Nitrogen utilization in the activated sludge process
by Curtis Kenneth Townsend

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE in Civil Engineering
Montana State University
© Copyright by Curtis Kenneth Townsend (1972)

Abstract:
Under man's influence the process of eutrophication can be greatly accelerated by the concentration of nutrients in wastewater discharges. Biological growth can be controlled in a body of water by limiting the amount of any element required for growth of microorganisms. Nitrogen is required for the formation of amino and nucleic acids which are essential parts of living cells.

This research involved construction of a pilot plant and a study of nitrogen removal by the activated sludge process with cell retention time as a variable. In the conventional activated sludge process nitrogen can be removed by wasting cells that contain nitrogen.

One way to optimize nitrogen removal is by maximum cell wasting. However, this is limited by the necessity to keep the food to microorganism ratio within the proper range for adequate sludge settling.

A second approach involves wasting cells with a maximum concentration of nitrogen. The results of this experiment indicate insignificant changes in the nitrogen concentration of the activated sludge with cell retention times from 1.72 to 10.15 days.

The results of this research indicate that fixation may add significantly to the nitrogen content in the effluents from activated sludge treatment. In processes with no cell wasting (extended aeration), the nitrogen concentration of the effluent may exceed that of the influent.

In presenting this thesis in partial fulfillment of the requirements for an advanced degree at Montana State University, I agree that the Library shall make it freely available for inspection. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by my major professor, or, in his absence, by the Director of Libraries. It is understood that any copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Signature Curtis L [illegible]

Date June 12, 1972

NITROGEN UTILIZATION IN THE ACTIVATED SLUDGE PROCESS

BY

Curtis Kenneth Townsend

A thesis submitted to the Graduate Faculty in partial
fulfillment of the requirements for the degree

of

MASTER OF SCIENCE

in

Civil Engineering

Approved:

Head, Major Department

R L Sanks
Chairman, Examining Committee

Henry L Parsons
Graduate Dean

MONTANA STATE UNIVERSITY
Bozeman, Montana

August 1972

ACKNOWLEDGEMENT

Financial support for this investigation was provided by the Water Quality Office of the Environmental Protection Agency. Facilities were provided by the Civil Engineering Department of Montana State University.

Special thanks are due Dr. A. A. Friedman of Tennessee Technological University, formerly of Montana State University, for suggesting the subject of this study and for his advice on the design of the pilot plant.

Also gratefully acknowledged is the guidance provided by the author's graduate committee: Dr. R. L. Sanks, chairman, and Dr. T. Asano of the MSU Civil Engineering Department, and Dr. K. L. Temple from the Department of Botany and Microbiology.

TABLE OF CONTENTS

TABLE OF CONTENTS
(Continued)

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Under man's influence the process of eutrophication can be greatly accelerated by the concentration of nutrients in wastewater discharges. Biological growth can be controlled in a body of water by limiting the amount of any element required for growth of microorganisms. Nitrogen is required for the formation of amino and nucleic acids which are essential parts of living cells.

This research involved construction of a pilot plant and a study of nitrogen removal by the activated sludge process with cell retention time as a variable. In the conventional activated sludge process nitrogen can be removed by wasting cells that contain nitrogen.

One way to optimize nitrogen removal is by maximum cell wasting. However, this is limited by the necessity to keep the food to microorganism ratio within the proper range for adequate sludge settling.

A second approach involves wasting cells with a maximum concentration of nitrogen. The results of this experiment indicate insignificant changes in the nitrogen concentration of the activated sludge with cell retention times from 1.72 to 10.15 days.

The results of this research indicate that fixation may add significantly to the nitrogen content in the effluents from activated sludge treatment. In processes with no cell wasting (extended aeration), the nitrogen concentration of the effluent may exceed that of the influent.

CHAPTER I

INTRODUCTION

ROLE OF NUTRIENTS

Eutrophication is a process which involves an increase in the biologic productivity of a body of water as a result of nutrient enrichment. This occurs naturally, but under man's influence excessive amounts of nutrients can enter an aquatic ecosystem through wastewaters resulting in an acceleration of the eutrophication process.

Until recently, the degree of wastewater treatment was usually measured relative to the oxygen requirement and suspended solids of the effluent from treatment processes. The effect of the effluent on the oxygen balance of the receiving waters was of particular interest. Biological treatment facilities were often capable of removing up to 95% of this oxygen demand as measured by the five day biological oxygen demand (BOD_5) test. This in turn represents a portion of the amount of oxygen required to convert any biologically oxidizable carbon in the wastewaters to CO_2. Even when this BOD_5 was reduced to 95% of its original value, there were many cases where considerable growth of plants and microorganisms was evident below wastewater discharge points in the stream. This growth was in excess of what could be supported by the remaining 5% of the carbon available.

When sufficient carbon, hydrogen, nitrogen, oxygen, phosphorous and other required elements are present in a body of water; bacteria, algae and other flora will proliferate. If these elements are introduced to a stream, river or lake, growth of plants and microorganisms will proceed to the extent that one of the elements becomes limiting. This phenomenon was first noted by Liebig (1), who demonstrated that growth is limited by the nutrient which is available in the least abundance relative to the nutritional requirements.

Excessive algal growths can occur in carbon rich receiving waters if triggered by a small amount of nutrients remaining in wastewater effluents even after the best secondary treatment. In recent years research in waste treatment has shifted toward optimizing processes for the removal of nutrients as well as carbon from wastewaters. The work of this thesis involves the study of nitrogen in the activated sludge waste treatment process with the aim of finding a means for optimizing nitrogen removal.

ROLE OF NITROGEN

Molecular nitrogen (N_2) comprises 78.084% by weight of the atmosphere, but it is chemically inert and cannot be used by most organisms. These organisms must obtain nitrogen from the environment in some combined form such as nitrate or ammonia. Nitrogen in these combined states seldom exceeds a few parts per million (ppm) in surface water and soil, and its concentration often becomes the limiting factor in the growth of living organisms (2). Infrequently, it may reach levels greater than 100 ppm in ground water where it is not subject to biological uptake (3).

Nitrogen is a requirement for the formation of amino and nucleic acids, which are essential parts of living cells. Therefore, the amount of microbial growth in any body of water can be controlled to the extent that the useable nitrogen content can be controlled.

Grundy (4) has stated that 1,035 to 4,210 million lbs of nitrogen enter the aquatic ecosystem in the United States annually through natural processes. Contributions to useable nitrogen from natural sources include decomposition of organic materials from benthal deposits in lakes and rivers, decomposition of organic materials in the soil and subsequent runoff to streams, rivers and lakes, dissolution of salts containing nitrogen, and fixation of molecular nitrogen. He estimated the additional nitrogen entering this same ecosystem annually under man's influence to be 3,990 million lbs. This 3,990 million lbs is approximately equal to the maximum amount (4,210 million lbs) which enters the aquatic ecosystem through natural causes. Were nitrogen always the limiting nutrient, the rate of eutrophication of the entire aquatic ecosystem would double. If excess nitrogen is provided to a receiving water, then algae and bacteria and other aquatic plants will continue to grow until some other nutrient such as phosphorous or the substrate, carbon, becomes limiting.

OBJECTIVES

Design and Construction of Pilot Plant

The first part of the research entailed the design and construction of an activated sludge pilot plant. This included a continuous-flow, stirred-tank reactor, a gravity clarifier and the necessary feed, waste and recycle pumps with all controls and other appurtenances required for operation.

Nitrogen Removal

The second part of this research involved measuring the nitrogen utilization by activated sludge organisms when varying the cell retention time i.e. sludge age while all other parameters were held constant. This experiment was carried out in a 6-liter pilot plant under constant conditions of feed, temperature and other variables.

The original intent in this part of the research was to measure the nitrogen utilization by activated sludge organisms with temperature as a variable, but the temperature control in the environmental cabinet was unreliable. Hence, it was expedient to investigate the nitrogen utilization by activated sludge as a function of the cell residence time.

CHAPTER II

ACTIVATED SLUDGE

PROCESS FOR WASTEWATER TREATMENT

Activated sludge is a process for removal of substrate carbon from wastewater by a community of microorganisms. Wastewater is introduced to an aeration tank containing an acclimated biological culture, called activated sludge. The culture utilizes the soluble and colloidal organic and inorganic carbon in the waste for respiration and growth. Competition between organisms, temperature, composition of the waste, cell retention time, and many other parameters tend to favor the dominance of certain species. In wastewater treatment the culture is continuously innoculated with new organisms from the influent waste, but this is not true in the pilot plant experiments herein reported because extensive effort was expended to keep the influent feed free from microorganisms.

The activated sludge process depends on the retention of the microorganisms in the aeration tank for a period longer than the hydraulic residence time. From the aeration tank the effluent flows to a settling basin where the microorganisms are separated from most of the remaining liquid by gravity. The settled microorganisms are then recycled back to the aeration tank. In most wastewater treatment plants a portion of the recycle microorganisms are wasted to maintain a steady population of activated sludge in the aeration tank. The amount of microorganisms wasted plus those carried out in the effluent from the settling basin must equal the growth rate to maintain a constant mass population.

The success of the activated sludge process is primarily dependent on the settleability of the microorganisms. The settleability of the organisms has been shown (5) to be dependent on their growth rate. The growth rate can be controlled by the concentration of feed and organisms. This is expressed as the food to microorganism ratio and is generally expressed as the total lbs of BOD_5 entering the reactor per day divided by the total mass of microorganisms in the reactor which is sometimes termed as loading rate:

$$\text{Loading rate} = \frac{\text{lbs BOD}_5}{\text{Day}} \cdot \frac{1}{\text{lbs MLSS}}$$

where

BOD_5 = The five day BOD of the waste

MLSS = Mixed liquor suspended solids which represents the dry weight of unfilterable solids and is nearly the same as dry weight of organisms

The microorganisms generally exhibit satisfactory settling characteristics if the food to microorganism ratio is between 0.2 and 0.5/day (6). The concentration of the influent and effluent was measured as total organic carbon in this experiment. BOD_5 was not measured since this test is time consuming and is not accurate.

ORGANISMS

Diaz et al (7) isolated over 300 bacterial strains by plating samples of activated sludge on sewage agar. Gram negative bacteria of the genera Zoogloea and Comamonas were predominant. Other workers including Allen (8), Jasewicz and Porges (9), and Rogovskoya and Lazareva (10) also studied activated sludge but were unable to find Zoogloea. Anderson and McCoy (11) showed that the dominant bacterial species of activated sludge was Pseudomonas.

Many of the bacteria isolated by Diaz et al (7) were tested for their ability to stabilize sterilized raw sewage. None of the isolates produced an effluent of equal quality to that of the entire activated sludge; however, Zoogloea did form flocs. They also speculated that the bacteria had a great advantage over protozoan in utilizing soluble substrate because the bacteria have a much larger surface to volume ratio.

Diaz et al (7) also found that many of the isolates contained poly-beta-hydroxy-butyric acid (PHB). From this they speculated that this might be the means by which the organic matter of sewage is rapidly removed during the early stages of activated sludge treatment and is then subsequently metabolized. PHB has been shown to act as a reserve material in many species (12, 13, 14, 15). This early removal could be the conversion of organics to stored PHB which is metabolized during subsequent aeration.

A study of 150 bacterial strains isolated from raw sewage indicated significant differences from activated sludge (7). Coliforms, which constitute nearly a quarter of the bacteria in sewage isolates, were rarely encountered in sludge.

Prakasam et al (16) demonstrated that the plating medium has a significant effect on the species isolated from activated sludge. They speculate that it is impossible to cultivate many of the bacteria from sludge on any known media. Thus, while there have been many studies on the composition of activated sludge, there are still many bacterial strains that have yet to be isolated. They attained their highest productivity using activated sludge extract agar.

CHAPTER III

NITROGEN METABOLISM

VALENCE STATES OF NITROGEN

Inorganic nitrogen can exist in the following seven states of valence (17):

-3	0	+1	+2	+3	+4	+5
NH_3	N_2	N_2O	NO	N_2O_3	NO_2	N_2O_5

The principal biological processes involving nitrogen are shown in Figure 1 (18). The individual processes are discussed in the sections of this chapter on "NITRIFICATION," "DENITRIFICATION," "CELL SYNTHESIS," and "FIXATION."

Fewson and Nicolas (19) have proposed the pathway shown in Figure 2 as the most likely in the assimilation and dissimilation of nitrogen. Nitrite (NO_2^-), nitric oxide (NO), and hydroxylamine (NH_2OH) are well established intermediates in the pathway. However, the evidence that nitroxyl (HNO) and nitrous oxide (N_2O), are intermediates is inconclusive (18).

Nitrogen in the -3 valence state is incorporated into organic compounds for cell synthesis. Some bacteria and algae are capable of reducing the higher valence states of nitrogen to the -3 state for this utilization, but most higher forms of animal life are not capable of this reduction and require their nitrogen supply in the NH_3 state

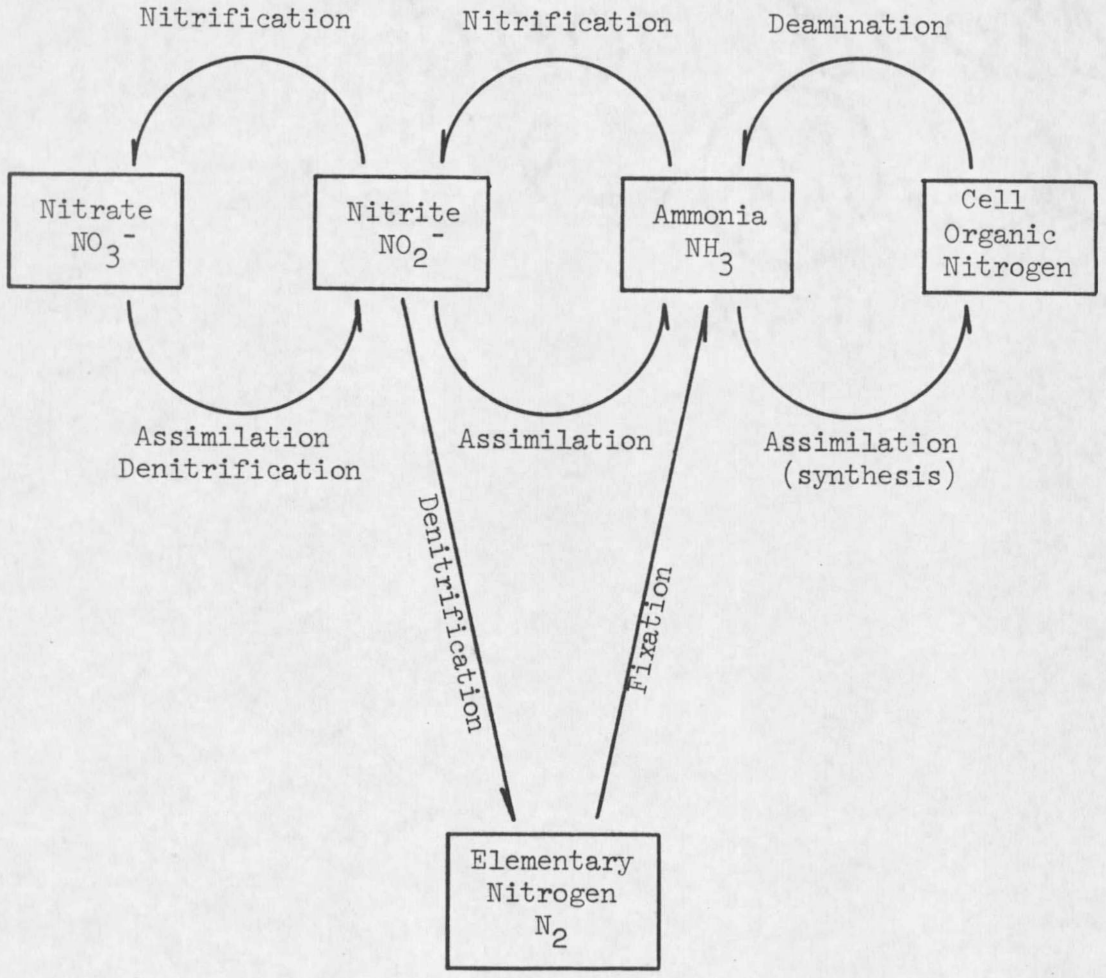


Figure 1. PRINCIPAL BIOLOGICAL PROCESSES INVOLVING NITROGEN (18)

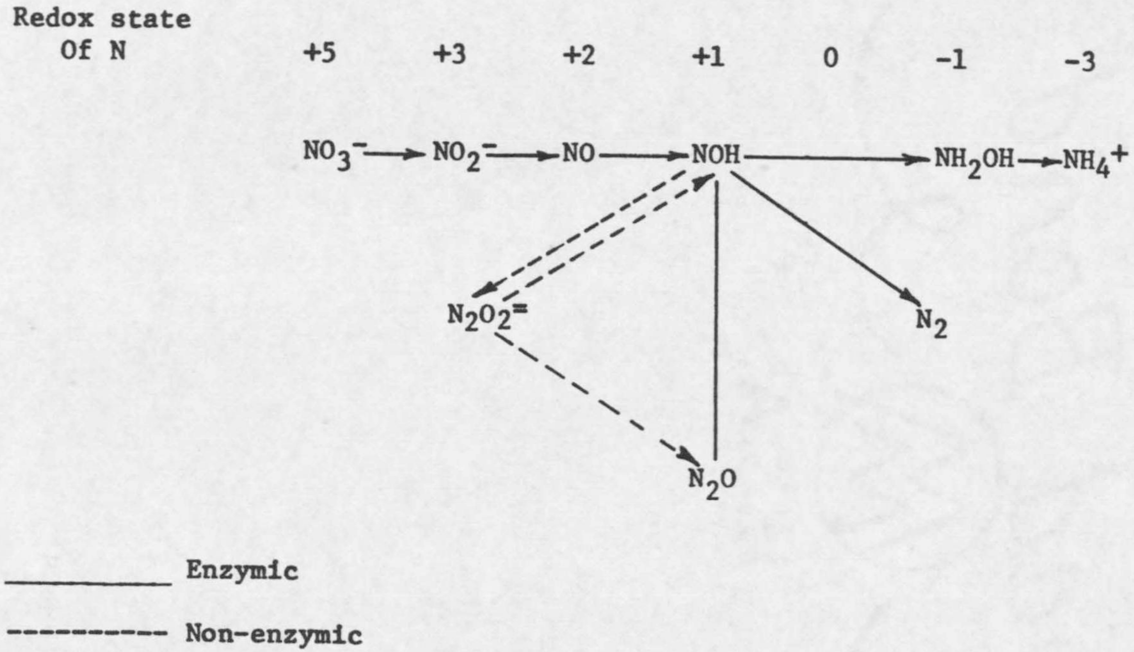


Figure 2. PROPOSED PATHWAYS OF NITRATE ASSIMILATION AND DISSIMILATION (18,19)

(2). Many organisms require nitrogen in previously synthesized compounds.

NITRIFICATION

Nitrification is the oxidation of reduced forms of nitrogen to nitrite or nitrate. The first part of this oxidation is carried out by *Nitrosomonas*, *Nitrosococcus* and possibly *Nitrosospira*, *Nitrosocystis*, and *Nitrosogloea*. These aerobic chemoautotrophic ammonia oxidizers derive their energy from the oxidation of NH_4^+ to NO_2^-. The final step of the oxidation is carried out by *Nitrobacter* and *Nitrocystis*. These aerobic chemoautotrophic nitrite oxidizers derive their energy from the oxidation of NO_2^- to NO_3^- (18, 20, 21) (Name change - See "APPENDIX D").

The complete oxidation of the nitrogen forms in activated sludge may be limited by the slow growth rate of both *Nitrosomonas* and *Nitrobacter*. Many of the bacteria in activated sludge reproduce at a higher rate than either *Nitrosomonas* or *Nitrobacter*. If the other organisms grow at such an accelerated rate that it is necessary to waste the nitrifiers faster than they reproduce, then they will be eliminated from the sludge and no nitrification will occur.

In 1917, Meyerhof (22) determined the relationship between the growth characteristics of *Nitrosomonas* and *Nitrobacter* and pH. The rates of oxidation of ammonia and nitrite by the respective organisms are shown in Figures 3 and 4. The lack of any apparent activity by *Nitrosomonas* below pH 7.6 would limit nitrification in many cases.

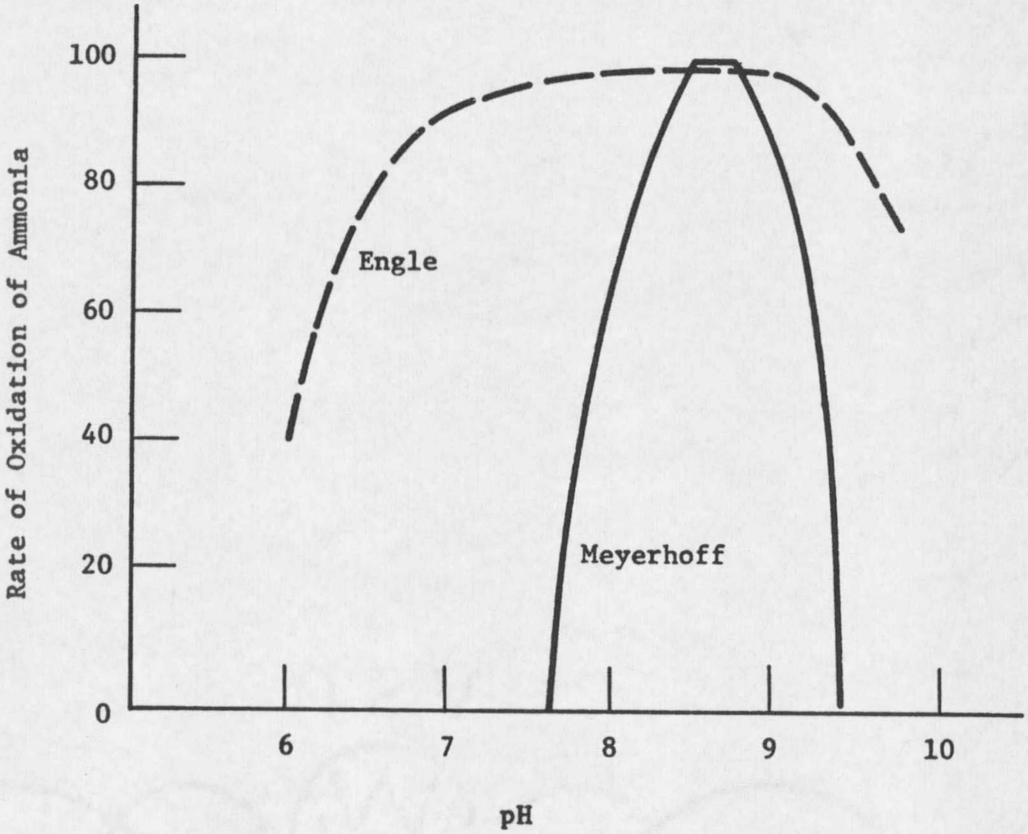


Figure 3. RATE OF OXIDATION OF AMMONIA BY NITROSOMONAS (23,24)

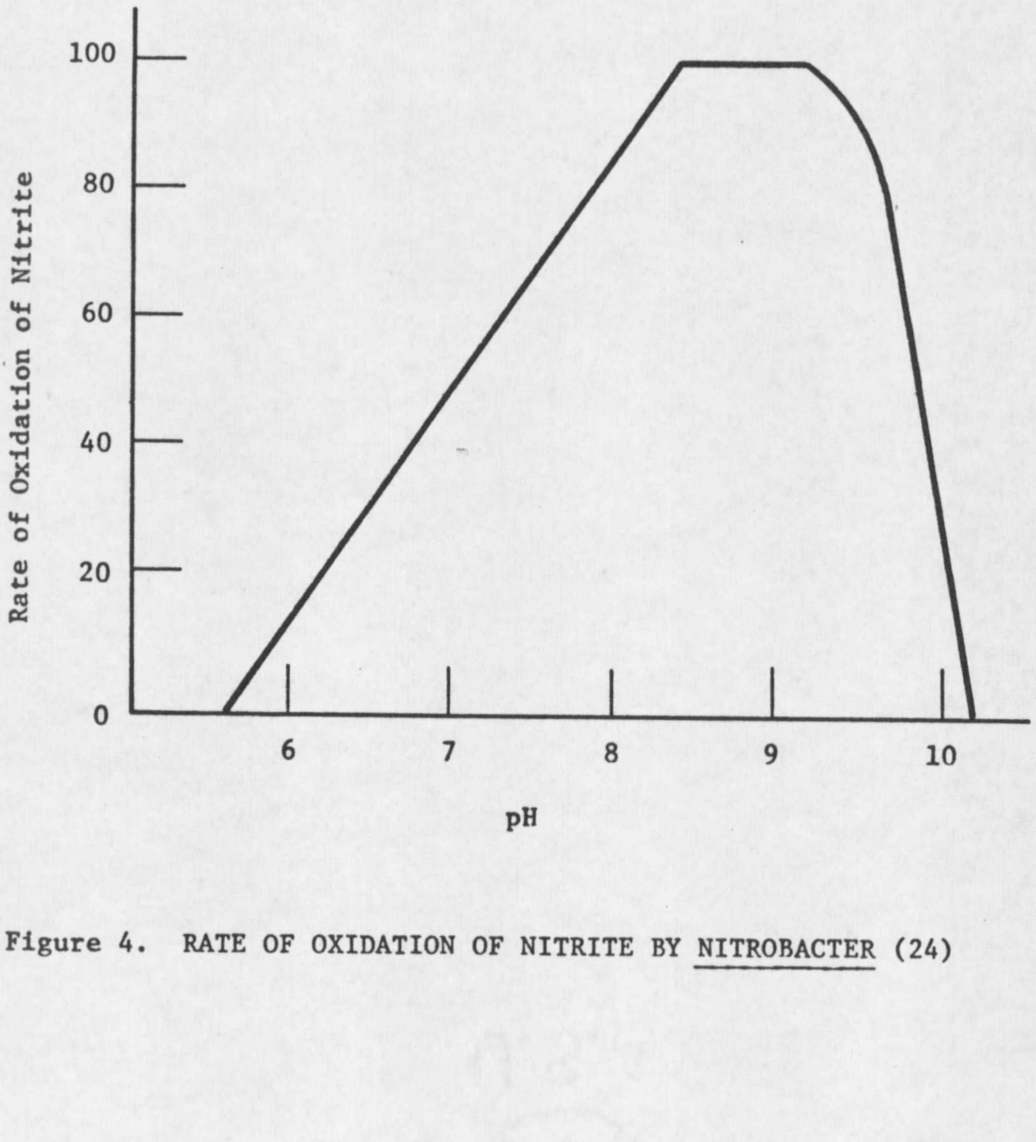


Figure 4. RATE OF OXIDATION OF NITRITE BY NITROBACTER (24)

In sharp contrast, Engle et al (25) showed that the rate of oxidation by Nitrosomonas varied with pH as shown in Figure 3 which was reasonably verified by Wild et al (24).

The growth rate of the nitrifiers is also temperature dependent. Wuhrman (26) has stated that at temperatures above 14°C, the population of nitrifiers will sustain itself if the cell retention time (total volume of sludge in the reactor/volume of sludge wasted per day) is maintained greater than two or three days. The cell retention time should be at least four to five days at temperatures of 8°-10°C.

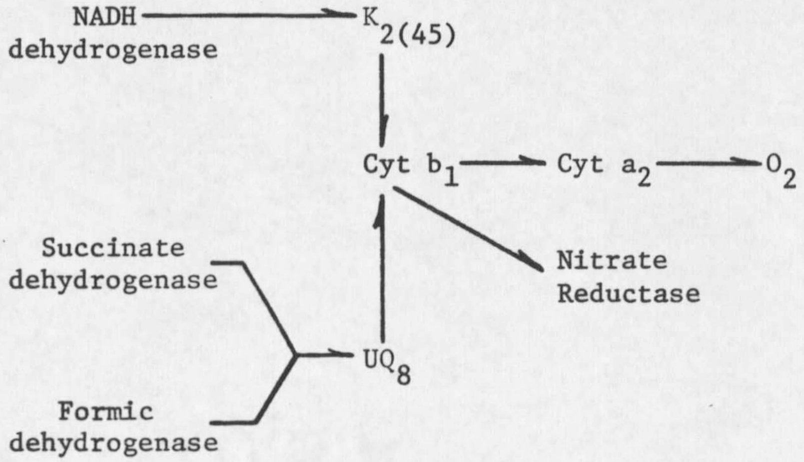
Stewart (6) and Slechta et al (27) have stated that nitrification does not take place in conventional activated sludge systems with food to microorganism ratios greater than 0.25 to 0.35 lb BOD_5/day/lb volatile suspended solids.

Wuhrman (26) has also stated that oxygen tension must be sufficient for this nitrification to occur. The normal oxygen concentration of activated sludge treatment plants is at least 1-1.5 ppm O_2 which is sufficient for this oxidation. With all the above conditions satisfied the oxidation should be complete and one would expect to find no lower forms of nitrogen in the soluble portion of the effluent from activated sludge treatment. The lower forms of nitrogen should all be converted to NO_2^- and NO_3^-.

DENITRIFICATION

Nitrate and nitrite are reduced by microorganisms for two distinct purposes. The first of these involves the reduction to the NH_4^+ valence state for utilization in cell synthesis. This reduction is referred to as assimilatory nitrate reduction and is not considered denitrification (28). This will be covered further under "CELL SYNTHESIS."

The second process for the reduction of nitrate is actually nitrate respiration or electron transport in which nitrate serves as the terminal electron acceptor (29). The product of nitrate respiration is usually nitrite but certain bacteria are capable of carrying the respiration further to produce nitrogen (N_2), nitrous oxide (N_2O), or nitric oxide (NO) (30). When nitrate is reduced to one of these gaseous end products, the process is referred to as denitrification since the gases are lost from the medium. Kashket and Brodie (31) and Itagaki (32) have shown the alternate pathways of electron transport to cytochrome b_1 from NADH and succinate in E. coli.



In the first case, the transfer proceeds via vitamin K_{2}(45) and in the second via UQ_8. From cytochrome b_1 the electrons are transported to nitrate reductase and then to nitrate in nitrate respiration. In oxygen respiration the electrons are transported from cytochrome b_1 to cytochrome a_2 and then to oxygen.

Taniguchi and Itagaki (33) isolated nitrate reductase from E. coli and found that the solubilized enzyme did not contain flavin or cytochromes but did contain one mole of molybdenum and about 40 moles of iron per mole of enzyme. Cytochrome-linked nitrate reductases have been purified from several other microorganisms (30).

It has been shown that oxygen is a strong competitive inhibitor of nitrate respiration (34). In competitive inhibition, the inhibitor and the substrate compete for the same active site on the enzyme. The inhibitor reacts with the enzyme reversibly to form an enzyme-inhibitor (EI) complex.

$$E + I \rightleftharpoons EI$$

The EI complex cannot break down to form reaction products as can the enzyme-substrate (ES) complex; therefore, the percent of inhibition of the enzyme is a function of the ratio of the concentrations of the inhibitor and substrate rather than a function of the concentration of the inhibitor alone (2). The overall reaction could be considered as follows:

$$E + S + I \rightleftharpoons \underset{\text{Active}}{ES} + \underset{\text{Inactive}}{EI} \rightleftharpoons E + P + \underset{\text{Inactive}}{EI}$$

Moore et al (35) states reports on the extent of inhibition by oxygen have varied but sufficient evidence exists to indicate oxygen causes a noncompetitive inhibition of nitrate reduction.

A noncompetitive inhibitor combines with either the free enzyme or the enzyme-substrate complex. The inhibitor generally combines with the enzyme at some place other than the active site. Consequently, the degree of inhibition is independent of the substrate concentration. The enzyme species available to combine with the noncompetitive inhibitor includes E and ES.

$$(E + ES) + I \rightleftharpoons EI + ESI$$

The forms EI and ESI are inactive; so the only enzyme available to carry out the reaction is E or ES which has not combined with I (2, 36).

Schroeder et al (28) stated that the inhibition of nitrate reduction by oxygen is primarily a matter of reaction rates.

CELL SYNTHESIS

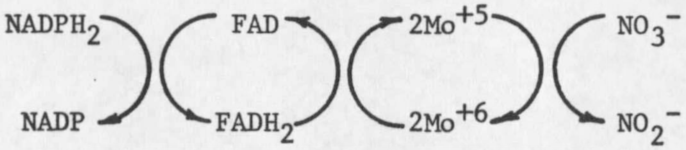
The organic constituents of microbial cells contain nitrogen in the -3 valence state or the same state as in ammonia, NH_3 (20). Ammonia is directly converted to organic form by the reductive amination of alpha-ketoglutaric acid which is an intermediate of the tricarboxylic acid (TCA) cycle (20). The products of this amination are glutamic acid and glutamine. Other nitrogenous compounds are formed by transamination of these compounds. For cell synthesis, energy requirements are lowest if nitrogen is supplied in the ammonia state as no valence change is required.

There was doubt as to the existence of the TCA cycle in bacteria for many years as whole cells were unable to oxidize key intermediates of the cycle (30). Barrett & Kallio (37) demonstrated enzyme preparations of Pseudomonas fluorescens were able to oxidize TCA cycle intermediates while the cells from which the extracts were made were unable to oxidize the same intermediates. They concluded the difficulty lay in the penetration of the highly polar TCA cycle intermediates into the cell. This observation was subsequently made with other bacteria (30).

Sokatch (30) has stated the requirements for establishing the existence of the TCA cycle in bacteria include detection of the enzymes of the cycle at a specific activity high enough to account for the growth of the organism and oxidation of radioactive acetate yielding tricarboxylic acid cycle intermediates labeled as predicted on the basis of the known reactions. He states it can be reasonably assumed that the cycle exists in those organisms for which such data are available. He has shown these activities for a number of species of bacteria and Saz and Krampitz (38) showed that Micrococcus lysodeikticus yielded labeled TCA cycle acids as predicted on a substrate of labeled acetate.

NO_3^- and N_2 may also be utilized by many organisms; however more energy is required as these forms must be reduced to the ammonia valence for incorporation in cellular material. This type of reduction is assimilatory nitrate reduction (20). The enzymes of nitrate assimilation are distinct from those involved in denitrification (nitrate respiration). They are differentiated operationally by the fact that enzymes of nitrate assimilation are pyridine nucleotide-linked while the enzymes of nitrate respiration are connected to the cytochrome system (30).

Assimilatory nitrate reduction is not inhibited by the presence of oxygen (35). Assimilatory nitrate reduction is limited by available nitrogen rather than available hydrogen acceptors. The first step is the reduction of nitrate to nitrite and is initiated by nitrate reductase, a flavoprotein which contains molybdenum. A schematic of the electron transfer is as follows (20, 28):



Nitrate Reductase

In this process electrons flow from an electron carrier of lower standard reduction potential to one of higher potential (2). Thus, the standard reduction potentials increase from NAD to FAD to Mo to NO_3^-, and $NADPH_2$ is oxidized to NADP as 2 electrons pass to FAD which is reduced to $FADH_2$. When Mo^{+6} is reduced to Mo^{+5}, 2 moles of molybdenum in the enzyme are reduced for each mole of $FADH_2$ that is oxidized. The 2 moles of molybdenum are then oxidized from a valence of +5 to +6 as one mole of nitrate is reduced to one mole of nitrite. In electron transport the carriers are specific in selecting other carriers with which they react. $NADH_2$ can transfer to FAD but cannot directly transfer to Mo^{+6} or NO_3^- (2).

Chemical energy is carried from oxidation reactions of catabolism to the energy-requiring reactions of anabolism or synthesis in the form of electrons as shown in Figure 5 (2). In this reaction $NADPH_2$ carries energy-rich electrons from catabolism to initiate the reduction of nitrate.

Stainer and Doudoroff (20) have stated the reduction of NO_2^- to NH_3 is not fully understood but is believed to involve three successive 2 electron transfers as follows:

	NO_2^-		(X)		NH_2OH		NH_3
Valence of N	+3		+1		-1		-3
Electron Transfer		-2		-2		-2	

The complexity of these reactions and the many enzymes required

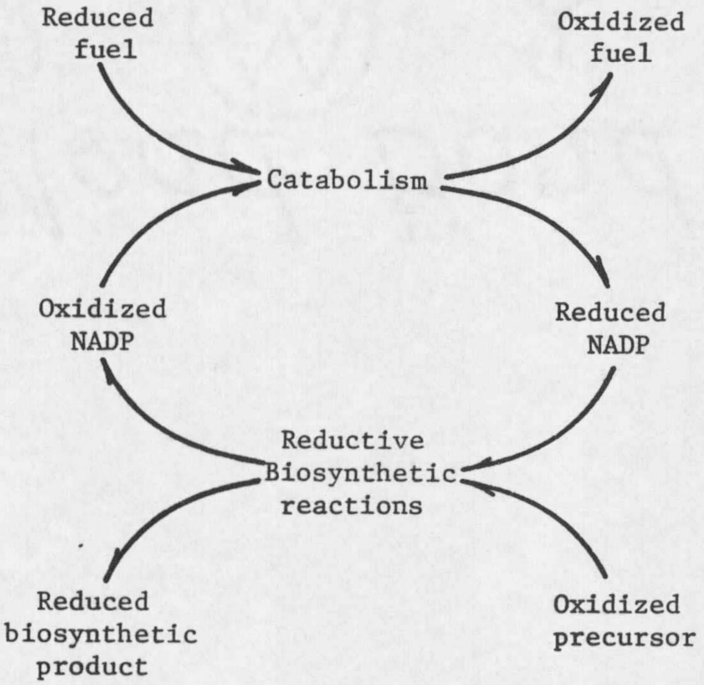


Figure 5. TRANSFER OF REDUCING POWER VIA THE NADP CYCLE (2)

for the reduction of nitrate help to explain the reason many organisms can grow with ammonia as a nitrogen source and yet are incapable of utilizing NO_3^- for cell synthesis (20).

FIXATION

Molecular nitrogen (N_2) comprises 78.084% of the atmosphere by weight, but it is chemically inert and can be used by only a few selected organisms. The remainder of the organisms must obtain their nitrogen in some combined form such as nitrate, ammonia or more complex compounds such as amino acids. Painter (18) has stated that nitrogen fixation occurs only during growth. Hence, one would assume that the N_2 fixed would be limited to growth, and there would be none fixed in conjunction with respiration.

Azotobacter is the most common bacterium known to fix nitrogen under aerobic conditions (20) or under the conditions existing in an activated sludge treatment process. Newton et al (39) has shown that nitrogen fixation by Azotobacter in pure culture is not inhibited by ammonia with NH_4-N concentrations of less than 100 mg/l. Above that concentration, inhibition of fixation is complete.

Certain filamentous blue green algae are also known to fix N_2 under aerobic conditions. Bacillus, Clostridium spp., and Aerobacter also fix nitrogen but only under anaerobic conditions or at very low oxygen tensions.

Painter (18) states that nitrogen fixation has yet to be proved to occur in normal sewage treatment processes and it seems unlikely to play more than a very small part in the nitrogen balance of such systems. However in bodies of natural or polluted water, fixation could play a significant role in supplying nitrogen requirements.

CHAPTER IV

NITROGEN REMOVAL FROM WASTEWATER

CELL WASTING

One way to remove nitrogen from wastewater is to waste activated sludge. The average chemical composition of the bacteria found in activated sludge has been shown by Hoover et al (40) to approximate $C_5H_7O_2N$. Middlebrooks et al (41) found the nitrogen content of activated sludge cells to be 9.22% with 95% confidence limits of 8.46-9.98%. Where growth is distorted by limiting nutrients, Symons et al (42) has shown the composition can vary from 1.8% to 9.9% nitrogen in the microorganisms of activated sludge.

A cell whose composition is $C_5H_7O_2N$ contains 53.1% carbon and 12.39% nitrogen. Average municipal sewage contains approximately 150 mg carbon per liter and 40 mg N per liter (27, 41). It is reasonable to assume 25% of the carbon is utilized for cell synthesis (6), so 0.25 x 150 or 37.5 mg/l of the carbon will be incorporated in new cells. Hence, 37.5 x 12.39/53.1 = 8.8 mg/l of nitrogen can be used. This means 8.8/40 or 22% of the nitrogen in average municipal sewage can be utilized in cell synthesis.

The two ways of optimizing the nitrogen removal are by achieving maximum cell wasting and trying to obtain cells which contain a greater percentage of nitrogen. The first of these processes is limited by the need to keep the F/M ratio within required limits to assure adequate sludge settleability. Eckenfelder (43) has shown the relationship in Figure 6 between sludge loading (F/M) in activated sludge and the sludge volume index (SVI) which is a measure of settleability. The settleability limitation could be reduced by using a more exotic means for cell separation than sedimentation.

The means for optimizing the second alternative would entail finding a growth stage in which the percentage of nitrogen in the cells was the greatest. The first approach is contrary to the normal operation of sewage treatment plants because it maximizes sludge, whereas in most plants an attempt is made to obtain maximum respiration and minimum growth to reduce the quantity of sludge requiring disposal.

The work of this thesis was directed toward the second approach by attempting to maximize the concentration of nitrogen in the microorganisms of an activated sludge culture by varying the cell retention time. If this can be done the percent nitrogen in the effluent of municipal sewage might be reduced to tolerable limits.

OTHERS

Shammas (44) has presented a comparison of the methods shown in Table I for the removal of nitrogen from wastewater effluents. The processes may be classified as biological, chemical or physical. Physical processes include reverse osmosis, distillation and land application. The most promising chemical processes include ammonia stripping, ion exchange, electrochemical treatment and electrodialysis

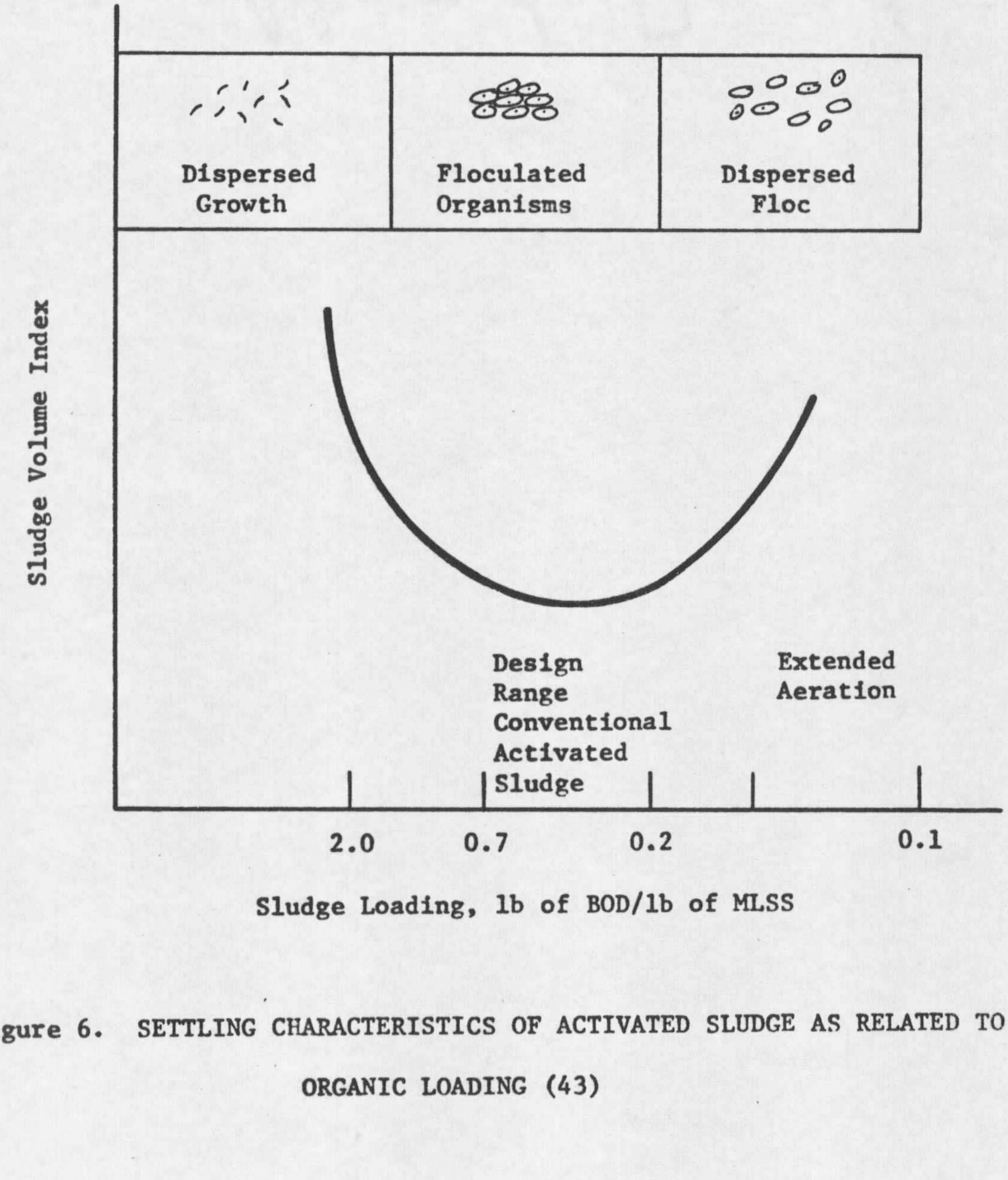


Figure 6. SETTLING CHARACTERISTICS OF ACTIVATED SLUDGE AS RELATED TO ORGANIC LOADING (43)

TABLE I

COMPARISON OF NITROGEN REMOVAL PROCESSES

Process	Class	Removal % Efficiency	Estimated Cost $/mg	Wastes to be Disposed of	Remarks
Ammonia Stripping	Chemical	80-98*	9-25	---	*Efficiency based on ammonia-N only
Anaerobic denitrification	Biological	60-95	25-30	none	--------
Algae harvesting	Biological	50-90	20-35	liquid & sludge	large land area
Conventional biol. treat.	Biological	30-50	30-100	sludge	--------
Ion exchange	Chemical	80-92	170-300	liquid	Efficiency & cost depends on degree of pretreatment
Electrochemical treatment	Chemical	80-85	4-8*	liquid & sludge	*Power cost only
Electrodialysis	Chemical	30-50	100-250	liquid	Cost based on 1-10 mgd capacity, 1000 p.p.m solids
Reverse osmosis	Physical	65-95	250-400	liquid	--------
Distillation	Physical	90-98	400-1000	liquid	--------
Land application	Physical	*	75-150	none	*Efficiency depends on form of nitrogen

Biological processes include algae harvesting and nitrification-denitrification. Probably the best process to be combined with activated sludge would be nitrification-denitrification. In most instances where nitrification-dentrification has been attempted, it has been necessary to add methanol to the anaerobic reactor (45) to obtain denitrification.

Mueller (46) has successfully experimented with a biological process based on the use of gaseous hydrocarbons (natural gas or methane) for microbial assimilation of mineral nutrients in wastewater effluent. By adding either nitrogen or phosphorous until the ratio of nitrogen to phosphorous was 10 or 12 to 1, he got virtually complete removal of both. His experiments were carried out aerobically by bubbling a mixture of 20% methane and 80% air through a reactor with retention times of 5 to 10 hours depending on effluent strength. It is possible that digester gas could also be used for the carbon source in an anaerobic denitrification chamber.

CHAPTER V

EXPERIMENTAL FACILITIES AND PROCEDURES

DESIGN OF PILOT PLANT

A schematic design of the pilot plant is shown in Figure 7. Details of the reactor, clarifier and related equipment are shown in Figures 8, 9, and 10 and Table II.

The pilot plant was constructed so that many of the physical parameters could be varied within the range shown in Table III. With the exception of the waste rate, which was used as the control variable, all other physical parameters were held constant during this experiment with values shown in Table III.

The clarifier volume can be varied by changing the level of the vacuum line from the clarifier. The reactor volume can be controlled by changing the elevations of both the clarifier and the vacuum line.

The feed, sludge recycle, and waste pumps operate by circumferentially forcing the solution through 1/8" ID by 3/16" OD Tygon tubing. The capacity of each pump is approximately 0.7 ml/revolution. The feed rate can be varied by changing the speed of the drive motor and the waste and recycle rates can be varied by changing the percentage of time the pumps run during each cycle. The length of each cycle for the recycle and waste can be varied by changing the gear ratios in the timers. The timers can be set for percent of time on or off by changing the wiring from the timers to the pumps.

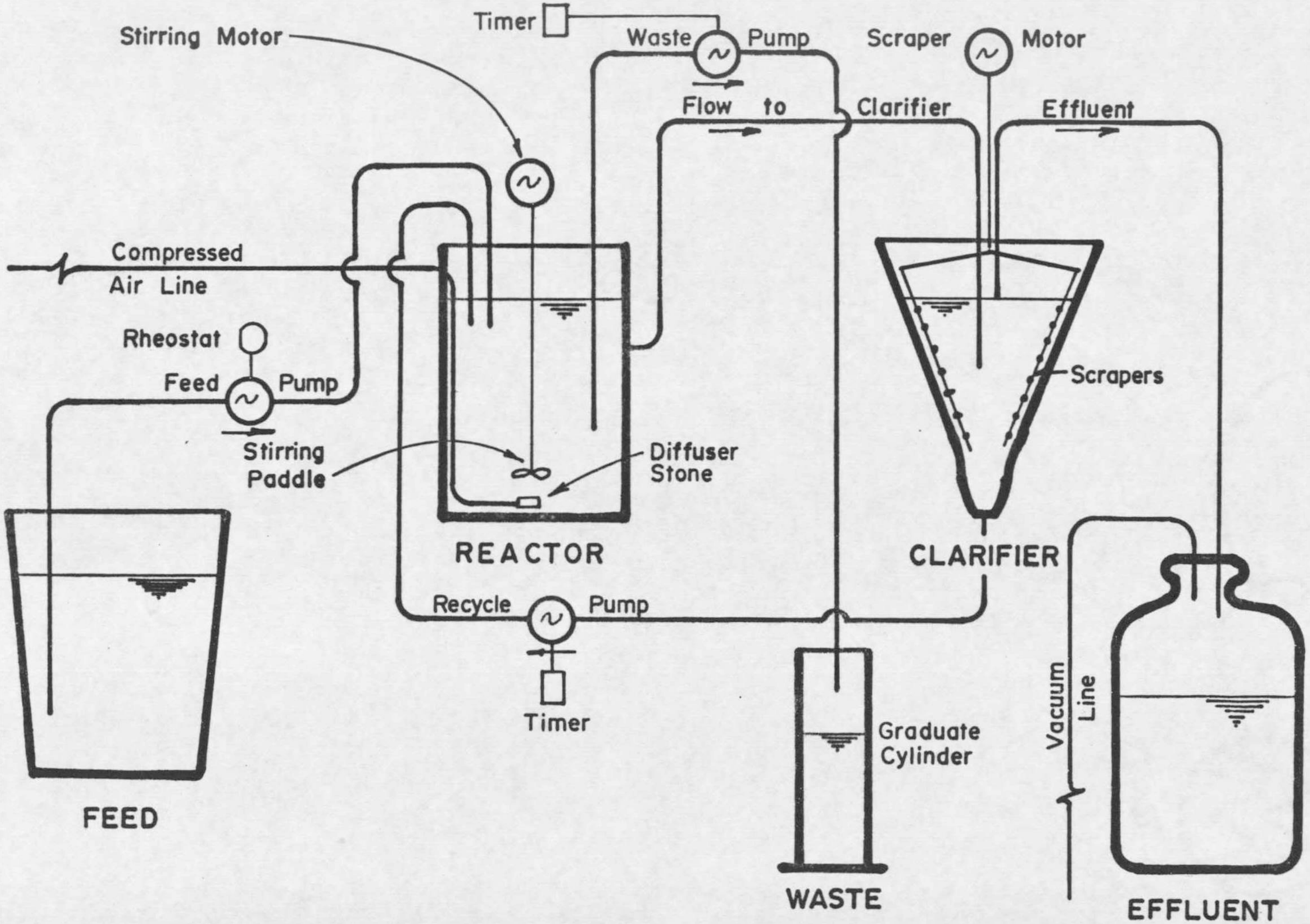


Figure 7. SCHEMATIC OF PILOT PLANT

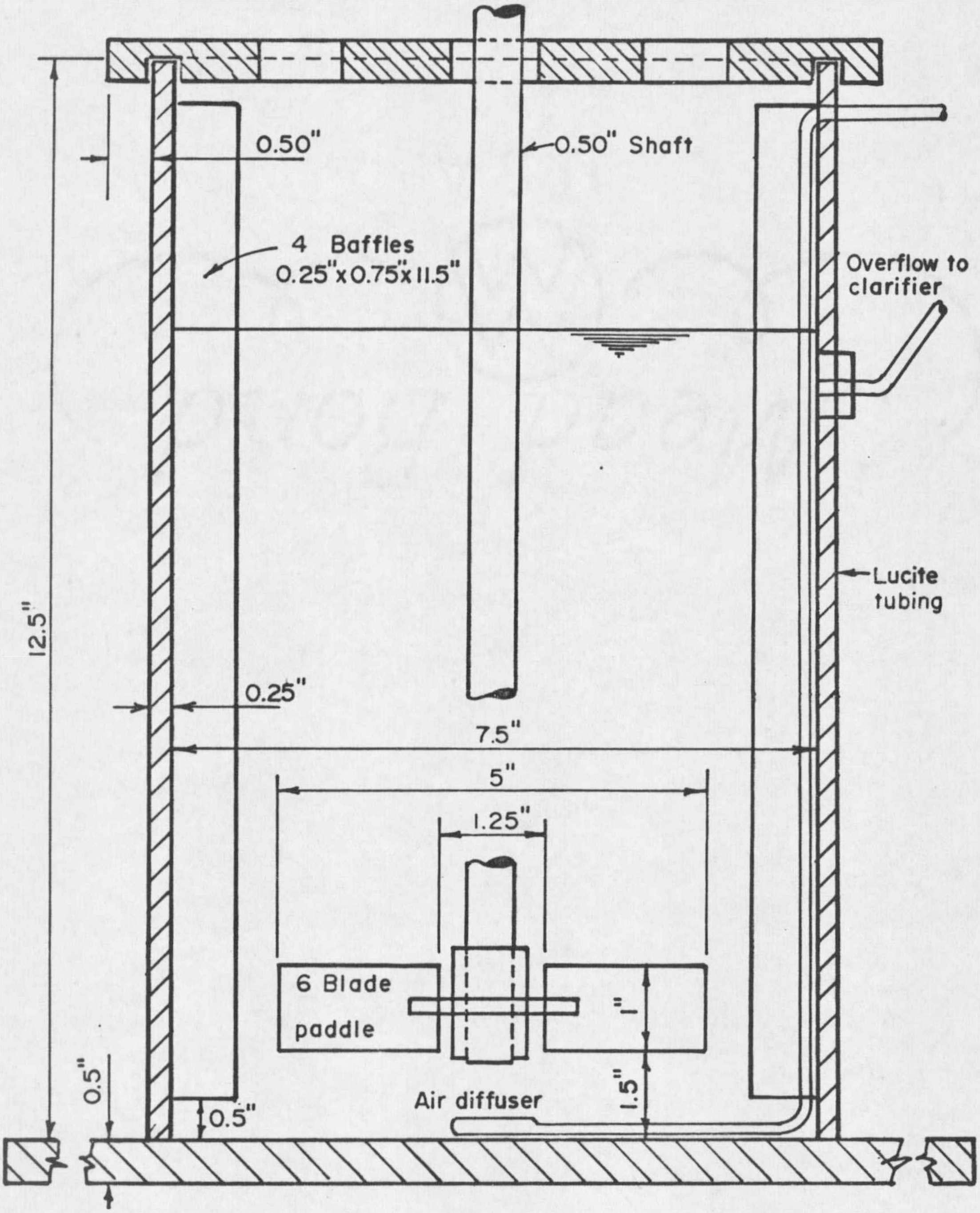
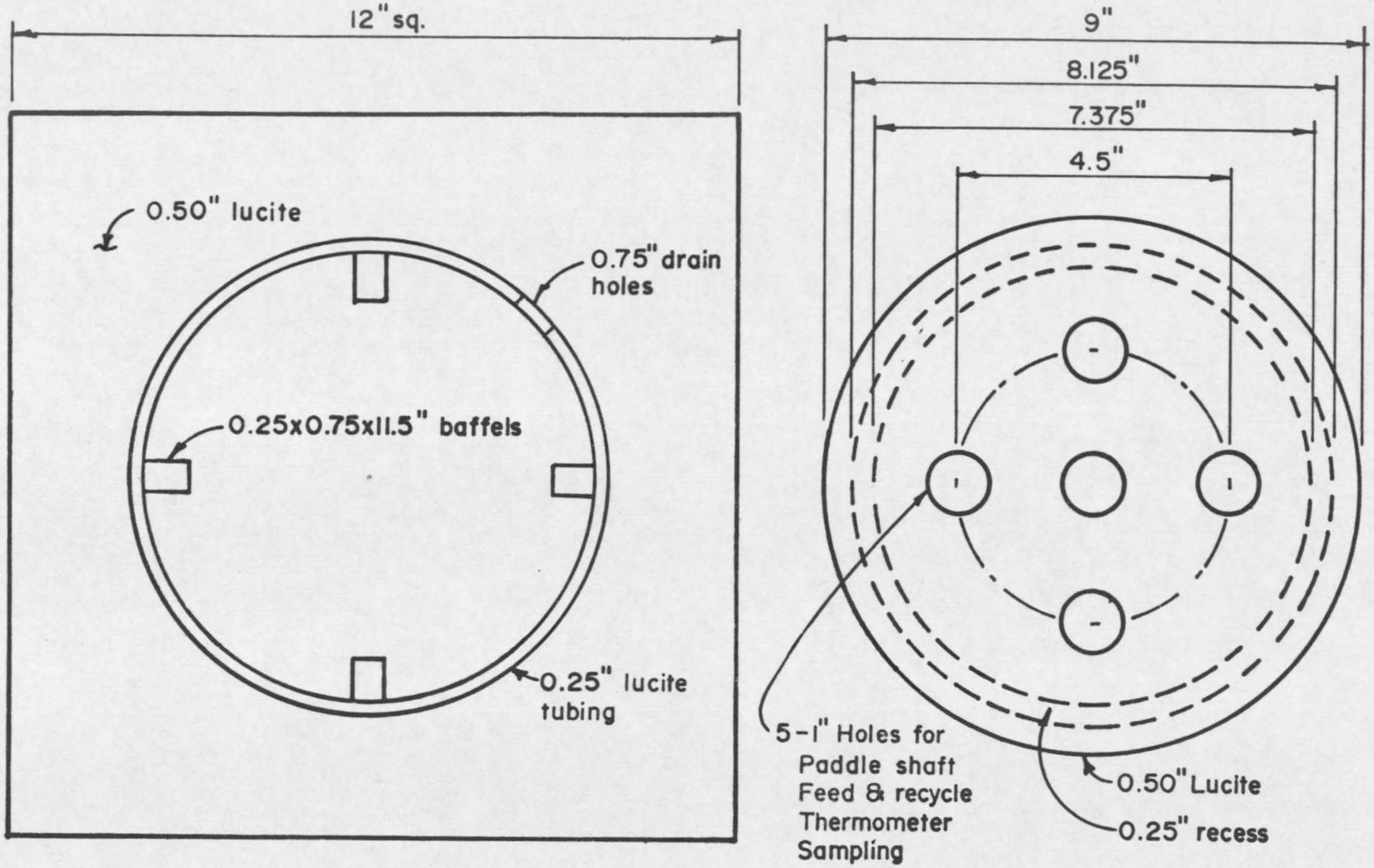


Figure 8. REACTOR AND PADDLE



REACTOR AND BASE PLATE

COVER PLATE

Figure 9. REACTOR BASE AND COVER

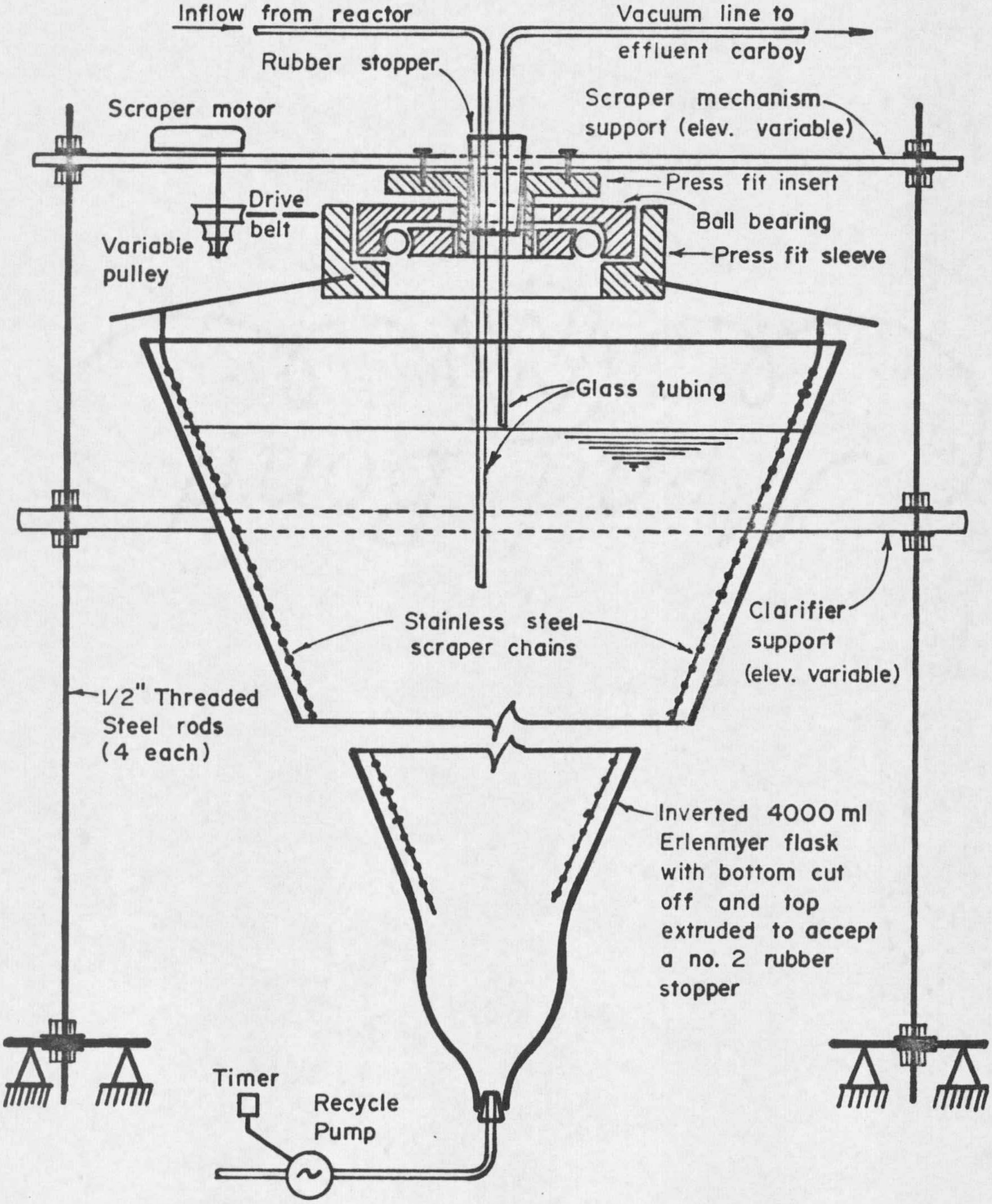


Figure 10. CLARIFIER AND SCRAPER

TABLE II

PILOT PLANT EQUIPMENT

Feed Pumps	Sigmamotor pumps powered with 1/50 H.P. direct current, variable speed Bodine motors controlled with Minarik SL-14 timers. Speed range of pumps approximately 2 to 120 rpm.
Recycle Pumps	100 rpm Model 4.100 Sigmamotor pumps. Percent of time pumps operate controlled with Singer Model CM-2 industrial timers on a 60 second cycle.
Waste Pumps	20 rpm Model 4.20 Sigmamotor pumps. Percent of time pumps on controlled with Singer Model CM-8 industrial timers on a 60 minute cycle.
Stirring Motors	Dual-shaft, variable speed, G.K. Heller, GT 21-18, 1/40 H.P., direct current motors controlled with G.K. Heller GT 21 motor controller. Speed range 0 to 6000 rpm.
Pressure Reducer	Sioux Industrial air pressure reducer and liquid trap.
Air Diffusers	Medium porosity, 45 mm diameter fritted disks.
Clarifier Scrapers	Powered with a 4 watt, 2 rpm Synchron motors and speed reducers.
Rotameter	Fisher and Porter Model 10A1034NFC with 4 tubes. Each tube is calibrated from 0 to 450 cc/min.

TABLE III

RANGE OF VARIABLES FOR COMPONENTS OF PILOT PLANT

Component	Units	Setting this experiment	Useable Range
Reactor			
Volume	liters	6	0-8
Clarifier			
Volume	liters	2.8	0-3.5
Feed Pump			
Capacity	ml/min	16.5	2-85
Recycle Pump			
Capacity	ml/min	8	0.5-70
Timer Cycle	seconds	60	10-90
Waste Pump			
Capacity	ml/day	500-1500	500-20,000
Timer Cycle	min	60	20-180
Stirring Paddle			
Velocity	rpm	4.3-4.7	0-6000
Clarifier Scraper			
Velocity	rpm	2.0	1-2
Aeration			
Capacity	cc/min	400	0-1800

Mixing in the reactors is accomplished by both aeration and stirring. The rate of aeration is measured with a rotameter and controlled with a needle valve. The stirring velocity is controlled by varying the speed of the direct current motor that rotates the paddles.

NUTRIENT MEDIUM

The carbon source utilized for these experiments was from a sucrose feed which contained the nutrients shown in Table IV. This feed solution was based on the work of Friedman (47). The feed was diluted with distilled water and therefore required the addition of the "micro nutrients" shown in Table V (20, 48).

The individual stock solutions for the feed were mixed in two liter volumes as required. These individual concentrated solutions were then mixed together daily to the required volumes as shown in Table IV and were diluted with distilled water to obtain a feed solution of the desired strength.

ACCLIMATION OF SLUDGE SEED

The activated sludge seed for this experiment was obtained from the aerator of the Bozeman, Montana activated sludge treatment plant. The seed was batch fed in a 5 gallon carboy and aerated without cell wasting for about 2 months prior to initiation of the continuous-flow, stirred-tank reactor (CFSTR) experimental work. Each morning the air was shut off and the activated sludge was allowed to settle for approximately 30 minutes. The supernatant was drawn off until the remaining

TABLE IV

FEED SOLUTION PROPORTIONS (47)

Reagent	Stock Solution Concentration g/1	Quantity Required to Mix 36 liters of Feed Solution ml	Final Molecular Concentration in Dilute Feed Solution mg/1
$FeCl_2$	0.25	50	0.35
$MgSO_4 \cdot 7H_2O$	45.0	50	62.50
K_2HPO_4	16.0	100	44.40
NH_4Cl	70.0	100	194.44
$C_{12}H_{22}O_{11}$(1)	180.0	20	100.00
Bacto-Peptone(2)	40.0	100	111.11

(1) Sucrose - pure cane sugar (42.1% carbon)

(2) Difco Laboratories #0118-01 (40.4% carbon)

TABLE V

MICRO NUTRIENTS ADDED TO FEED SOLUTION

Component	Stock Solution Concentration mg/1	Quantity Required for 36 liters of Feed Solution ml	Final molecular Concentration in Dilute Feed Solution ug/1
H_3BO_3	180	2	10
$CuSO_4$	14	2	0.78
KI	35	2	1.9
$MnSO_4$	140	2	7.8
Na_2MoO_4	71	2	4.0
$ZnSO_4$	140	2	7.8
$CaCl_2$	35	2	1.9

volume was approximately 6 liters. An amount of concentrated feed, which was equivalent to what would be fed to one 6 liter CFSTR reactor during a 24 hour period, was added to the microorganisms in the carboy. The volume in the carboy was then made up to approximately 15 liters with tap water and the system was then aerated for the next 24 hours. The air volume introduced was sufficient to provide stirring as well as the required oxygen.

HYDRAULIC LOADING

Feed

The rate of application of the feed solution was intended to be one liter per hour. The rate varied from this because of mechanical variations and the inability to set the speed of the pumps exactly. During the testing period the maximum variation from this was 7.5% with an average of 2.5%. All calculations were based on physically measured volumes of feed to minimize the effects of minor variations.

The feed solution was mixed daily in a 32 gallon garbage can with a clean plastic liner inserted. The plastic liners were discarded after each day's use. They were used to avoid having to acid-wash the feed containers daily. Concentrated solutions of the individual feed components were added to the container daily and then the feed was diluted to the required total weight with distilled water.

The time and weight were recorded at the time that the feed was started each day and again at the time that it was shut off the next day. The feed was shut off for approximately 30 minutes each day while the new solution was mixed. During this time the feed tubing was acid-washed and the activated sludge was allowed to settle in the reactor for 15 minutes for a modified SVI measurement. A new 8"-10" piece of Tygon tubing was placed in the feed pump every day when the feed solution was changed to reduce changes in the feed rate caused by a "set" in the tubing. After the feed pump was started each day, the rate of feed was checked by pumping into a graduated cylinder for 2 or 3 minutes.

Waste

The cell waste rate was the major control for this system. In activated sludge plants the waste is normally taken from the recycle line where the microorganism concentration is the greatest. In this experiment, however, the waste was taken from the reactor to reduce the measurements required. Since the MLSS of the reactor was measured daily, the amount of microorganisms wasted could be calculated by multiplying the MLSS by the volume wasted.

The waste was collected in a 1000 ml or 2000 ml graduated cylinder depending on the waste rate. The amount of waste was measured every day at 9:00 a.m. Then the graduated cylinder was emptied and replaced to collect the next day's waste. The volume wasted also included any samples taken from the reactor for testing.

When the system had reached steady state and intensive testing had begun, it was necessary to shut the waste pump off for a certain amount of time to compensate for the additional sample volumes required from the reactor for testing. The waste rate was set at a certain amount and the system was allowed to adjust to a constant MLSS in the reactor. Then nitrogen samples were taken for 5 or 6 days as described elsewhere.

The waste rate was then changed and the system was allowed to attain steady state at another MLSS.

Recycle

The rate of recycle from the clarifier to the reactor was set at approximately 50% of the feed rate. The recycle rate was checked every 3 or 4 days by pumping into a graduate cylinder for 4 or 5 cycles. The Tygon tubing in this pump was changed only when it seemed necessary to maintain a constant recycle ratio. This rate was adjusted as required by changing the on-off ratio of the timer.

MEASUREMENT OF BIOLOGICAL PARAMETERS

TOC

The total organic carbon (TOC) of the feed solution and the soluble material in the reactor was measured daily. The filters used were Schleicher and Schuell number 25 glass fiber filters. These S & S filters had a diameter of particle efficiency of 99.99% for particles larger than 0.3 microns. The feed was measured to verify a proper mixture and also to indicate that no bacterial respiration had taken place in the feed solution. The TOC in the reactor was measured to indicate the efficiency of the process in removing carbon.

TOC was determined with a Beckman Model No 915 Carbon Analyzer. The samples were taken daily and deep frozen until quite a few had been collected. The carbon analyzer was calibrated using prepared standards for both total carbon and inorganic carbon. The organic carbon is the difference between the two.

Each measurement of organic carbon by this method required 200 microliters at the most. The small sample volumes required by this test are a definite asset. Large sample volume requirements often upset this type of biological experiment.

MLSS

In sewage treatment the mixed liquor suspended solids (MLSS) test measures any suspended solids in the liquid which may include bacteria, organic and inorganic solids. Since the influent to the pilot plant used in this experiment contained only soluble material, the MLSS measured only the bacterial flocs of the activated sludge. For each reactor four S & S filters were dried at 104°C, cooled, and weighed to the nearest 0.01 mg. Then two measured volumes of sample from the reactor and two from the effluent were passed through individual filters. The filters were then dried for 24 hours at 104°C, cooled and weighed. The MLSS was expressed as mg/l of dry weight solids.

The values for MLSS were corrected for filter weight loss as follows. Filters were dried and weighed as above. An amount of distilled water equivalent to the sample size was passed through the filters. They were then dried and weighed again and the weight loss was recorded. This correction was then applied to each MLSS value.

Nitrogen

When the system reached steady state at certain cell retention times (CRT), samples were taken daily for 5 or 6 days to measure Kjeldahl, ammonia, and nitrate plus nitrite nitrogen. Sufficient sulfuric acid was added to each sample to raise the concentration above 2500 mg/1 H_2SO_4. Then the samples were deep frozen until they were tested. The reagents used for these tests were prepared as described in Standard Methods (49). The exact procedures and sample sizes are described in Appendix C.

Kjeldahl (ammonia plus organic) nitrogen was measured in the feed, an unfiltered reactor sample, and a filtered effluent sample. The procedures followed were those described in Standard Methods (49) and "Aminco Reprint No 104" (50).

Ammonia nitrogen was determined using a distillation-titration procedure for the feed and a filtered effluent sample. The procedures followed were those described in Standard Methods (49) and "Aminco Reprint No 104" (50).

Nitrate concentration in the feed and a filtered effluent sample was measured using the phenoldisulfonic acid method described in Standard Methods (49). This method includes both nitrate plus nitrite and no attempt was made to differentiate between the two.

Cell Retention Time

The cell retention time (CRT) is defined as the total mass of microorganisms divided by the rate at which they are lost from the system.

CRT was computed using the following equation which was derived for the system shown in Figure 11. All of the variables on the right hand side of the equation were measured daily.

$$\text{CRT} = \frac{(V_r+V_c)X_r}{Q_eX_e+Q_wX_r}$$

where

Q_i	= Influent feed rate	1/day
X_i	= Microorganisms in feed	mg/1
a	= Fraction of Q_i recycled	
X_r	= Conc. of microorganisms in reactor	mg/1
Q_e	= Effluent flow rate	1/day
X_e	= Conc. of microorganisms in effluent	mg/1
B	= Compression ratio of recycle	
Q_w	= Waste rate	1/day

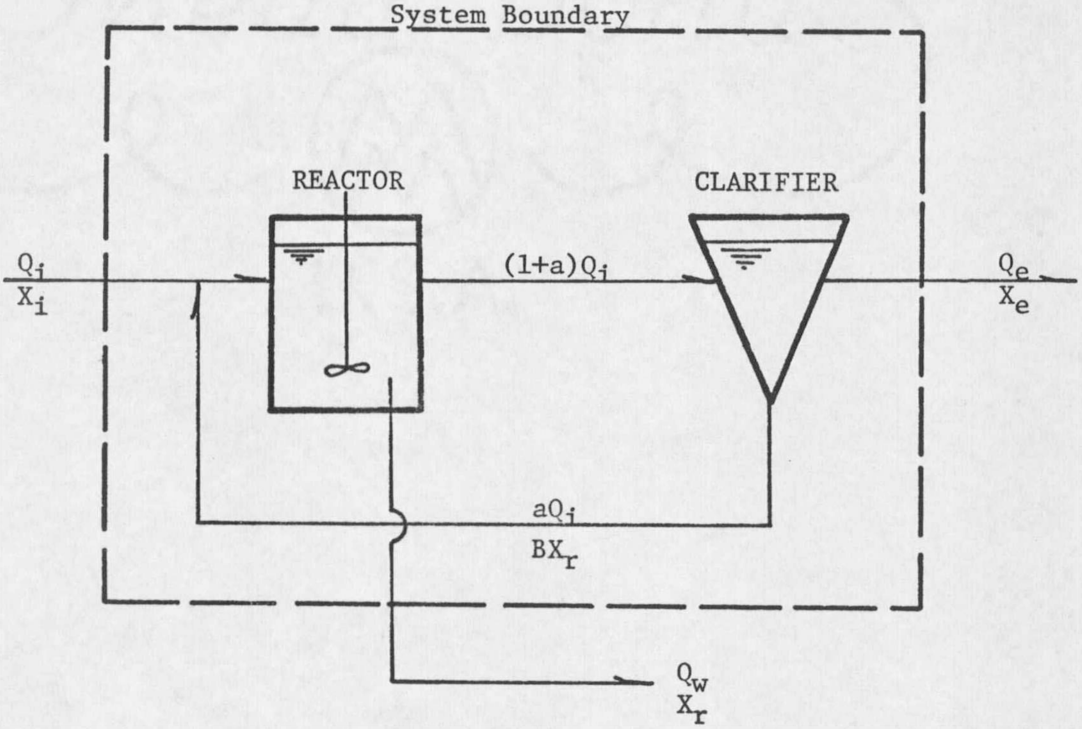


Figure 11. MICROORGANISM BALANCE

V_r = Reactor volume	liters
V_c = Clarifier volume	liters

The term for microorganisms in the influent, X_i, in this experiment was assumed equal to zero and extensive effort was expended to keep the feed sterile. When the system was at steady state, the mass of microorganisms in the system was constant. With the assumption that the concentration of microorganisms in the clarifier was the same as in the reactor, the total mass of microorganisms in the system was $(V_r+V_c)X_r$. Microorganisms were lost from the system in the waste and the effluent.

CHAPTER VI

NITROGEN UTILIZATION

NITROGEN BALANCE

For each period of testing at different values of CRT a nitrogen balance was computed using the system shown in Figure 12 and the following equation:

NITROGEN IN = NITROGEN OUT

$$QN + V_R a X_R K_f = Q_E N_E + Q_w N_R + (NH_3\text{-}N)\uparrow + (N_2\text{-}N)\uparrow$$

where

Q	= Rate of feed	1/day
N	= Nitrogen concentration in feed	mg/1
V_R	= Volume of reactor	1
a	= Ratio of X_R that are nitrogen fixers	dimensionless
X_R	= Concentration of microorganisms in the reactor	mg/1
K_f	= Rate constant for nitrogen fixation	day^{-1}
Q_E	= Rate of effluent	1/day
N_E	= Soluble + organic nitrogen concentration in the effluent	mg/1
Q_w	= Rate of waste	1/day
N_R	= Soluble + organic nitrogen concentration in the reactor	mg/1

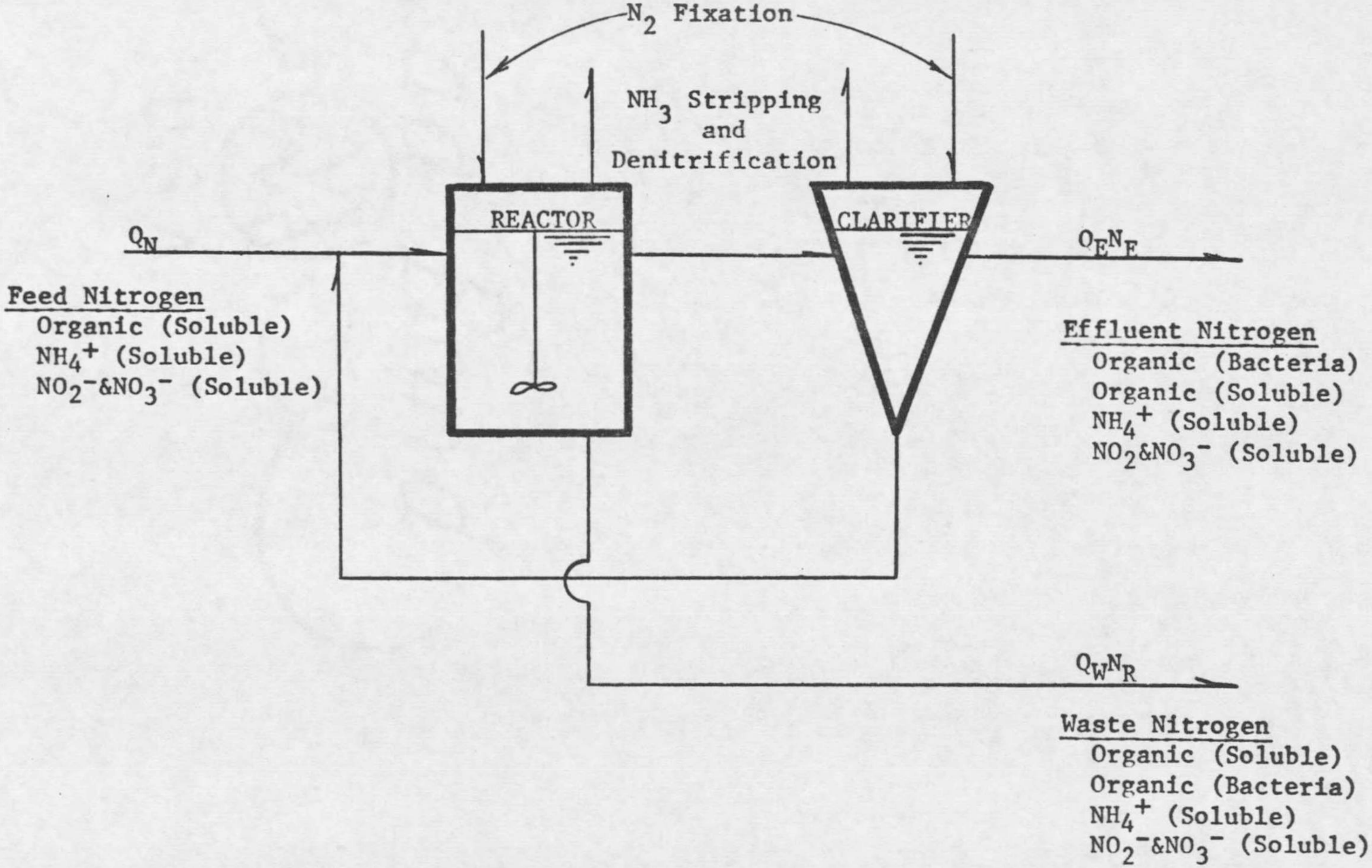


Figure 12. NITROGEN FLOW THROUGH PILOT PLANT

(NH_3-N) = Nitrogen removed through ammonia stripping mg/day

(N_2-N) = Nitrogen removed through denitrification mg/day

There was no nitrogen accumulation term in the equation since the calculations were based on an assumption of steady state conditions. The assumption of steady conditions was verified experimentally as shown in Tables XII, XIII and XIV in "APPENDIX A."

The nitrogen introduced to the system through the feed consists of ammonium ion in HN_4Cl and organic and inorganic nitrogen in the Bacto-Peptone. The constituents of the latter are shown in Table VI. The total influent nitrogen concentration was as follows:

NH_4Cl - N	51.00 mg/l
Bacto-Peptone N	17.25 mg/l
Total Influent N	68.25 mg/l

Since the reactor was continually aerated, nitrogen could have been introduced to the system by fixation, but it was originally thought this would be negligible (18). However, the experimental results indicated significant nitrogen fixation.

When the system reached steady state for a certain cell retention time, samples were taken from the feed, reactor and effluent. Three samples were taken from the feed and three filtered samples from the

TABLE VI

TYPICAL ANALYSIS OF BACTO-PEPTONE (48)

Ash	4.0%
Total Nitrogen	15.5%
Amino Nitrogen	3.0%

Amino Acids (in percentages)

Arginine	8.0%
Aspartic Acid	6.0%
Glutamic Acid	11.0%
Glycine	23.0%
Histidine	1.0%
Isoleucine	2.0%
Leucine	3.5%
Lysine	4.5%
Methionine	1.0%
Phenylalanine	2.5%
Threonine	1.5%
Tyrosine	1.0%
Valine	3.0%

Vitamin Factors (micrograms per gram)

Pyridoxine	2.5
Biotin	0.3
Threonine	0.5
Nicotinic Acid	35.0
Riboflavin	4.0

effluent. Ammonia, Kjeldahl and nitrate plus nitrite nitrogen were measured in the soluble fraction of the samples. Total Kjeldahl Nitrogen was measured in a single, unfiltered reactor sample. Any ammonia, nitrate or nitrite in the reactor was assumed to be the same as in the effluent sample. This reduced the necessary volume of sample required from the reactor and reduced the possibility of upsetting the system.

The results of these measurements are shown in Tables XII, XIII, and XIV in "APPENDIX A" and are plotted in Figure 13. In each run, there was more nitrogen leaving the system than was introduced through the feed. The only reasonable explanation appears to be nitrogen fixation occurred. This conclusion is reinforced by the fact that at higher MLSS or longer CRT values the amount of fixation was significantly greater. An increase in nitrogen was shown by Clark and Dostal (51) on a study of nitrogen in an aerated lagoon. However, they attributed the increase in nitrogen to the possibility of non-representative influent samples.

The average nitrogen entering the system in the feed was approximately 1638 mg/day. With an MLSS concentration of 2000 mg/1 in the reactor, approximately 96 mg of nitrogen was fixed per day. This fixation increased the nitrogen entering the system by 5.85%.

Nitrogen fixation could also occur in domestic sewage treatment. However, this should be verified by procedures other than the nitrogen balance used in this experiment. With longer detention times, higher MLSS and smaller influent nitrogen concentrations, fixation could far exceed the 5.85% found in this pilot plant study.

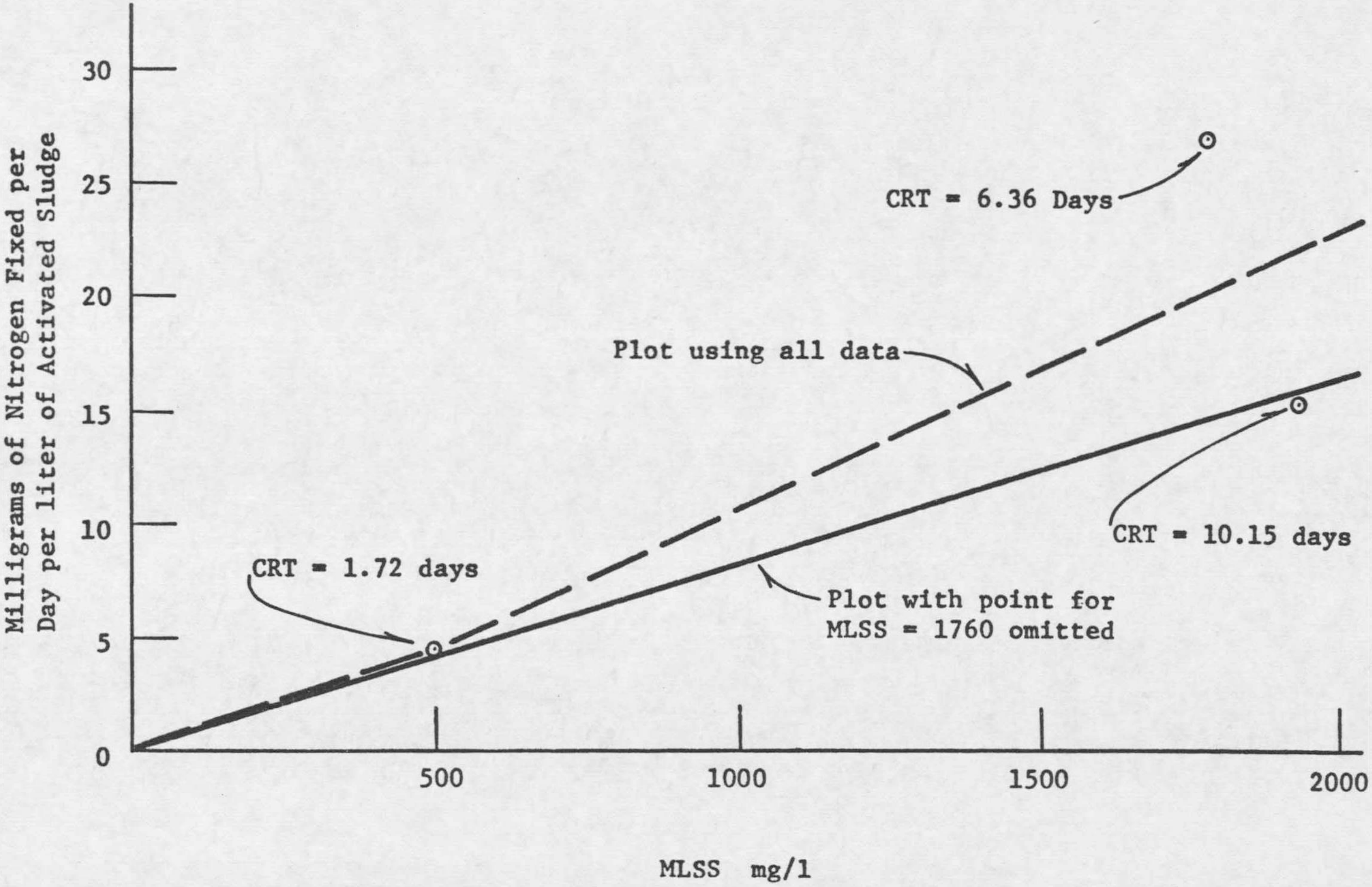


Figure 13. EFFECT OF MLSS AND CRT ON NITROGEN FIXED PER DAY

NITROGEN UTILIZATION VERSUS CELL RETENTION TIME

Nitrogen is utilized by bacteria for growth and multiplication. A plot of the percent nitrogen incorporated in activated sludge with respect to CRT is shown in Figure 14. The data were taken from Tables XII, XIII and XIV and the nitrogen balance calculations in "APPENDIX A." This plot reveals that CRT, within the range explored, has very little effect on the nitrogen content of cells. Therefore to attain maximum nitrogen removal in the activated sludge process the cell wasting rate should be as high as possible.

If this system were operated in such a way that the wasted sludge was completely removed from the system (not digested with supernatant return to the system), then the nitrogen removal would be as shown in Table VII. This information was compiled from Tables XII, XIII and XIV, and the nitrogen balance calculations in "APPENDIX A." The removals were 6.16% and 7.02% at CRT values of 1.72 and 10.15 days respectively. At a CRT of 6.36 days there was an actual gain of nitrogen. The only reasonable explanation for the gain in nitrogen would be fixation. If the first two values were corrected to a nitrogen concentration common in sewage (40±mg/1)(27), then the removal would be approximately 11% assuming that carbon was still limiting.

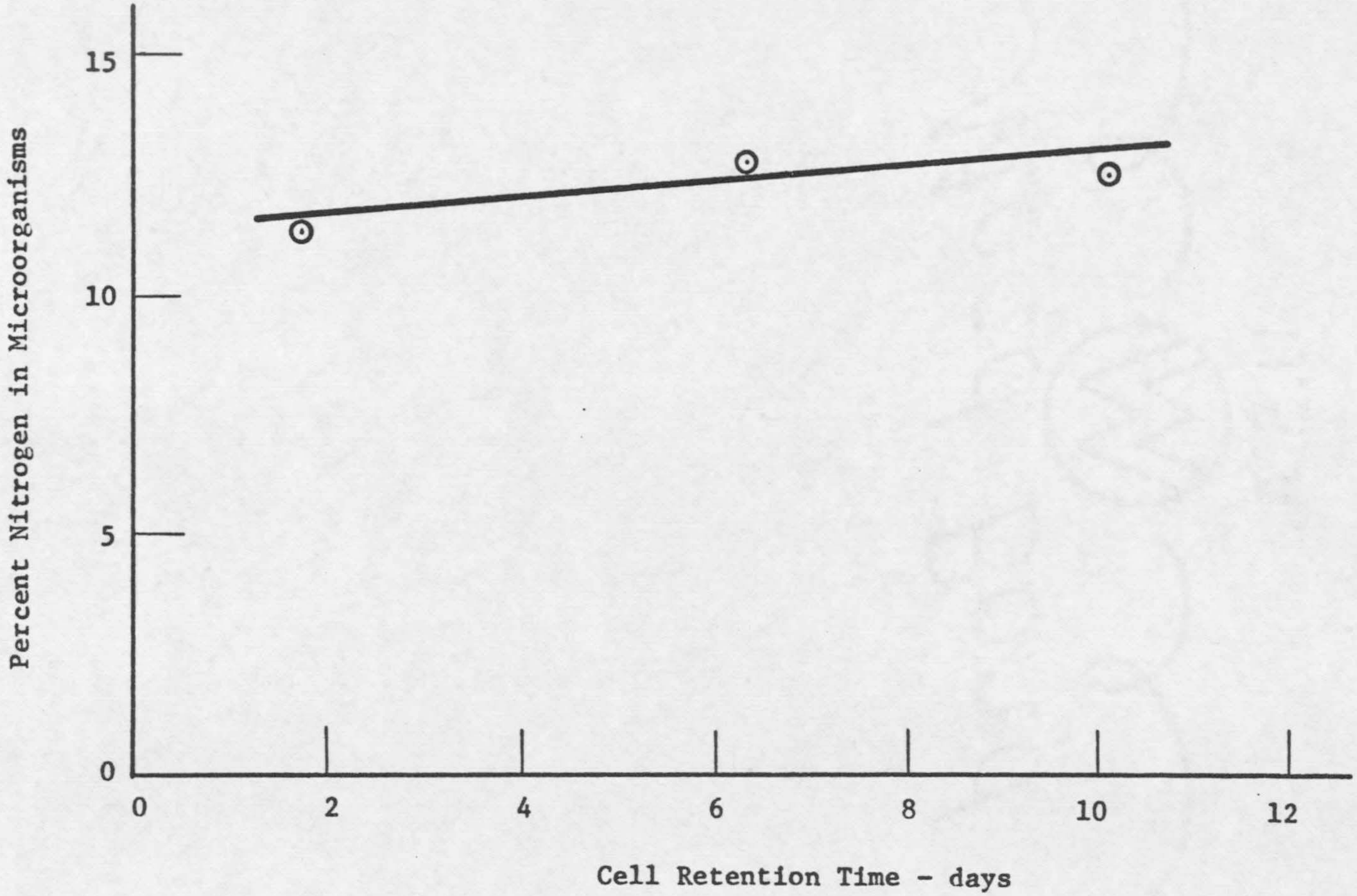


Figure 14. NITROGEN IN ACTIVATED SLUDGE MICROORGANISMS WITH RESPECT TO CELL RETENTION TIME

TABLE VII

NITROGEN REDUCTION THROUGH PILOT PLANT

Daily Average - Four Day Test

CRT days	Influent Nitrogen mg/day	Effluent Nitrogen mg/day	Reduction mg/day	Percent Nitrogen Reduction
1.72	1590	1492	98	6.16
6.36	1682	1689	-7	-
10.15	1509	1403	106	7.02

CHAPTER VII

SETTLEABILITY

During the course of this investigation the settleability of the activated sludge was measured daily. The compressed air, stirring paddle and pumps were shut off each morning while changing the feed lines. The activated sludge in the reactor was allowed to settle for 15 minutes and the volume occupied by the settled activated sludge organisms was measured in milliliters.

This test is similar to the sludge volume index (SVI) described in Standard Methods (49), but, the results are not the same because there are significant differences in the physical dimensions and the settling time was only 15 minutes. To distinguish it from SVI, this parameter was called the settled sludge volume (SSV). It was plotted against the food to microorganism (F/M) ratio. The F/M ratio was expressed as

$$F/M = \frac{\text{TOC fed/day}}{\text{Reactor MLSS}}$$

where TOC is total organic carbon. This is different from the F/M ratio normally used in waste treatment literature. It is usually expressed in terms of BOD_5 rather than TOC.

The plot of F/M versus SSV is shown in Figure 15. The plotted points were determined by selecting 5 day periods when the SSV was

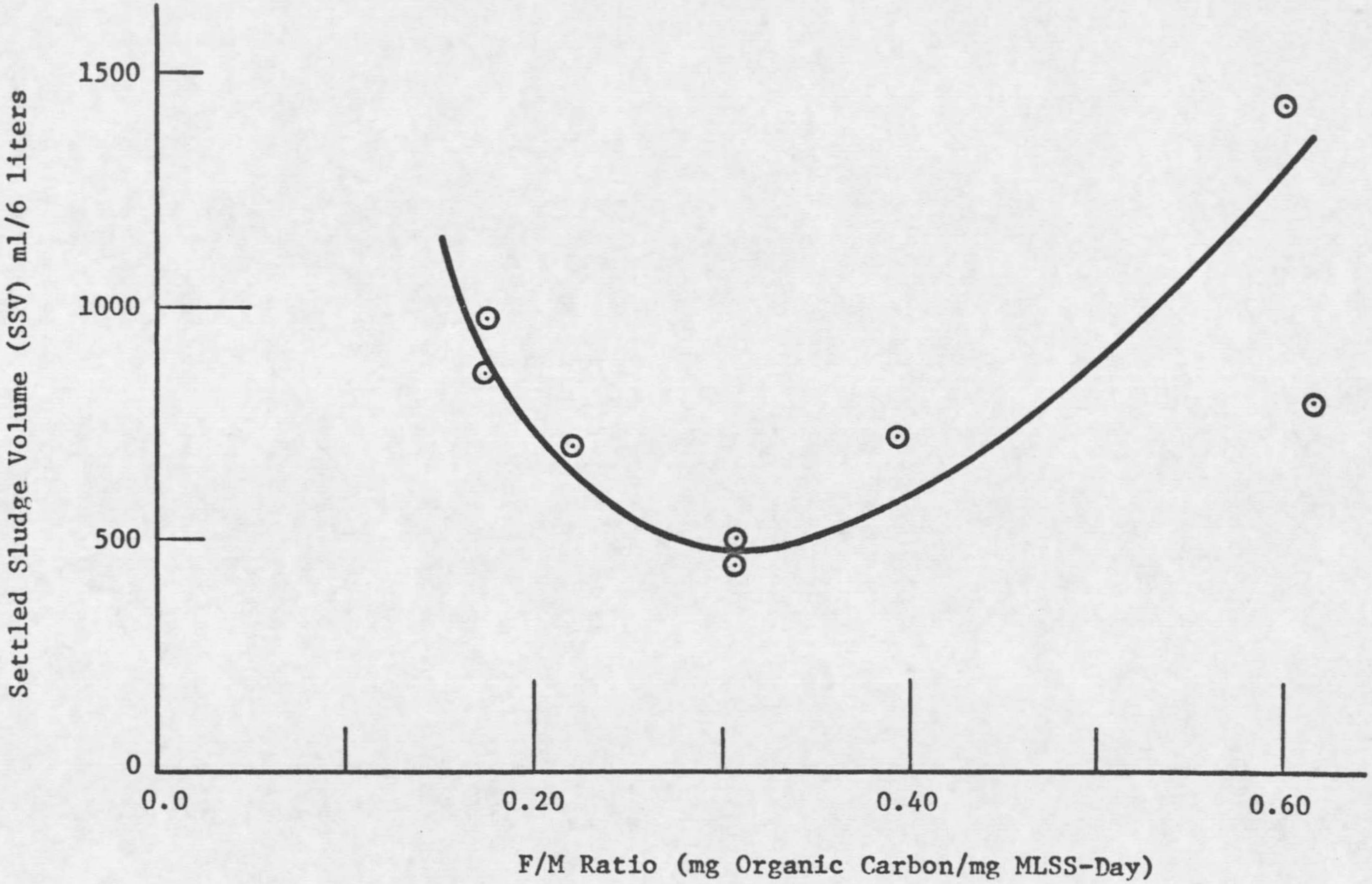


Figure 15. SETTLEABILITY OF ACTIVATED SLUDGE WITH RESPECT TO FOOD TO MICROORGANISM RATIO

relatively constant. Both the SSV and F/M ratios were averaged for each 5 day period. The curve is well defined for loading rates from 0.16 to 0.32/day but the values at higher loading rates do not agree well because the system was very unstable in this range. This curve resembles that prepared by Eckenfelder (43). However, the numerical values are not the same because the means of expressing F/M and SSV are different.

CHAPTER VIII

DISCUSSION

CLARIFIER PLUGGING

The CFSTR shown in Figure 7 was used throughout this experiment. At the outset of the experiments, the CFSTR was operated without wasting as explained in Chapter V. The first attempts to operate the CFSTR were unsuccessful because the activated sludge was so sticky that it could not be pumped from the bottom of the clarifier. A number of modifications were made in the operating procedure and physical apparatus in attempts to overcome this. No success was attained until the clarifier was rebuilt with no obstructions in the lower portion.

When the waste pumps were put in operation, the characteristics of the sludge changed. The stickiness of the sludge disappeared and it readily recycled from the bottom of the clarifier. This writer believes that many of the revisions made to the pilot plant may have been unnecessary if sludge had been wasted both when the organisms were batch fed and when the CFSTR was first activated.

TEST PROCEDURE MODIFICATIONS

The original intent was to allow the system to arrive at steady state as measured by a daily MLSS test and then take samples for testing the system at 6 hour intervals for 2 or 3 days. The first time this was attempted it was noted there was a large hourly variation in the MLSS of the system. This was attributed to the feed and air being shut off while the feed tubing was cleaned and the organisms were allowed to settle for the SSV test. Because of this variation the testing procedure was modified by taking samples at the same time daily for a number of days. This was done with the realization that the system was not at steady state but with the hope that the system was at approximately the same state every day. The reason for testing for 5 days was to obtain average values and to reduce experimental error.

INSTABILITY AT LOW MLSS

When the waste rate was set at a high value in order to obtain a short CRT, the system was extremely unstable. This instability was apparent with MLSS values below 600-700 mg/1. Under normal daily operating conditions the system could be maintained in a stable condition, but when additional samples were taken to test nitrogen and phosphorous, the MLSS would decrease daily until testing was ended. The waste pump was shut off for a period long enough to compensate for the samples taken, but the variation in the rate at which the microorganisms were removed must have upset the system. At higher MLSS values this was not critical.

PUMP ADJUSTMENTS

The pumps used for the feed, recycle and waste required very critical tolerance so that the tubing passing through them was completely flattened when pumping. However, if the clearance was set too close, the threads on the shaft stripped. This caused much difficulty until all of the pumps had been properly adjusted. The greatest problem was encountered with the feed pump since it was working against the highest head.

NITROGEN TESTS

The tests used for ammonia and total Kjeldahl nitrogen presented no particular problems. The only variation from the methods described in the test procedures (49, 50) was the addition of a small amount of H_2SO_4 to the distillation water to convert any NH_3 to NH_4^+ ion (52).

For this experiment there was no reason to separate NO_3^- from NO_2^-. The phenoldisulfonic acid test was used for the sum of the two states. This test was used because it was the only one listed in Standard Methods (49) which would compensate for the interfering substances present. This method required the use of a large amount of glassware and was extremely time consuming.

An attempt was made to utilize an alternate method devised by Bremner (52). In this method NO_3^- and NO_2^- are reduced to NH_4^+ using Devarda's alloy. The ammonia is then measured by a distillation procedure. No success was attained on samples with known concentrations of NO_3^- and NO_2^-.

NITRIFICATION

At no time during the course of this experiment was any of the ammonia from the feed oxidized to NO_2^- or NO_3^-. The first assumption was that the nitrate test procedure was faulty. To check this possibility, known quantities of NO_2^- and NO_3^- were added to samples of the feed and effluent. The minimum recovery was within 92% which indicates the test procedure was reasonably accurate.

The next assumption was that the pH was too low for activity of the nitrifying bacteria. The phosphate buffer used in the feed solution maintained the pH in the reactor between 6.8 and 7.0. The pH of the feed was raised to 9.7 using 6N NaOH. This resulted in a reactor pH of 8.0. The feed pH was maintained at this level for 10 days to obtain an active nitrifying culture of bacteria, but still no nitrification occurred.

Further research indicated that the F/M ratio may have been too high to attain nitrification. The exploration of this possibility was complicated by the fact that all previous work on the required F/M ratios was reported in terms of BOD_5. The BOD_5 of the feed solution used in this experiment was not known, so tests were run for its determination. The results of these BOD_5 determinations varied widely. The results of eighteen tests indicate that the BOD_5 is probably between 125 mg/l and 175 mg/l. The total organic carbon in the feed was both calculated and physically measured as approximately 90 mg/l. Downing, et al (53) have indicated that the ratio of BOD_5 to organic carbon in sewage is normally around 1.7. If this factor were applied to the feed used in this experiment, a BOD_5 value of 1.7 x 90 or

153 mg/l would be obtained. The F/M ratio for BOD_5 values of 125, 153 and 175 mg/l would be as shown for an MLSS of 1900 mg/l. These would be the minimum loading ratios possible since 1900 mg/l was the maximum MLSS value attained during the experiment.

$$F/M \text{ (for } BOD_5 = 125) = \frac{(125 \text{ mg/l})\ (24 \text{ liters/d})}{(1900 \text{ mg/l})\ (6 \text{ liters})} = 0.263/\text{day}$$

$$F/M \text{ (for } BOD_5 = 153) = \frac{(153 \text{ mg/l})\ (24 \text{ liters/d})}{(1900 \text{ mg/l})\ (6 \text{ liters})} = 0.321/\text{day}$$

$$F/M \text{ (for BOD5} = 175) = \frac{(175 \text{ mg/l})\ (24 \text{ liters/day})}{(1900 \text{ mg/l})\ (6 \text{ liters})} = 0.368/\text{day}$$

Schlecta and Culp (27) and Stewart (6) have stated nitrification will not occur at loadings over 0.25 to 0.35/day. In order to decrease the loading factor below 0.25 while keeping the feed constant, it would be necessary to increase the MLSS. The pumps and timers used on the pilot plant could not be set to decrease the waste to a low enough quantity to maintain a higher MLSS. This eliminated the possibility of testing at higher CRT value and of attaining nitrification. If smaller tubing can not be used to reduce the capacity of the pumps, then the gear ratios should be changed for a smaller capacity.

NITROGEN FIXATION

The results of this experiment indicate that nitrogen fixation by activated sludge is a definite occurrence under the test conditions.

The presence of excess ammonia does not appear to inhibit fixation as one might expect. Consequently, in extended aeration (or other processes in which there is no sludge wasting), the effect of biological treatment will be to increase the amount of nitrogen due to nitrogen fixation. In processes with longer detention times the amount would probably be much greater than the 5.85% increase indicated in this experiment.

If the effluent from treatment is discharged to nitrogen-limited waters, the effect of fixation could be quite significant and should be considered. On the other hand, nitrogen-fixation could be beneficial for the treatment of nitrogen-deficient wastewaters.

NITROGEN UTILIZATION

When corrected to an influent nitrogen concentration of 40 mg/l (as might be encountered in domestic sewage) the nitrogen removal of approximately 11% is lower than values normally reported for domestic sewage (44). The BOD_5 of the feed in this experiment is approximately 150 mg/l whereas that reported for normal domestic sewage ranges from 200-400 mg/l. BOD_5 and organic carbon are correlated as shown by Downing et al (53). Therefore a higher BOD_5 indicates a higher concentration of organic carbon available for cell synthesis. If the removals were normalized to BOD_5 values of 200-400 mg/l the respective nitrogen removals would be 15-30% which correlate better with values reported by Shammas (44).

CHAPTER IX

SUMMARY AND CONCLUSIONS

Eutrophication of bodies of water is a natural process. However, under man's influence, this process can be greatly accelerated by the concentration of nutrients in wastewater discharges.

Biological growth can be controlled in a body of water by limiting the amount of any element required for growth of microorganisms. Nitrogen is required for the formation of amino and nucleic acids which are essential parts of living cells.

An activated sludge pilot plant was constructed and experiments were carried out to optimize nitrogen removal from the effluent of the activated sludge waste treatment process. The activated sludge process is based on the utilization of nutrients and substrate by microorganisms for respiration and growth.

Nitrogen is incorporated in living cells in the -3 valence state. Some microorganisms are capable of reducing more highly oxidized states to the ammonia valence. However, many are not capable of this reduction and require their nitrogen supply as ammonia or in more complex molecules such as vitamins.

A number of aerobic chemoautotrophic bacteria derive their energy from the oxidation of the more reduced states of nitrogen to nitrite and nitrate.

Nitrate and nitrite are reduced by microorganisms for two distinct purposes. The first is for incorporation of ammonia in new cells and the second is for respiration in which the nitrate serves as the terminal electron acceptor in anaerobic systems.

Combined states of nitrogen (such as ammonia or nitrate) seldom exceed concentrations of a few parts per million in surface waters. Molecular nitrogen (N_2) exists in vast quantities in the atmosphere, but it is chemically inert and can be used by only a few selected nitrogen-fixing organisms.

In the conventional aerobic activated sludge process at neutral pH values, the only way to remove nitrogen from the effluent is by wasting microorganisms that contain nitrogen. Nitrogen removal could be optimized by maximum cell wasting and by wasting cells with a maximum nitrogen concentration.

In this experiment the following concentrations of nitrogen were found in the activated sludge for various cell retention times:

Cell Retention Time	Percent Nitrogen-N
1.72	11.19
6.37	12.80
10.15	12.59

The results of this experiment indicate nitrogen fixation may play a significant role in the amount of nitrogen introduced to waters receiving effluents from biological treatment.

The following conclusions are presented based on the previously described experiment:

1. Cell retention times of 1.72 to 10.15 days have very little effect on the amount of nitrogen incorporated in the microorganisms of activated sludge.
2. The amount of nitrogen that can be removed by biological treatment is limited by the amount of other substrates and nutrients required for growth of activated sludge.
3. The amount of cells that can be wasted is limited by the necessity to keep the F/M ratio within the required range for adequate sludge settleability. More exotic means of cell separation would extend this range.
4. Within the CRT range tested there is a strong indication of a straight line relationship between nitrogen fixation and MLSS concentration. This would indicate no selectivity for the predominance or repression of the nitrogen fixers within this CRT range.

APPENDICES

APPENDIX A

NITROGEN DATA AND CALCULATIONS

TABLE VIII

NITROGEN TEST DATA REACTOR NO. 1
(All data as mg/l N)

Source of sample	Component tested	Date				
		11/22	11/23	11/24	11/25	11/26
Feed (Not Filtered)	Kjeldahl	71.2	71.8	70.9	71.8	69.5
	NH_3	51.7	51.2	51.7	53.5	51.7
	Organic	19.5	20.6	19.2	18.3	17.8
	NO_3^-	0.3	0.3	0.3	0.3	0.3
	Total N	71.5	72.1	71.2	72.1	69.8
Reactor (Not Filtered)	Kjeldahl	302.0	302.0	282.0	293.0	274.0
Effluent (Filtered)	Kjeldahl	67.8	65.5	64.2	63.5	66.2
	NH_3	64.5	61.9	63.5	62.9	62.6
	Organic	3.3	3.6	0.7	0.6	3.6
	NO_3^-	0.0	0.0	0.0	0.0	0.0
	Total N	67.8	65.5	64.2	63.5	66.2

TABLE IX

NITROGEN TEST DATA REACTOR NO. 1
(All data as mg/l N)

Source of sample	Component tested	Date				
		10/25	10/26	10/27	10/28	10/29
Feed (Not Filtered)	Kjeldahl	57.2	64.0	70.1	67.1	68.5
	NH_3	46.7	49.8	50.7	48.6	49.7
	Organic	10.5	14.2	19.4	18.5	18.8
	NO_3^-	0.3	0.2	0.2	0.1	0.1
	Total N	57.5	64.2	70.3	67.2	68.6
Reactor (Not Filtered)	Kjeldahl	296.0	284.0	315.0	305.0	318.0
Effluent (Filtered)	Kjeldahl	57.7	57.5	59.4	60.7	70.5
	NH_3	58.5	53.4	48.2	57.4	60.0
	Organic	-0.8	4.1	11.2	3.3	10.5
	NO_3^-	0.1	0.1	0.0	0.0	0.0
	Total N	57.8	57.6	59.4	57.4	70.5

TABLE X

NITROGEN TEST DATA REACTOR NO. 2
(All data as mg/l N)

Source of sample	Component tested	Date				
		10/25	10/26	10/27	10/28	10/29
Feed (Not Filtered)	Kjeldahl	62.5	65.3	66.3	67.1	71.5
	NH_3	50.5	49.8	50.0	51.7	51.2
	Organic	12.0	15.5	16.3	15.4	20.3
	NO_3^-	0.5	0.3	0.5	0.8	0.6
	Total N	63.0	65.6	66.8	67.8	72.1
Reactor (Not Filtered)	Kjeldahl	121.0	104.0	117.0	111.0	104.0
Effluent (Filtered)	Kjeldahl	49.9	55.0	57.8	57.4	62.5
	NH_3	60.5	55.9	57.4	56.9	50.2
	Organic	-10.6	-0.9	0.4	0.5	12.3
	NO_3^-	0.0	0.0	0.1	0.1	0.0
	Total N	49.9	55.9	57.9	57.5	62.5

TABLE XI

TOTAL ORGANIC AND SOLUBLE ORGANIC NITROGEN

Date	Soluble + Insoluble Org + NH_3 N mg/1	NH_3-N mg/1	Soluble Insoluble Org-N mg/1	Soluble Org + NH_3 N mg/1	Soluble NH_3-N mg/1	Soluble Org-N mg/1
REACTOR #1						
10/25	296.0	58.5	237.5	57.7	58.5	-0.8
10/26	284.0	53.4	230.6	57.5	53.4	4.1
10/27	315.0	48.2	266.8	59.4	48.2	11.2
10/28	305.0	57.4	247.6	60.7	57.4	3.3
10/29	318.0	60.0	258.0	70.5	60.0	10.5
Total	1518.0	277.5	1240.5	305.8	277.5	28.3
Average	303.6	55.5	248.1	61.2	55.5	5.7
11/22	302.0	64.5	237.5	67.8	64.5	3.3
11/23	302.0	61.9	240.1	65.5	61.9	3.6
11/24	282.0	63.5	218.5	64.2	63.5	0.7
11/25	293.0	62.9	231.1	63.5	62.9	0.6
11/26	274.0	62.6	211.4	66.2	62.6	3.6
Total	1453.0	315.4	1138.6	327.2	315.4	11.8
Average	290.6	63.1	227.7	65.4	63.1	2.4

TABLE XI (cont.)

Date	Soluble + Insoluble Org + NH_3 N mg/l	NH_3-N mg/l	Soluble Insoluble Org-N mg/l	Soluble Org + NH_3 N mg/l	Soluble NH_3-N mg/l	Soluble Org-N mg/l
REACTOR #2						
10/25	121.0	60.5	60.5	49.9	60.5	-10.6
10/26	104.0	55.9	49.0	55.0	55.0	-0.9
10/27	117.0	57.4	59.6	57.8	57.4	0.4
10/28	111.0	56.9	54.1	57.4	56.9	0.5
10/29	104.0	50.2	53.8	62.5	50.2	12.3
Total	557.0	280.9	277.0	282.6	280.9	1.7
Average	111.4	56.2	55.4	56.5	56.2	0.3

TABLE XII

NITROGEN DATA FOR CELL RETENTION TIME EQUALS 1.72 DAYS

Date	Influent		Reactor					Effluent			CRT
	Total N mg/l	Q liters	Sol + Insol Org-N mg/l	Sol Org N mg/l	Insol Org N mg/l	MLSS mg/l	Waste Q liters	Tot Sol N mg/l	MLSS mg/l	Q mg/l	days
10/25	63.0		60.5	-10.6	71.1	554		49.9	69		
		23.0					1.13				2.22
10/26	65.6		49.0	-0.9	49.9	505		55.9	68		
		24.8					1.09				1.85
10/27	66.8		59.6	0.4	59.2	439		57.9	78		
		23.4					1.11				1.47
10/28	67.8		54.1	0.5	53.6	417		57.5	110		
		23.6					1.12				1.35
10/29	72.1		53.8	12.3	41.5	346		62.5	92		
Total	335.3	94.8	277.0	1.7	275.3	2261	4.45	283.7	417	90.35	6.89
Avg	67.1	23.7	55.4	0.3	55.1	492	1.11	56.7	83	22.59	1.72

TABLE XIII

NITROGEN DATA FOR CELL RETENTION TIME EQUALS 6.36 DAYS

Date	Influent		Reactor					Effluent			CRT
	Total N mg/l	Q liters	Sol + Insol Org-N mg/l	Sol Org N mg/l	Insol Org N mg/l	MLSS mg/l	Waste Q liters	Tot Sol N mg/l	MLSS mg/l	Q mg/l	days
11/22	71.5		237.5	3.3	234.2	1938		67.8	58		
		22.15					0.58			21.57	6.52
11/23	72.1		240.1	3.6	236.5	1809		65.5	82		
		24.05					0.46			23.59	5.78
11/24	71.2		218.5	0.7	217.8	1732		64.2	76		
		24.05					0.52			23.53	6.12
11/25	72.1		231.1	0.6	230.5	1628		63.5	53		
		24.05					0.59			23.46	7.03
11/26	69.8		211.4	3.6	207.8	1693		66.2	38		
Total	356.7	94.30	1138.6	11.8	1126.8	8800	2.15	327.2	307	92.15	25.45
Avg	71.3	23.58	227.7	2.4	225.3	1760	0.54	65.4	61	23.04	6.36

TABLE XIV

NITROGEN DATA FOR CELL RETENTION TIME EQUALS 10.15 DAYS

Date	Influent		Reactor					Effluent			CRT
	Total N mg/l	Q liters	Sol + Insol Org-N mg/l	Sol Org N mg/l	Insol Org N mg/l	MLSS mg/l	Waste Q liters	Tot Sol N mg/l	MLSS mg/l	Q mg/l	days
10/25	57.5	22.8	237.5	-0.8	238.3	1970	0.63	57.8	16	22.17	10.12
10/26	64.2	24.1	230.6	4.1	226.5	1910	0.645	57.6	26	23.45	9.89
10/27	70.3	22.3	266.8	11.2	255.6	1914	0.66	59.4	13	21.64	10.49
10/28	67.2	22.7	247.6	3.3	244.3	1959	0.67	57.4	18	22.04	10.09
10/29	68.6		258.0	10.5	247.5	1876		70.5	18		
Total	327.8	91.9	1240.5	28.3	1212.2	9629	2.61	302.7	91	89.30	40.59
Avg	65.6	23.0	248.1	5.7	242.4	1926	0.65	60.5	18	22.33	10.15

NITROGEN BALANCE CALCULATIONS (SEE TABLES XI, XII, XIII, XIV)

Nitrogen In = Nitrogen Out

$$(Q_IT)(N_I) + N_F = (Q_ET)(N_E) + (Q_WT)(N_W)$$

where

$\%N$ = Nitrogen Concentration of activated sludge

$$= \frac{\text{insoluble organic nitrogen concentration in reactor}}{\text{microorganism concentration in reactor}}$$

N_E = Soluble + insoluble nitrogen concentration in effluent

= Soluble nitrogen in effluent plus nitrogen in effluent microorganisms

N_W = Soluble + insoluble nitrogen in the waste

N_F = Nitrogen fixed

N_I = Nitrogen in feed

Q_IT = Total feed during test period

Q_ET = Total effluent during test period

Q_WT = Total waste during test period

Reactor #1 Test Period 10/25-10/29 (Table XIV) CRT = 10.15 days

$\%N = (248.1-5.7)/1926 = 242.4/1926 = 0.1259 = 12.59\%$

$N_E = 60.5 + (18)(0.1259) = 60.5 + 2.3 = 62.8$

$N_F = (89.34)(62.8) + (2.60)(303.6) - (65.66)(91.90)$

$= 5611 + 789 - 6034$

= 366 mg/4 days

= 91.5 mg/day

Nitrogen fixed/day/liter MLSS

= 91.5/6

= 15.3 mg/day/liter MLSS

Reactor #1 Test Period 11/22-11/26 (Table XIII) CRT = 6.36 days

$\%N = (227.7-2.4)/1760 = 12.80\%$

$N_E = 65.4 + (61)(0.1280) = 65.4 + 7.9 = 73.3$

$N_F = (73.3)(92.15) + (2.15)(290.6) - (71.34)(94.30)$

$= 6754 + 625 - 6727$

N_F = 652 mg/4 days

= 163 mg/day

Nitrogen fixed/day/liter of MLSS

= 163/6

= 27.2 mg/day/liter MLSS

Reactor #2 Test Period 10/25-10/29 (Table XII) CRT = 1.72 days

%N = (55.4-0.3)/(492) = 11.19%

N_E = (56.7) + (0.1119)(83) = 56.7 + 9.3 = 66.0

N_F = (66.0)(90.4) + (4.5)(111.4) - (94.8)(67.1)

= 5966 + 501 - 6361

= 106 mg/4 days

= 27 mg/day

Nitrogen fixed/day/liter MLSS

= 27/6

= 4.5 mg/day/liter MLSS

APPENDIX B

SETTLEABILITY DATA

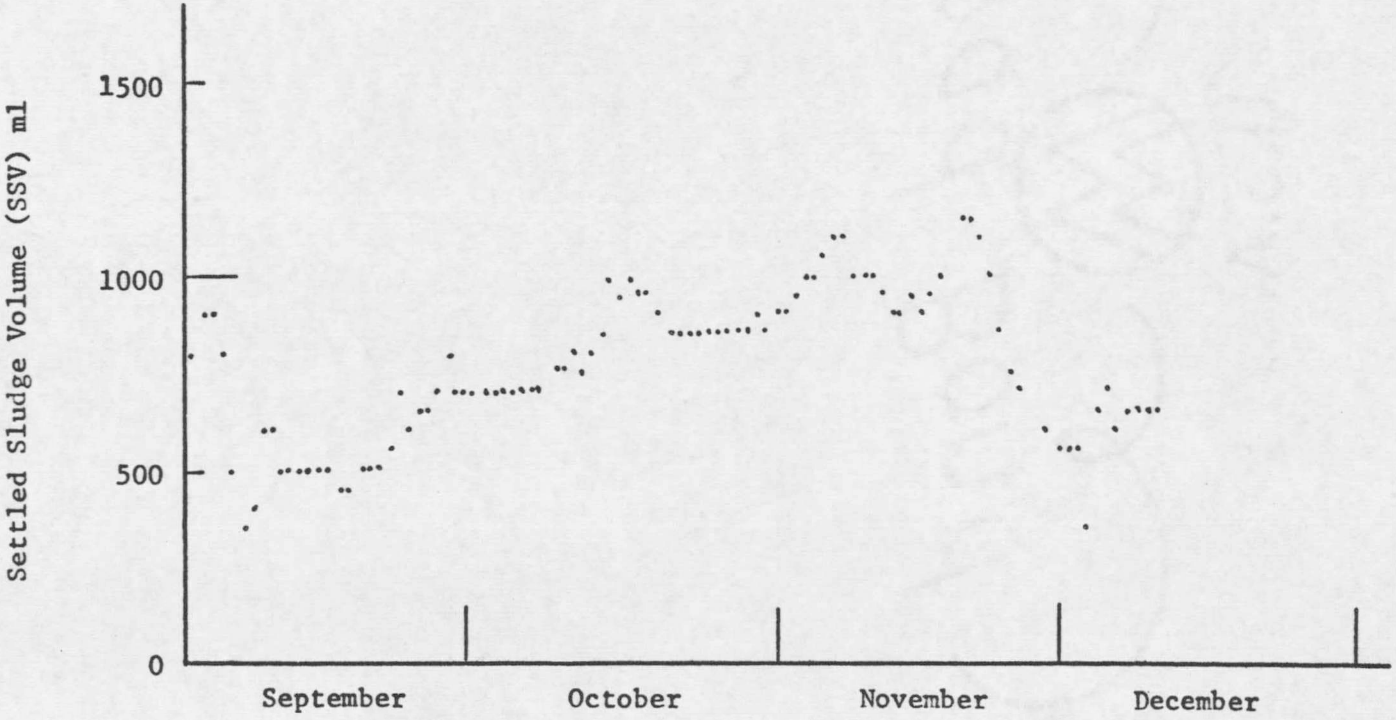


Figure 16. REACTOR NO. 1 SLUDGE SETTLEABILITY

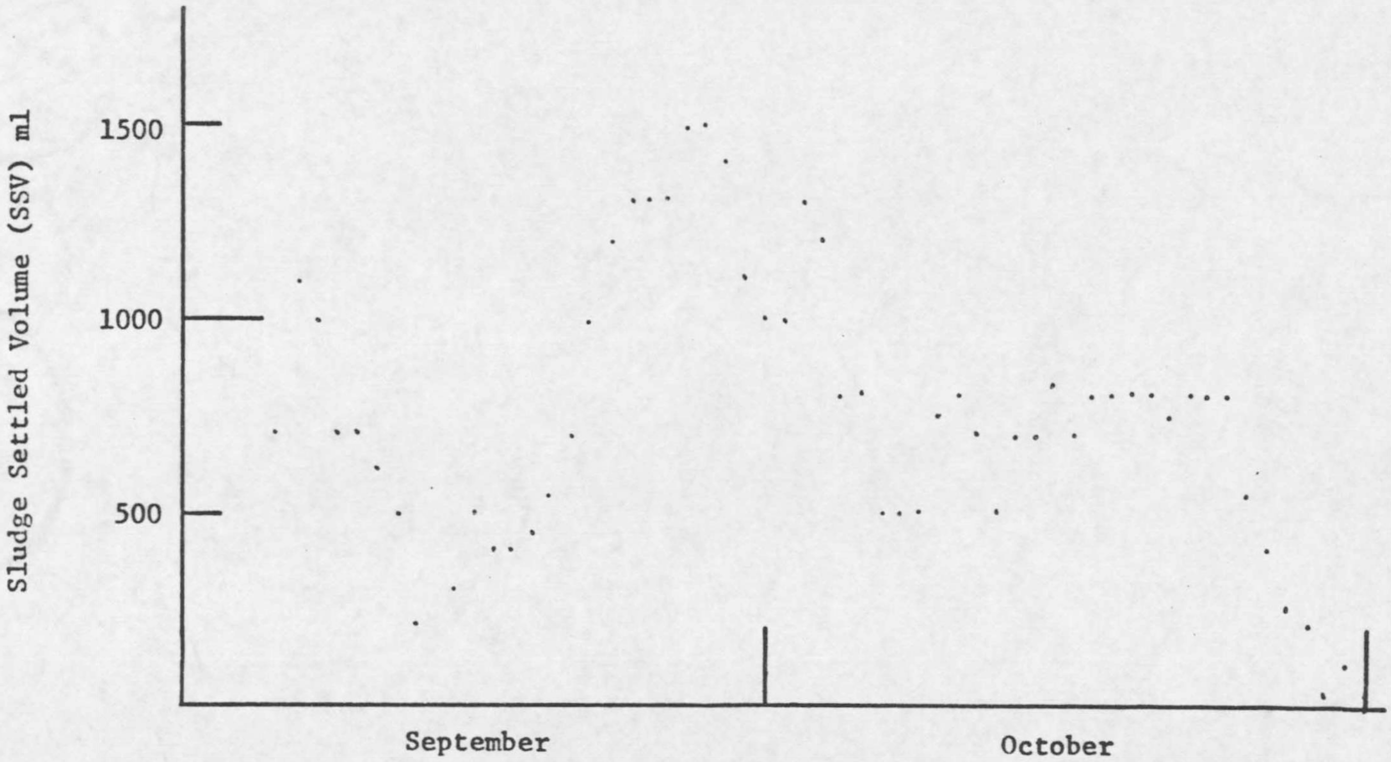


Figure 17. REACTOR NO. 2 SLUDGE SETTLEABILITY

TABLE XV

F/M RATIO AND SLUDGE SETTLING DATA

Date	Reactor			Feed	F/M	SVI	
	No	Volume	MLSS			Daily	Avg
1971		liters	mg/1	liters		ml	ml
9/11	1					500	
		6.00	1112	20.20	0.272		500
9/12	1					500	
		6.05	1077	22.95	0.318		500
9/13	1					500	
		6.05	1075	23.15	0.320		500
9/14	1					500	
		6.05	1111	23.45	0.314		500
9/15	1					500	
Avg					0.308		500
10/2	1					700	
		6.05	1603	22.84	0.212		700
10/3	1					700	
		6.00	1621	24.32	0.225		700
10/4	1					700	
		6.00	1581	22.88	0.217		700
10/5	1					700	
		6.00	1590	23.92	0.225		700
10/6	1					700	
Avg					0.220		700
10/22	1					850	
		6.00	1920	22.60	0.177		850
10/23	1					850	
		6.00	1923	22.10	0.173		850
10/24	1					850	
		6.00	1958	22.80	0.175		850
10/25	1					850	
		6.00	1940	22.80	0.176		850

TABLE XV (cont.)

Date	Reactor			Feed	F/M	SVI	
	No	Volume	MLSS			Daily	Avg
1971		liters	mg/l	liters		ml	ml
10/26	1					850	
Avg					0.175		850
10/15	1					1000	
		6.00	1823	24.00	0.197		975
10/16	1					950	
		6.00	1866	21.90	0.176		975
10/17	1					1000	
		6.00	1895	22.80	0.180		975
10/18	1					950	
		6.00	1866	18.20	0.146		950
10/19	1					950	
Avg					0.175		969
9/3	1					900	
		6.00	909	23.85	0.394		900
9/4	1					900	
		6.00	919	23.40	0.387		850
9/5	1					800	
		6.00	840	22.80	0.408		650
9/6	1					500	
		6.00	739	19.10	0.388		425
9/7	1					350	
Avg					0.394		706
9/15	2					500	
		6.10	1263	22.70	0.264		450

TABLE XV (cont.)

Date	Reactor			Feed	F/M	SVI	
	No	Volume	MLSS			Daily	Avg
1971		liters	mg/l	liters		ml	ml
9/16	2					400	
		6.10	1144	18.30	0.236		400
9/17	2					400	
		6.10	932	20.10	0.318		425
9/18	2					450	
		6.10	817	22.30	0.402		500
9/19	2					550	
Avg					0.305		444
9/24	2					1300	
		6.18	567	23.60	0.615		1300
9/25	2					1300	
		6.20	590	26.00	0.640		1400
9/26	2					1500	
		6.20	612	24.20	0.573		1500
9/27	2					1500	
		6.17	513	20.20	0.575		1450
9/28	2					1400	
Avg					0.601		1412.5
10/17	2					800	
		5.98	563	22.90	0.612		800
10/18	2					800	
		6.02	578	22.80	0.590		800
10/19	2					800	
		6.07	529	23.10	0.648		800
10/20	2					800	
		6.10	545	22.40	0.607		775
10/21	2					750	
Avg					0.614		793.75

APPENDIX C

NITROGEN TEST PROCEDURES

TOTAL KJELDAHL NITROGEN TEST (49, 50)

APPARATUS

Aminco micro Kjeldahl rotary digestion rack and steam distillation apparatus.

REAGENTS

See Standard Methods (49) pages 245 and 246.

SAMPLES

Analyze samples that contain between 0.1 to 0.9 mg of nitrogen.

	Size Required for Analysis ml	Size Sample Taken ml
Feed	10	20
Reactor	5	10
Effluent (Filtered)	10	20

When the above samples are taken, add 1 drop of 18N H_2SO_4 and deep freeze until time of analysis.

TEST PROCEDURE

1. Place the above required samples in 100 ml micro-Kjeldahl digestion-distillation flasks.
2. Add 10 ml of the digestion reagent and one carborundum boiling chip to each sample.

3. Place the samples on the digestion apparatus with the heaters adjusted so that the samples come to a rolling boil in four to five minutes. Samples will turn black, give off copious white fumes and then clear. When the mixture clears, allow it to boil for 30 minutes more to insure complete digestion.
4. Allow samples to cool and add 20 ml of ammonia free water to prevent the formation of K_2SO_4 crystals.
5. Add two drops of phenolphthalein indicator to each digested sample.
6. Steam out distillation flask for approximately five minutes.
7. Add 10 ml of 2% boric acid solution to a 50 ml Erlenmyer flask and place on distillation apparatus so that tip of distillate tube extends all the way down into the boric acid.
8. Insert the lightly greased, ground glass end of the digestion-distillation flask in the distillation apparatus. Add 10 ml of NaOH, Na_2SO_4 reagent to the bottom of the flask with the special entrance tube.
9. Open steam valve just enough to mix the digested solution with the NaOH, Na_2SO_4 reagent. If the mixture does not turn pink, add 5 ml more of the base reagent.
10. Distill with steam for four minutes after the first drop of distillate goes into the distillate tube or until 25 ml has been distilled into the boric acid solution. Then lower the boric acid away from the distillate tube, and allow distillation

to continue for 1 minute to clear the tip of the distillate tube.

11. Carry a blank using 10 ml of digestion reagent and 10 ml of NaOH, Na_2SO_4 reagent through the same procedure.

12. Titrate the NH_3 in the indicating boric acid solution with $0.02 \pm N$ H_2SO_4 for a pale lavender end point.

13. (Organic + NH_3) - N is calculated as follows:

$$(\text{Organic} + NH_3) - N = \frac{(A - B)\cdot(14{,}007)\ (N)}{\text{ml sample}}$$

where

A = ml $0.02 \pm N$ H_2SO_4 required for sample

B = ml $0.02 \pm N$ H_2SO_4 required for blank

N = Normality H_2SO_4 titrant

AMMONIA NITROGEN TEST (49, 50)

APPARATUS

Aminco micro Kjeldahl steam distillation apparatus.

REAGENTS

See Standard Methods (49) page 225.

SAMPLES

Take 25 ml samples of the feed and of the filtered effluent. Add one drop of 18N H_2SO_4 to each sample and deep freeze until the time of analysis.

TEST PROCEDURE

1. Pipette 10 ml of the sample into a 125 ml Erlenmyer flask with a stirring bar. Add 2 drops phenolphthalein indicator. Determine the amount of 1N NaOH required to raise the pH to the phenolphthalein end point. Discard this sample.
2. Pipette 10 ml of the sample into a 100 ml micro-Kjeldahl digestion-distillation flask. Add 10 ml phosphate buffer solution and mix. Then add the same amount of 1N NaOH as required in 1 above. Do not add buffer or acid until ready to complete step 5 below.
3. Steam out distillation apparatus for approximately 5 minutes.
4. Add 10 ml of 2% boric acid solution to a 50 ml Erlenmyer flask and place on distillation apparatus so that tip of distillate tube extends all the way down into the boric acid.

5. Insert the lightly greased, ground glass end of the digestion-distillation flask in the distillation apparatus, and distill with steam for 4 minutes after the first drop of distillate goes into the distillate tube or until 25 ml has been distilled into the boric acid solution. Then lower the boric acid away from the distillate tube and allow distillation to continue for 1 minute to clear the tip of the distillate tube.
6. Check the pH of the solution after distillation. If the pH falls outside the 7.2-7.6 range, discard and repeat with more phosphate buffer.
7. Titrate the NH_3 in the indicating boric acid solution with $0.02 \pm N$ H_2SO_4 for a pale lavender end point.
8. Carry a blank using 5 ml phosphate buffer through the same procedure.
9. Ammonia nitrogen is calculated as follows:

$$NH_3\text{-}N = \frac{(A - B)\ (14{,}007)\ (N)}{\text{ml sample}}$$

where

A = ml $0.02 \pm N$ H_2SO_4 required for sample

B = ml $0.02 \pm N$ H_2SO_4 required for blank

N = Normality H_2SO_4 titrant

NITRATE PLUS NITRITE TEST (49)

METHOD

Phenoldisulfonic Acid.

REAGENTS

See <u>Standard Methods</u> (49) pages 235, 236 and 465.

SAMPLES

At the time of sampling take 25 ml sample, add 1 drop 18N H_2SO_4 and deep freeze until time of analysis.

TEST PROCEDURE

1. Pipette 10 ml samples into each of 2-125 Erlenmyer flasks and dilute to 100 ml with distilled water. Use one sample for a chloride determination and the other for the NO_3-N determination.
2. Add 1.0 ml of acid indicator solution, which is required in the Mercuric Nitrate procedure, to the sample used for the chloride determination. The sample should be blue-green at this point to indicate a pH of 2.5±0.1. If the color is not blue-green, see <u>Standard Methods</u>, part 1120.
3. Determine the amount of 0.0141N $Hg(NO_3)_2$ required to bring the sample to a definite purple end point. Carry a blank through the above procedure and determine the chloride concentration as follows:

$$\text{mg/l - Cl} = \frac{(A - B)\ (N)\ (35{,}450)}{\text{ml sample}}$$

where

A = ml titrant for sample

B = ml titrant for blank

N = Normality of $Hg(NO_3)_2$

4. To the other diluted sample from step 1 add 1 ml 1N H_2SO_4 and stir. Add $KMnO_4$ dropwise while stirring until a faint pink color persists for 15 minutes.
5. Add enough $AgSO_4$ to the sample from step 4 to exactly equal the chloride concentration as determined in step 3.
6. Filter the sample through a 0.45 micron filter into an evaporating dish and wash through with approximately 20 ml of distilled water.
7. Neutralize the sample to pH 7 with 1N NaOH and evaporate to dryness over a hot water bath.
8. Add 2 ml of phenoldisulfonic acid reagent to the residue, heat over a water bath and rub with a glass rod to insure the dissolution of all solids. Then dilute the mixture with 20 ml of distilled water.
9. Add 7 ml concentrated NH_4OH to attain full color development.
10. Filter the sample into a 100 ml volumetric flask and wash through with distilled water. Fill the flask to the mark

with distilled water. Carry a blank through the above procedure.

11. Make readings at 410 millimicrons on a Spectronic 20 with 100% transmittance for distilled water.
12. Determine the NO_3-N concentration by comparison with readings from standards that were prepared using appropriate quantities of 100 mg/1 NO_3-N standard solution and carried through steps 7-11 above.

APPENDIX D

BACTERIAL NOMENCLATURE REVISION

BACTERIAL NOMENCLATURE REVISION (54)

Ammonia Oxidizers	Nitrite Oxidizers
Nitrosomonas	*Nitrobacter*
Nitrosococcus	*Nitrospina*
Nitrosospira	*Nitrococcus*
Nitrosolobus	

REFERENCES

1. Liebig, J. Chemistry and its Relation to Agriculture and Physiology, (John Wylie and Sons, New York). (Not read).

2. Lehninger, A. L. Biochemistry, (Worth Publishers Inc., New York, 1970).

3. McCarty, P. L. et al Committee Report. "Chemistry of Nitrogen and Phosphorous in Water." Journal American Water Works Association 62: 127-140 (Feb. 1970).

4. Grundy, R. D. "Strategies for Control of Man Made Eutrophication." Environmental Science and Technology 5: 1184-1190 (Dec. 1971).

5. Eckenfelder, W. W. "A Theory of Activated Sludge Design for Sewage." The Activated Sludge Process in Sewage Treatment Theory and Application, (University of Michigan, Department of Civil Engineering) 72-86 (1966).

6. Stewart, M. J. "Activated Sludge System Variations: Specific Applications." Water and Sewage Works: R-7 to R-22 (1971).

7. Diaz, F. F. and J. V. Bhat. "Microbial Ecology of Activated Sludge: I Dominant Bacteria." Applied Microbiology 12(5): 412-417 (Sept. 1964).

8. Allen, L. A. "The Bacteriology of Activated Sludge." Journal of Hygiene 43: 424-431 (1943).

9. Jasewicz, L. and N. Porges. "Biochemical Oxidation of Dairy Wastes: VI Isolation and Study of Sludge Microorganisms." Sewage and Industrial Wastes 28: 1130-1136 (1956).

10. Rogovskaya, Z. I. and M. F. Lazareva. "Intensification of Biochemical Purification of Industrial Sewage: I A Microbiological Specification of Activated Sludges Purifying Various Industrial Sewages." Mikrobiologiya 28: 565-573 (1959). (Not read).

11. Anderson, R. and E. McCoy. "Floc-Forming Bacteria from Activated Sludge." Bacteriological Proceedings: 162 (1963).

12. Williamson, D. H. and J. F. Wildinson. "The Isolation and Estimation of Poly-Beta-Hydroxybutyrate Inclusions of Bacillus Species." Journal of General Microbiology 19: 198-209 (1958).

13. Doudoroff, M. and R. Y. Stanier. "Role of Poly-Beta-Hydroxybutyric Acid in the Assimilation of Organic Carbon by Bacteria." Nature 183: 1440-1442 (1959).

14. Rouf, M. A. and J. L. Stokes. "Isolation and Identification of the Sudanophilic Granules of Sphaerotilus natans." Journal of Bacteriology 83: 343-347 (1962).

15. Sierra, G. and N. E. Gibbons. "Role and Oxidation Pathway of Poly-Beta-Hydroxybutyric Acid in Micrococcus halodenitrificans." Canadian Journal of Microbiology 8: 255-269 (1962).

16. Prakasam, T. B. S. and N. C. Dondera. "Aerobic Heterotrophic Bacterial Populations of Sewage and Activated Sludge." Applied Microbiology 15(3): 461-467 (May 1967).

17. Sawyer, C. N. and P. L. McCarty. Chemistry for Sanitary Engineers, Second Edition, (McGraw-Hill, New York, 1967).

18. Painter, H. A. "A Review of the Literature on Inorganic Nitrogen Metabolism in Microorganisms." Water Research 4(6): 393-450 (June 1970).

19. Fewson, C. A. and D. J. D. Nicholas. "Utilization of Nitrate by Microorganisms." Nature, London 190: 2-7 (1961).

20. Stanier, R. Y., Doudoroff, M., and E. A. Adelberg. The Microbial World, Third Edition, (Prentice-Hall, Inc., Englewood Cliffs, N. J., 1970).

21. Borchardt, J. A. "Nitrification in the Activated Sludge Process." The Activated Sludge Process in Sewage Treatment Theory and Application. (University of Michigan, Department of Civil Engineering, 1966).

22. Meyerhof, O. Arch f. die ges Physiologie. 164, 416 (1916). (Not read).

23. Engel, M. S. and M. Alexander. "Growth and Autotrophic Metabolism of Nitrosomonas Europea." Journal Bacteriology 76: 217 (1958).

24. Wild, H. E. Jr., Sawyer, C. N., and T. C. McMahon. "Factors Affecting Nitrification Kinetics." Journal Water Pollution Control Federation 43(9): 1845-1854 (Sept. 1971).

25. Engel, M. S. and M. Alexander. "Growth and Autotrophic Metabolism of Nitrosomonas Europea." Journal Bacteriology 76: 217 1958).

26. Wuhrman, K. "Objectives, Technology, and Results of Nitrogen and Phosphorous Removal Processes." Advances in Water Quality Improvements, (University of Texas Press, Austin and London, 1968).

27. Slechta, A. F. and G. L. Culp. "Water Reclamation Studies at the South Tahoe Public Utility District." Journal Water Pollution Control Federation 39(5): 787-814 (May 1967).

28. Schroeder, E. D. and A. W. Rusch. "The Role of Nitrate Nitrogen in Bio-oxidation." (The Laboratory of Environmental Science and Engineering, William Marsh Rice University, Houston, Texas, 1967).

29. Nason, A. "Exzymatic Pathways of Nitrate, Nitrite and Hydroxylamine Metabolisms." Bacteriological Review 26(1): 16 (1962).

30. Sokatch, J. R. Bacterial Physiology and Metabolism, (Academic Press, London and New York, 1969).

31. Kashket, E. and A. Brodie. "Oxidative Phosphorylation in Fractional Bacterial Systems." Journal of Biological Chemistry 238(7): 2564-2570 (July 1963).

32. Itagaki, E. "The Role of Lipophilic Quinones in the Electron Transport System of Escheria Coli." Journal of Biochemistry 55: 432-445 (1964).

33. Taniguchi, S. and E. Itagaki. "Nitrate Reductase of Nitrate Respiration Type from E. Coli: I. Solubilization and Purification from the Particulate System with Molecular Characterization as a Metallo-protein." Biochemica Et Biophysica Acta 44(1): 263-279 (October 1960).

34. Gel'man, N. S., Luboyanova, M. A. and D. N. Ostrovskii. Respiration and Phosphorylation of Bacteria (Translated from Russian, Plenum Press, New York, 1967).

35. Moore, S. F. and E. D. Schroeder. "An Investigation of the Effects of Residence Time on Anaerobic Bacterial Denitrification." (Unpublished paper).

36. Segel, I. H. Biochemical Calculations, (John Wiley and Sons, Inc., New York, 1968).

37. Barrett, J. T. and R. E. Kallio. "Terminal Respiration in Pseudomonas Fluorescens: Component Enzymes of the Trycarboxylic Acid Cycle." Journal of Bacteriology 66(5): 517-525 (November 1953).

38. Saz, H. J. and L. O. Drampitz. "The Oxidation of Acetate by Micrococcus Lysodeikticus." Journal Bacteriology 67(4): 409 (April 1954).

39. Newton, J. W., Wilson, P. W. and R. H. Burris. "Direct Determination of Ammonia as an Intermediate in Nitrogen Fixation By Azotobacter." Journal of Biological Chemistry 204: 445-451 (1953).

40. Hoover, S. R. and N. Porges. "Assimilation of Dairy Wastes by Activated Sludge: II The Equation of Synthesis and Rate of Oxygen Utilization." Sewage and Industrial Wastes 24(3): 306-312 (March 1952).

41. Middlebrooks, E. J., Jenkens, D., Neal, R. C., and J. L. Phillips. "Kinetics and Effluent Quality in Extended Aeration." Water Research 3: 39-46 (1969).

42. Symons, J. M. and R. E. McKinney. "The Biochemistry of Nitrogen in the Synthesis of Activated Sludge." Sewage and Industrial Wastes 30(7): 874-890 (July 1958).

43. Eckenfelder, W. W. Jr. Water Quality Engineering for Practicing Engineers, (Barnes and Noble, Inc., New York, 1970).

44. Shammas, N. K. Optimization of Biological Nitrification, Dr. of Engineer Dissertation, (Civil Engineering, University of Michigan, 1971).

45. Mulbarger, M. C. "Nitrification and Denitrification in Activated Sludge Systems." Journal Water Pollution Control Federation 43(10): 2059-2070 (Oct. 1971).

46. Mueller, J. C. "Nutrient Removal by Natural Gas Fermentation." Journal Water Pollution Control Federation 44(1): 25-33 (Jan. 1972).

47. Friedman, A. A. Temperature Effects on Growth Rate and Yield for Activated Sludge, Dr. of Engineer Dissertation, (University of California at Davis, Davis, California, 1970).

48. Difco Manual, Ninth Edition, (Difco Laboratories, Inc., Detroit 1, Michigan, 1953).

49. Standard Methods for the Examination of Water and Wastewater, Thirteenth Edition, (American Public Health Association, Washington D. C., 1971).

50. Aminco Reprint No. 104, "The Determination of Nitrogen by the Kjeldahl Procedure Including Digestion, Distillation and Titration." (American Instrument Co., Inc., Silver Springs, Maryland, 1959).

51. Clark, B. D. and K. A. Dostal. Evaluation of Waste Treatment System: Chemawa Indian School, (Federal Water Pollution Control Administration, Pacific Northwest Water Laboratory, Report No. FR-6, June 1968).

52. Bremner, J. M. "Inorganic Forms of Nitrogen in Methods of Soil Analysis." C. A. Black edition - American Society of Agronomy. (Madison, Wisconsin, 1965).

53. Downing, A. L., Painter, H. A., and G. Knowles. "Nitrification in the Activated Sludge Process." Journal Institute of Sewage Purification 2: 130-154 (1964).

54. Watson, S. W. "Taxonomic Considerations of the Family Nitrobacteraceae Buchanan." International Journal of Systematic Bacteriology 21(3): 254-270 (July 1971).

MONTANA STATE UNIVERSITY LIBRARIES

3 1762 10005244 6

[redacted]

N378
T663
cop.2

Townsend, Curtis K
Nitrogen utilization in the activated sludge process

NAME AND ADDRESS

MAY 4 19[redacted] George A. Murgel 1109 Roste
R
MAY 22 1977 AG

[redacted]

N378
T663
Cop. 2

COLLEGE PL
BINDE
COLLEGE PLACE, W