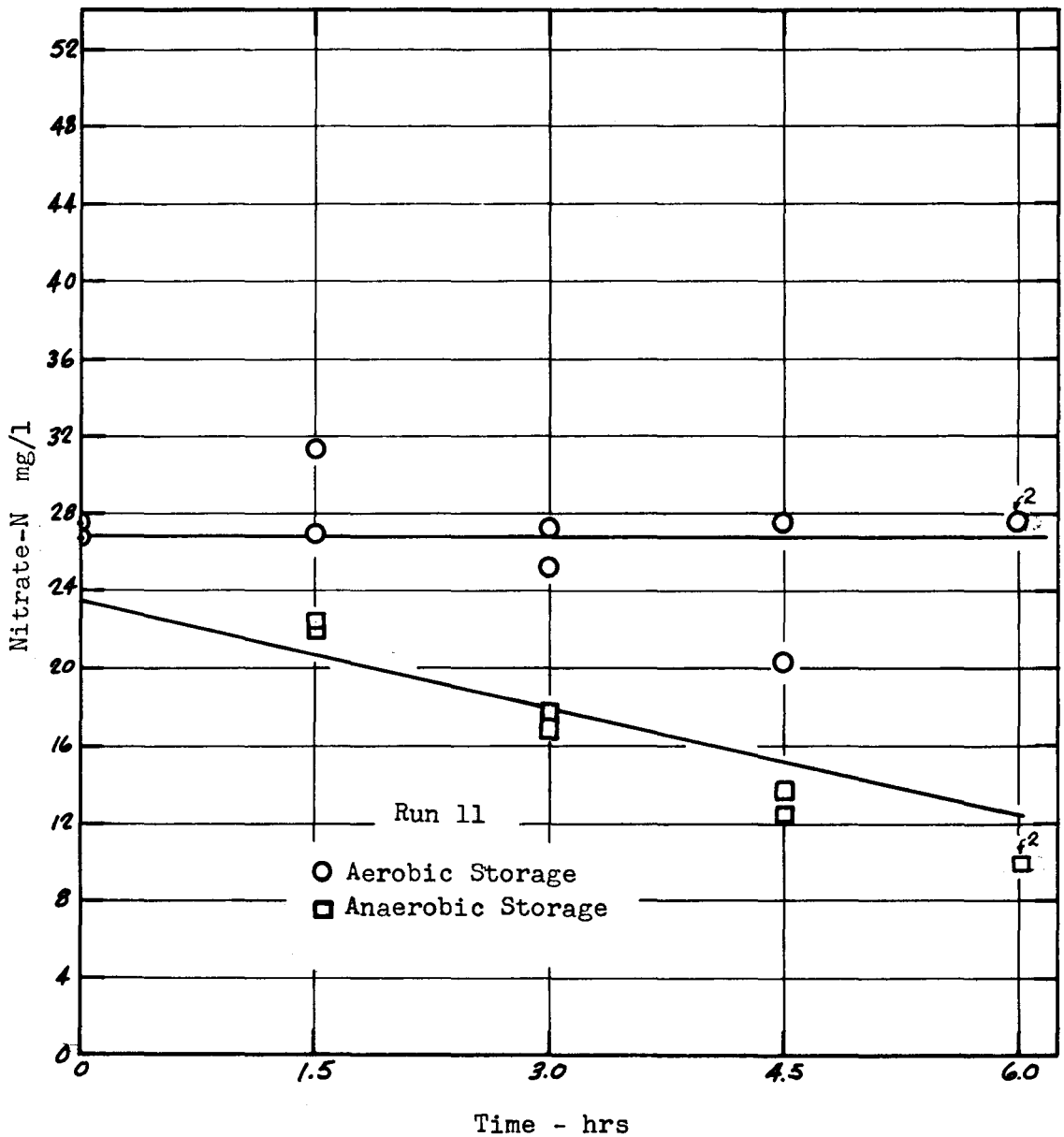



FIG.10 TYPICAL NITRATE-N PROGRESSION

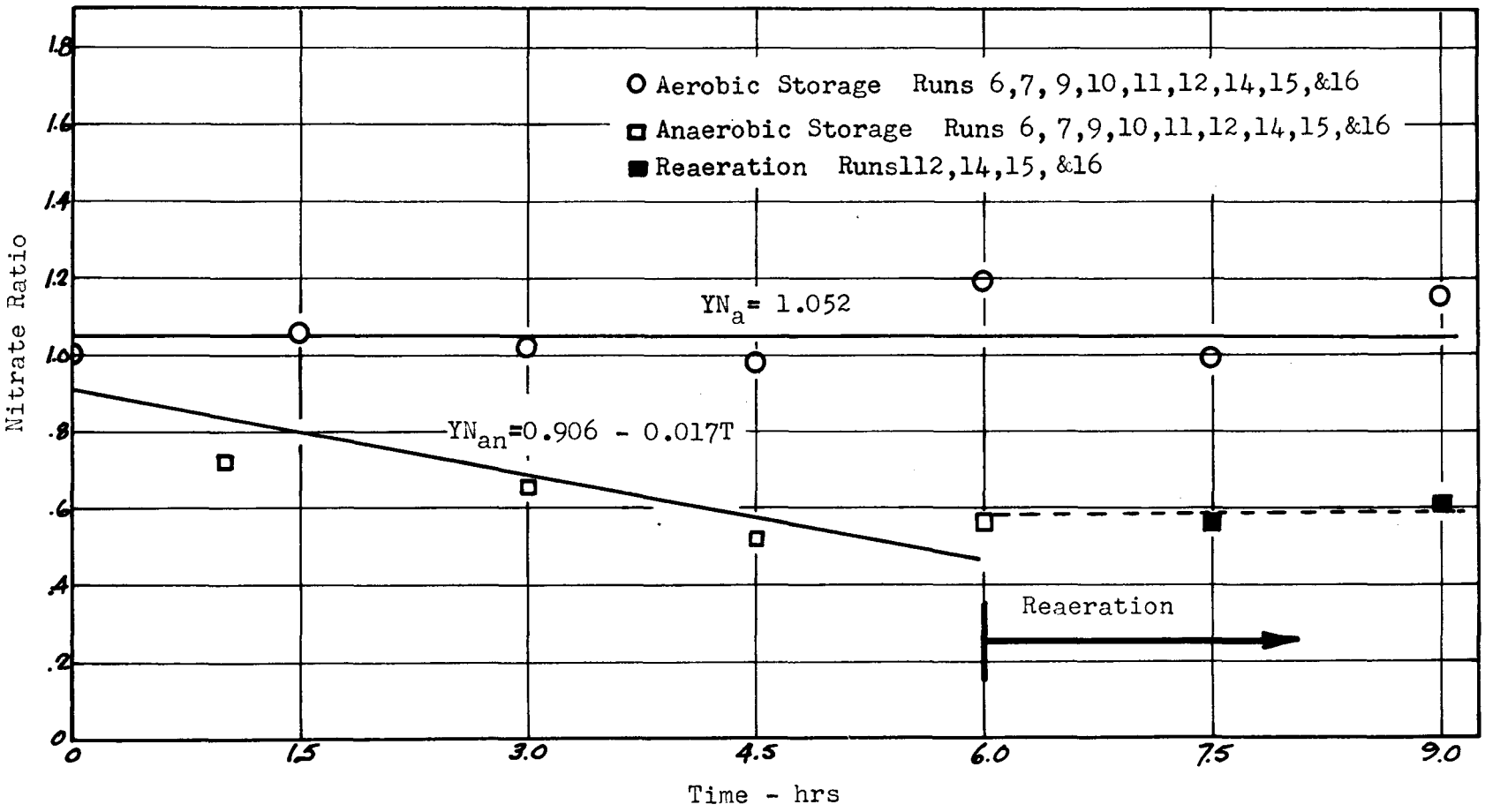



FIG.11 PROGRESSION OF NITRATE-N RATIO

aerobically stored sludge. An F-test to determine the significance of the equation as compared to a horizontal line through the mean indicated no significance at the 0.01 level. The plotted values could be equally represented by a horizontal line:

$$YN_a = 1.052 \qquad (0 \leq T \leq 9)$$

No decrease in nitrate-nitrogen occurred during aerobic storage.

The nitrate ratio mean values plotted for anaerobic storage in Figure 11 indicate that there was a possibility of a curvilinear trend. A linear approximation as evaluated by the least squares method was selected:

$$YN_{an} = 0.906 - 0.071T \qquad (0 \leq T \leq 6)$$

An F-test indicated that the above equation was significant at the 0.01 level as compared to a horizontal line. Although the equation tends to indicate the possibility of a 9% error in the initial mean ratio value, the equation does suggest that there was a definite reduction in nitrate-nitrogen during anaerobic storage.

Upon reaeration of the anaerobically stored sludge, there was a definite discontinuity in the trend line and the nitrate regression tended to level off. This observation agreed with the fact that there was no difference in the oxygen uptake following both aerobic and anaerobic storage

In conjunction with the nitrate determinations, nitrite-nitrogen determinations were also carried out and the mean nitrite ratio values are plotted in Figure 12. Using the method of least squares an equation was developed for sludge stored aerobically. The equation was tested using an F-test and it was determined that the equation was not significant at the 0.01 level as compared to a horizontal line through the mean. Hence the nitrite data for aerobically stored sludge can be represented by the horizontal line:

$$NI_a = 0.991 \qquad (0 \leq T \leq 9)$$

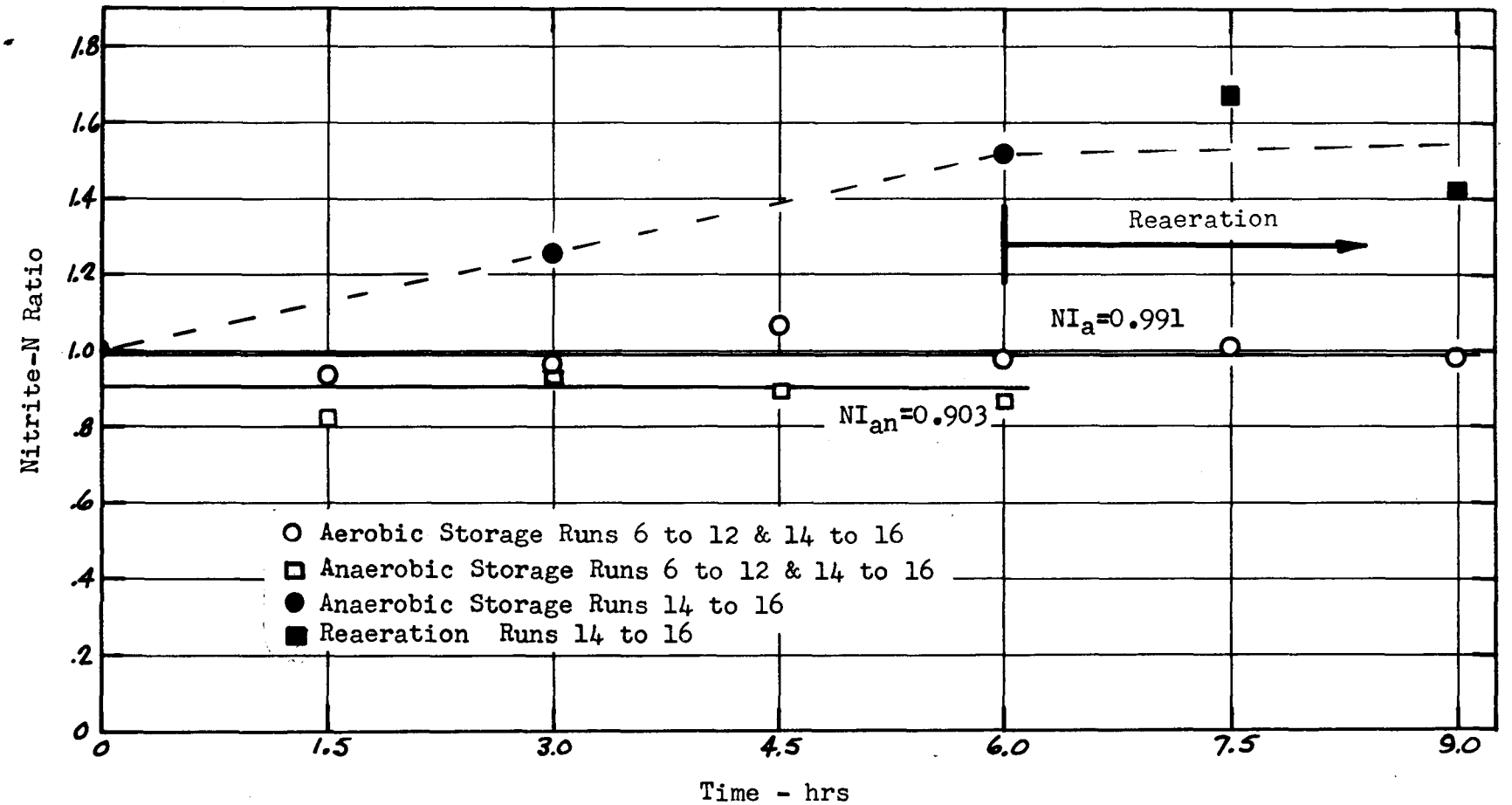



FIG.12 PROGRESSION OF NITRITE-N RATIO

This would indicate that there was little or no change in nitrite-nitrogen concentration during aerobic storage.

An equation for the nitrite-nitrogen progression in anaerobically stored sludge was developed and evaluated by an F-test. Although the equation indicated the possibility of a decrease in the nitrite level with time, the gross variance in the data did not permit the equation to be statistically significant. Hence, the data can only be represented by a horizontal line through the mean:

$$NI_{an} - 0.903 \qquad (O \leq T \leq 9)$$

The dotted line plotted on Figure 12 indicates the average of nitrite-nitrogen determinations during runs 14 to 16. Although there is a possibility that nitrite-nitrogen is produced during anaerobic storage (where sludge has been subjected to high aeration rates) this observation is not conclusive because of insufficient data.

Ammonia determinations were carried out during runs 12 to 16 in order to supplement the nitrate and nitrite data. The data plotted in Figure 13 was typical of ammonia results.

The ammonia for runs 12 to 16 have been plotted in the form of mean value ratios in Figure 14. It was observed that there was an increase in the ammonia level during both aerobic and anaerobic storage. There did not appear to be any discontinuity in the linear relationship due to the reaeration of the anaerobically stored sludge. The equations developed for the progression of ammonia in terms of ratios for both aerobically and anaerobically stored sludge were almost identical and hence could be replaced by the single equation:

$$AM = 1.012 + 0.016T$$

The F-test indicated that the above equation was significant in removing the residual error as compared to a horizontal line through the mean.

Based on the above equation, the ammonia-nitrogen concentration increased by 14.4% over a period of 9 hours. Using average values of the

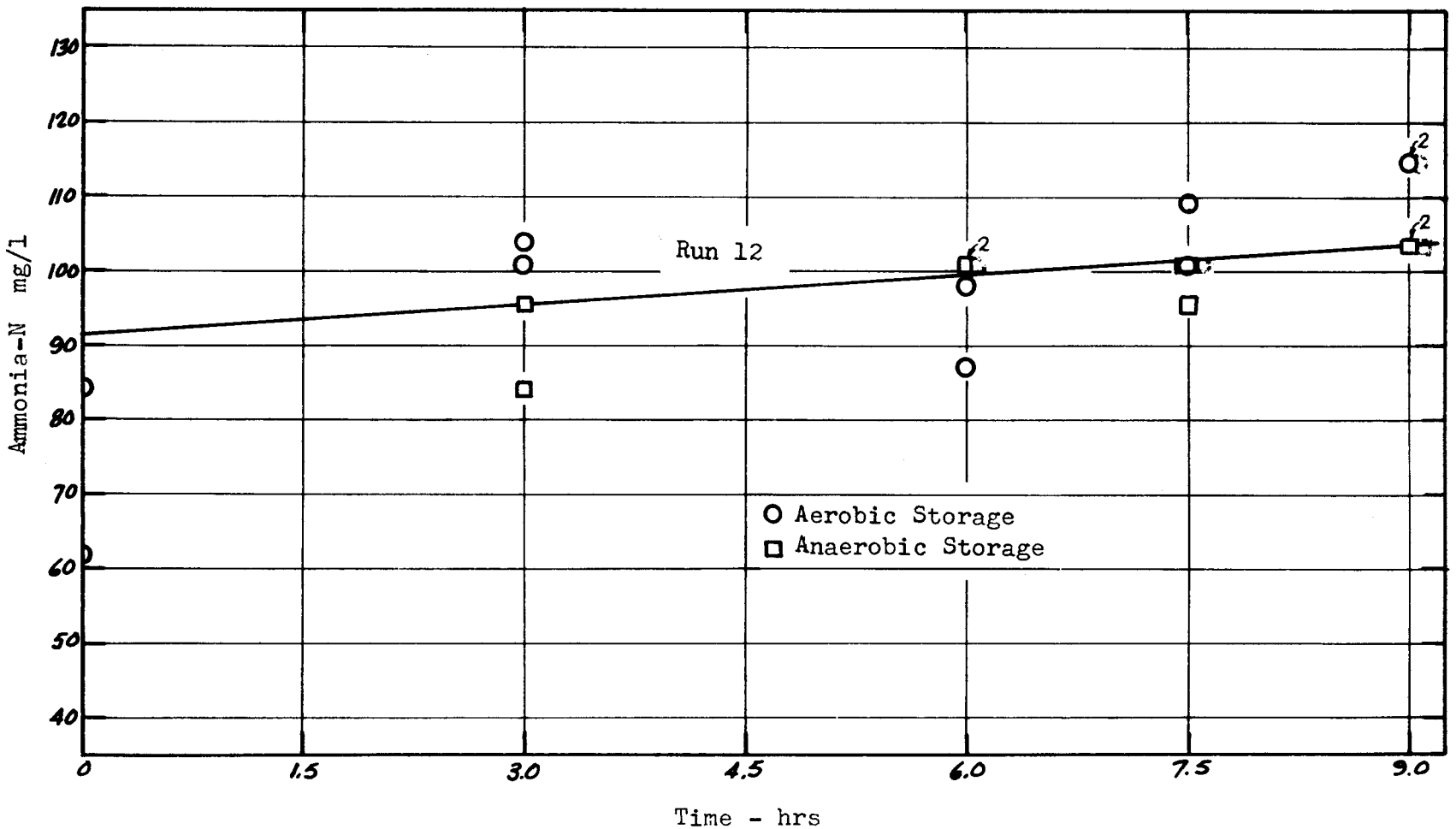



FIG.13 TYPICAL AMMONIA-N PROGRESSION

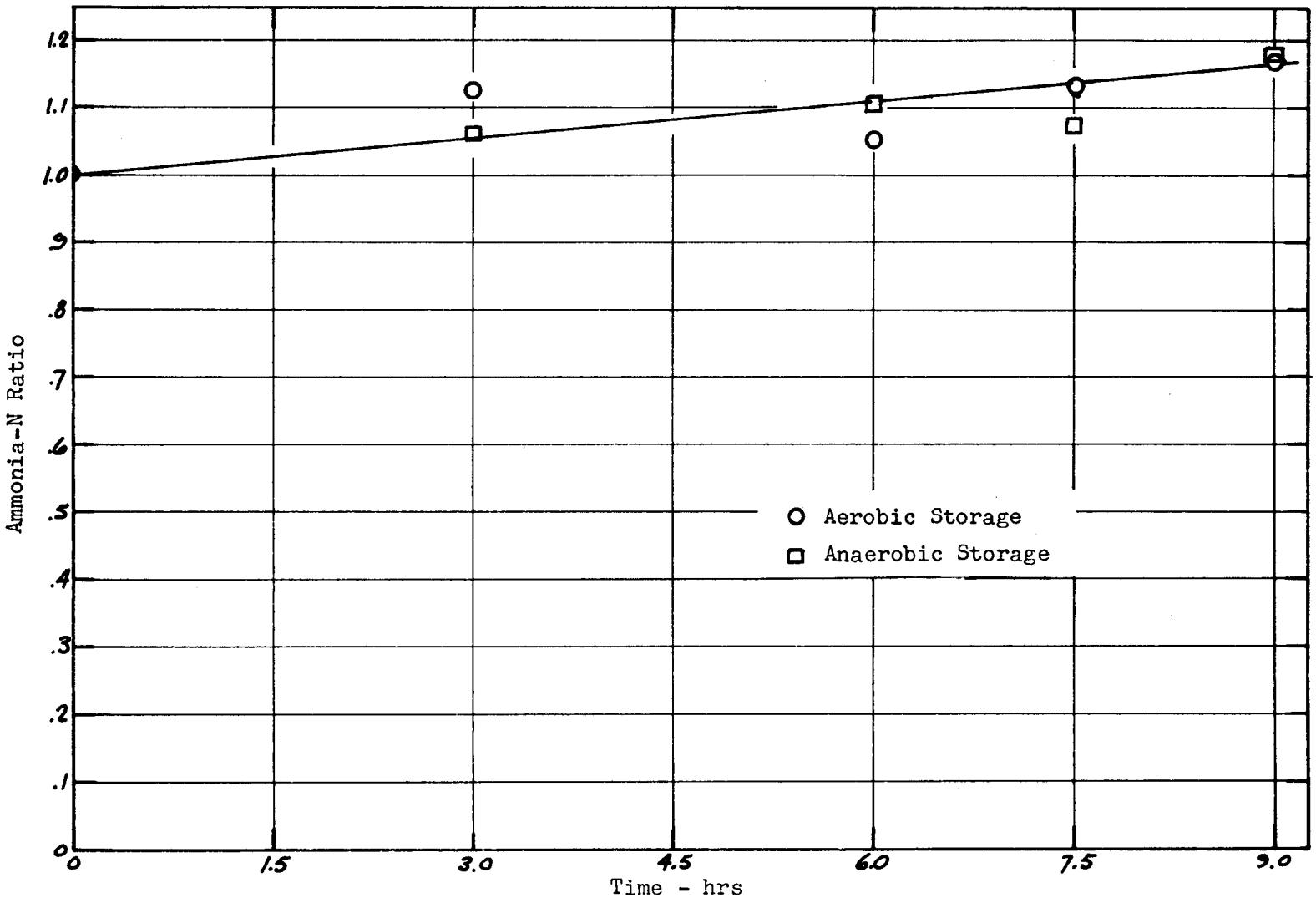



FIG.14 PROGRESSION OF AMMONIA-N RATIO

ammonia concentrations for runs 12 to 16, this is equivalent to an increase of approximately 18 mg/l. The loss of solids over 9 hours for the same runs was 5.4% which is equivalent to 2.8 mg/20 ml sample or 140 mg/l. It would appear that the production of ammonia was a result of this loss of solids.

Gas Evolution

Manometric recordings were made of the samples stored anaerobically in the Warburg reaction flasks. Each flask had a KOH boat to absorb any carbon dioxide that might be produced. It was found that in almost all cases, a gas was being evolved during anaerobic storage.

Gas chromatographic studies were carried out during run 17. The glass stopcocks on the Warburg flasks were replaced by a rubber septum and an extra long #23 hypodermic needle during the flushing of the flasks with argon. The needles were removed after approximately one hour of flushing. After a sufficient amount of gas had evolved, 1 ml samples of the gas were taken and subjected to analysis using a Fisher Scientific Clinical Gas Partitioner. No peaks of any type were noted in the analysis of the Warburg contents, however, the range of the recorder was such that the small amount of gas evolved, even if completely nitrogen, could not be detected.

# CHAPTER 5

## DISCUSSION, CONCLUSIONS & RECOMMENDATIONS

### Discussion

McLellan (1964) in his studies found that sludge which had been stored anaerobically utilized more oxygen during subsequent endogenous respiration than aerobically stored sludge. Following 3 hour storage, the difference was 1.06 mg $O_2$ per gm solids per hour which McLellan considered significant. Wuhrmann (1960) found after 125 minutes of anaerobic storage that the endogenous respiration rate was 1.00 mg $O_2$ per gm solids per hour higher than that following aerobic storage. Wuhrmann considered that this difference in respiration rate was insignificant. In this study, it was found that the endogenous respiration rates were dependent upon the oxygen supplied to the reactor. Using a 3 litre reactor, it was found that when the air flow was 750 ml per minute, the difference in the endogenous respiration rate of 6 hour anaerobically stored sludge was 2.3 mg $O_2$ per gm solids per hour more than for the sludge stored aerobically. Later when the air rate was increased to 975 ml per minute, the average difference in the endogenous respiration rate for 4 runs was insignificant.

The use of argon gas to simulate anaerobic conditions produced results similar to that of McLellan (1964) who used nitrogen. Thus, in this study it was assumed that there was no difference between argon and nitrogen in simulating anaerobic conditions.

McLellan found a slight decrease in solids during anaerobic storage and none during aerobic storage. In this study there was a slight decrease in solids during storage, although it was independent of the type of storage.

Little or no increase in organic carbon during anaerobic storage

was observed. Although McLellan found an increase in carbon concentration during anaerobic storage, he used the COD test to measure carbon. No nitrite correction was included which could have an appreciable effect on his results. For example, in McLellan's study, after 6 hours storage, the COD liberated was 36. 5 mg/l which is equivalent to approximately 32 mg/l of nitrite. Although verification is still required, a liberation of approximately 40 mg/l of nitrite-nitrogen has been noted in this study. The inorganic nitrogen investigations in this study were carried out on a mixed culture of micro-organisms. Although much work has been done by biochemists and microbiologists on pure culture studies, there is a scarcity of nitrogen investigations using mixed cultures. Accordingly there is very little work reported in the literature with which the results of this investigation can be compared.

Wuhrmann (1960) has made some attempt to balance the nitrogen present throughout an activated sludge process and has succeeded in some cases. In another case, he obtained an under balance of 26% and assumed that the nitrogen had been lost to the system. Taniguchi et al (1956) found that by measuring the accumulation of nitrite, hydroxylamine and ammonia, they could recover 80-90% of the reduction products of nitrate during anaerobic storage.

The nitrate investigations carried out in this study indicated that there was a decrease in the nitrate level during anaerobic storage. The nitrate concentration tended to level off upon subsequent reaeration indicating no nitrification. During aerobic storage the nitrate level tended to be approximately constant.

The disappearance of the nitrate ion during anaerobic storage could not be accounted for and it appears that other forms of nitrogen, eg. hydroxylamine and some form of the hyponitrite ion, should be measured.

Lewis and Busch (1964) have demonstrated that the nitrate form of nitrogen is not a suitable nutrient source in the BOD test as with any nitrogen form not

of the ammonia valence, lower values of BOD will be observed when there is a requirement for nutrient nitrogen. This finding tends to support the hypothesis that the nitrate ion can be used as a substitute hydrogen acceptor. In this study, it is possible that the nitrate ion was used as an hydrogen acceptor to satisfy the endogenous respiration requirements of an anaerobically stored sludge and resulted in the formation of nitrite ions. However, the utilization of nitrite ions as hydrogen acceptors during anaerobic storage could not be conclusively determined because of the great variations in the nitrite determinations. It should be noted that in all runs, there was always some nitrate ions left which could be used as hydrogen acceptors. The reaeration of the anaerobically stored sludge did not increase the nitrate level. This would indicate that part of the oxygen during reaeration was used for nitrification and is verified by experimental data (Figure 5).

The ammonia levels during storage tended to increase irrespective of the type of storage and could be accounted for by the decrease in the solids level (eg. deamination of cellular material).

Manometric measurements indicated that a gas other than carbon dioxide was produced during anaerobic storage. However, with the equipment used, this gas could not be identified.

The initial measurements varied from run to run. This variance was noted in all measurements whether solids, carbon, nitrites etc. Simultaneous studies had noted that the microbial population within the reactor was dynamic and, during the course of our study, had not attained steady state. Cassell et al (1966) considered that a continuous mixed culture was a dynamic system in that the biological activity fluctuated continuously. They assumed that these fluctuations are the result of various microbial interactions occurring in a mixed culture.

Conclusions

(1) The endogenous respiration rates of activated sludge following storage

appeared dependent upon the oxygen supplied to the reactor. Thus with low oxygen input to the reactor, there was a greater rate of oxygen uptake by the sludge following anaerobic storage as compared to aerobic storage.

(2) There was no significant difference in the liberation of organic carbon between aerobically or anaerobically stored sludge. The increase in oxygen uptake by endogenously respiring sludge following anaerobic storage could not be attributed to a liberation of carbon.

(3) The nitrate ion was apparently used as a hydrogen acceptor by endogenously respiring sludge during anaerobic storage.

(4) Upon reaeration of the anaerobically stored sludge, the nitrate concentration tended to level off.

(5) There was a slight decrease in the solids concentration which is independent of the type of storage. This decrease in solids possibly accounts for the increase in the ammonia concentration during both aerobic and anaerobic storage.

Recommendations

The development of a steady state laboratory model activated sludge process would be beneficial in a study of this type. All operating parameters should be defined so that comparisons with other studies could be made.

Further work should be carried out to improve the techniques for analyzing the various forms of nitrogen. Specifically, a new method for nitrite determinations should be developed as the present diazotization method tended to produce erratic results. The use of Technicon Auto-Analyzer should greatly improve the repeatability and accuracy of the results.

It would appear that in addition the nitrogen forms studied here, hydroxylamine and hyponitrite or perhaps a dimerized form of hyponitrite should be studied to obtain an overall picture of nitrogen metabolism by a mixed culture.

It is apparent that most sanitary engineers sadly lack knowledge of biochemistry

especially with respect to enzymology. This knowledge is necessary to adequately explain the metabolism of activated sludge.

It was assumed in this study, that argon could replace nitrogen gas in order to simulate anaerobic conditions. This assumption should be verified as it is possible that nitrogen gas may have an effect on nitrogen transformations. It is recommended that argon or some other inert gas (other than nitrogen) be used to simulate anaerobic conditions.

## BIBLIOGRAPHY


1. American Public Health Association, Standard Methods for the Examination of Water and Waste Water, 11th Edition, New York, (1960).

2. American Public Health Association, Standard Method for the Examination of Water and Waste Water, 12th Edition, New York, (1965).

3. Ardern, E. and Lockett, W. T., "Experiments on the Oxidation of Sewage without the Aid of Filters". J. Soc. Chem. Ind. 33, 523, (1914) Reprinted in J. Inst. Sew. Purif. 3, 175-188, (1954).

4. Bragstead, R. E. and Bradney, L. "Packinghouse Waste and Sewage Treatment at Sioux Falls, South Dakota". Sew. Works Jour., 9, 959-969, (1937).

5. Bringmann, G., Kuhn, R., and Wagner, B., "Laboratory Experiments with Biological Removal of Nitrogen from Waste Water in the Form of Nitrogen Gas". Gesundh-Ing 80, 364, (1959). Abs., Jour. WPCF 33, 786, (1961).

6. Campbell, L., "Uptake of Dissolved Organic Carbon by Activated Sludge". Water and Poll. Control 104, 9, 31-33, (1966).

7. Cassell, E. A., Sulzer, F. T., and Lamb, J. C., III, "Population Dynamics and Selection in Mixed Cultures". Jour. WPCF 38, 1398-1409, (1966).

8. Clesceri, L. S., "Effect of Fermentation Variables on Properties of Activated Sludge". Ph. D. Thesis, University of Wisconsin, (1963).

9. Conn, E. E., and Stumpf, P. K., Outlines of Biochemistry, John Wiley & Sons, New York, (1963).

10. Eckenfelder, W. W., and O'Connor, D. J., Biological Waste Treatment, Pergamon Press, New York, (1961).

11. Hawkes, H. A., The Ecology of Waste Water Treatment, MacMillan Co., New York, (1963).

12. Jaworski, C. A., and Stankey, S. A., "Excessive Nitrite Nitrogen in Activated Sludge," Jour. WPCF, 33, 1286 (1961).

13. Krishnan, P., and Gaudy, A. F., "Mechanism and Kinetics of Substrate Utilization at High Biological Solids Concentrations," 21st Industrial Waste Conference, Purdue University, Proceedings (1966).

14. Lewis, J. W., and Busch A. W., "BOD Progression in Soluble Substrates, VIII, The Quantitative Error Due to Nitrate as a Nitrogen Source, "19th Industrial Waste Conference, Purdue University, Proceedings, (1964) and Water and Sewage Works, 112, 106, (1965).

15. Ludzack, F. S., and E. Hinger M. B., "Controlling Operation to Minimize Activated Sludge Effluent Nitrogen", Jour. WPCF 34, 920-931, (1962).

16. Keefer, C. E., and Meisel J., "Activated Sludge Studies I Effect of Sludge Age on Oxidizing Capacity." Sew. & Ind. Wastes, 22, 117-1122, (1950).

17. McElroy, W. D., and Spencer, D., "Normal Pathways of Assimilation of Nitrate and Nitrite". Inorganic Nitrogen Metabolism, Edited W. D. McElroy and B. Glass, John Hopkins, Baltimore, (1956).

18. McLellan, J. C., "Anaerobiosis in the Activated Sludge Process". M. Eng. Thesis, McMaster University, (1964).

19. Murphy, K. L., and McLellan, J. C., "Anaerobiosis in the Activated Sludge Process," Proc. 5th Annual Industrial Water and Waste Conference, Houston, Texas, (1965).

20. Nason, A., "Enzymatic Steps in the Assimilation of Nitrate and Nitrite in Fungi and Green Plant." Inorganic Nitrogen Metabolism, Edited by W. D. McElroy and B. Glass, John Hopkins, Baltimore, (1956).

21. Nason, A., and Takahashi, H., "Inorganic Nitrogen Metabolism." Annual Reviews of Microbiology, 12, 203. Edited by C. E. Clifton, S. Raffel and M. P. Starr, Annual Reviews, Inc., Palo Alto, California, (1958).

22. Ridenour, G. M., and Henderson C. N., "Stale Return Sludge as a Factor in the Activated Sludge Process." Sew. and Ind. Wastes 6, 36-41, (1934).

23. Sawyer, C. N., "Bacterial Nutrition and Synthesis." Paper, Conference on Biological Waste Treatment, Manhattan College, New York (1955) and Biological Treatment of Sewage and Industrial Wastes, Edited by J. McCabe and W. W. Eckenfelder, Reinhold Publishing Corp., New York, (1956).

24. Sawyer, C. N., and Bradney, L., "Rising of Activated Sludge in Final Settling Tanks." Sew. Works Jour. 17, 1191-1209, (1945).

25. Taniguchi, S., Sato, R., and Egami, F., "The Enzymatic Mechanisms of Nitrate and Nitrite Metabolism in Bacteria." Inorganic Nitrogen Metabolism, Edited by W. D. McElroy and B. Glass, John Hopkins, Baltimore, (1956).

26. Umbreit, W. W., Burris, R. H., and Stauffer, J. F., Manometric Techniques, Burgess Publishing Co., Minneapolis, Minn., (1957).

27. Verhoeven, W., "Some Remarks on Nitrate and Nitrite Metabolism in Micro-organisms." Inorganic Nitrogen Metabolism, Edited by W. D. McElroy and B. Glass, John Hopkins, Baltimore, (1956).

28. Water Pollution Control Federation, Manual of Practice No. 8, Sewage Treatment Plant Design, (1959).

29. Westgarth, W. C., "Anaerobiosis in the Activated Sludge Process." Ph. D. Thesis, University of North Carolina, (1963).

30. Wooldridge, W. R., and Standfast, A. F. B., "The Use of the Barcroft Differential Manometer in the Estimation of the Oxygen Absorption of Sewage." Biochem. Jour. 30, 141-147, (1936).

31. Wuhrmann, K., "Factors Affecting Efficiency and Solids Production in the Activated Sludge Process, "Paper, Conference on Biological Waste Treatment, Manhattan College, New York, (1955), and Biological Treatment of Sewage and Industrial Wastes Vol I, Edited by W. W. Eckenfelder and J. McCabe, Reinhold Publishing Corp., New York, (1956).

32. Wuhrmann, K., "Effect of Oxygen Tension on Biochemical Reactions in Sewage Purification Plants, "Paper, Third Conference on Biological Waste Treatment, Manhattan College, New York, (1960), and Advances in Biological Waste Treatment, Edited by W. W. Eckenfelder and J. McCabe, Pergamon Press, New York, (1963).

## APPENDIX A

a) Preparation of Synthetic Sewage

| | | |
|---|---|---|
| Dextrose | ($C_6H_{12}O_6$) | 4.0 gm/l |
| Bacto-Peptone (Difco) | | 2.5 gm/l |
| Sodium Oleate | | 0.8 gm/l |
| Urea | ($NH_2CONH_2$) | 0.7 gm/l |
| $K_2HPO_4$ | | 0.3 gm/l |
| Na Cl | | 0.2 gm/l |

The above feed was usually made up in 4 or 6 litre quantities and refrigerated. The diluent used was tap water. The pH of the feed varied between 7.4 and 7.7. The 5-day Biochemical Oxygen Demand was determined to be approximately 5400 mg/l while the Chemical Oxygen Demand (without the sulphamic acid correction) was approximately 8950 mg/l.

b) Calculation of Feed to Reactor:

Assumptions:

Load Factor (L. F.) = 0.5 mg BOD/day/mg MLSS

Yield Coefficient (Y. C.) = 0.4

Sludge Age (S. A.) = 5 days

Mixed Liquor Suspended Solids (MLSS) = 2000 mg/l

$$L.\ F. = \frac{L_a Q}{S_a V}$$

Feed required for reactor solids

mg BOD/day = 0.5 (2000)3 = 3000

Solids produced

mg Solids produced/day = 0.4 (3000) = 1200

Volumes of Waste and Feed

$$\frac{1200 \text{ mg/day}}{2000 \text{ mg/l}} \times 1000 \text{ ml/l} = 600 \text{ ml/day}$$

$$\text{Feed concentration} = \frac{3000 \text{ mg BOD/day}}{600 \text{ ml}} = 5000 \text{ mg BOD/l}$$

$$\text{Sludge Age} = \frac{\text{Total Solids in reactor}}{\text{Solids produced/day}}$$

$$= \frac{2000 \text{ mg/l } (3 \text{ l})}{1200 \text{ mg/day}}$$

$$= 5 \text{ days}$$

## APPENDIX B

## REACTOR OPERATING DATA

| | |
|---|---|
| Reactor | Virtis Magnetic Drive Fermenter Model 40-B 4 as manufactured by The Virtis Company Inc., Gardiner, New York |
| Liquid Volume | 3 litres |
| Spacing of Vanes on Rotor | top vane 3/4" from top<br>bottom vane 4" from top |
| Speed of rotation | 500 rpm |
| Sludge Age | 5 days (i.e. 600 ml feed/day into 3000 ml) |
| Feeding and Wasting Intervals | 6 hours |
| Temperature | 28°C to 32°C |
| Initial Air flow | 750 ml/min at 14.7 psi at 70°F |
| Final Air flow | 975 ml/min at 14.7 psi at 70°F<br>(Runs #13 to #16) |

## APPENDIX C

## CARBON AND ORGANIC NITROGEN CONTENT OF MICRO-ORGANISMS

### Preparation of Solids

Samples of the mixed liquor were taken and centrifuged. The medium was then decanted while the solids were retained. Double distilled water was used to wash the solids by resuspending the solids. The samples were again centrifuged and the medium again decanted. This procedure was repeated 4 times. The washed solids were then resuspended in double distilled water and the samples were mixed together to ensure an even distribution of solids. Aliquots were taken of the resuspended solids for suspended solids, carbon and organic nitrogen determinations.

### Carbon Determination

Ultrasonic vibrations were applied to the aliquots to break up the solids such that the solids would pass through a 50 micro-litre syringe. Carbon determinations were made using a Beckman Carbonaceous Analyzer.

Average Carbon Content = 0.4539 mg Carbon/mg Solids
i.e. 45.4%

Sawyer (1955) reported that an empirical formula $C_{118}\ H_{170}\ O_{51}\ N_{17}\ P$ for sludge was obtained on the basis of work by Helmers. This formula indicates that the carbon content of microbial solids was approximately 53.05%.

### Organic Nitrogen Determination

Organic nitrogen (as ammonia-nitrogen) was determined on aliquots of washed solids using an Aminco-Koegel Micro-Kjeldahl Apparatus.

Average Organic Nitrogen Content = 0.088 mg $NH_3N$/mg Solids
i.e. 8.8%

An average organic nitrogen content as ammonia-nitrogen of 8.0% was obtained by Helmers as reported by Sawyer.

# APPENDIX D

## COD OF NITROGENOUS COMPOUNDS

50 ml samples of nitrogenous compounds were placed into the 500 ml reaction flasks. 25 ml of 0.05N dichromate solution plus 75 ml of sulphuric acid were added to the samples. Silver sulphate was not used because of the relatively simple structure of the nitrogenous compounds. Sulphamic acid and mercuric sulphate corrections also were not used.

The following table is a summary of the results obtained:

| Nitrogenous compound | Concentration of Nitrogen as N mg/l | Average mg COD/mg N of compound |
|---|---|---|
| $NaNO_3$ | 100 | 0.29 |
| $NaNO_2$ | 100 | 1.27 |
| $NH_4Cl$ | 175 | 0.28* |
| $NH_4OH$ | 190 | 0.00 |

* Value includes chloride correction

# APPENDIX E

## DATA

TABLE I

OXYGEN UPTAKE DATA

Following 6 Hours Storage

| | Time | Run 14 mg | Run 15 mg | Run 15 (1) mg/gm Solids | |
|---|---|---|---|---|---|
| Aerobic Storage | 0.00 | 0.000 | 0.000 | 0.000 | |
| | .25 | 0.012 | 0.037 | 1.85 | |
| | .50 | 0.030 | 0.061 | 3.10 | |
| | .75 | 0.056 | 0.069 | 3.50 | |
| | 1.0 | 0.076 | 0.094 | 4.75 | |
| | 1.25 | 0.120 | 0.102 | 5.17 | |
| | 1.50 | 0.133 | 0.114 | 5.78 | |
| | 1.75 | 0.109 | 0.147 | 7.43 | |
| | 2.00 | 0.182 | 0.147 | 7.43 | |
| | 2.25 | 0.144 | 0.172 | 8.77 | |
| | 2.50 | 0.217 | 0.196 | 9.91 | |
| | 2.75 | 0.189 | 0.208 | 10.52 | |
| | 3.0 | 0.234 | 0.233 | 11.76 | |
| Anaerobic (2) Storage | 0.25 | | 0.000 | 0.00 | (3) |
| | 0.50 | 0.000 | 0.053 | 2.67 | |
| | 0.75 | 0.040 | 0.061 | 3.08 | |
| | 1.00 | 0.075 | 0.102 | 5.14 | |
| | 1.25 | 0.111 | 0.122 | 6.16 | |
| | 1.50 | 0.155 | 0.151 | 7.60 | |
| | 1.75 | 0.155 | 0.203 | 10.27 | |
| | 2.00 | 0.210 | 0.191 | 9.68 | |
| | 2.25 | 0.211 | 0.240 | 12.12 | |
| | 2.50 | 0.280 | 0.285 | 14.39 | |
| | 2.75 | 0.248 | 0.317 | 16.02 | |
| | 3.0 | 0.310 | 0.341 | 17.26 | |

Notes:

(1) Average Solids for Run 15 is 19.8 mg for 10 ml sample. Solids data not available for Run 4 (10 ml samples),

(2) Argon gas used to simulate anaerobic conditions,

(3) Data for Anaerobic Storage requires extrapolation for comparison with Aerobic Storage.

TABLE II

OXYGEN UPTAKE DATA

Following 6 Hours Storage

| | Time | Run 13 mg | Run 14 mg | Run 15 mg | Run 16 mg | Average (1) |
|---|---|---|---|---|---|---|
| Aerobic Storage | 0.0 | 0.000 | 0.000 | 0.000 | 0.000 | 0.00 |
| | 0.5 | 0.163 | 0.105 | 0.102 | 0.149 | 2.54 |
| | 1.0 | 0.270 | 0.219 | 0.229 | 0.307 | 4.97 |
| | 1.5 | 0.422 | 0.298 | 0.331 | 0.456 | 6.49 |
| | 2.0 | 0.566 | 0.410 | 0.480 | 0.615 | 10.07 |
| | 2.5 | 0.680 | 0.517 | 0.578 | 0.762 | 12.32 |
| | 3.0 | 0.813 | 0.612 | 0.705 | 0.916 | 14.78 |
| Anaerobic Storage (4) | 0.5 | 0.000 | 0.000 | 0.000 | 0.000 | 0.00 (3) |
| | 1.0 | 0.075 | 0.121 | 0.147 | 0.168 | 2.43 |
| | 1.5 | 0.194 | 0.219 | 0.292 | 0.333 | 4.96 |
| | 2.0 | 0.317 | 0.334 | 0.439 | 0.510 | 7.66 |
| | 2.5 | 0.432 | 0.431 | 0.555 | 0.656 | 9.96 |
| | 3.0 | 0.537 | 0.533 | 0.679 | 0.821 | 12.34 |
| Average (2) Solids mg | | 45.5 | 52.4 | 55.0 | 54.6 | |

Notes:

(1) Average $O_2$ Uptake mg/gm Solids,
(2) Sample Size 20 ml,
(3) Data for Anaerobic Storage requires extrapolation for comparison with Aerobic Storage,
(4) Nitrogen gas used to simulate anaerobic conditions.

## TABLE III

DATA AEROBIC STORAGE

| RUN NO | TIME HR | SOLIDS MG | | CARBON MG/L | | NITRATE MG/L | | NITRITE MG/L | |
|---|---|---|---|---|---|---|---|---|---|
| 1 6 | 0.0 | 67.3 | 71.6 | 94.2 | 103.4 | 2.2 | 2.9 | 19.0 | 19.9 |
| 1 6 | 1.5 | 64.2 | 66.6 | 96.9 | 113.6 | 2.5 | 2.7 | 19.7 | 19.7 |
| 1 6 | 3.0 | 64.3 | 65.2 | 115.0 | 107.8 | 2.2 | 2.2 | 18.0 | 18.3 |
| 1 6 | 6.0 | 64.1 | 63.9 | 108.4 | 116.2 | 3.0 | . | 19.4 | 19.7 |
| 1 6 | 7.5 | 65.7 | 65.7 | 98.2 | 110.0 | 0.6 | 0.8 | 22.3 | 21.6 |
| 1 7 | 0.0 | 35.8 | 32.2 | 115.2 | 117.2 | 1.0 | 1.2 | 25.0 | 24.0 |
| 1 7 | 1.5 | 34.1 | . | 119.2 | . | 1.2 | . | 30.6 | . |
| 1 7 | 3.0 | 35.3 | 34.2 | 117.4 | 123.6 | 1.1 | 1.1 | 23.7 | 23.2 |
| 1 7 | 4.5 | 32.4 | 33.3 | 119.8 | 121.2 | 1.1 | 1.1 | 25.1 | 22.4 |
| 1 7 | 6.0 | 32.0 | 32.1 | 116.0 | 116.2 | 1.0 | 0.8 | 22.3 | 21.0 |
| 1 8 | 0.0 | 73.5 | 72.4 | 86.2 | 108.6 | . | . | 65.3 | 63.7 |
| 1 8 | 1.5 | 73.4 | . | 89.4 | . | . | . | 61.0 | . |
| 1 8 | 3.0 | 72.0 | 70.8 | 89.2 | 103.2 | . | . | 58.8 | 57.2 |
| 1 8 | 4.5 | 72.0 | 72.1 | 97.0 | 100.0 | . | . | 59.7 | 62.5 |
| 1 8 | 6.0 | 74.0 | 74.4 | 83.4 | 105.8 | . | . | 50.3 | 53.3 |
| 1 9 | 0.0 | 62.2 | 57.2 | 98.2 | 99.8 | 45.1 | 44.7 | 108.8 | 101.0 |
| 1 9 | 1.5 | 56.4 | 56.8 | 96.2 | 97.2 | 45.1 | 45.9 | 71.5 | 105.5 |
| 1 9 | 3.0 | 56.3 | 56.1 | 96.6 | 96.2 | 43.3 | 49.7 | 136.5 | 139.0 |
| 1 9 | 4.5 | 55.3 | . | 99.0 | . | 44.7 | . | 127.0 | . |
| 1 9 | 6.0 | 56.4 | 61.6 | 94.8 | 98.4 | 44.7 | 47.7 | 120.0 | 102.0 |
| 110 | 0.0 | 48.4 | 48.5 | 82.2 | 82.6 | 14.8 | 15.4 | 189.0 | 192.0 |
| 110 | 1.5 | 48.0 | 48.4 | 81.6 | 83.2 | 15.3 | 17.3 | 193.0 | 198.0 |
| 110 | 3.0 | 48.0 | 47.8 | 80.0 | 80.4 | 15.7 | 18.3 | 210.0 | 199.0 |
| 110 | 4.5 | 48.5 | 48.7 | 80.0 | 82.2 | 15.0 | 15.7 | 196.0 | 198.0 |
| 110 | 6.0 | 49.3 | 48.9 | 88.8 | 84.4 | 32.0 | 35.3 | 202.0 | 196.0 |
| 111 | 0.0 | 53.4 | 54.4 | 83.6 | 78.2 | 26.7 | 27.5 | 129.8 | 143.5 |
| 111 | 1.5 | 52.7 | 53.5 | 83.4 | 85.0 | 31.3 | 26.9 | . | 150.0 |
| 111 | 3.0 | 52.7 | 53.2 | 82.2 | 86.6 | 27.3 | 25.1 | . | 145.0 |
| 111 | 4.5 | 52.6 | 53.2 | 87.8 | 83.2 | 20.3 | 27.5 | 162.0 | 153.0 |
| 111 | 6.0 | 53.1 | 53.0 | 83.4 | 84.2 | 27.5 | 27.4 | 165.0 | 159.5 |

**NOTE: Sample size is 20 ml.**

## TABLE IV

DATA ANAEROBIC STORAGE

| RUN NO | TIME HR | SOLIDS MG | | CARBON MG/L | | NITRATE MG/L | | NITRITE MG/L | |
|---|---|---|---|---|---|---|---|---|---|
| 206 | 0.0 | 67.3 | 71.6 | 94.2 | 103.4 | 2.2 | 2.9 | 19.0 | 19.0 |
| 206 | 1.5 | 65.2 | . | 94.8 | . | 1.0 | . | 11.4 | 12.0 |
| 206 | 3.0 | 64.5 | 63.8 | 109.8 | 121.6 | 0.9 | 0.9 | 3.5 | 3.5 |
| 206 | 6.0 | 64.1 | 63.9 | 108.4 | 116.2 | 0.8 | 0.9 | 0.0 | 0.1 |
| 206 | 7.5 | 65.9 | 64.1 | 105.6 | 114.0 | 1.0 | 1.0 | 0.0 | 0.0 |
| 207 | 0.0 | 35.8 | 32.2 | 115.2 | 117.2 | 1.0 | 1.2 | 25.0 | 24.0 |
| 207 | 1.5 | 35.5 | 34.8 | 98.2 | 111.4 | 0.8 | 0.8 | 25.8 | 22.8 |
| 207 | 3.0 | 38.7 | 34.4 | 118.0 | 106.0 | 0.8 | 0.9 | 17.2 | 16.4 |
| 207 | 4.5 | 33.6 | . | 118.0 | 106.0 | 0.7 | 0.9 | 14.4 | 16.0 |
| 207 | 6.0 | 33.5 | 32.6 | 117.4 | 136.4 | 0.7 | 0.6 | 10.2 | 11.6 |
| 208 | 0.0 | 73.5 | 72.4 | 86.2 | 108.6 | . | . | 65.3 | 63.7 |
| 208 | 1.5 | 72.0 | 69.0 | 91.6 | 93.2 | . | . | 57.3 | . |
| 208 | 3.0 | 70.8 | 60.8 | 106.6 | 94.8 | . | . | 56.0 | 62.0 |
| 208 | 4.5 | 70.7 | 70.7 | 107.2 | 109.2 | . | . | 57.2 | 55.8 |
| 208 | 6.0 | 74.4 | 74.1 | 106.0 | 105.4 | . | . | 60.2 | 46.5 |
| 209 | 0.0 | 62.2 | 57.2 | 98.2 | 99.8 | 45.1 | 44.7 | 108.8 | 101.0 |
| 209 | 1.5 | 56.8 | 61.1 | 91.8 | 97.8 | 36.3 | 35.3 | 62.5 | 47.0 |
| 209 | 3.0 | 55.8 | 55.9 | 88.6 | 101.6 | 32.4 | 30.0 | 106.0 | 104.0 |
| 209 | 4.5 | 56.1 | 55.4 | 99.0 | 95.6 | 32.7 | 25.7 | 112.0 | 85.0 |
| 209 | 6.0 | 55.6 | 56.1 | 97.8 | 97.4 | 19.0 | 21.3 | 63.0 | 58.5 |
| 210 | 0.0 | 48.4 | 48.5 | 82.2 | 82.6 | 14.8 | 15.4 | 189.0 | 192.0 |
| 210 | 1.5 | 48.0 | 47.8 | 80.0 | 84.4 | 12.0 | 14.0 | 190.0 | 192.0 |
| 210 | 3.0 | 47.6 | 47.6 | 86.4 | 86.0 | 7.3 | 5.5 | 206.0 | 202.0 |
| 210 | 4.5 | 48.2 | 47.9 | 86.0 | 88.0 | 2.3 | 4.0 | 194.5 | 191.0 |
| 210 | 6.0 | 47.5 | 46.1 | 84.6 | 88.6 | 32.5 | 2.5 | 206.0 | 187.0 |
| 211 | 0.0 | 53.4 | 54.4 | 83.6 | 78.2 | 26.7 | 27.5 | 129.8 | 143.5 |
| 211 | 1.5 | 53.2 | 53.8 | 83.4 | 86.0 | 22.0 | 22.3 | 107.0 | 144.5 |
| 211 | 3.0 | 51.8 | 52.6 | 89.4 | 88.0 | 17.7 | 16.8 | 139.0 | 140.0 |
| 211 | 4.5 | 52.7 | . | 91.2 | . | 13.7 | 12.5 | 143.5 | 139.0 |
| 211 | 6.0 | 52.5 | 52.3 | 88.0 | 86.0 | 9.9 | 10.0 | 131.5 | 130.0 |

**NOTE: Sample size is 20 ml.**

## TABLE V

DATA AEROBIC STORAGE

| RUN NO | TIME HR | SOLIDS MG | | CARBON MG/L | | NITRATE MG/L | | NITRITE MG/L | | AMMONIA MG/L | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 112 | 0.0 | 45.1 | 45.8 | 117.2 | 114.0 | 145.0 | 146.0 | 46.4 | 43.3 | 61.6 | 94.0 |
| 112 | 3.0 | 43.4 | 43.3 | 113.2 | 110.6 | 123.0 | 131.6 | 40.6 | 45.2 | 100.8 | 103.6 |
| 112 | 6.0 | 42.0 | 42.2 | 114.4 | 114.6 | 155.0 | 172.0 | 33.7 | 33.5 | 98.0 | 96.8 |
| 112 | 7.5 | 42.1 | 41.1 | 115.2 | 113.2 | 158.0 | 163.0 | 29.0 | 36.2 | 109.2 | 100.8 |
| 112 | 9.0 | 41.6 | 41.6 | 112.6 | 112.0 | 162.4 | 155.0 | 30.5 | 37.7 | 114.0 | 114.8 |
| 113 | 0.0 | 47.1 | 47.9 | 118.6 | 111.6 | • | • | • | • | 103.6 | 95.2 |
| 113 | 3.0 | 47.4 | 48.2 | 118.6 | 110.6 | • | • | • | • | 120.4 | 123.4 |
| 113 | 6.0 | 45.7 | 47.2 | 111.4 | 112.6 | • | • | • | • | 99.4 | 96.6 |
| 113 | 9.0 | 45.8 | 47.2 | 122.6 | 111.6 | • | • | • | • | 117.6 | 123.2 |
| 114 | 0.0 | 54.4 | 54.8 | 126.0 | 117.2 | 148.4 | 152.0 | 43.2 | 44.1 | 129.4 | 126.0 |
| 114 | 3.0 | 54.0 | 51.2 | 116.2 | 110.8 | 168.0 | 157.6 | 47.5 | 49.0 | 129.8 | 126.0 |
| 114 | 6.0 | 53.4 | 54.0 | 97.0 | 107.4 | 157.6 | 145.6 | 42.8 | 42.2 | 136.0 | 131.6 |
| 114 | 7.5 | 53.2 | 53.1 | 114.0 | 108.8 | 139.0 | 148.0 | 43.6 | 43.8 | 137.2 | 140.0 |
| 114 | 9.0 | 53.4 | 54.1 | 118.8 | 115.4 | 152.6 | 148.6 | 44.0 | 44.3 | 140.0 | 131.6 |
| 115 | 0.0 | 57.3 | 57.0 | 94.0 | 84.0 | 43.0 | 39.8 | 144.5 | 140.9 | 179.2 | 190.4 |
| 115 | 3.0 | 58.1 | 55.5 | 96.2 | 110.0 | 44.7 | 40.6 | 142.5 | 147.0 | 184.8 | 192.2 |
| 115 | 6.0 | 56.2 | 56.2 | 117.4 | 96.4 | 43.2 | 43.4 | 149.0 | 148.5 | 187.6 | 193.2 |
| 115 | 7.5 | 56.7 | 61.6 | 88.2 | 94.4 | 44.8 | 46.0 | 153.5 | 153.5 | 182.0 | 190.4 |
| 115 | 9.0 | 54.8 | 54.2 | 103.6 | 103.8 | 42.3 | 43.4 | 150.0 | 148.0 | 190.4 | 193.2 |
| 116 | 0.0 | 57.5 | 57.5 | 100.0 | 100.0 | 61.6 | 62.2 | 225.0 | 219.0 | 154.0 | 152.6 |
| 116 | 3.0 | 54.9 | 55.9 | 110.0 | 108.4 | 71.0 | 69.8 | 198.0 | 173.0 | 147.0 | 144.2 |
| 116 | 6.0 | 55.3 | • | 109.2 | • | 82.5 | • | 234.0 | • | 142.8 | • |
| 116 | 7.5 | 55.9 | 55.0 | 106.0 | 112.0 | 92.6 | 91.9 | 242.0 | 208.0 | 144.2 | 147.0 |
| 116 | 9.0 | 55.1 | 55.3 | 106.0 | 113.6 | 90.2 | 88.2 | 222.0 | 226.0 | 145.6 | 145.6 |

**NOTE: Sample size is 20 ml.**

## TABLE VI

DATA ANAEROBIC STORAGE

| RUN NO | TIME HR | SOLIDS MG | | CARBON MG/L | | NITRATE MG/L | | NITRITE MG/L | | AMMONIA MG/L | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 212 | 0.0 | 45.1 | 45.8 | 117.2 | 114.0 | 145.0 | 146.0 | 46.4 | 43.3 | 61.6 | 84.0 |
| 212 | 3.0 | 42.4 | 43.5 | 118.0 | 114.6 | 135.8 | 132.4 | 30.7 | 31.0 | 84.0 | 95.2 |
| 212 | 6.0 | 42.4 | 42.2 | 124.0 | 124.4 | 136.0 | 135.8 | 11.5 | 11.3 | 100.8 | 100.8 |
| 212 | 7.5 | 41.9 | 41.4 | 123.8 | 123.6 | 127.8 | 148.0 | 13.4 | 13.0 | 95.2 | 100.8 |
| 212 | 9.0 | 41.9 | 42.4 | 121.2 | 123.8 | 135.6 | 140.0 | 15.5 | 15.1 | 103.6 | 103.6 |
| 213 | 0.0 | 47.1 | 47.9 | 118.6 | 111.6 | • | • | • | • | 103.6 | 95.2 |
| 213 | 3.0 | 46.8 | 48.9 | 119.0 | 112.0 | • | • | • | • | 120.4 | 102.2 |
| 213 | 6.0 | 36.6 | 47.0 | 123.0 | 114.4 | • | • | • | • | 133.0 | 123.0 |
| 213 | 9.0 | 46.1 | 46.8 | 108.0 | 106.0 | • | • | • | • | 151.2 | • |
| 214 | 0.0 | 54.4 | 54.8 | 126.0 | 117.2 | 148.4 | 152.0 | 43.2 | 44.1 | 120.4 | 126.4 |
| 214 | 3.0 | 51.7 | 54.0 | 111.8 | 114.6 | 146.8 | 126.4 | 67.0 | 68.0 | 128.8 | 128.8 |
| 214 | 6.0 | 53.5 | 53.0 | 114.0 | 115.2 | 108.0 | 94.0 | 85.8 | 87.5 | 126.0 | 126.0 |
| 214 | 7.5 | 52.3 | 53.6 | 111.2 | • | 115.9 | • | 83.3 | • | 131.6 | • |
| 214 | 9.0 | 53.9 | 53.7 | 114.0 | 117.4 | 114.4 | 126.0 | 81.0 | 82.6 | 117.6 | 128.0 |
| 215 | 0.0 | 57.3 | 57.0 | 94.0 | 84.0 | 43.0 | 39.8 | 144.5 | 140.9 | 179.2 | 190.4 |
| 215 | 3.0 | 56.5 | 56.6 | 113.0 | 112.6 | 23.5 | 25.7 | 158.0 | 160.0 | 187.6 | 183.4 |
| 215 | 6.0 | 55.9 | 56.0 | 110.0 | 98.0 | 6.7 | 15.8 | 176.0 | 176.5 | 187.6 | 168.0 |
| 215 | 7.5 | 48.3 | 56.9 | 92.0 | • | 7.0 | • | 183.5 | • | 179.2 | • |
| 215 | 9.0 | 55.4 | 55.3 | 104.4 | 106.0 | 6.0 | 7.9 | 155.5 | 166.0 | 190.4 | 187.6 |
| 216 | 0.0 | 57.5 | 57.5 | 100.0 | 100.0 | 61.6 | 62.2 | 225.0 | 219.0 | 154.0 | 152.6 |
| 216 | 3.0 | 55.6 | 56.0 | 115.6 | 112.2 | 37.4 | 39.7 | 251.0 | 244.5 | 138.6 | 140.0 |
| 216 | 6.0 | 55.6 | 55.3 | 110.2 | 106.0 | 18.1 | 19.6 | 288.0 | 296.0 | 135.8 | 127.4 |
| 216 | 7.5 | 54.9 | 55.4 | 113.6 | 114.0 | 19.8 | 20.8 | 266.0 | 280.0 | 140.0 | 138.6 |
| 216 | 9.0 | 54.8 | 55.2 | 111.0 | 112.6 | 29.0 | 27.5 | 298.0 | 264.0 | 141.4 | 141.4 |

**NOTE: Sample size is 20 ml.**

# APPENDIX F

## COMPUTER PROGRAMS

NOTE:

The computer program for solids and carbon analysis may be modified for nitrate, nitrite, and ammonia analyses by altering the READ and WRITE statements (data input and output).

TABLE VII

```
$JOB            003324 E CZARNECKI      100    010    030
$IBJOB          NODECK
$IBFTC
C      O2 UPTAKE CALCULATIONS          ANAEROBIC STORAGE     RUN 14
C      FLASK NO'S ARE 13, 14, 15            ANAEROBIC STORAGE  RUN 14
C      CORRESPONDING I'S ARE 1,2, 3                          RUN 14
C      NT= MAXIMUM NO. OF READINGS
C      ABC(M,I) IS A COUNTER
C      DENSITY OF OXYGEN AT 20'C IS 0.001331 MG/UL
       DIMENSION NFLK(50),FLKCON(50),T1RDG(50),T2RDG(50),RDGMAN(50,20),
      1RDGINT(50,20),THBCOR(50),ACTCHG(50,20),ACTCAL(50,20),O2INT(50,20),
      2TOTO2(50,20),SUMTOT(50),ABC(50,20),FACTOR(50),AVERGE(50),AVEMG(20)
       N=3
       NRUN=14
       NT=19
       DENSTY=0.001331
       READ (5,1) (NFLK(I),FLKCON(I), I=1,N,1)
       WRITE (6,1) (NFLK(I),FLKCON(I),I=1,2,1)
     1 FORMAT (I3,F7.2)
       READ (5,3) (T1RDG(J),T2RDG(J), J=1,NT,1)
       WRITE (6,3) (T1RDG(J),T2RDG(J),J=1,3,1)
     3 FORMAT (2F7.3)
       READ (5,4) ((RDGMAN(M,I), M=1,20,1),I=1,N,1)
       WRITE (6,4) ((RDGMAN(M,I),I=1,8,1),M=1,1,1)
     4 FORMAT (10F8.3)
       DO 501 I=1,N,1
       WRITE (6,5) NRUN
       WRITE (6,6) NFLK(I),FLKCON(I)
       WRITE (6,7) NT
     5 FORMAT (1H1,10HRUN NUMBER,I3)
     6 FORMAT  (3X,13HFLASK NUMBER ,I3,4X,15HFLASK CONSTANT ,F7.2,1X,
      15HUL/CM)
     7 FORMAT (3X,16HNO. OF READINGS ,I3)
       WRITE (6,101)
       WRITE (6,102)
       WRITE (6,103)
       WRITE (6,104)
   101 FORMAT (1H0,2X,4HTIME,5X,4HTIME,5X,8HINTERVAL,4X,8HINTERVAL,5X,
      17HTHERMO-,5X,6HACTUAL,3X,8HINTERVAL,4X,5HTOTAL)
   102 FORMAT (3X,4H(HR),3X,8HINTERVAL,3X,9HMANOMETER,3X,9HMANOMETER,3X,
      110HBAROMETRIC,3X,6HCHANGE,4X,6HOXYGEN,4X,6HOXYGEN)
   103 FORMAT (12X,4H(HR),6X,7HREADING,6X,6HCHANGE,4X,10HCORRECTION,4X,
      14H(CM),5X,6HUPTAKE,4X,6HUPTAKE)
   104 FORMAT (24X,4H(CM),8X,4H(CM),8X,4H(CM),17X,4H(UL),6X,4H(UL))
       M=1
       J=14
       THBCOR(J)=0.0
       RDGINT(M,I)=0.0
       O2INT(M,I)=0.0
       ACTCHG(M,I)=0.0
       TOTO2(M,I)=0.0
       ABC(M,I)=1.0
       T=6.5
       DT=0.5
       WRITE (6,8) T,RDGMAN(M,I),RDGINT(M,I),THBCOR(J),ACTCHG(M,I),
      1O2INT(M,I),TOTO2(M,I)
     8 FORMAT (1H0,F6.2,9X,3F12.2,F11.2,2F10.2)
       DO 500 M=2,6,1
       IF (RDGMAN(M,I).EQ.0.0) GO TO 400
```

```
      GO TO 300
  400 ABC(M,I)=0.0
      TOTO2(M,I)=0.0
      GO TO 501
  300 T=T+DT
      J=J+1
      THBCOR(J)=(T1RDG(J)-T1RDG(J-1)+T2RDG(J)-T2RDG(J-1))/2.0
      RDGINT(M,I)=RDGMAN(M,I)-RDGMAN(M-1,I)
      ACTCHG(M,I)=RDGINT(M,I)-THBCOR(J)
      ACTCAL(M,I)=(ACTCHG(M,I))*(-1.0)
      O2INT(M,I)=ACTCAL(M,I)*FLKCON(I)
      TOTO2(M,I)=O2INT(M,I)+TOTO2(M-1,I)
      ABC(M,I)=1.0
      WRITE (6,9) T,DT,RDGMAN(M,I),RDGINT(M,I),THBCOR(J),ACTCHG(M,I),
     1O2INT(M,I),TOTO2(M,I)
    9 FORMAT (2X,F5.2,F9.2,3F12.2,F11.2,2F10.2)
  500 CONTINUE
  501 CONTINUE
      WRITE (6,201)
      WRITE (6,202)
      WRITE (6,203)
      WRITE (6,204)
  201 FORMAT (1H1,2X,4HTIME,6X,4HTIME,6X,9HSUMMATION,3X,6HNUMBER,3X,
     17HAVERAGE,4X,7HAVERAGE)
  202 FORMAT (3X,4H(HR),4X,8HINTERVAL,6X,6HOXYGEN,6X,2HOF,6X,6HOXYGEN,
     15X,6HOXYGEN)
  203 FORMAT(13X,4H(HR),8X,6HUPTAKE,4X,6HFLASKS,4X,6HUPTAKE,5X,6HUPTAKE)
  204 FORMAT (26X,4H(UL),16X,4H(UL),7X,4H(MG))
      TX=6.5
      DO 601 M=1,6,1
      SUMTOT(M)=0.0
      FACTOR(M)=0.0
      DO 600 I=1,N,1
      SUMTOT(M)=SUMTOT(M)+TOTO2(M,I)
      FACTOR(M)=FACTOR(M)+ABC(M,I)
  600 CONTINUE
      AVERGE(M)=SUMTOT(M)/FACTOR(M)
      AVEMG(M)=AVERGE(M)*DENSTY
      WRITE (6,10)TX,DT,SUMTOT(M),FACTOR(M),AVERGE(M),AVEMG(M)
   10 FORMAT (2X,F5.2,F10.2,F13.2,F9.2,F11.2,F14.5)
      TX=TX+DT
  601 CONTINUE
      STOP
      END
$ENTRY
 13 112.15
 14 113.30
 15 113.36
  15.0    15.0
  14.8    14.85
  14.4    14.5
  14.0    14.1
  13.8    13.8
  13.2    13.3
  13.3    13.4
  13.0    13.1
  12.6    12.7
  12.6    12.7
```

```
  12.5    12.6
  12.0    12.2
  11.8    11.8
  11.4    11.45
  11.0    11.1
  10.4    10.5
  10.0    10.1
   9.6     9.7
   9.1     9.25
  15.00   13.75   12.60   11.40   10.30    9.20

  15.00   13.80   12.50   11.40   10.40    9.20

  15.00   13.90   12.60

$IBSYS

                                CD TOT   0133
```

TABLE VII (A)

NOMENCLATURE

| Program Symbol | Meaning or Equivalent |
|---|---|
| ABC | Counter |
| ACTCAL | -(ACTCHG) |
| ACTCHG | Actual change in manometer reading |
| AVEMG | Average oxygen uptake (Gravimetric) |
| AVERGE | Average oxygen uptake (Volumetric) |
| DENSTY | Density of gas |
| DT | Time Interval |
| FACTOR | Number of flasks |
| FLKCON | Flask constant |
| J, M, N | Counters |
| NFLK | Flask number |
| NRUN | Run number |
| NT | Maximum number of readings |
| O2INT | Interval oxygen uptake |
| RDGMAN | Manometric reading |
| RDGINT | Interval change in manometer reading |
| SUMTOT | Summation of cumulative oxygen uptake |
| T, TX | Time |
| T1RDG | Thermobarometer #1 reading |
| T2RDG | Thermobarometer #2 reading |
| THBCOR | Thermobarometer correction |
| TOTO2 | Cumulative oxygen uptake |

TABLE VIII

```
$JOB    WATFOR  003324 E CZARNECKI       100    010    030
$IBJOB          NODECK
$IBFTC
C       SOLIDS AND CARBON ANALYSIS
        DIMENSION IATM(5),IRUN(5),T(5),SOL1(5),SOL2(5),CARB1(5),CARB2(5),S
       1OL(5),SOLS(5),CAR(5),CARB(5),TK(10),SUMSOL(10),SQSOL(10),SQCAR(10)
       2,SUMCAR(10),VARS(10),VARC(10),STERR(10),SDCAR(10),A(10),AVSOL(10),
       3AVCAR(10),TK2(10),XY(10),XZ(10),AVSOL2(10),AVCAR2(10),YC(10),
       4ZC(10),YIM(10),ZIM(10),YCM(10),ZCM(10),YIM2(10),ZIM2(10),YCM2(10),
       5ZCM2(10)
        DO  15 K=1,7
        SUMSOL(K)=0.0
        SQSOL(K)=0.0
        SUMCAR(K)=0.0
        SQCAR(K)=0.0
   15   A(K)=0.0
        TK(1) =0.0
        DO 16 K=2,7
   16   TK(K) = TK(K-1) +1.5
  500   READ(5,1)(IATM(I),IRUN(I),T(I),SOL1(I),SOL2(I),CARB1(I),CARB2(I),
       1I=1,5,1)
    1   FORMAT (I1,I2,F5.1,4F6.1)
        IF(IATM(1).EQ.1) GO TO 10
        IF(IATM(1).EQ.2) GO TO 11
        IF (IATM(1).EQ.3) GO TO 501
   10   WRITE(6,2)
    2   FORMAT (1H-,4X,15HAEROBIC STORAGE)
        GO TO 12
   11   WRITE(6,3)
    3   FORMAT(1H-,4X,17HANAEROBIC STORAGE)
   12   WRITE(6,4) IRUN(1)
    4   FORMAT (1H0,4X,10HRUN NUMBER,I3)
        WRITE(6,5)
        WRITE(6,6)
    5   FORMAT (1H0,4X,4HTIME,3X,6HSOLIDS,3X,6HCARBON,3X,6HSOLIDS,3X,
       16HCARBON)
    6   FORMAT(5X,4H(HR),4X,4H(MG),4X,6H(MG/L),4X,5HRATIO,4X,5HRATIO)
        DO 400 I=1,5
        IF (T(I).EQ.5.5) GO TO 400
        X1=2.0
        X2=2.0
        C=SOL1(I)+SOL2(I)
        B=CARB1(I)+CARB2(I)
        IF(C.LE.(1.5*SOL1(I)))X1=1.0
        IF(B.LE.(1.5*CARB1(I)))X2=1.0
        IF(C.EQ.0.0) GO TO 200
        SOL(I)=C/X1
        SOLS(I)=SOL(I)/SOL(1)
  101   IF(B.EQ.0.0) GO TO 210
        CAR(I)=B/X2
        CARB(I)=CAR(I)/CAR(1)
        GO TO 100
  200   SOL(I)=0.0
        SOLS(I)=0.0
        GO TO 101
  210   CAR(I)=0.0
        CARB(I)=0.0
  100   WRITE(6,7) T(I),SOL(I),CAR(I),SOLS(I),CARB(I)
    7   FORMAT (1H0,2F8.1,F9.1,2F9.2)
```

```
      K=1
      IF (T(I).EQ.1.5) K=2
      IF (T(I).EQ.3.0) K=3
      IF (T(I).EQ.3.0) K=3
      IF (T(I).EQ.4.5) K=4
      IF (T(I).EQ.6.0) K=5
      IF (T(I).EQ.7.5) K=6
      IF (T(I).EQ.9.0) K=7
      A(K)=A(K)+1.0
      Z=SUMSOL(K)+SOLS(I)
      SUMSOL(K)=Z
      ZA=SQSOL(K)+(SOLS(I)**2)
      SQSOL(K)=ZA
      ZB=SUMCAR(K)+CARB(I)
      SUMCAR(K)=ZB
      ZCC=SQCAR(K) + (CARB(I)**2)
      SQCAR(K)=ZCC
400   CONTINUE
      GO TO 500
501   WRITE(6,20)
      WRITE(6,21)
      WRITE(6,22)
      WRITE(6,23)
 20   FORMAT(1H1,20X,20HSTATISTICAL ANALYSIS)
 21   FORMAT(1H0,19X,22HCARBON AND SOLIDS DATA)
 22   FORMAT(1H0,4X,4HTIME,4X,6HSOLIDS,3X,8HVARIANCE,3X,8HSTANDARD,5X,
     16HCARBON,3X,8HVARIANCE,3X,8HSTANDARD)
 23   FORMAT(5X,4H(HR),5X,5HRATIO,13X,9HDEVIATION,6X,5HRATIO,13X,
     19HDEVIATION)
      DO 505 K=1,7
      AVSOL(K) = SUMSOL(K) / A(K)
      VARS(K) = (SQSOL(K)/A(K))-((SUMSOL(K)/A(K))**2)
      STERR(K) = SQRT(VARS(K))
      AVCAR(K) = SUMCAR(K) / A(K)
      VARC(K) = (SQCAR(K)/A(K))- ((SUMCAR(K)/A(K))**2)
      SDCAR(K) = SQRT(VARC(K))
      XY(K)=AVSOL(K) * TK(K)
      XZ(K)=AVCAR(K)*TK(K)
      TK2(K)=TK(K) ** 2
      AVSOL2(K)=AVSOL(K) **2
      AVCAR2(K)=AVCAR(K) **2
      WRITE( 6,8) TK(K), AVSOL(K), VARS(K),STERR(K), AVCAR(K), VARC(K),
     1SDCAR(K),TK2(K),XY(K),XZ(K),AVSOL2(K),AVCAR2(K)
  8   FORMAT (1H0,F8.1,F10.3,2F11.4,F13.3,2F11.4,10X,5F8.4 )
505   CONTINUE
      XN=7.0
      SUMX=0.0
      SUMY=0.0
      SUMZ=0.0
      SUMX2=0.0
      SUMXZ=0.0
      SUMXY=0.0
      SUMY2=0.0
      SUMZ2=0.0
      DO 601 K=1,7
      SUMX=TK(K) + SUMX
      SUMX2=TK2(K) + SUMX2
      SUMY=AVSOL(K) + SUMY
```

```
      SUMXY=XY(K) + SUMXY
      SUMZ=AVCAR(K) + SUMZ
      SUMXZ=XZ(K) + SUMXZ
      SUMY2=AVSOL2(K) + SUMY2
      SUMZ2=AVCAR2(K) + SUMZ2
  601 CONTINUE
      A01=(SUMY*SUMX2) - (SUMX * SUMXY)
      A02=(XN * SUMX2) - (SUMX ** 2)
      A0=A01/A02
      A11=(XN * SUMXY) - (SUMX * SUMY)
      A12=(XN * SUMX2) - (SUMX ** 2)
      A1=A11/A12
      B01=(SUMZ * SUMX2) - (SUMX *SUMXZ)
      B11=(XN * SUMXZ) - (SUMX * SUMZ)
      B0=B01/A02
      B1=B11/A12
      XMEAN=SUMX / XN
      YMEAN=SUMY / XN
      ZMEAN=SUMZ / XN
      WRITE(6,40)
   40 FORMAT (1H-,5X,4HSUMX,6X,4HSUMY,6X,4HSUMZ,5X,5HSUMX2,5X,5HSUMY2,
     15X,5HSUMZ2,5X,5HSUMXY,5X,5HSUMXZ )
      WRITE(6,41) SUMX,SUMY,SUMZ,SUMX2,SUMY2,SUMZ2,SUMXY,SUMXZ
   41 FORMAT (1H-,8F10.3  )
      WRITE(6,42) XMEAN, YMEAN
      WRITE(6,43) XMEAN, ZMEAN
   42 FORMAT (1H0,3X,23HMEAN OF  SOLIDS DATA IS ,F6.3,2H, ,F6.3 )
   43 FORMAT (1H0,3X,23HMEAN OF  CARBON DATA IS ,F6.3,2H, ,F6.3 )
      WRITE(6,30)
   30 FORMAT(1H-,4X,48HLINE OF TREND (LEAST SQUARES) FOR SOLIDS DATA IS)
      WRITE(6,31) A0,A1
   31 FORMAT(1H0,10X,2HY=,F6.3,2H+ ,F6.3,2HX )
      WRITE(6,32)
   32 FORMAT(1H-,4X,48HLINE OF TREND (LEAST SQUARES) FOR CARBON DATA IS)
      WRITE(6,33) B0,B1
   33 FORMAT(1H0,10X,2HZ=,F6.3,2H+ ,F6.3,2HX )
      SUMYI2=0.0
      SUMYC2=0.0
      SUMZC2=0.0
      SUMZI2=0.0
      WRITE(6,44)
   44 FORMAT(1H1,1HX,10X,2HYC,10X,2HZC,8X,4HY-YM,8X,4HZ-ZM,8X,4HY-YC,8X,
     14HZ-ZC )
   45 FORMAT (1H0,F4.1,6F12.5)
      DO 701 K=1,7
      ZC(K)= B0 + B1 * TK(K)
      YC(K)= A0 + A1 * TK(K)
      YIM(K)=AVSOL(K) - YMEAN
      YCM(K)=AVSOL(K) - YC(K)
      ZIM(K)=AVCAR(K) - ZMEAN
      ZCM(K)=AVCAR(K) - ZC(K)
      YIM2(K)=YIM(K) ** 2
      YCM2(K)=YCM(K) ** 2
      ZIM2(K)=ZIM(K) ** 2
      ZCM2(K)=ZCM(K) ** 2
      SUMYI2= YIM2(K) + SUMYI2
      SUMYC2= YCM2(K) + SUMYC2
      SUMZC2= ZCM2(K) + SUMZC2
```

```
      SUMZI2= ZIM2(K) + SUMZI2
      WRITE(6,45) TK(K),YC(K),ZC(K),YIM(K),ZIM(K),YCM(K),ZCM(K)
  701 CONTINUE
      WRITE(6,46)
   46 FORMAT (1H0,1X,6HSUMYI2,2X,6HSUMYC2,2X,6HSUMZI2,2X,6HSUMZC2 )
      WRITE(6,47) SUMYI2,SUMYC2,SUMZI2,SUMZC2
   47 FORMAT (4F8.5 )
      SUMCY2= SUMYI2 - SUMYC2
      SUMCZ2= SUMZI2 - SUMZC2
      RY2= 1.0 - (SUMYC2 / SUMYI2 )
      RZ2= 1.0 - (SUMZC2 / SUMZI2 )
      RY = SQRT(RY2)
      RZ = SQRT(RZ2)
      YMSC = SUMCY2
      YSSE = SUMYC2
      YMSE = YSSE / (XN-2.0)
      ZMSC = SUMCZ2
      ZSSE = SUMZC2
      ZMSE = ZSSE / (XN-2.0)
      FY = YMSC/YMSE
      FZ = ZMSC/ZMSE
      WRITE (6,48)
   48 FORMAT(1H1,10X,2HRY,8X,4HYMSC,8X,4HYSSE,8X,4HYMSE,8X,2HFY )
      WRITE (6,49) RY,YMSC,YSSE,YMSE,FY
   49 FORMAT (1H0,5F12.5)
      WRITE (6,51)
   51 FORMAT(1H-,10X,2HRZ,8X,4HZMSC,8X,4HZSSE,8X,4HZMSE,8X,2HFZ )
      WRITE (6,50) RZ,ZMSC,ZSSE,ZMSE,FZ
   50 FORMAT (1H0,5F12.5)
      STOP
      END
$ENTRY
1 7   0.0   35.8   32.2  115.2  117.2    1.0    1.2   25.0   24.0
1 7   1.5   34.1     .   119.2     .     1.2     .    30.6     .
1 7   3.0   35.3   34.2  117.4  123.6    1.1    1.1   23.7   23.2
1 7   4.5   32.4   33.3  119.8  121.2    1.1    1.1   25.1   22.4
1 7   6.0   32.0   32.1  116.0  116.2    1.0    0.8   22.3   21.0
1 8   0.0   73.5   72.4   86.2  108.6     .      .    65.3   63.7
1 8   1.5   73.4     .    89.4     .      .      .    61.0     .
1 8   3.0   72.0   70.8   89.2  103.2     .      .    58.8   57.2
1 8   4.5   72.0   72.1   97.0  100.0     .      .    59.7   62.5
1 8   6.0   74.0   74.4   83.4  105.8     .      .    50.3   53.3
1 9   0.0   62.2   57.2   98.2   99.8   45.1   44.7  108.8  101.0
1 9   1.5   56.4   56.8   96.2   97.2   45.1   45.9   71.5  105.5
1 9   3.0   56.3   56.1   96.6   96.2   43.3   49.7  136.5  139.0
1 9   4.5   55.3     .    99.0     .    44.7     .   127.0     .
1 9   6.0   56.4   61.6   94.8   98.4   44.7   47.7  120.0  102.0
110   0.0   48.4   48.5   82.2   82.6   14.8   15.4  189.0  192.0
110   1.5   48.0   48.4   81.6   83.2   15.3   17.3  193.0  198.0
110   3.0   48.0   47.8   80.0   80.4   15.7   18.3  210.0  199.0
110   4.5   48.5   48.7   80.0   82.2   15.0   15.7  196.0  198.0
110   6.0   49.3   48.9   88.8   84.4   32.0   35.3  202.0  196.0
111   0.0   53.4   54.4   83.6   78.2   26.7   27.5  129.8  143.5
111   1.5   52.7   53.5   83.4   85.0   31.3   26.9     .   150.0
111   3.0   52.7   53.2   82.2   86.6   27.3   25.1     .   145.0
111   4.5   52.6   53.2   87.8   83.2   20.3   27.5  162.0  153.0
111   6.0   53.1   53.0   83.4   84.2   27.5   27.4  165.0  159.5
112   0.0   45.1   45.8  117.2  114.0  145.0  146.0   46.4   43.3   61.6   84.0
```

```
112   3.0   43.4   43.3 113.2 110.6 123.0 131.6   40.5   45.2 100.8 103.6
112   6.0   42.0   42.2 114.4 114.6 155.0 172.0   33.7   32.5  98.0  86.8
112   7.5   42.1   41.1 115.2 113.2 158.0 163.0   39.0   36.2 109.2 100.8
112   9.0   41.6   41.6 112.6 112.0 162.4 155.0   39.5   37.7 114.8 114.8
113   0.0   47.1   47.9 118.6 111.6     .     .      .      . 103.6  95.2
113   3.0   47.4   48.2 118.6 110.6     .     .      .      . 120.4 123.4
113   6.0   45.7   47.2 111.4 112.6     .     .      .      .  99.4  96.6
113   9.0   45.8   47.2 122.6 111.6     .     .      .      . 117.6 123.2
113   5.5
116   0.0   57.5   57.5 100.0 100.0  61.6  62.2  225.0  219.0 154.0 152.6
116   3.0   54.9   55.9 110.0 108.4  71.0  69.8  198.0  173.0 147.0 144.2
116   6.0   55.3      . 109.2     .  82.5     .  234.0      . 142.8     .
116   7.5   55.9   55.0 106.0 112.0  92.6  91.9  242.0  208.0 106.0 112.0
116   9.0   55.1   55.3 106.0 113.6  90.2  88.2  222.0  226.0 145.6 145.6
3333333333
```

```
$IBSYS
```

```
CD TOT    0253
```

TABLE VIII (A)

NOMENCLATURE

| Program Symbol | Meaning or Equivalent |
|---|---|
| A | Counter |
| AVCAR | Average carbon ratio, z |
| AVCAR2 | $z^2$ |
| AVSOL | Average solid ratio, y |
| ALSOL2 | $y^2$ |
| CAR | Average of carbon data (run) |
| CARB | Carbon ratio, $C_t/C_o$ |
| CARBI, CARB2 | Carbon data (run) |
| FY | YMSC/YMSE, F value, solids |
| FZ | ZMSC/ZMSE, F value, carbon |
| IATM | Atmospheric identification |
| IRUN | Run identification |
| K | Counter |
| RY | $r_y$, correlation coefficient |
| RY2 | $(r_y)^2$ |
| RZ | $r_z$, correlation coefficient |
| RZ2 | $(r_z)^2$ |
| SDCAR | Standard deviation of carbon ratio |
| SOL | Average of solids data (run) |
| SOL1, SOL2 | Solids data (run) |
| SOLS | Solids ratio, $S_t/S_o$ |
| SQCAR | Carbon ratio squared |
| SQSOL | Solids ratio squared |
| STERR | Standard deviation of solids ratio |
| SUMCAR | Sum of carbon ratios |

| | |
|---|---|
| SUMSOL | Sum of solids ratios |
| SUM X | $\sum t$ |
| SUM Y | $\sum y$ |
| SUMZ | $\sum z$ |
| SUM X2 | $\sum t2$ |
| SUM XY | $\sum ty$ |
| SUM XZ | $\sum tz$ |
| SUMY 2 | $\sum y^2$ |
| SUM Z2 | $\sum z^2$ |
| SUMYI2 | $\sum(y-\bar{y})^2$ |
| SUMYC2 | $\sum(y-\hat{y})^2$ |
| SUMZC2 | $\sum(z-\hat{y})^2$ |
| SUM ZI2 | $\sum(z-\bar{z})^2$ |
| TK | Time, t, T |
| TK2 | $t^2$ |
| VARC | Variance of carbon ratios |
| VARS | Variance of solids ratios |
| XMEAN | Mean of storage time, $\bar{t}$ |
| XN | Number of time points |
| XY | tz |
| XZ | ty |
| YC | $\hat{y}$, estimate of y |
| YCM | $y-\hat{y}$ |
| YCM2 | $(y-\hat{y})^2$ |
| YIM | $(y-\bar{y})$ |
| YIM2 | $(y-\bar{y})^2$ |

| | |
|---|---|
| YMEAN | $\bar{y}$, mean of solids ratios |
| YMSC | Estimate of variance removed by fitting straight lines (solids ratio) |
| YMSE | Residual variance or error (solids ratio) |
| YSSE | Difference of sum of squares |
| ZC | $\hat{z}$, estimate of z |
| ZCM | $z-\hat{z}$ |
| ZCM2 | $(z-\hat{z})^2$ |
| ZIM | $z-\bar{z}$ |
| ZIM2 | $(z-\bar{z})^2$ |
| ZMEAN | $\bar{z}$, mean of carbon ratios |
| ZMSC | Estimate of variance removed by citting straight line (solids ratio) |
| ZMSE | Residual variance or error (carbon ratio) |
| ZSSE | Difference of sum of squares (carbon ratio) |

# APPENDIX G

# CALIBRATION CURVES

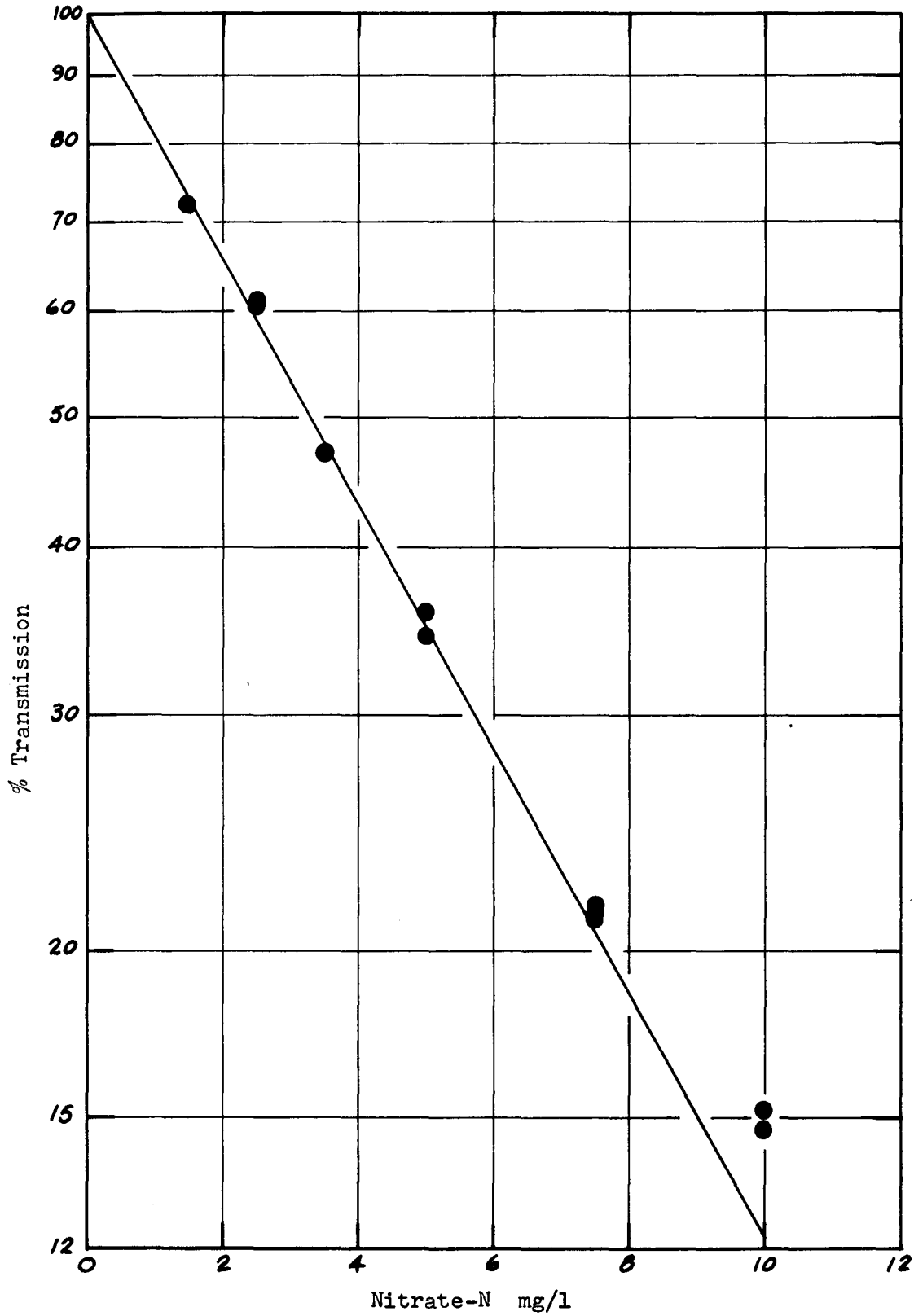



FIG.15 NITRATE CALIBRATION CURVE

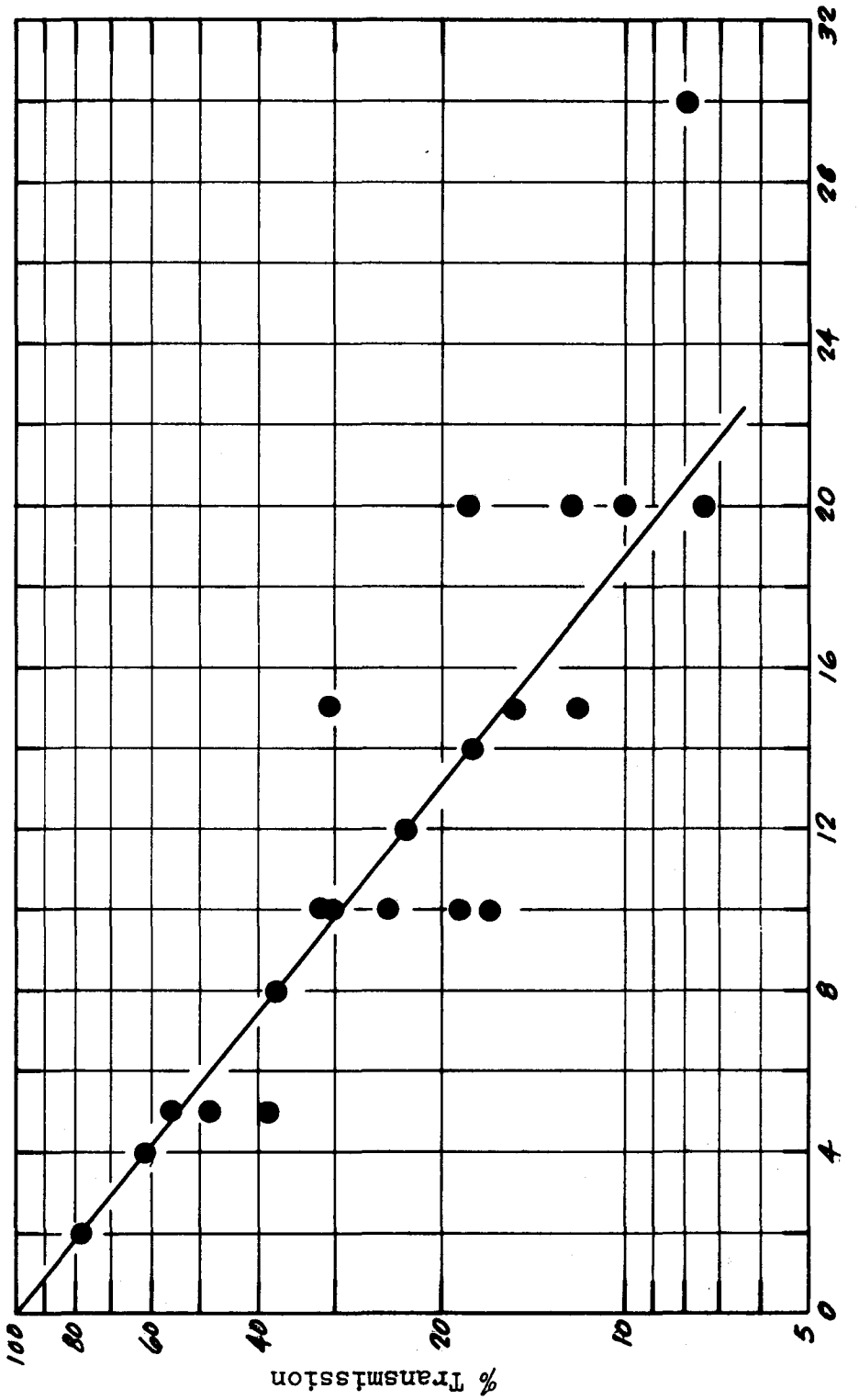



FIG.16 NITRITE CALIBRATION CURVE