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

Abstract:
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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.

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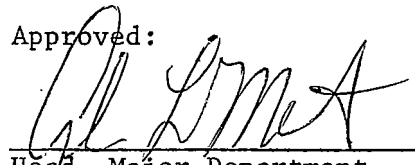

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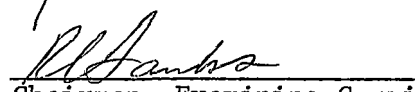

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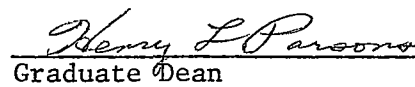

Graduate Dean

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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.

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ABSTRACT

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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

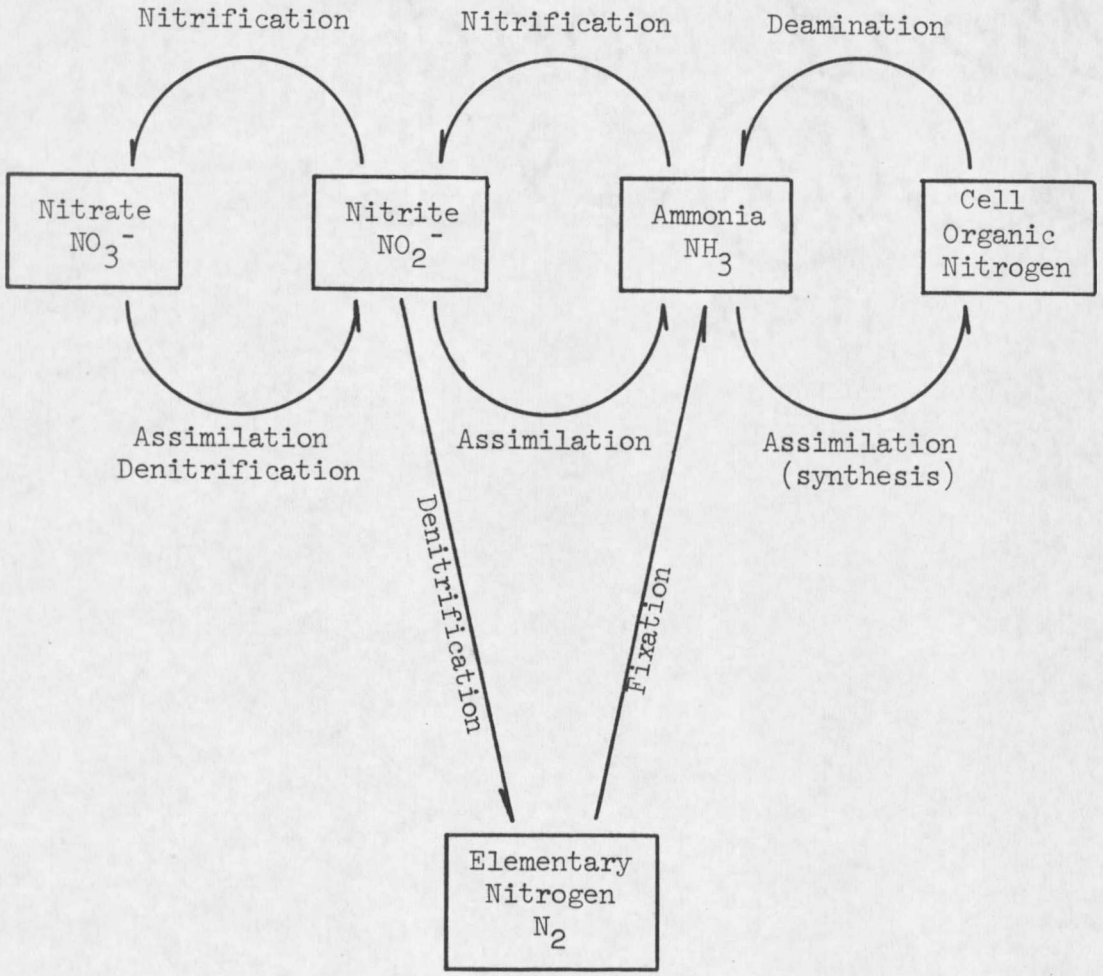
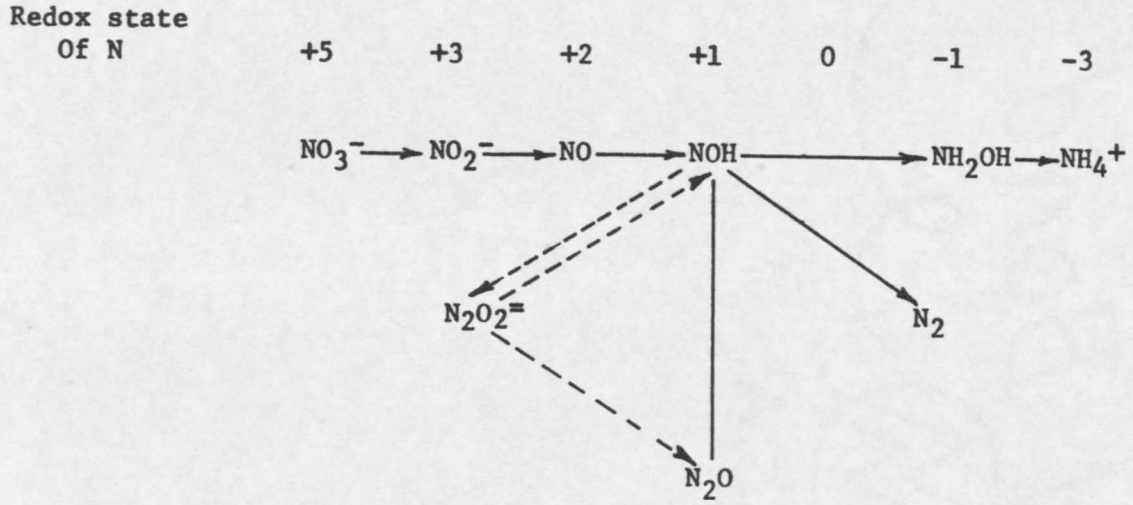


Figure 1. PRINCIPAL BIOLOGICAL PROCESSES INVOLVING NITROGEN (18)



_____ Enzymic

---------- Non-enzymic

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.

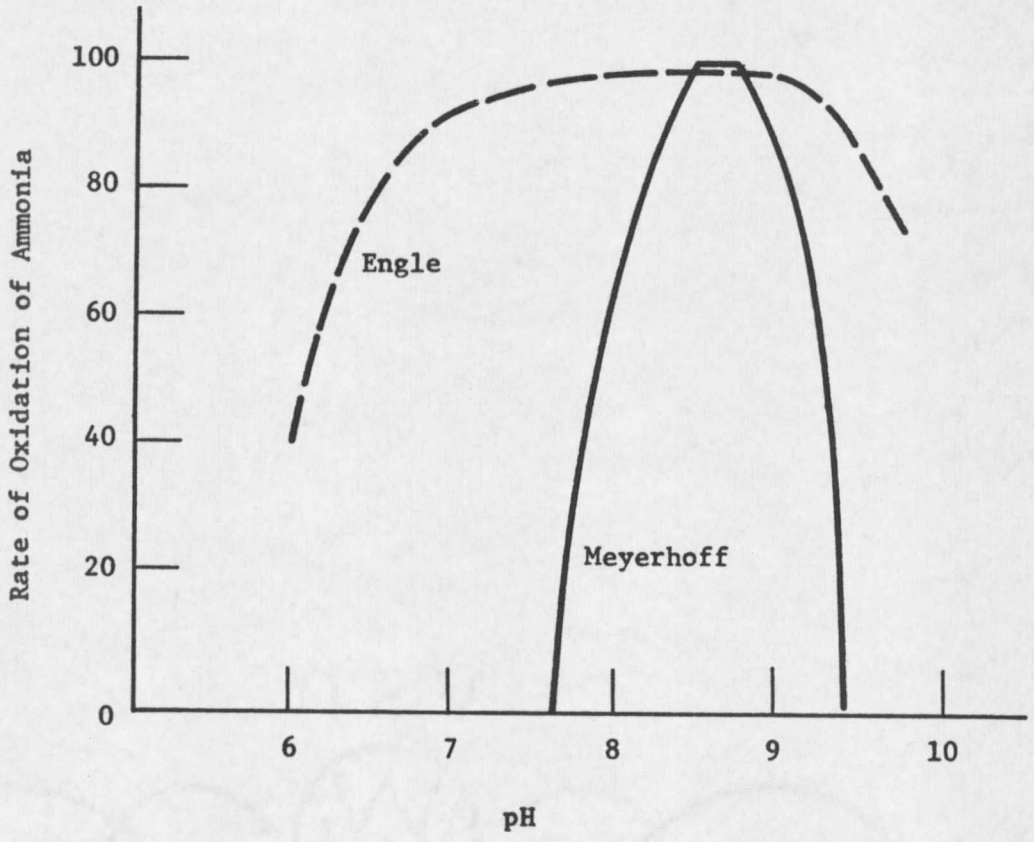


Figure 3. RATE OF OXIDATION OF AMMONIA BY NITROSOMONAS (23,24)

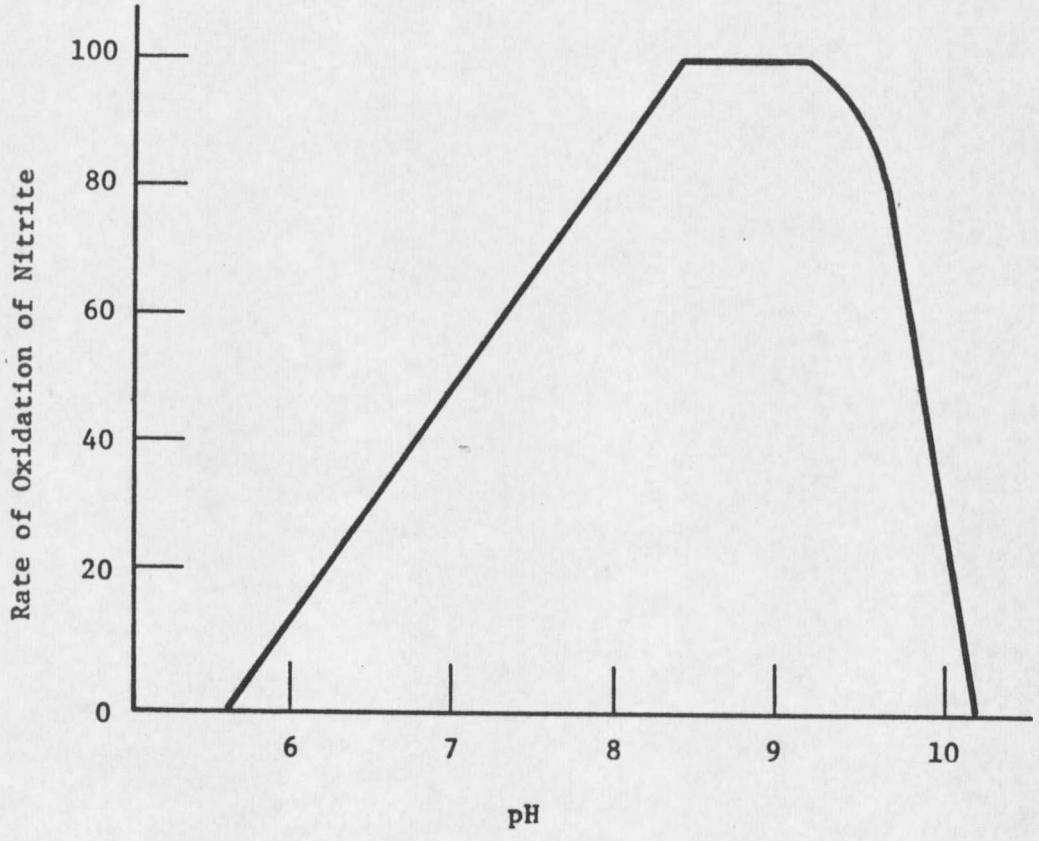


Figure 4. RATE OF OXIDATION OF NITRITE BY NITROBACTER (24)

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.

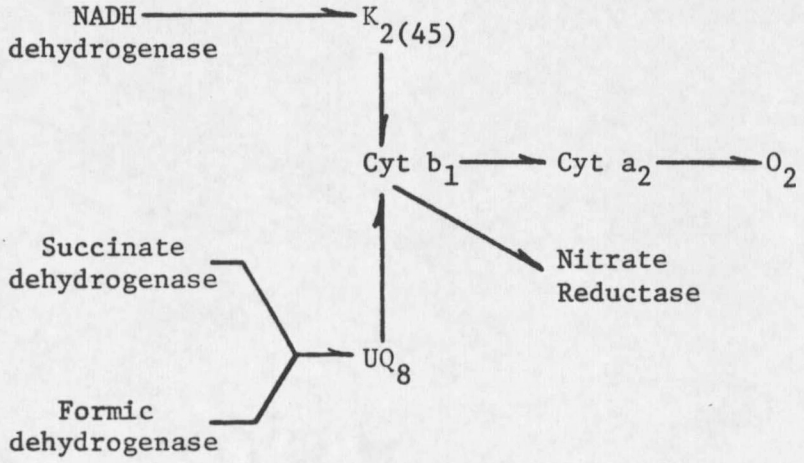
Wuhrman (26) has also stated that oxygen tension must be sufficient for this nitrification to occur. The normal oxygen concentration of activated sludge treatment plants is at least 1-1.5 ppm O_2 which is sufficient for this oxidation. With all the above conditions satisfied the oxidation should be complete 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.

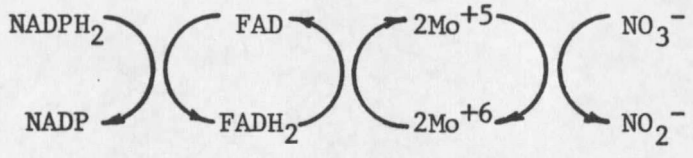
There was doubt as to the existence of the TCA cycle in bacteria for many years as whole cells were unable to oxidize key intermediates of the cycle (30). Barrett & Kallio (37) demonstrated enzyme preparations of Pseudomonas fluorescens were able to oxidize TCA cycle intermediates while the cells from 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

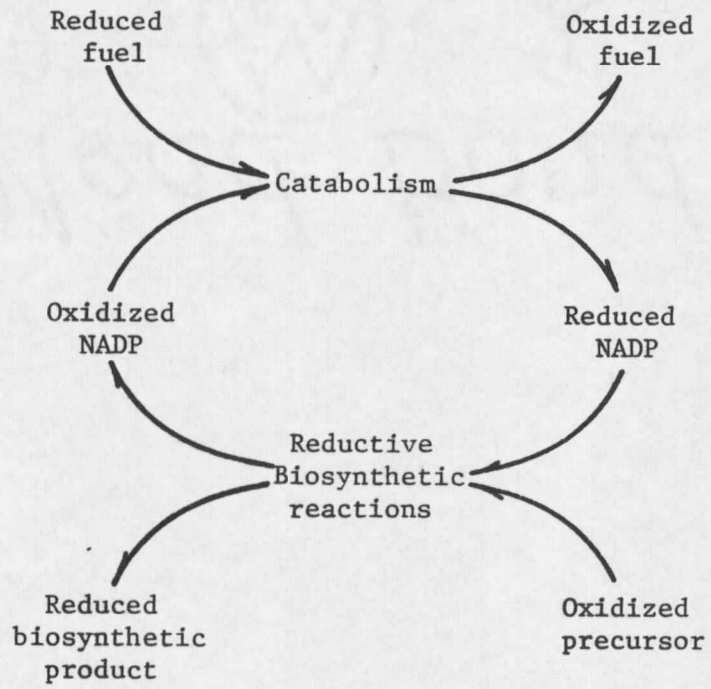


Figure 5. TRANSFER OF REDUCING POWER VIA THE NADP CYCLE (2)

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/1. 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

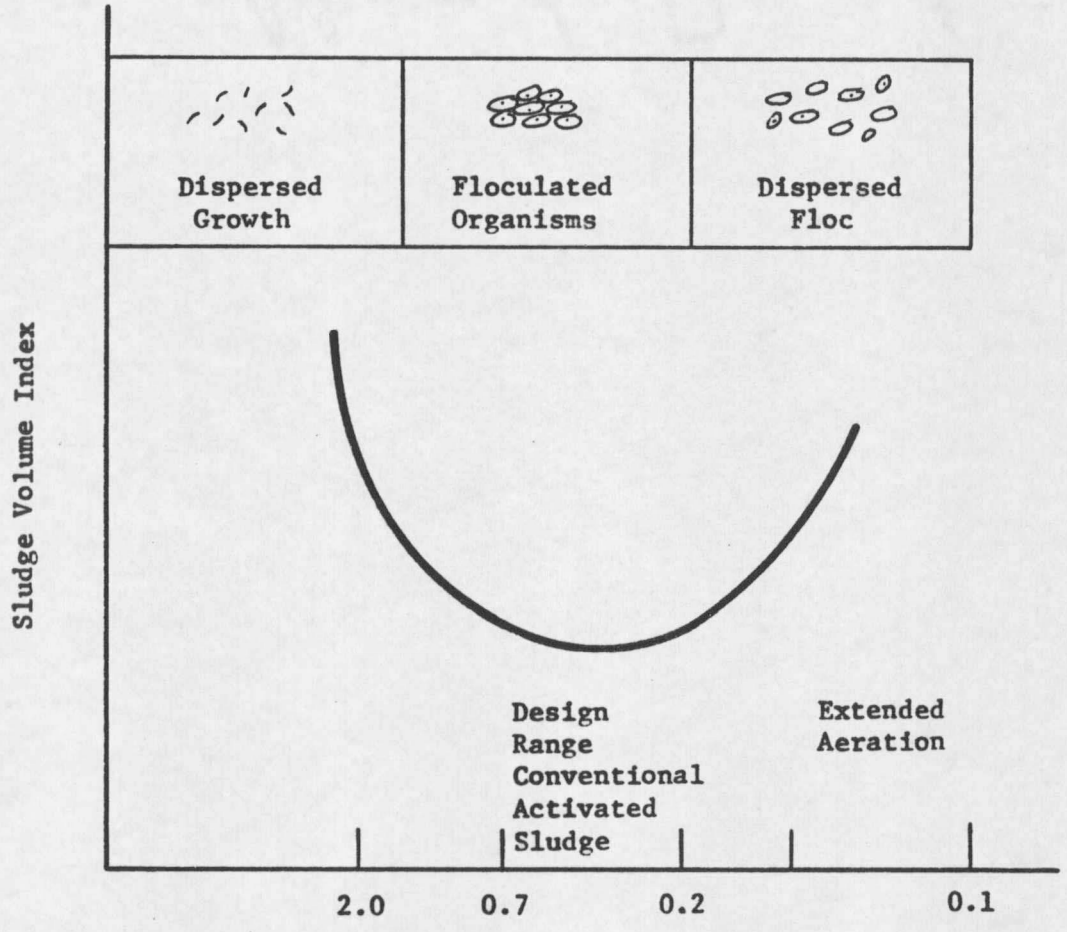


Figure 6. SETTLING CHARACTERISTICS OF ACTIVATED SLUDGE AS RELATED TO ORGANIC LOADING (43)

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.

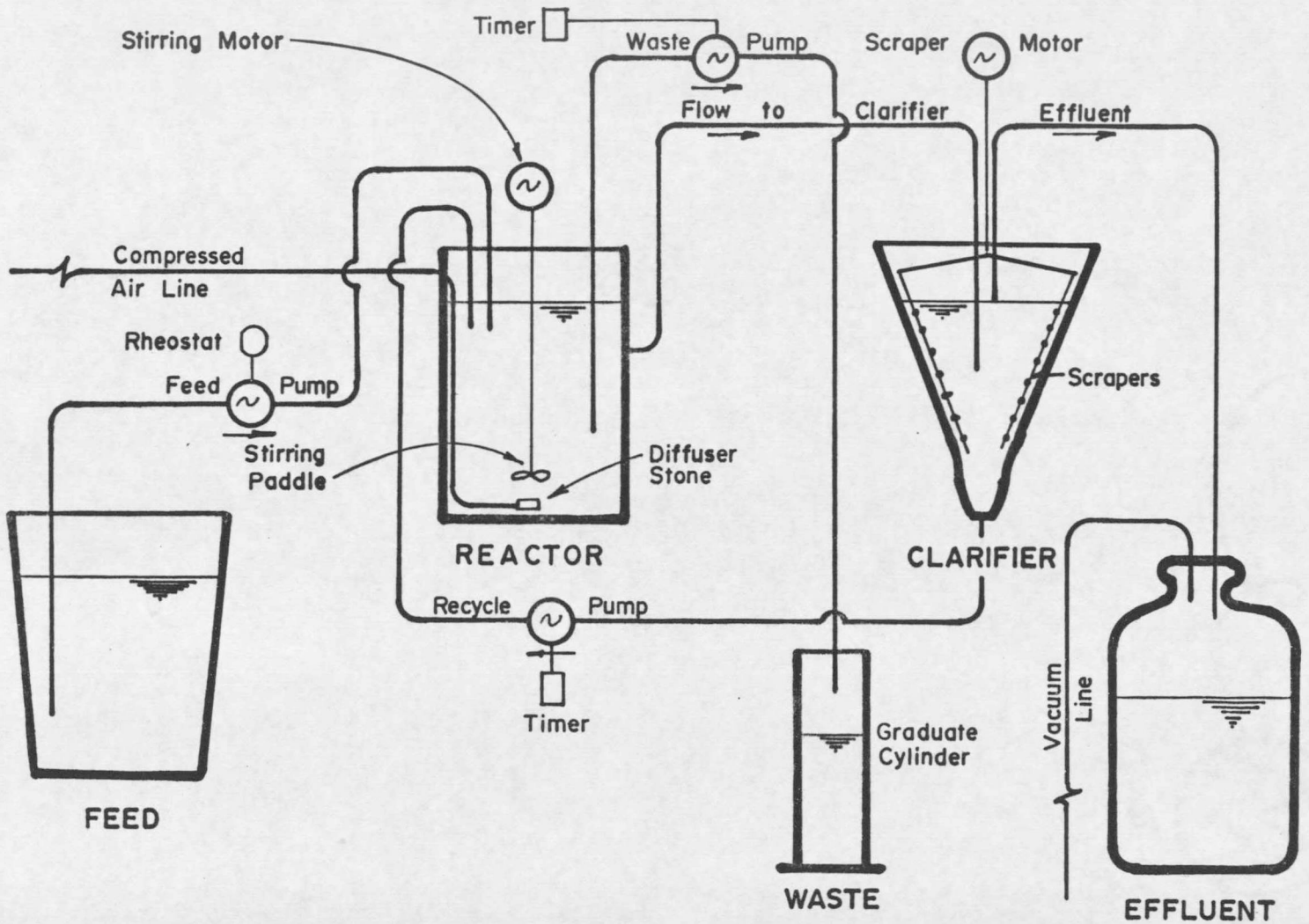


Figure 7. SCHEMATIC OF PILOT PLANT

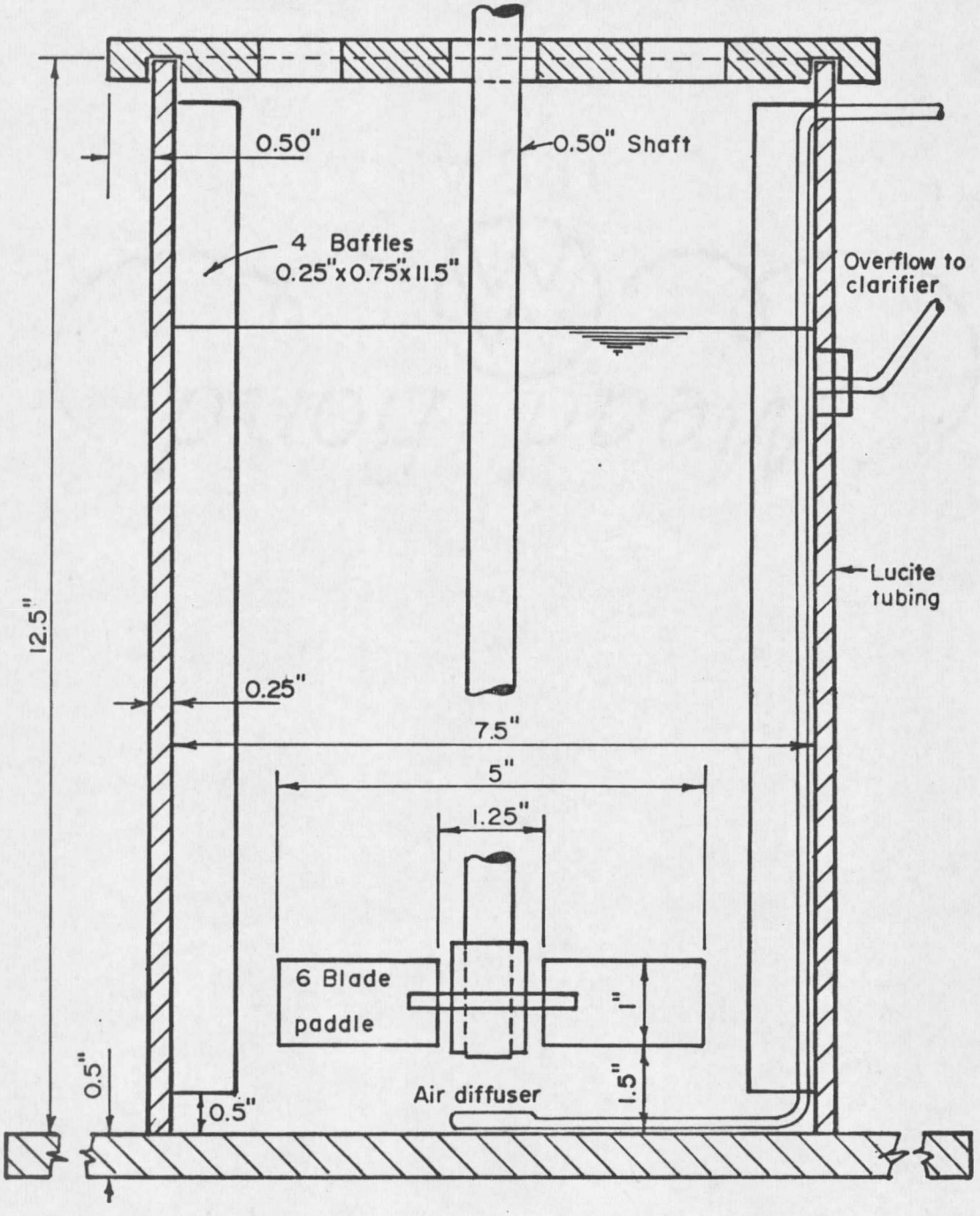


Figure 8. REACTOR AND PADDLE