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UNION CARBIDE CORPORATION
NUCLEAR DIVISION
POST OFFICE BOX X
OAK RIDGE, TENNESSEE 37830

ORNL/MIT-233

DATE: April 26, 1976

COPY NO

SUBJECT: Denitrification of Nitrate Waste Solutions

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ABSTRACT

Growth rates for the denitrifying bacteria Pseudomonas Stutzeri were studied to minimize the time necessary to start up a bacterial denitrification reactor. Batch experiments were performed in nine 250-ml Erlenmeyer flasks, a 7-liter fermentor, and a 67-liter fermentor. All reactors maintained an anaerobic environment. Initial microorganism inoculum concentration was varied over four orders of magnitude. Initial nitrate and substrate carbon concentrations were varied from 200 to 6000 ppm and from 56 to 1596 ppm, respectively, with a carbon-to-nitrogen weight ratio of 1.18. In all experiments, except those with the highest initial substrate-to-bacteria ratio, no growth was observed due to substrate depletion during the lag period. In those experiments which did exhibit an increase in bacterial population, growth also stopped due to substrate depletion. A model simulating microbe growth during the induction period was developed, but insufficient data were available to properly adjust the model constants. Because of this, the model does not accurately predict microbe growth. The metabolism of Pseudomonas Stutzeri was studied in detail. This resulted in a prediction of the denitrification stoichiometry during steady state reactor operation. Iron was found to be an important component for bacterial anabolism.

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CONTRACT NO. W-7405-ENG-26

Contents

1. SUMMARY

The waste stream from the solvent extraction uranium purification process contains high nitrate concentration levels. It has been previously determined that bacterial reduction of aqueous nitrate to nitrogen represents the best method of nitrate removal. It is possible that the heavy metals occasionally found in this waste stream may result in poisoning and destruction of the bacterial population. Therefore, the effect of the system variables on the bacterial growth during a subsequent startup procedure is of interest.

The objective is to model bacterial growth during the startup period. This model may then be used in developing the reactor startup procedure. Experiments have been performed with calcium acetate as the carbon source, calcium nitrate as the nitrate waste, and Pseudomonas Stutzeri as the denitrifying bacteria. Experiments were conducted to determine the effect of initial bacteria, acetate, and nitrate concentrations and reactor volume on bacterial growth during the induction period in batch reactors. The currently accepted initial feed carbon-to-nitrogen weight rates of 1.18 was maintained in all experiments. Reactors of 0.25-, 7-, and 67-liter volume were available. Nitrate feed concentrations were varied between 200 and 6000 ppm and the carbon concentrations between 56 and 1596 ppm. The initial bacteria concentration was varied over four orders of magnitude.

Results show that, in general, the carbon level in solution dropped to zero well before the nitrate level. This indicates that the initial carbon-to-nitrogen weight ratio of 1.18 is too low to satisfy the bacterial requirements during the induction period in a batch reactor. It has been suggested that substrate inhibition occurs for carbon levels above 4000 ppm. It is therefore recommended that experiments be performed to determine the optimum carbon-to-nitrogen feed ratio for both batch and flow reactors.

Appreciable growth was observed only in those reactors containing the highest initial substrate-to-bacteria ratio. The substrate level dropped to zero before any bacterial growth could occur in all of the remaining reactors. It is therefore concluded that a large excess of substrate must be present for microbe growth in batch reactors to avoid substrate depletion.

As part of the modeling effort, an attempt to establish the denitrification reaction stoichiometry by studying bacterial metabolism was made. The stoichiometry for the stationary growth phase was considered to be

$$0.5Ca(C_2H_3O_2)_2 + 0.8Ca(NO_3)_2 + 2H_2O + H^+ \rightarrow 1.3Ca^{+2} + 2CO_2 + 0.8N_2 + 3.2H_2O + 1.6\ OH^-$$

This is equivalent to the stoichiometry found from experimentation performed at Y-12. The reaction stoichiometry for the induction period cannot be determined from the biochemical concepts of metabolism since the partitioning of a carbon source between the tricarboxylic acid and the glyoxylate cycle is not known. The study of metabolism showed that iron is a key component in the denitrification process since it is required by the nitrate reductase enzyme and cytochromes. It is recommended that iron be added to the reactor in sufficient amounts to satisfy bacterial anabolic activities. Experimentation should be conducted to determine the amount required.

The bacterial growth model developed is based on the kinetic rate expressions for change in carbon, nitrate, and microbe concentrations with time. Due to limited data, the values for the rate constants have been assumed to remain constant with substrate concentrations. The model as it now stands does not accurately predict microbe growth during the induction period. It is recommended that experiments be performed to obtain more accurate values of the rate constants and their variation with substrate concentration.

The bacteria population seemed to behave similarly for duplicate experiments performed in 250-ml and 67-liter reactors. It may tentatively be concluded that reactor volume has no effect on bacterial growth during the induction period.

All experiments were run as batch processes. Since the startup procedure currently being considered for the Y-12 reactor calls for flow to begin when the nitrate level reaches 200 ppm, it is recommended that all experiments be repeated as flow experiments.

2. INTRODUCTION

Uranium purification at the Oak Ridge Y-12 Plant is accomplished by a solvent extraction process. The effluent from the purification process contains high nitrate concentrations with nitrate being in the form of acidic evaporation condensates or aluminum nitrate-containing extraction raffinates (1). New water quality regulations require modifications of traditional methods for disposal of these nitrate wastes. Bacterial reduction of aqueous nitrate to nitrogen has been proposed as a possible disposal method.

The ability of Pseudomonas Stutzeri to reduce nitrate to nitrogen gas has been studied by C.W. Francis of ORNL. These studies were conducted on a column denitrification unit packed with anthracite coal particles and on stirred reactors. Denitrification of calcium nitrate, sodium nitrate, ammonium nitrate, and uranyl nitrate wastes was investigated. An induction period of three to five days after the bacteria has been introduced into the system was necessary for the seed bacteria to reproduce at a rate sufficient to process the nitrate in the waste stream (2, 3). There

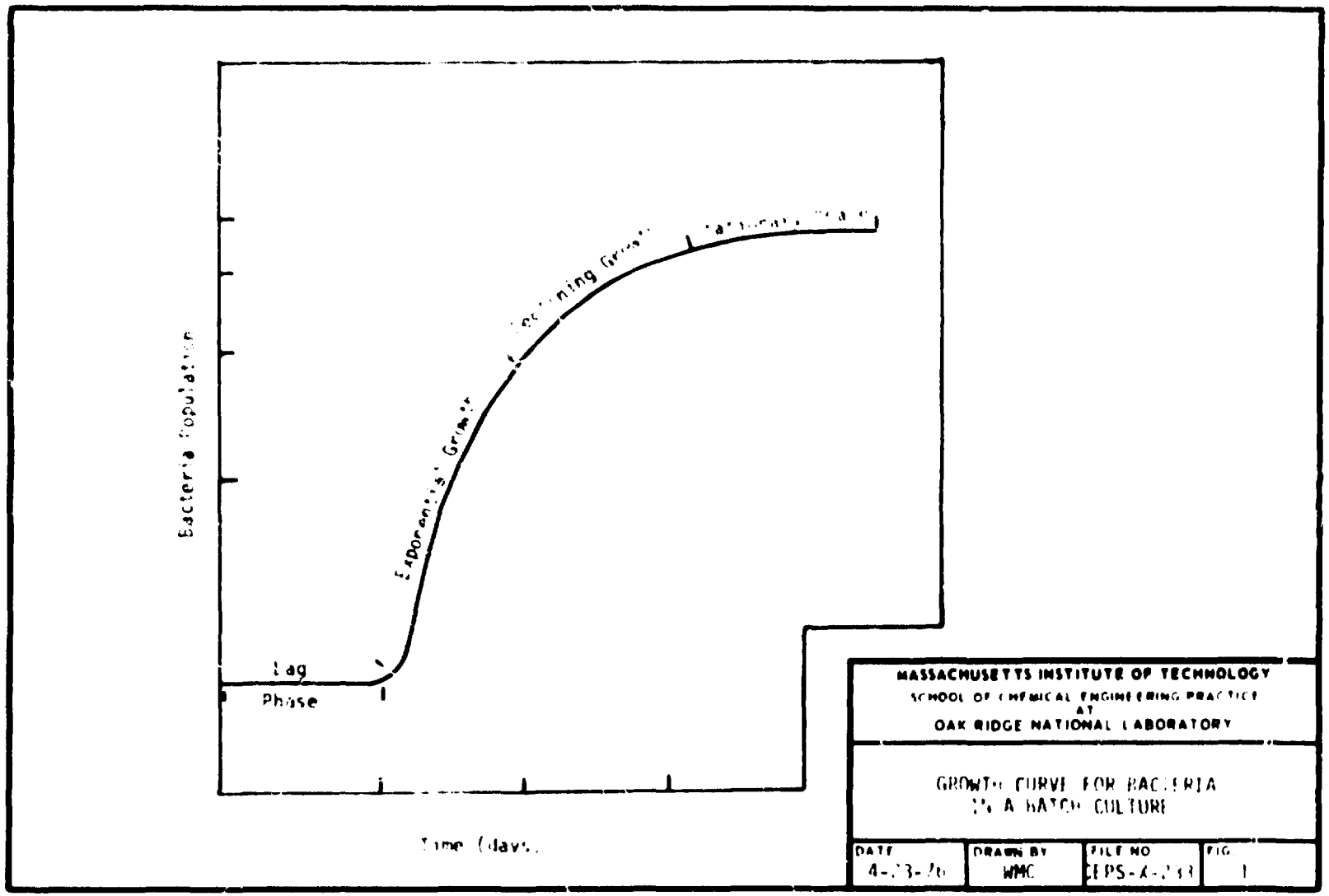
are three distinct phases of biological growth during this induction period as shown in Fig. 1. During the lag period, there is no change in the bacteria population. The lag period ends when the bacteria have become acclimated to the system and start to reproduce at an exponential rate. The declining growth period is marked by a dramatic decrease in bacteria production, ending at a point where the bacteria count attains a stationary phase value. The induction period is considered to be complete once the bacteria count reaches this stationary phase level.

It is possible that the heavy metals occasionally found in the Y-12 waste stream may cause the biological denitrification process to cease due to the adverse effect on Pseudomonas Stutzeri. This would result in a reactor shutdown while new bacteria are introduced. It is therefore desirable to be able to predict the kinetics of bacterial growth during the ensuing startup period.

Successful operation of the bacterial denitrification process requires that the microorganism, Pseudomonas Stutzeri, be maintained in an oxygen-free environment (4). Carbon, in the form of calcium acetate, and trace nutrients must be supplied for metabolic activities. Initial bacteria concentration, acetate and nitrate feed concentrations, carbon-to-nitrogen mole ratio, pH, and temperature have been identified as influencing the duration of the induction period (5).

The proposed startup procedure for the denitrification production plant allows up to five days for the induction period. An improved startup procedure may be suggested by experimentally determining the effect of bacteria concentration, initial acetate concentration, and initial nitrate concentration on bacterial growth during the induction period. The goal is to develop a model simulating bacterial growth during the induction period as a function of the aforementioned variables. To obtain this objective, a literature survey was performed to determine the extent of previous modeling efforts for bacterial growth during denitrification. Information on bacterial metabolism and denitrification reaction stoichiometry was also obtained. The effect of initial bacteria concentration on the induction period in a batch reactor was then experimentally determined by a set of experiments conducted in 250-ml flasks. The presently accepted carbon and nitrate concentrations of 133 and 500 ppm were maintained for the above experiments. The initial acetate and nitrate concentrations were varied, at a fixed carbon-to-nitrogen weight ratio of 1.18, in 250-ml flasks to determine the effect on the induction period.

It was desired to obtain scaleup factors for continuously-stirred tank reactors during the induction growth period. Experiments were performed in reactor volumes of 0.25, 7, and 67 liters for this purpose. A mathematical model simulating the change in bacteria concentration during the induction period was developed based on information obtained in the literature survey and on the experimental results. This model will aid in developing the startup procedure for the Y-12 denitrification production reactor.



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GROWTH CURVE FOR BACTERIA
IN A BATCH CULTURE

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3. APPARATUS AND PROCEDURE

3.1 Experimental Procedure

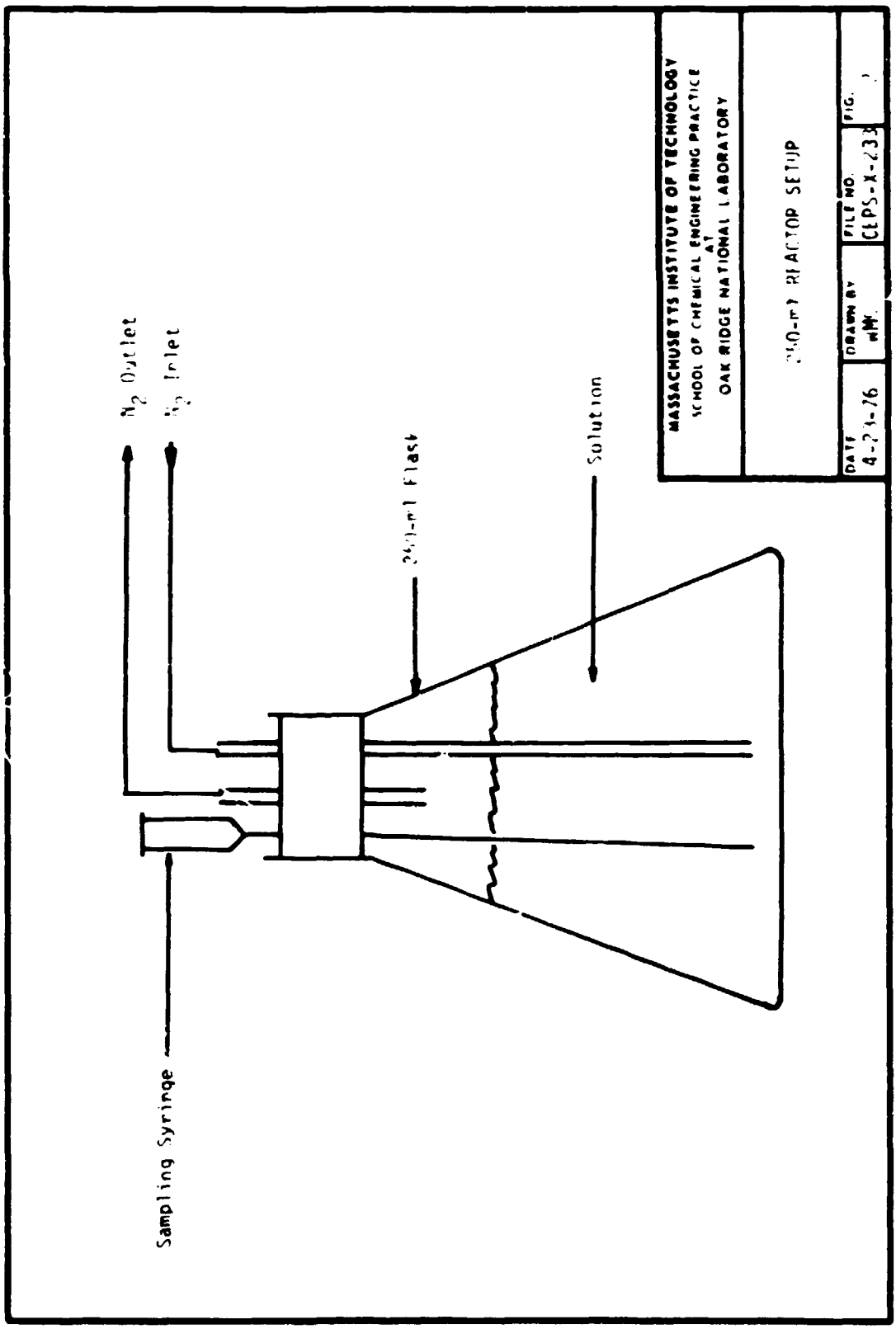
The experiments to determine the effect of initial nitrate and acetate concentrations and the effect of initial inoculum size were conducted in 0.25-liter Erlenmeyer flasks as shown in Fig. 2. To maintain an anaerobic environment, samples were drawn with a syringe through a length of capillary tubing which had its end submerged in the solution. The anaerobic environment was initiated by filling the flask with water and the necessary nutrient chemicals. Nitrogen was then bubbled through the solution in the flasks to remove any dissolved oxygen in the water. After approximately 10 min, the stopper was removed from the flask, the microbe inoculum added, and the stopper was replaced. Nitrogen was bubbled through the solution for another 5 min and the nitrogen inlet and outlet lines were clamped.

The 250-ml flasks were agitated and maintained at a constant temperature, 30°C, in a New Brunswick Scientific Aquatherm Water Shaker Bath. The bath was equipped with a Plexiglas hood which allowed the flasks to be blanketed in nitrogen at all times. During sampling, the Plexiglas hood had to be removed. When drawing samples, a nitrogen stream from a tank stored nearby was bubbled through the solution to eliminate the possibility of oxygen entering the flasks.

Experiments to determine the effect of reactor size were performed in 7- and 67-liter fermentors. A schematic of the 7-liter fermentor is shown in Fig. 3. The large fermentors were air-tight and were flushed with nitrogen after being purged by bubbling nitrogen gas through the solution. The gas produced by the bacteria was first scrubbed with sodium hydroxide solution to remove carbon dioxide. The volume of nitrogen which was left was collected and measured.

Approximately 8 ml of solution were drawn twice a day from each experimental apparatus. After measuring the pH, the samples were centrifuged at 4000 rpm, or about 1800 g's, for 4 min. Past results have shown that centrifuging removes practically all of the calcium carbonate suspended in solution. The original sample was then divided into three equal volume samples so microbe population, acetate concentration, and nitrate concentrations could be measured.

The microbe concentration was determined with a Coulter Electronics Model Z Counter. This method was found to be much faster and less tiresome than the standard microscopic technique. The Coulter Counter measures the solid particles in solution and operates on the principle of electrical conductivity differences between microbes and an electrolyte solution used in diluting each sample. The microorganism, suspended in the electrolyte, was forced through a small aperture in which an electrical current path had been established. As each microbe, which acts as an insulator, displaced electrolyte in the aperture, an electrical pulse proportional to


N_2 Outlet
N_2 Inlet
Solution
Sampling Syringe
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AT
OAK RIDGE NATIONAL LABORATORY
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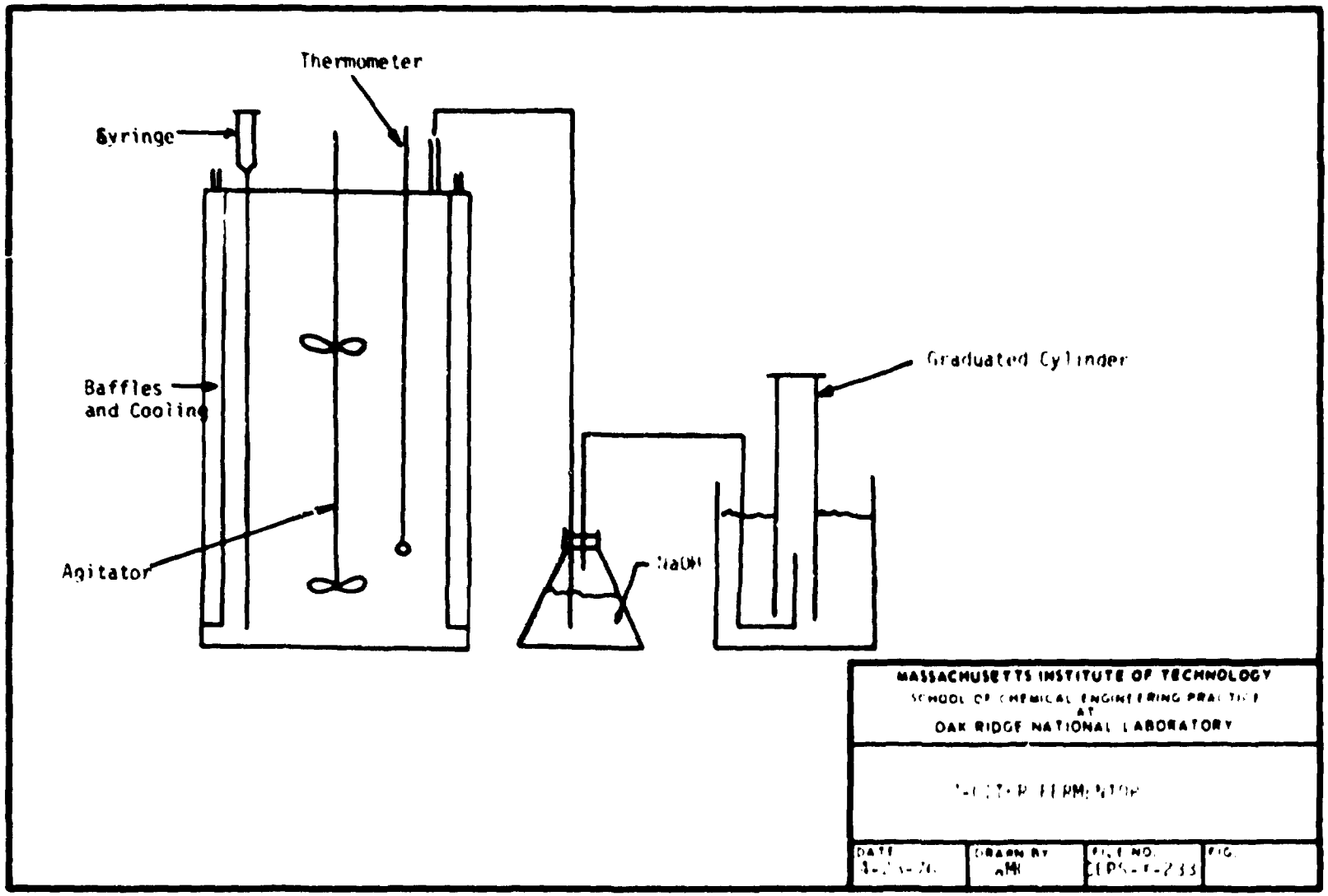
FIG.


Thermometer
Syringe
Baffles
and Cooling
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NaOH
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the microbe size was produced and counted. Since the volume drawn by the Coulter Counter was known and one pulse was produced for each microbe, a microbe concentration could be calculated. The electrolyte used in the dilution of the sample was Cutter Laboratories' sodium chloride solution.

It was impossible to measure the carbon in the sample by spectrophotometric techniques due to the absorbance of nitrate ions. The acetate concentration in the sample was therefore measured with a Dohrmann Envirotech Organic Carbon Analyzer. The sample was first filtered through a 0.45 micrometer Millipore filter to remove microbes, leaving acetate as the only organic carbon matter. It was experimentally determined that this method removed over 99% of the biomass from samples with high initial microbe population.

A sample was filtered through a 0.11 micrometer filter, but it was found that this filter added organic carbon to the solution due to its polymer composition. For this experiment the Envirotech Organic Analyzer was used to measure the carbon content of the sample before and after filtering. The sample was then acidified to a pH of two with hydrochloric acid. This was done so that the inorganic carbon in the sample could be easily removed in the form of carbon dioxide. After injecting the sample into the analyzer, the water in the sample was removed in a vaporization zone. The acetate was then pyrolyzed to carbon dioxide. This carbon dioxide was then reacted with hydrogen over a nickel catalyst producing methane which was burned in a flame ionization detector producing an electrical signal proportional to the carbon in the methane. This value in turn was proportional to the carbon content in the original sample.

The nitrate concentration of the sample was measured with an Orion Model 93-07 Nitrate Electrode. It was impossible to measure the nitrate concentration by spectrophotometric techniques due to the absorption, at the same wavelength, of the other species in the sample. After adjusting the pH of the sample to 4.5 with acetic acid, the nitrate electrode and a reference electrode were immersed in the sample solution. The voltage difference between the two electrodes, which is proportional to the nitrate concentration of the sample, was measured with a digital voltmeter. The nitrate concentration could then be read from a calibration curve prepared with standard solutions of known nitrate concentrations.

3.2 Experiments

A list of the experiments conducted is given in Table 1. Experiment 1, performed in a 250-ml flask, was run following the startup procedure currently being developed at the Y-12 Plant. In experiments 2, 3, and 4, the initial inoculum size was varied while maintaining the initial carbon and nitrate concentrations at 133 and 500 ppm. In experiments 5, 6, and 7, the initial nitrate and carbon concentrations were varied while maintaining the currently accepted carbon-to-nitrogen weight ratio of 1.18. Experiment 8, a duplication of experiment 1, was conducted in a 7-liter fermentor for the purpose of studying the effect of reactor volume on bacterial growth during the induction period.

Table 1. Experiments

No.	Volume	Nitrate (ppm)	Carbon (ppm)	Inoculum Size (ml/l)
1	250 ml	500	133	8.6
2	250 ml	500	133	0.86
3	250 ml	500	133	86.0
4	250 ml	500	133	860.0
5	250 ml	200	53	8.6
6	250 ml	1000	266	8.6
7	250 ml	6000	1596	8.6
8	7 liter	500	133	8.6
9	250 ml	500	133	8.6
10	250 ml	6000	1596	8.6
11	67 liter	6000	1596	8.6

In experiments 1 through 8, iron, which is necessary for cell growth, was supplied in the form of a paper clip placed in the reactor. The iron is a key component in the bacterial electron transport system; for further explanation see Sect. 4.1. The paper clip simulated the steel wall of the reactor which is the iron source in the proposed Y-12 system. For experiments 9, 10, and 11, an iron concentration of 50 ppm was supplied to the reactors in the form of ferric sulfate to determine if additional iron had to be added to the solution to increase bacterial growth.

In all experiments 20 ppm of phosphate, 10 ppm of magnesium sulfate, and 1 ppm of sodium molybdate were provided as trace nutrients for metabolic activities.

4. THEORY

4.1 Metabolism of Bacteria

The development of a comprehensive model of bacterial growth during the induction period requires the knowledge of the reaction stoichiometry. There are two approaches to obtain the stoichiometry of interest: through

the experimental measurements of the chemical species involved and through a theoretical investigation of the metabolic processes of the bacteria.

From the considerable amount of experimental data available, past groups have suggested reaction equations for both the induction period and the constant population phase (6). It was impossible to perform an assay of all chemical species present. Instead, a theoretical approach has been taken to verify the accuracy of the reaction equations proposed by Clark et al.(6).

The metabolic processes of a bacteria accomplish two primary functions: supply of energy to maintain the organism and synthesis of biomass for reproduction. The tricarboxylic acid cycle (TCA), or Krebs cycle, was assumed responsible for supply of energy through the production of ATP and the glyoxylate cycle for the synthesis of biomass. Each of these cycles has been studied to obtain the stoichiometry of the reactions.

When bacterial growth reaches the stationary, constant population phase, there is no net change of the number of bacteria. Since it was assumed the TCA cycle involves no consumption of carbon for growth, but energy transfer alone, theory predicts that an overall mass balance over the cycle will result in the steady state reaction equation.

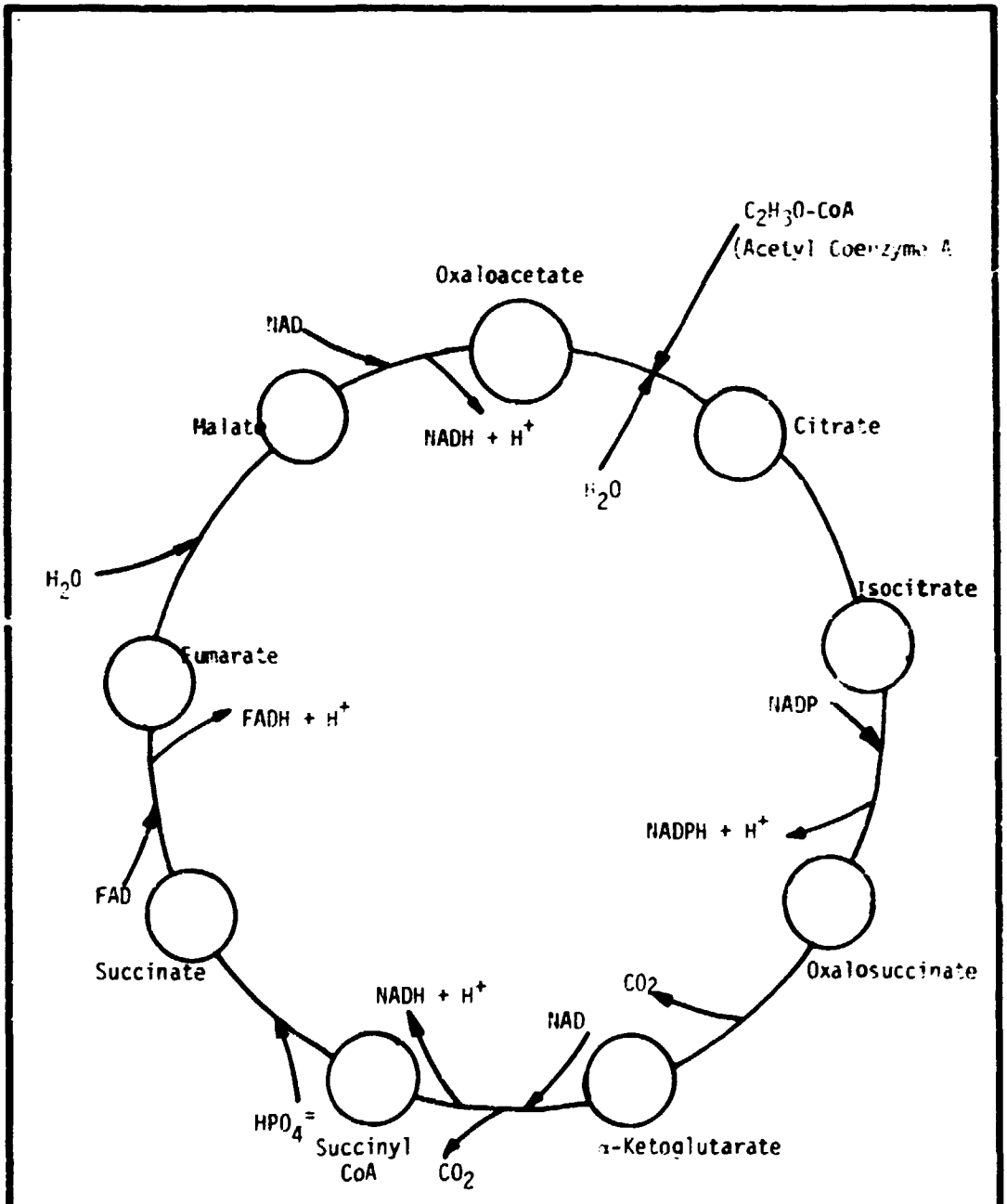
Pseudomonas Stutzeri is a facultative organism; i.e., an organism able to respire with or without molecular oxygen as the terminal electron acceptor for the electron transport system. In this experiment, the bacteria uses acetate as both an energy and carbon source. Energy is produced when acetate is consumed in the TCA cycle. Acetate enters the cycle after reacting with ATP and coenzyme A to form acetyl-coenzyme A. The different reactions of the TCA cycle are shown in Fig. 4, and the corresponding equations of each component reaction are found in Appendix 10.1.

The overall mass and charge balance on the TCA cycle yields the following equation for the calcium-acetate feed:

$$\frac{1}{2}Ca(C_2H_3O_2)_2 + 2H_2O + H^+ \rightarrow \frac{1}{2}Ca^{+2} + 2CO_2 + 8(H^+ + e^-) \qquad (1)$$

Actually, the hydrogen atoms of the righthand side are "transported" by coenzymes NAD^+, $NADP^+$, or FAD from the protoplasm to the cellular membrane where the electron transport system (ETS) is located.

Energy is stored when ADP reacts with phosphate, HPO_4^{-2}, to form ATP. This occurs only once during the TCA cycle. Most of the energy is made available by the oxidation of NADH, NADPH, and $FADH_2$ given off by the cycle in the electron transport system (1). A good deal of free energy from such oxidations is stored in ATP, as the reaction of ADP to ATP is coupled with the oxidations. For the process to be possible, there must be a terminal electron acceptor for the ETS in the surrounding solution. In this experiment, nitrate acts as the electron acceptor, and thus,



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TRICARBOXYLIC ACID CYCLE

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denitrification of a solution containing NO_3^- to $\frac{1}{2}N_2$ is accomplished by the ETS. Figure 5 shows a schematic of the ETS, and the chemical equations governing the ETS are elaborated on in Appendix 10.1.

Coupling the TCA-cycle with the ETS results in a general reaction equation for steady state reaction. The equation obtained is

$$\frac{1}{2}Ca(C_2H_3O_2)_2 + \frac{4}{5}Ca(NO_3)_2 + 2H_2O + H^+ \rightarrow \frac{13}{10}Ca^{+2} + 2CO_2 + \frac{4}{5}N_2 + \frac{16}{5}H_2O + \frac{8}{5}OH^- \quad (2)$$

If allowance is made for the formation of calcium carbonate,

$$1.3\ Ca^{+2} + 1.3\ CO_2 + 1.3\ H_2O \rightarrow 1.3\ CaCO_3 + 2.6\ H^+ \quad (3)$$

the equation reduces to

$$0.5Ca(C_2H_3O_2)_2 + 0.8Ca(NO_3)_2 \rightarrow 1.3CaCO_3 + 0.8N_2 + 0.7CO_2 + 1.5\ H_2O \quad (4)$$

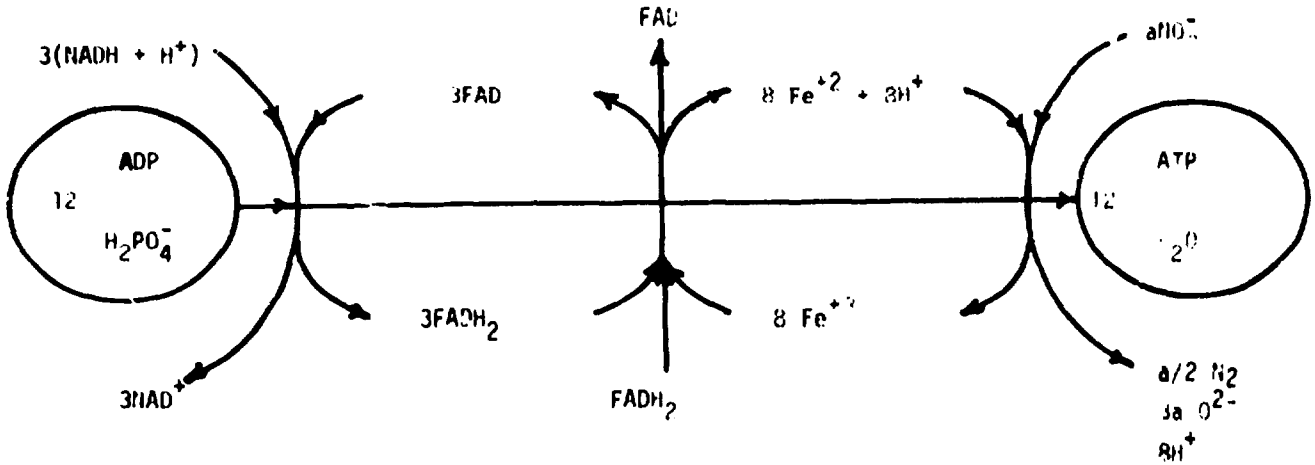
This is precisely the equation arrived at by Napier et al. from experimental data.

For the growth phase, the reactions of both the TCA and glyoxylate cycles occur. In addition to the production of ATP, the bacteria uses the carbon from acetate to form biomass. The glyoxylate cycle is also known as the TCA bypass since the step of α-ketoglutarate synthesis from isocitrate is skipped as shown in Fig. 6.

Two acetates enter into the glyoxylate cycle as acetyl-coenzyme A. No CO_2 is given off as all the carbon is employed in protein synthesis. Thus, the four-carbon compound released by the cycle is oxaloacetate (8). The overall reaction scheme for one glyoxylate cycle is

$$Ca(C_2H_3O_2)_2 + H_2O + 2H^+ \rightarrow Ca^{+2} + \underset{\text{oxaloacetate}}{C_4O_5H_6} + 6(H^+ + e^-) \quad (5)$$

Six $(H^+ + e^-)$'s are also attached to NAD^+ in the form $NADH + H^+$. If all six NADH are used in the ETS for production of energy, and thereby reduction of NO_3^-, the overall equation from combining reactions of the glyoxylate cycle with the reactions of the ETS gives



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ELECTRON TRANSPORT SYSTEM			
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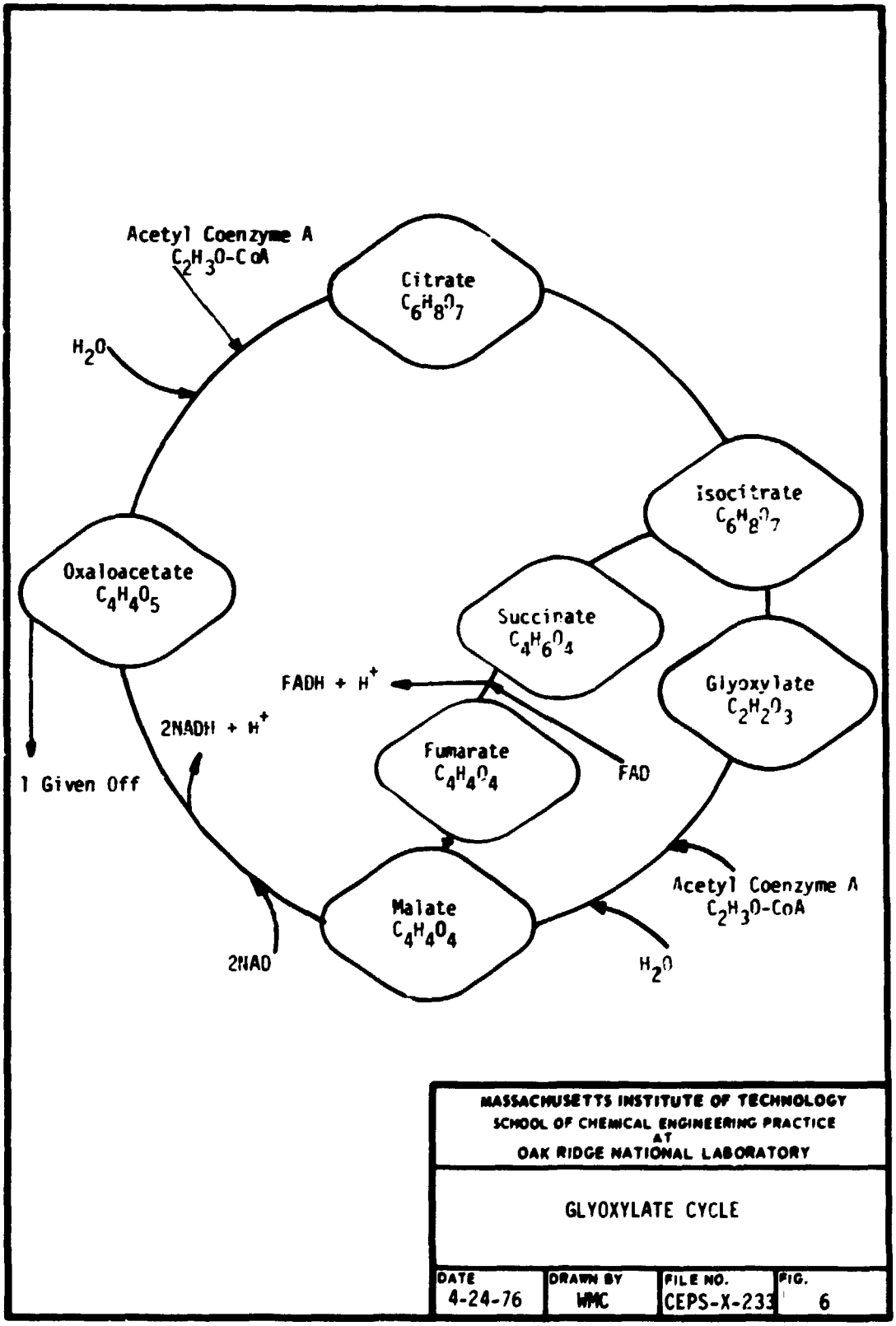
Acetyl Coenzyme A
C_2H_3O-CoA
H_2O
Citrate
$C_6H_8O_7$
Isocitrate
$C_6H_8O_7$
Oxaloacetate
$C_4H_4O_5$
Succinate
$C_4H_6O_4$
Glyoxylate
$C_2H_2O_3$
FADH + H^+
2NADH + H^+
Fumarate
$C_4H_4O_4$
FAD
1 Given Off
Malate
$C_4H_4O_4$
Acetyl Coenzyme A
C_2H_3O-CoA
2NAD
H_2O
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GLYOXYLATE CYCLE
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FIG. 6

$$Ca(C_2H_3O_2)_2 + \frac{3}{5}Ca(NO_3)_2 + 2H^+ \rightarrow C_4O_5H_4 + \frac{7}{5}H_2O + \frac{3}{5}N_2 + \frac{6}{5}OH^- + \frac{8}{5}Ca^{+2} \quad (6)$$

The derived equation cannot, however, represent the induction period of growth because carbon, nitrogen, phosphate, and other minerals to a lesser degree, are required in the reproduction of microbes. During the induction period, the assumption that all NADH's from the glyoxylate cycle are involved in the ETS NO_3^- to N_2 reaction is not a good one, as NADH's are known to be required by other enzymatic reactions that reduce nitrates to ammonia which is necessary for peptide and other anabolic needs (9). Furthermore, the number of TCA cycles per glyoxylate cycle, or vice versa, must be known to obtain the reaction equation of induction period. Rather than one general equation as in the steady state, the independent reaction equations cannot be added to give one equation. This is not allowed when the kinetics of the reactions are time-dependent. Hence the reaction equation for the induction period cannot be predicted theoretically.

To arrive at one equation for the startup period, Napier et al. (6) averaged the measurements of reacting species taken during this period. The suggested equation is

$$0.55\ Ca(C_2H_3O_2)_2 + 0.8\ Ca(NO_3)_2 \rightarrow 0.04\ C_5H_7O_2N(\text{biomass}) + 0.65CO_2 + 0.78N_2 + 1.35CaCO_3 + 1.45H_2O + 0.12(OH) \quad (7)$$

The biomass formula, obtained by Napier from a literature source, is incomplete as phosphorus, and trace amounts of other minerals are consumed during bacteria growth and represent 4.6% of the wet weight of a bacteria (11).

4.2 Bacterial Growth Model

The Monod model is the best known and most widely used model for simulating bacterial growth during the induction period. The model does not include the lag period. Monod's model is based on the stoichiometry

$$R_s = -a\ R_p \quad (8)$$

Monod suggested that the growth rate dependence on the substrate concentration follows the Michaelis-Menton form (12):

$$R_p = \frac{\mu C C_s}{K + C_s} \tag{9}$$

In batch growth with

$$\left.\begin{aligned} C &= C_o \\ C_s &= C_{s_o} \end{aligned}\right\} \text{ at } t = 0$$

and

the following equation applies

$$\frac{dC}{dt} = -\frac{1}{a}\frac{dC_s}{dt} = \frac{\mu C C_s}{K + C_s} \tag{10}$$

Integrating the first part of Eq. (10) yields

$$C_{s_o} - C_s = a(C - C_o) \tag{11}$$

This implies that all of the acetate consumed goes into biomass production. This is clearly not the case for the Pseudomonas Stutzeri denitrification process. The acetate reacts to form both biomass and carbon dioxide. Therefore, the Monod model for bacterial growth is not applicable for the bacteriological process in question.

Since acetate reacts to form biomass and carbon dioxide, and since acetate is the only carbon source in the system, kinetic equations describing the rate of change of carbon, nitrate, and microorganism concentrations in solution can be proposed.

$$\frac{dC_s}{dt} = -K_1 C C_s - K_2 C_a \tag{12}$$

$$\frac{dC}{dt} = K_1 C C_s \tag{13}$$

$$\frac{dC_N}{dt} = -K_2 C \tag{14}$$

The constants γ and a are stoichiometric conversion factors relating the amount of carbon which reacts to form biomass and the carbon which reacts

to form other products. Therefore, the units of γ are ppm of carbon per ppm of biomass and the units of α are ppm of carbon per ppm of nitrate.

Equations (12) through (14) apply once the microorganism growth has started and do not apply to the lag period. No change in the bug count occurs during the lag period. Equations (12) and (14) are valid during the lag period when dC/dt is set equal to zero. The duration of the lag period can be determined by experimentation. The value of dC/dt can be set equal to zero for this period and equal to Eq. (13) once the growth begins. This procedure results in a model which will predict carbon, nitrate, and microorganism concentrations from the start of the experiment until the beginning of the stationary phase.

A program package entitled Bugs is available at ORNL for performing numerical integration on systems of differential equations in the form of equations (12) through (14). The program inputs are K_1, K_2, initial carbon, nitrate, and microorganism concentrations. The program outputs are carbon, nitrate, and microorganism concentrations at any time, t. The values of K_1 and K_2 may be determined from the data of an experiment showing a substantial bacteria count increase. Once determined, the values of K_1 and K_2, which should be constant, together with Eqs. (12) through (14) should predict bacterial growth during the induction period for all experiments.

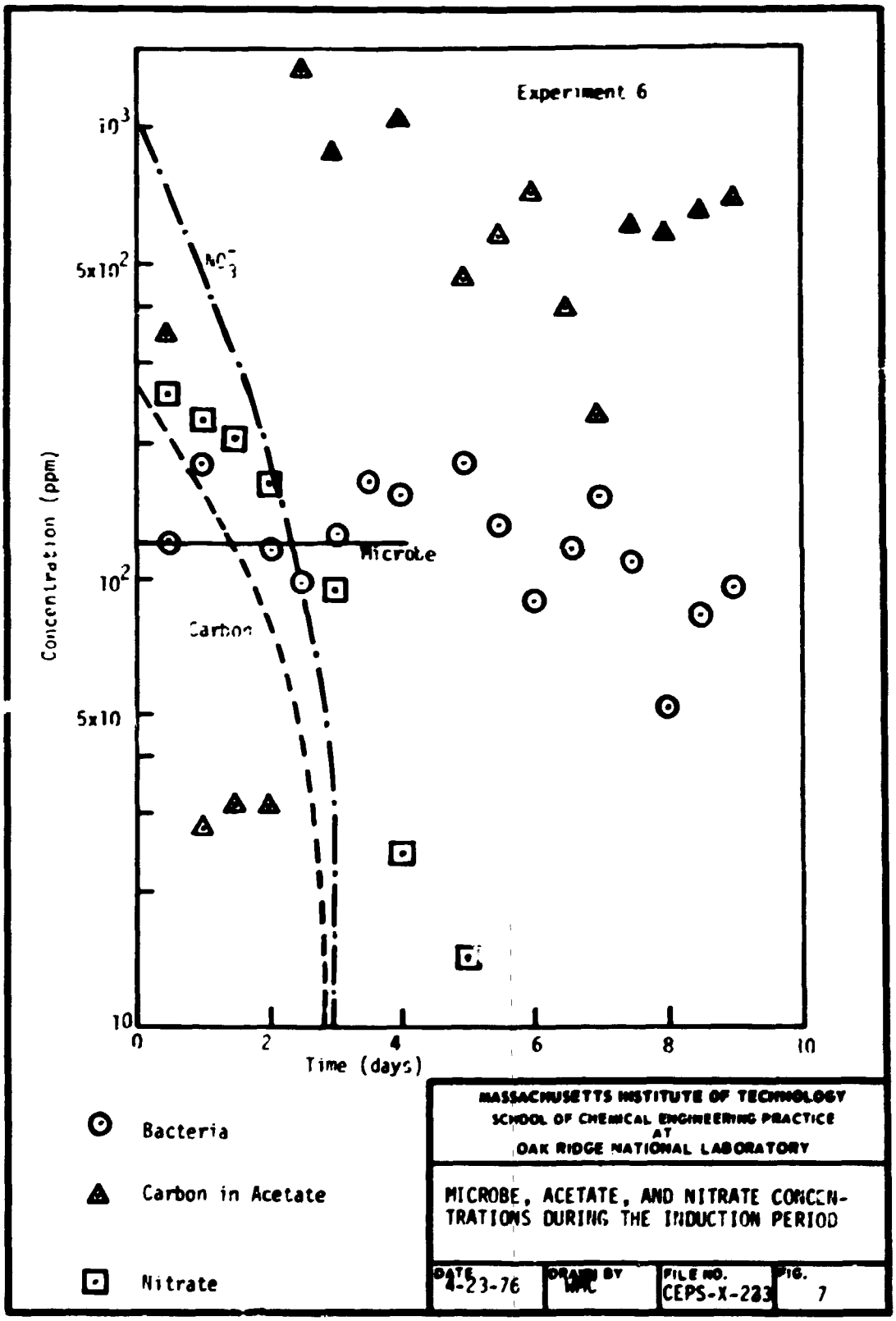
K_1 is determined by plotting $(dC/dt)/C$ as a function of C_S for the experiment in question. K_1 is the slope of the curve obtained. K_2 is the slope of the curve obtained when (dC_N/dt) is plotted as a function of C

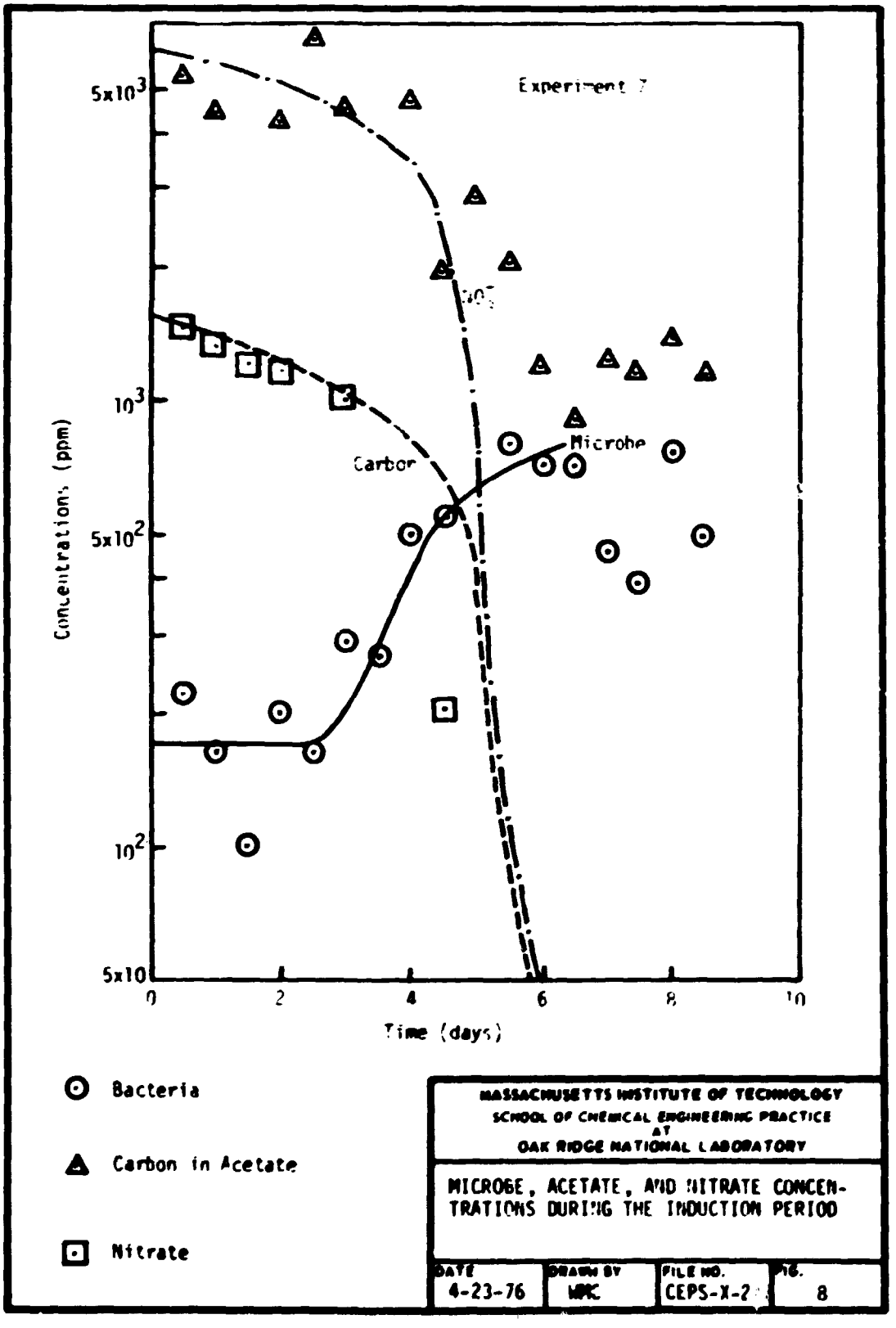
5. RESULTS

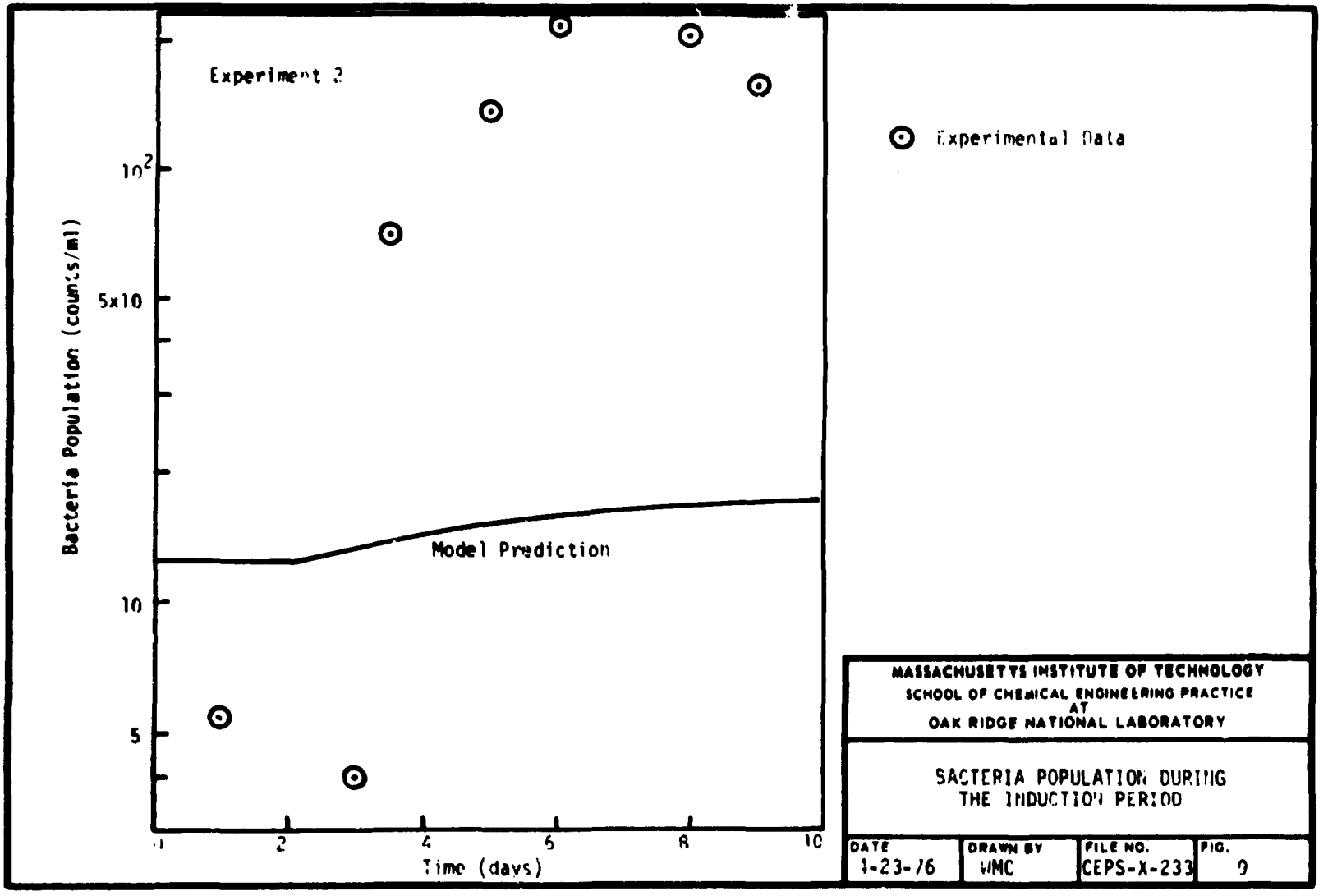
The majority of experiments exhibited no microorganism growth. The results of experiment 6 are typical of this outcome and are presented in Fig. 7. The circular data points represent microbe growth, the triangular points represent nitrate concentrations, and the substrate carbon levels are plotted as squares. These conventions will be used on all figures presenting experimental data. The lines plotted on the figures with experimental results represent the predictions of the mathematical model for microbe growth.

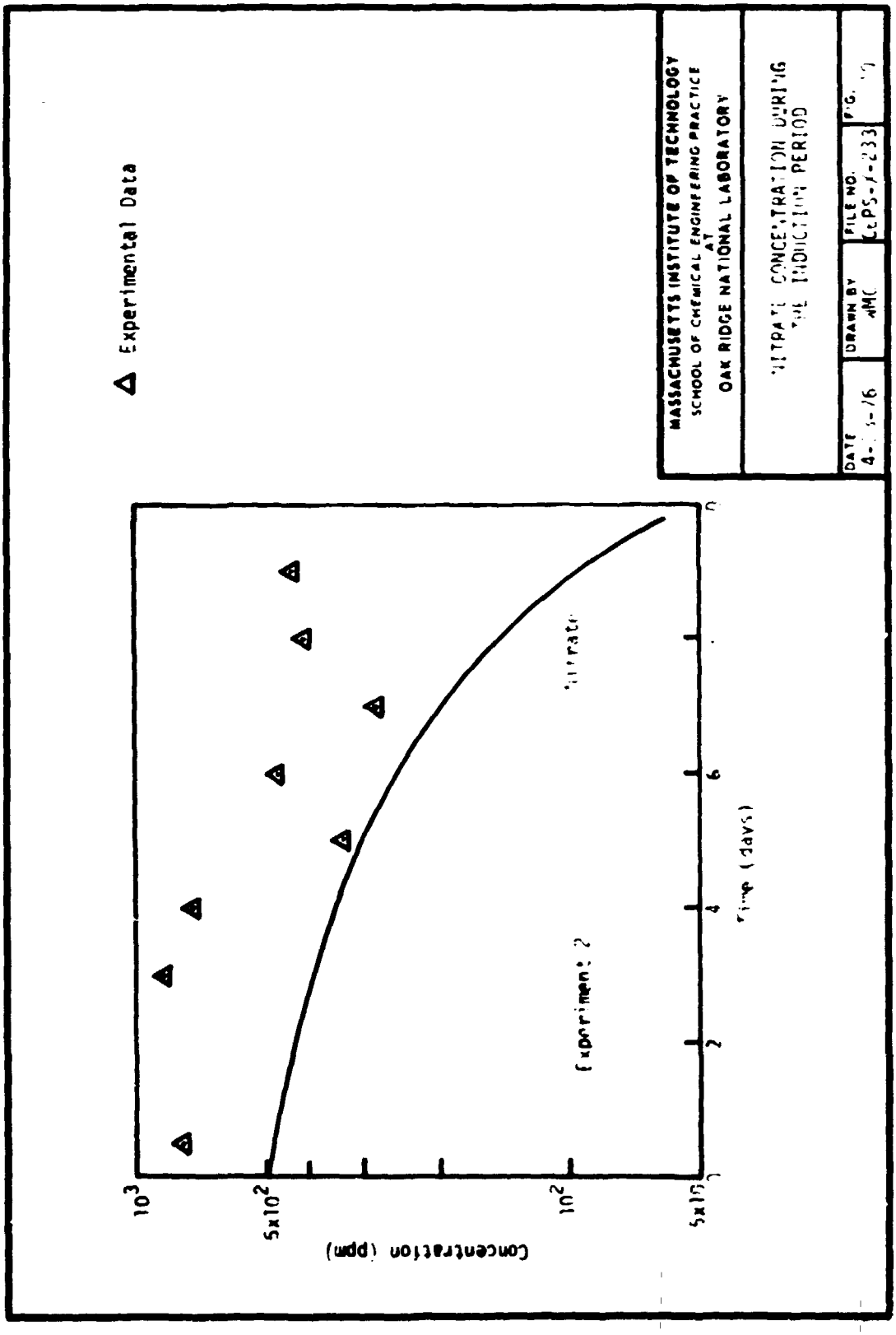
The data for experiment 7, which exhibited a typical growth pattern, are given in Fig. 8. This experiment was used to determine the constants used in the microbe growth model. The only other experiment clearly exhibiting growth was experiment 2. The results for microbe growth, nitrate concentration, and substrate carbon concentration are presented in Figs. 9, 10, and 11, respectively.

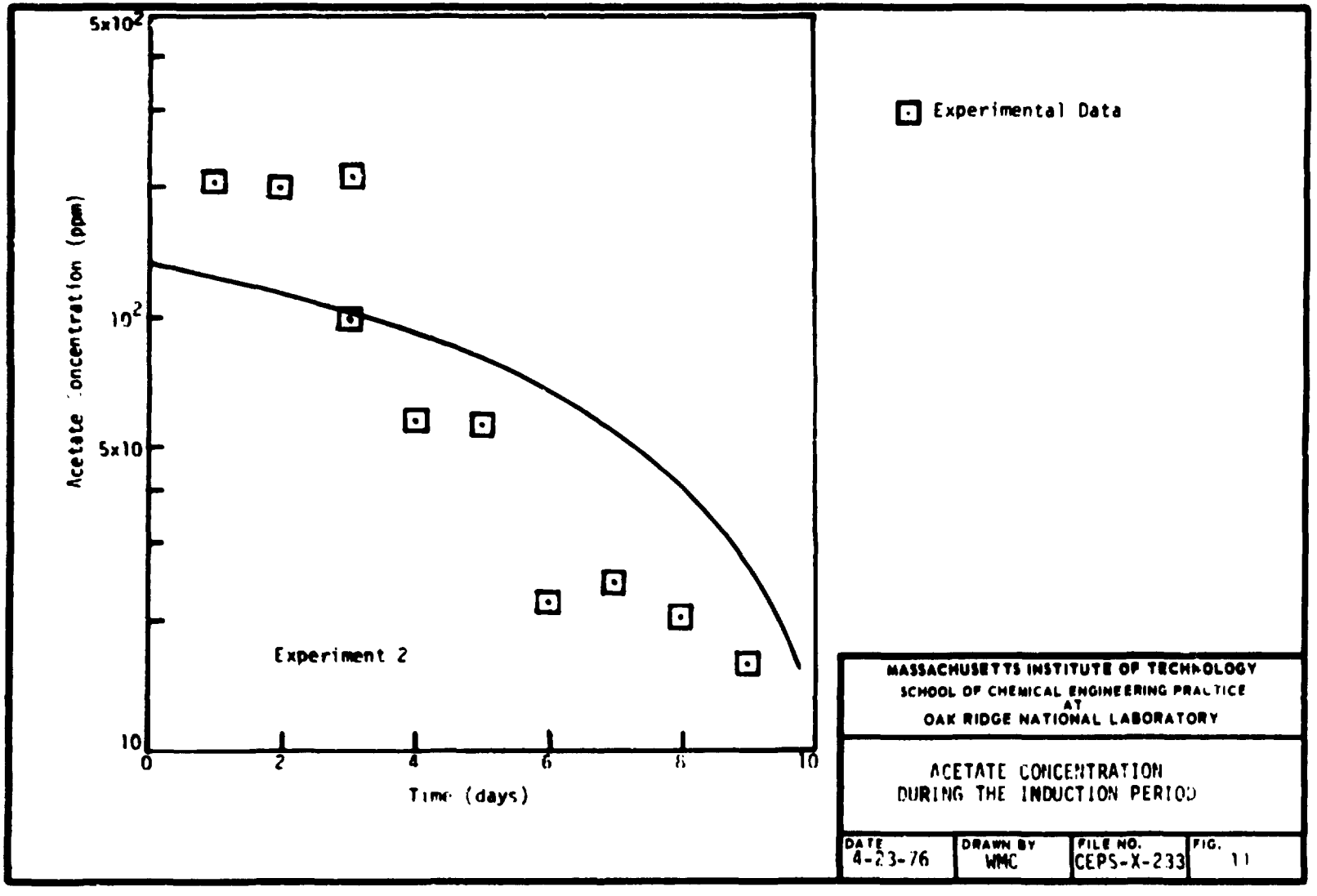
The data for experiments 2, 6, and 7 are also presented in tabular form in Tables 3, 7, and 8, respectively, and are found in Appendix 10.5. Tables 11 and 12 contain the data for experiments 10 and 11, which were made to determine the effect of reactor volume on microbe growth. These two experiments were still underway when this report was written. The data and model predictions for all experiments are given in the Appendix.


Experiment 6
10^3
$5x10^2$
10^2
$5x10$
10
NO_3^-
Nitrate
Carbon
Concentration (ppm)
0
2
4
6
8
10
Time (days)
Bacteria
Carbon in Acetate
Nitrate
MASSACHUSETTS INSTITUTE OF TECHNOLOGY
SCHOOL OF CHEMICAL ENGINEERING PRACTICE
AT
OAK RIDGE NATIONAL LABORATORY
MICROBE, ACETATE, AND NITRATE CONCENTRATIONS DURING THE INDUCTION PERIOD
DATE 4-23-76
DRAWN BY WMC
FILE NO. CEPS-X-283
FIG. 7


Experiment 7
Carbon
Microbe
Concentrations (ppm)
Time (days)
5x10^3
10^3
5x10^2
10^2
5x10
0
2
4
6
8
10
Bacteria
Carbon in Acetate
Nitrate
MASSACHUSETTS INSTITUTE OF TECHNOLOGY
SCHOOL OF CHEMICAL ENGINEERING PRACTICE
AT
OAK RIDGE NATIONAL LABORATORY
MICROBE, ACETATE, AND NITRATE CONCENTRATIONS DURING THE INDUCTION PERIOD
DATE 4-23-76
DRAWN BY WRC
FILE NO. CEPS-X-2
FIG. 8


Experiment 2
Experimental Data
Bacteria Population (counts/ml)
10^2
5x10
10
5
Model Prediction
2
4
6
8
10
Time (days)
MASSACHUSETTS INSTITUTE OF TECHNOLOGY
SCHOOL OF CHEMICAL ENGINEERING PRACTICE
AT
OAK RIDGE NATIONAL LABORATORY
BACTERIA POPULATION DURING
THE INDUCTION PERIOD
DATE 1-23-76
DRAWN BY WMC
FILE NO. CEPS-X-233
FIG. 9


Δ Experimental Data
MASSACHUSETTS INSTITUTE OF TECHNOLOGY
SCHOOL OF CHEMICAL ENGINEERING PRACTICE
AT
OAK RIDGE NATIONAL LABORATORY
DATE
DRAWN BY
FILE NO.
FIG.
Concentration (ppm)
10^3
$5x10^2$
10^2
2
4
6
Time (days)
Experimental


5x10²
10²
5x10
10
Acetate concentration (ppm)
0
2
4
6
8
10
Time (days)
Experiment 2
Experimental Data
MASSACHUSETTS INSTITUTE OF TECHNOLOGY
SCHOOL OF CHEMICAL ENGINEERING PRACTICE
AT
OAK RIDGE NATIONAL LABORATORY
ACETATE CONCENTRATION
DURING THE INDUCTION PERIOD
DATE 4-23-76
DRAWN BY WMC
FILE NO. CEPS-X-233
FIG. 11

6. DISCUSSION OF RESULTS

In seven of the eleven experiments, no appreciable microbe growth was observed. The seven experiments with no growth, experiments 1, 3, 4, 5, 6, 8, and 9, all exhibited similar concentration histories for microbes and substrates. Thus the data for experiments 6 are presented in Fig. 7 as representative of these experiments. As can be seen on Fig. 7, the substrate carbon level rapidly falls off toward zero before any growth occurs in the microorganism population. The nitrate data taken up to day five are probably inaccurate due to equipment problems.

It was originally thought that potassium flouride had to be added to the selective ion electrode inner chamber to reduce the interference from the carbonate ions. During the first five days of analyses, the inner chamber as well as the sample contained potassium fluoride. It was found that the inner chamber should contain acetic acid adjusted to a pH of 4.5. On day five this equipment problem was corrected. After day five, the nitrate readings remained fairly constant at about 500 ppm. By this time, the substrate carbon concentration was very low. In effect, microbe growth did not occur because of substrate depletion. This is supported by the fact that growth only occurred in the experiments with the highest substrate to microbe ratios. The substrate carbon levels continued to decline to about zero while the nitrate concentration leveled out at a fixed value in most of the experiments. This indicates that the carbon consumption is higher than the 1.18 carbon-to-nitrogen mass ratio suggested in a previous report (3). The pH of the bacteria solution dropped very slightly over the course of the experiment as shown in Table 8 in Appendix 10.5.

The proposed startup procedure for the Y-12 Plant Denitrification Reactor calls for starting the reactor as a batch process with an initial substrate carbon level of 133 ppm and an initial nitrate concentration of 500 ppm. When the nitrate concentration in the reactor falls to 200 ppm, a feed flow of nitrate and acetate will be introduced which should prevent substrate depletion. Since the experiments performed for this study were all batch experiments, they are not comparable with the proposed Y-12 reactor startup instructions.

Microbe growth occurred in experiments 2, 7, 10, and 11. Experiments 10 and 11 had only been underway for 4.5 days at the writing of this report; therefore the growth reported in these experiments is tentative. The data for experiments 10 and 11 are given in Tables 11 and 12 in Appendix 10.5, respectively.

The microbe growth exhibited in experiments 2 and 7 show the three phases expected for a population increase. After the start of the experiment, a lag phase of about 2.5 days occurs during which no microorganism growth occurs. Following the lag phase is a period of exponential growth terminating in a declining growth period. The population levels off at a final stationary phase value. The substrate concentrations fall slightly during the lag period and then decrease rapidly during the exponential growth phase. It appears that the bacteria consume substrate even when

there is no appreciable microbe growth. During growth, the pH of the microorganism solution falls substantially, as shown in Tables 3 and 8 of Appendix 10.5. This may be due to the production of acidic waste products from CO_2 produced by the bacteria.

The populations in experiments 2 and 7 may have leveled out because the microbe concentration has reached its stationary phase value, or because the substrates have been depleted. In both experiments 2 and 7, the carbon substrate concentration falls to a very low level at the start of the declining growth phase. Thus, the reason for the end of microorganism growth appears to be the depletion of the carbon substrate.

In experiments 1 through 8, iron, which is necessary for bacterial metabolism, was supplied by a paper clip which was introduced into each flask after the experiments had run one day. After examining the data from experiments 1 and 7, these experiments were repeated using ferric sulfate as an iron source rather than the paper clip. In experiments 2 and 7, the total lag phase was about 2.5 days which was about 1.5 days after placing the wire in the flask. As seen in Tables 11 and 12 (Appendix 10.5), the lag period for the experiments with ferric sulfate added as an iron source is also about 1.5 days. The difficulty in estimating the transfer of iron from the paper clip suggests that ferric sulfate should be used in the future to simulate the operation at the Y-12 Plant since in addition to the steel reactor wall the waste stream fed to the reactor contains aqueous iron salts (13).

The effect of reactor size was studied in two sets of experiments. In experiments 1 and 8, the proposed Y-12 startup procedure was run in both a 250-ml Erlenmeyer flask and a 7-liter batch fermentor. Since no growth occurred in either experiment, it is impossible to accurately determine the effect of reactor size on microbe growth. However, the data for experiments 10 and 11 tentatively show no effect of reactor volume on the microorganism growth rate. Experiments 10 and 11 were started using a high substrate-to-microbe ratio, as in experiment 7, with experiment 10 in an Erlenmeyer flask and experiment 11 in a 67-liter batch fermentor. The microbe growth measured to date, as given in Tables 11 and 12, show close agreement, indicating no scaleup effects. It should be noted that these experiments were not completed at the writing of this report, and thus these conclusions are based on incomplete experiments.

The microbe growth model constants were determined using the data from experiment 7. The values of the constants were: $K_1 = 2.7 \times 10^{-5}$ $(hr)^{-1}$ $(ppm\ carbon)^{-1}$; $K_2 = 0.12$ $(hr)^{-1}(ppm\ nitrate)(ppm\ microbe)^{-1}$. As shown in Fig. 7, the model accurately predicts the microbe and substrate concentrations up until the end of the exponential growth phase, as it should, since the data from experiment 7 was used to evaluate the model constants. However, the model predictions for the other ten experiments compare poorly with the experimental data as shown in Figures 6, 8, 9, and 10. It seems that values of the model constants should depend on the initial microbe and substrate concentrations, as assumed in some of the more complicated models for microbe growth (12). There was insufficient data from these experiments to determine the dependence of the model constants on substrate and microbe concentration.

7. CONCLUSIONS

1. The experimental results obtained apply only to a batch denitrification process. They are not applicable to the proposed Y-12 denitrification reactor startup procedure, since the Y-12 startup instructions call for a nitrate and acetate feed to be started after the nitrate concentration has fallen to a fixed level.

2. A large excess of substrates must be present for microbe growth in batch reactors.

3. No firm conclusions can be drawn about the effect of iron on microbe growth. However, iron is a key component in the denitrification process in that it is required in the electron transport system (16).

4. The model does not accurately predict microbe growth during the induction period. The constants in the model were developed from one set of experimental data, and thus do not account for any dependency of the model constants on substrate concentration, microbe concentration, or reactor volume.

8. RECOMMENDATIONS

1. All experiments should be repeated with a feed flow started when the nitrate concentration reaches a fixed level as in the proposed Y-12 startup procedure. The experiments should be performed in 1-liter flasks to provide ample volume for sampling, using pumps to provide the feed flow.

2. The experimental data obtained from the flow experiments should be used to determine the effect of substrate and microbe concentrations on the constants used in the model.

3. Iron salts should be added to the Y-12 reactor if the iron content of the feed stream is insufficient to satisfy bacterial population growth rates.

4. Experiments should be performed to determine the optimum substrate-to-microbe ratio for growth promotion.

5. The addition of casamino acids may promote microbe growth (16). Experiments to determine the effect of casamino acid addition should be performed.

6. Experiments should also be performed to determine whether the reactor startup is quicker if the microbe population is grown under aerobic conditions, and then switched over to an anaerobic environment at the desired population level.

9. ACKNOWLEDGMENT

The authors wish to thank D.R. Stallions, C.W. Francis, J.M. Napier, and S.E. Shumate for their assistance.

10. APPENDIX

10.1 Mass and Charge Balance Over the TCA Cycle

In calculating the stoichiometry for the steady state reaction, mass and charge balances were carried out for each step of the Krebs cycle. The compounds involved are shown in Fig. 4.

The reactions occurring in the cycle are as follows:

$$\tfrac{1}{2}Ca(C_2H_3O_2)_2 \rightleftharpoons \tfrac{1}{2}Ca^{+2} + C_2H_3O_2^- \quad (15)$$

$$C_2H_3O_2^- + \underset{\text{ATP}}{[C_{10}N_5O_{13}P_3H_{11}]^{-4}} \xrightarrow[Mg^{+2}]{} C_2H_3O\text{-}\underset{\text{AMP}}{[C_{10}N_5O_7PH_{11}]^{-1}} + P_2O_7^{-4} \quad (16)$$

$$C_2H_3O\text{-}[C_{10}N_5O_7PH_{11}]^{-1} + \underset{\text{coenzyme A}}{\text{H-CoA}} \rightarrow C_2H_3O\text{-CoA} + \underset{\text{AMP-H}}{\text{H-}[C_{10}N_5O_7PH_{11}]^{-1}} \quad (17)$$

Entry to the cycle:

$$C_2H_3O\text{-CoA} + C_4H_4O_5 + H_2O \rightarrow C_6H_8O_7 + \text{H-CoA} \quad (18)$$

$$C_6H_8O_7 \text{ (citrate)} \rightarrow C_6H_8O_7 \text{ (isocitrate)} \quad (19)$$

$$C_6H_8O_7 + NAD^+ \rightarrow C_6H_6O_7 + NADH + H^+ \quad (20)$$

$$C_6H_6O_7 \rightarrow CO_2 + C_5H_6O_5 \quad (21)$$

$$C_5H_6O_5 + NADP^+ + \text{H-CoA} \rightarrow C_4H_5O_3\text{-CoA} + CO_2 + NADPH + H^+ \quad (22)$$

$$C_4H_5O_3\text{-CoA} + H^+ + HPO_4^{=} + \underset{\text{ADP}}{[C_{10}N_5O_{10}P_2H_{11}]^{-3}} \rightarrow C_4H_6O_4 + \underset{\text{ATP}}{[C_{10}N_5O_{13}P_3H_{11}]^{-4}} + \text{H-CoA} \quad (23)$$

$$C_4H_6O_4 + FAD \rightarrow C_4H_4O_4 + FADH_2 \quad (24)$$

$$C_4H_4O_4 + H_2O \rightarrow C_4H_6O_5 \quad (25)$$

$$C_4H_6O_5 + NAD^+ \rightarrow NADH + H^+ + C_4H_4O_5 \quad (26)$$

The following identity is used in cancelling reactant and product species:

$$\underset{ADP}{[C_{10}N_5O_{10}P_2H_{11}]^{-3}} + HPO_4^{-2} = \underset{AMP\text{-}H}{H\text{-}[C_{10}N_5O_7PH_{11}]^{-1}} + P_2O_7^{-4} \quad (27)$$

Thus, the overall equation for TCA-cycle is

$$\tfrac{1}{2}Ca(C_2H_3O_2)_2 + 2H_2O + H^+ \rightarrow \tfrac{1}{2}Ca^{+2} + 2CO_2 + 8(H^+ + e^-) \quad (28)$$

The electron associated with the hydrogen atom produced is used to reduce nitrate via the electron transport system. Hydrogen is attached to NAD^+, $NADP^+$, or FAD, each of which performs the function of transporting the electron to the nitrate. In the process of oxidizing one NADH, the free chemical energy given off is utilized to synthesize three ATP's from ADP's and HPO_4^{-2}'s with a net gain of energy into the organism.

Three $NADH+H^+$ and one $FADH_2$ are produced in one TCA cycle, and the steps leading to reduction of nitrate to nitrogen follows:

$$NADH+H^+ \rightarrow NAD^+ + 2[H^+ + e^-] \quad (29)$$

$$FAD + 2[H^+ + e^-] \rightarrow FADH_2 \quad (30)$$

A summation of these two gives,

$$NADH+H^+ + FAD \rightarrow NAD^+ + FADH_2 \quad (31)$$

which is followed by

$$FADH_2 + 2Fe^{+3} \rightarrow 2Fe^{+2} + 2H^+ + FAD \quad (32)$$

or,

$$NADH+H^+ + 2Fe^{+3} \rightarrow NAD^+ + 2Fe^{+2} + 2H^+ \quad (33)$$

For one cycle, the last step occurs four times. The next step is the reduction of nitrate.

$$8Fe^{+2} + 8H^+ + xNO_3^- \rightarrow 8[H^+] + 8Fe^{+3} + \frac{x}{2}N_2 + 3xO^{-2} \quad (34)$$

where the NO_3^- is introduced by

$$Ca(NO_3)_2 \rightarrow Ca^{+2} + 2NO_3^-$$

Coefficient x is found by electron balance as follows,

$$xN^{+5} + 5xe^- \rightarrow \frac{x}{2}N_2^\circ \quad (35)$$

$$5x = 8$$

Therefore,

$$x = \frac{8}{5}$$

By combining TCA cycle with ETS, the result is

$$\frac{1}{2}Ca(C_2H_3O_2)_2 + \frac{4}{5}Ca(NO_3)_2 + 2H_2O+H^+ \rightarrow \frac{13}{10}Ca^{+2} + 2CO_2 + \frac{4}{5}N_2 + \frac{16}{5}H_2O + \frac{8}{5}OH^- \quad (36)$$

Further reactions and simplifications are

$$1.3Ca^{+2} + 1.3CO_2 + 1.3H_2O \rightarrow 1.3CaCO_3 + 2.6H^+ \quad (37)$$

$$1.6H^+ + 1.6OH^- \rightarrow 1.6H_2O \quad (38)$$

If these equations are substituted into the original equation, the final equation is

$$0.5Ca(C_2H_3O_2)_2 + 0.8Ca(NO_3)_2 \rightarrow 1.3CaCO_3 + 0.8N_2 + 0.7CO_2 + 1.5H_2O \quad (39)$$

10.2 Mass and Charge Balance Over the Glyoxylate Cycle

For the growth period, both TCA and glyoxylate cycles occur. After going through the glyoxylate cycle, it was determined that a general stoichiometric equation cannot be calculated theoretically. The kinetics of reactions participating in the growth phase can only be determined from experimental data.

Reactions associated with the glyoxylate cycle are as follows:

$$Ca(C_2H_3O_2)_2 \rightarrow Ca^{+2} + 2C_2H_3O_2^- \quad (40)$$

$$2C_2H_3O_2^- + 2\underset{ATP}{[C_{10}N_5O_{13}P_3H_{11}]^{-4}} \xrightarrow[Mg^{+2}]{} 2C_2H_3O\text{-}\underset{-AMP}{[C_{10}N_5O_7PH_{11}]^{-1}} + 2P_2O_7^{-4} \quad (41)$$

$$2C_2H_3O\text{-}[C_{10}N_5O_7PH_{11}]^{-1} + 2H\text{-}CoA \rightarrow 2C_2H_3O\text{-}CoA + 2H\text{-}\underset{AMP\text{-}H}{[C_{10}N_5O_7PH_{11}]^{-1}} \quad (42)$$

Entering the cycle,

$$C_2H_3O\text{-}CoA + C_4H_4O_5 + H_2O \rightarrow C_6H_8O_7 + H\text{-}CoA \quad (43)$$

$$C_6H_8O_7 \rightarrow C_6H_8O_7 \text{ (isomerization)} \quad (44)$$

$$C_6H_8O_7 \rightarrow C_4H_6O_4 + C_2H_2O_3 \quad (45)$$

$$C_4H_6O_4 + FAD \rightarrow C_4H_4O_4 + FADH_2 \quad (46)$$

$$C_4H_4O_4 + H_2O \rightarrow C_4H_6O_5 \quad (47)$$

$$C_2H_2O_3 + C_2H_3O\text{-}CoA + H_2O \rightarrow C_4H_6O_5 + H\text{-}CoA \quad (48)$$

$$2(C_4H_6O_5) + 2(NAD^+) \rightarrow 2(C_4H_4O_5) + 2(NADH+H^+) \quad (49)$$

After simplification, the reaction equation for the TCA-bypass is

$$Ca(C_2H_3O_2)_2 + H_2O + 2H^+ \rightarrow Ca^{+2} + C_4O_5H_6 + 6(H^+ + e^-) \quad (50)$$

If the hydrogens attached to NAD^+ are assumed to reduce nitrate and undergo the ETS reactions, the oxidation of three NADH's give the equation

$$6Fe^{+2} + 6H^+ + \frac{6}{5}NO_3^- \rightarrow \frac{3}{5}N_2 + \frac{18}{5}O^{-2} + 6Fe^{+3} + 6H^+ \quad (51)$$

After the last equation has been simplified and combined with the glyoxylate cycle reaction equation, the following equation emerges:

$$Ca(C_2H_3O_2)_2 + \frac{3}{5}Ca(NO_3)_2 + 2H^+ \rightarrow C_4O_5H_4 + \frac{7}{5}H_2O + \frac{3}{5}N_2 + \frac{6}{5}OH^- + \frac{8}{5}Ca^{+2} \quad (52)$$

10.3 Sample Calculations

It is necessary to adjust the solutions in the 250-ml, 7-liter, and 67-liter reactors to the desired concentrations. Adjust the nitrate level to 6000 ppm for experiment 7. Basis: 1 liter.

$$\frac{6.0 \text{ g } NO_3^-}{\text{liter}} \cdot \frac{236 \text{ g } Ca(NO_3)_2 \cdot 4H_2O}{124 \text{ g}(NO_3)} = 11.419 \text{ g/}\ell \text{ of } Ca(NO_3)_2 \cdot 4H_2O$$

Adjust carbon level to 1596 ppm for experiment 7.

$$\frac{1.59 \text{ g C}}{\text{liter}} \cdot \frac{176 \text{ g } Ca(C_2H_3O_2)_2 \cdot H_2O}{48 \text{ g C}} = 5.852 \text{ g/}\ell \text{ of } Ca(C_2H_3O_2)_2 \cdot H_2O$$

Experiment 7 resulted in appreciable bacterial growth and is therefore used to find K_1 and K_2.

$$K_1 = (dC/dt)/CC_S$$

or plotting (dC/dt)/C as a function of C_S gives K_1 as the slope.

Point	C_{S_0} = 1216	C_0 = 107	(dC/dt)/C
1	1183	200	0.695
2	1000	289	0.484
3	373	504	0.159
4	206	553	0.128
5	25	392	-
6	6	776	0.0051

The slope of a line drawn through these points equals K_1. Thus, K_1 was found to be 0.000019 $ppm^{-1}hr^{-1}$.

To convert bacteria population to ppm, the following calculation is performed:

$$(10.7\times10^{7} \text{ bacteria/cc})(10^{-12} \text{ g/bacteria}) = 10.7\times10^{-5} \text{ g/cc}$$

$$= 107 \text{ ppm}$$

To obtain the microbe population, the readings from the Coulter Counter must be multiplied by factors which account for the necessary dilution of the sample to obtain the reading. The Coulter Counter draws a 50 µl sample. A factor must be included for any necessary external dilution. For example, if a 0.1-ml sample of microbe solution is added to 19.9 ml of diluent, the external dilution is

$$(19.9 + 0.1 \text{ ml})/0.1 \text{ ml} = 200$$

Therefore, if a Coulter Counter reading of 12,000 was obtained, the microbe population would be:

$$(12{,}000 \text{ counts}/50\ \mu l)(200)(1\times10^{+3}\ \mu l/ml) = 4.8 \times 10^{7} \text{ microbes/ml}$$

10.4 Nomenclature

- a stoichiometric coefficient describing biomass production per mole of substrate consumed
- C biomass concentration, bacteria/cc
- C_N nitrate concentration, ppm
- C_0 initial biomass concentration, bacteria/cc
- C_s carbon concentration, ppm
- C_{s0} initial carbon concentration, ppm
- K Monod rate constant for biomass production
- K_1 rate constant for biomass production, $ppm^{-1}\text{-}day^{-1}$
- K_2 rate constant for nitrate removal, day^{-1}
- α 0.266 ppm C/ppm NO_3^-
- γ 0.086 ppm C/ppm biomass
- μ maximum rate of biomass production, ppm/day

10.5 Data

Tables 2 through 12 present the time variation of pH, nitrate, carbon, and bacteria concentration for experiments 1 through 11.

Table 2. Data for Experiment 1

Day*	pH	NO_3^- (ppm)	Microbes (10^7 cells/cc)	Carbon (ppm)
0.5	7.9	47.5	12.1	197
1.0	7.7	25.9	24.1	239
1.5	7.8	22.0	11.4	259
2.0	7.7	19.1	11.6	174
2.5	7.9	1374	15.1	-
3.0	7.7	1113	13.3	295
3.5	7.4	-	13.4	-
4.0	7.2	750	8.5	27
5.0	7.4	250	10.7	55
5.5	-	308	22.1	-
6.0	7.3	417	12.6	-
6.5	7.7	261	12.5	-
7.0	7.4	230	8.7	-
7.5	6.9	219	9.1	-
8.0	7.2	340	7.6	44
8.5	7.7	329	8.2	-
9.0	7.7	314	15.8	123

*24-hr day

Table 3. Data for Experiment 2

Day	pH	NO_3^- (ppm)	Microbes (10^7 cells/cc)	Carbon (ppm)
0.5	8.0	780	1.27	419
1.0	7.7	27.5	0.55	203
1.5	7.6	24.9	0.35	232
2.0	7.7	26.4	0.25	199
2.5	7.9	2130	0.48	-
3.0	7.9	860	0.40	98
3.5	7.9	-	7.1	-
4.0	7.5	743	71.0	57
5.0	7.6	333	13.9	56
5.5	-	400	15.5	-
6.0	7.3	475	21.8	-
6.5	7.5	305	23.5	-
7.0	7.4	283	24.2	-
7.5	7.6	449	8.1	-
8.0	7.4	311	20.5	46.5
8.5	7.6	445	7.7	-
9.0	7.5	441	15.7	17

Table 4. Data for Experiment 3

Day	pH	NO_3^- (ppm)	Microbes (10^7 cells/cc)	Carbon (ppm)
0.5	8.0	62.5	186.0	212
1.0	7.3	10.5	116.0	115
1.5	7.2	1.05	125.0	113
2.0	7.1	1.0	119.0	97
2.5	7.5	667	80.8	-
3.0	7.3	1	91.5	34
3.5	7.5	-	95.2	-
4.0	7.3	582	80.5	58
5.0	7.4	-	82.7	44
5.5	-	-	82.5	-
6.0	7.0	8.7	76.0	-
6.5	7.2	-	70.8	-
7.0	7.0	15	77.6	-
7.5	7.3	20	70.9	-
8.0	7.0	13	51.0	25

Table 5. Data for Experiment 4

Day	pH	NO_3^- (ppm)	Microbes (10^7 cells/cc)	Carbon (ppm)
0.5	8.1	47.5	981.0	518
1.0	7.3	2.0	603.0	361
1.5	7.2	1.0	781.0	391
2.0	7.0	1.1	1120.0	488
2.5	7.3	661	833.0	-
3.0	7.2	1.1	761.0	618
3.5	7.2	-	1590.0	-
4.0	7.0	452	985.0	421
5.0	7.1	-	1530.0	673
5.5	-	-	737.0	-
6.0	6.8	14.7	740.0	-
6.5	7.1	-	760.0	-
7.0	6.9	-	445.0	44
7.5	7.1	28	716.0	-
8.0	6.9	22	500.0	102

Table 6. Data for Experiment 5

Day	pH	NO_3^- (ppm)	Microbes (10^7 cells/cc)	Carbon (ppm)
0.5	8.1	8.75	10.4	286
1.0	7.9	5.75	22.4	115
1.5	7.8	5.60	6.7	104
2.0	7.7	4.25	9.8	79
2.5	7.9	629	19.3	-
3.0	7.8	16.9	70.1	31
3.5	7.9	-	9.4	-
4.0	8.0	556	9.7	30
5.0	8.1	139	10.9	21
5.5	-	184	11.4	-
6.0	7.6	175	13.8	-
6.5	8.0	131	10.1	-
7.0	8.1	134	8.1	-
7.5	8.1	160	12.5	-
8.0	8.1	161	7.6	25
8.5	8.2	160	8.4	-
9.0	8.2	154	9.3	14

Table 7. Data for Experiment 6

Day	pH	NO_3^- (ppm)	Microbes (10^7 cells/cc)	Carbon (ppm)
0.5	8.0	350	12.0	255
1.0	7.7	27.5	18.0	223
1.5	7.5	31.3	8.7	207
2.0	7.7	31.4	11.7	163
2.5	7.9	1349	9.8	-
3.0	7.6	888	12.3	93
3.5	7.8	-	14.3	-
4.0	7.6	1060	15.4	24
5.0	7.7	464	17.2	14
5.5	-	574	13.3	-
6.0	7.6	700	8.9	-
6.5	7.8	390	11.5	-
7.0	7.5	231	15.2	-
7.5	7.6	602	10.7	-
8.0	7.5	580	5.1	23
8.5	7.5	637	8.3	-
9.0	7.9	677	9.7	40

Table 8. Data for Experiment 7

Day	pH	NO_3^- (ppm)	Microbes (10^7 cells/cc)	Carbon (ppm)
0.5	7.8	5750	22.6	1474
1.0	7.4	4536	16.6	1311
1.5	7.4	5292	10.7	1215
2.0	7.2	4267	20.0	1183
2.5	7.3	6710	16.3	-
3.0	6.8	4658	28.9	1000
3.5	6.7	-	27.6	-
4.0	6.6	4875	50.4	373
5.0	6.6	1950	55.3	206
5.5	-	2815	42.4	-
6.0	6.5	2046	80.2	-
6.5	6.7	1200	20.3	-
7.0	6.6	900	71.7	-
7.5	6.7	1244	46.3	-
8.0	6.7	1158	39.2	25
8.5	6.7	1402	77.6	-
9.0	6.8	1158	48.8	6

Table 9. Data for Experiment 8

Day	pH	NO_3^- (ppm)	Microbes (10^7 cells/cc)	Carbon (ppm)
0.5	7.8	20.0	2.5	651
1.0	8.2	16.3	7.5	440
1.5	8.1	14.5	14.4	433
2.0	8.1	10.0	13.8	312
2.5	8.2	1052	10.9	-
3.0	7.8	297	13.1	297
3.5	8.1	-	18.4	-
4.0	8.0	362	16.8	299
5.0	7.9	98	12.2	205
5.5	-	116	9.0	-
6.0	7.7	110	10.0	-
6.5	7.8	89	21.4	-
7.0	7.6	91	37.7	-
7.5	7.6	105	47.6	-
8.0	7.6	204	19.9	27
8.5	7.6	93	15.1	-
9.0	7.5	108	18.2	4

Table 10. Data for Experiment 9

Day	pH	NO_3^- (ppm)	Microbes (10^7 cells/cc)	Carbon (ppm)
0.5	7.7	446	3.6	184
1.0	7.8	498	5.2	400
1.5	7.6	502	2.5	211
2.0	7.4	355	3.3	292
2.5	7.3	260	4.4	137
3.0	6.6	140	10.4	135
3.5	-	140	3.6	135
4.0	7.2	100	5.4	128
4.5	7.7			

Table 11. Data for Experiment 10

Day	pH	NO_3^- (ppm)	Microbes (10^7 cells/cc)	Carbon (ppm)
0.5	7.2	6117	3.5	1656
1.0	6.9	6400	4.8	1168
1.5	7.3	6221	3.4	1622
2.0	6.8	9723	10.6	1522
2.5	6.8	4769	12.6	1370
3.0	6.6	4367	15.6	1295
3.5	-	3898	18.9	1202
4.0	6.3	3117	17.1	1160
4.5	6.5	-	-	-

Table 12. Data for Experiment 11

Day	pH	NO_3^- (ppm)	Microbes (10^7 cells/cc)	Carbon (ppm)
0.5	6.7	6440	2.8	1656
1.0	7.2	6780	3.9	1550
1.5	7.5	6697	3.5	1628
2.0	7.2	6746	4.4	1680
2.5	7.2	5411	7.2	1622
3.0	6.9	4845	16.6	1465
3.5	-	4436	17.3	1274
4.0	6.4	3823	26.9	1180
4.5	6.8	-	-	-

10.6 Location of Original Data

The original data may be found in ORNL Databook A-7745-G assigned to S.E. Shumate.

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