FINAL REPORT

Development of an Unconventional Approach to Nitrification-Denitrification

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Prepared for

Office of Water Research and Technology
U.S. Department of the Interior

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CHAPTER I

INTRODUCTION

The concern for nitrogen in wastewaters has grown considerably during the last ten years. The magnitude of this interest becomes immediately evident when one contemplates the number of national and international conferences, symposia, and workshops held, as well as the increased volume of publications reporting the research performed on water-related nitrogen problems.

This recent and relatively intensive scrutiny is being focused on the removal of nitrogen and nitrogenous compounds from wastewaters because the discharge of these contaminants manifests itself primarily in nitrogenous oxygen demand on streams, public health problems and eutrophication. The public health problem most often associated with nitrogen pollution of water is methemoglobinemia or "blue baby disease." This blood disorder is characterized by the fact that excessive concentrations of nitrate and/or nitrite in drinking water is toxic to infants and other young animals. But perhaps the single most important water problem resulting from nitrogen pollution is eutrophication, the over-fertilization of lakes and estuaries with plant nutrients. Investigators have recognized that nitrogen removal may be as significant as phosphorus reduction for eutrophication control.

Unless steps are taken to control the discharge of nitrogenous contaminants into receiving waters, nitrogen-related water problems

will continue to increase, especially with the multiple reuse of water. The problem of increased pollutional loads on our natural waters coupled with increased water requirements for industrial, municipal, and recreational use has become so acute and its persistence so menacing that regulatory action requiring removal of nitrogen from sewage effluent is becoming more common.

In fact, the increasingly stringent water quality standards which have already been implemented by state and federal regulatory authorities coupled with increased public awareness have resulted in a reevaluation of wastewater treatment objectives. An example of one of these standards is found in the Federal Water Pollution Control Administrations' publication Water Quality Criteria for Fish, Other Aquatic Life and Wildlife (1). It states that the naturally occurring ratios and amounts of nitrogen (particularly nitrate and ammonium) to total phosphorus should not be radically changed by the addition of water pollutants.

A more profound standard is found in Public Law 92-500. This law not only outlines the objectives and the general approach for cleaning the nation's waters but also establishes a National Pollution Discharge Elimination System (NPDES). Under this law, municipalities and private industry must apply for and receive NPDES permits before discharging wastestreams into receiving waters. Many of these waste effluents will require treatment beyond secondary treatment (i.e., nitrogen removal retrofit facilities) in order to meet NPDES effluent limitations.

Anticipated future NPDES effluent standards (issued by 1979 and later) are expected to be more stringent than those now imposed. In

fact, the National Commission on Water Quality suggested that certain effluent discharges must be of equal or better quality than that of the original water supply. The NPDES permits place effluent limitations on the discharge of wastewater but do not dictate methods of achieving these limitations.

Nitrogen can be removed from wastewater through conventional processes, but the methods are relatively expensive and obviously all the alternatives available for nitrogen removal are not appropriate in each situation. Experience has shown that for many applications nitrogen removal via biological nitrification-denitrification should be the method of choice.

The purpose of the experimental work presented in this study is to evaluate the feasibility of using an unconventional system for nitrifying and denitrifying sewage effluent. The research plan was to stop the nitrification process at the nitrite step, at which point denitrification was promoted. Theoretically, in this manner, less oxygen is used to convert the ammonia than is required in conventional systems, and less supplemental carbon will have to be supplied for denitrification from the nitrite form. The results and conclusions presented in this dissertation indicate that this type of unconventional nitrification-denitrification system exhibits potential for the development of an economic and effective method of nitrogen control.

CHAPTER II

REVIEW OF LITERATURE

Nitrogen Sources

The major origins of nitrogen entering fresh water are domestic and municipal sewage, industrial effluent, atmospheric sources, rural sources, and miscellaneous sources.

Domestic and Municipal Sewage Sources of Nitrogen

Domestic wastewaters are composed primarily of urine, feces, ground organic garbage and cellulose dispersed in relatively large volumes of water, although additional types of waste may also be present (2). Municipal wastewater is composed of domestic and industrial waste effluents.

Daily per capita excretion of urine is 1,500 ml and daily per capita excretion of fecal matter is 150 to 200 g (wet) or 25 to 50 g (dry). This results in a total nitrogen content of human waste of from 8 to 12 pounds/capita/year (3). Of this daily per capita excretion, approximately 80 percent is in the form of urea, a by-product of the breakdown of urine (4). Urea is readily hydrolyzed to ammonia by the enzyme urease (5). Because it undergoes hydrolysis to ammonia so rapidly, urea is seldom found in other than very fresh wastewater (4). Therefore, approximately 50 percent of the nitrogen content of

domestic sewage is in the ammonia form. The other 50 percent is in the form of organic nitrogen. Symons (3) reported that of the organic nitrogen portion of human waste, 50 percent is biodegradable while 50 percent is refractory to conventional wastewater treatment. Assuming a domestic waste return flow of 50 gallons/capita/day and a nitrogen waste load of 11 pounds/capita/year, Symons (3) calculated that the total nitrogen content of domestic sewage would be 73 mg/1. The ammonia-nitrogen content would be approximately 36 mg/1, the degradable organic-nitrogen concentration about 18 mg/1, and the refractory organic-nitrogen content about 18 mg/1.

Table 1 lists ranges of values for nitrogenous components for typical domestic wastewater (4).

TABLE 1

VALUES FOR NITROGENOUS COMPONENTS
FOR TYPICAL DOMESTIC WASTEWATER

Component	Range
Nitrogen (total as N)	20 - 85 ppm
Organic - N	8 - 35 ppm
Free Ammonia - N	12 - 50 ppm
Nitrite - N	0 ppm
Nitrate - N	0 ppm

McCarty, et al. (3), estimates that the total nitrogen content in sewage treatment plant effluents ranges from 18 to 28 mg/1. This

total nitrogen content is essentially all inorganic with only a small percentage associated with the organic suspended solids in the effluent (6). However, some of this nitrogen is refractory organic nitrogen (3). The predominant inorganic form of nitrogen in secondary effluents includes ammonia--ammonium ions, nitrate ions, and nitrate ions.

Operational and design parameters of the secondary treatment process determine which of the three nitrogen species predominates. Ammonia-ammonium is more prevalent in high-rate system effluents while nitrate and nitrite are more commonly associated with extended-aeration system effluents (7).

Dean (7) states that there exists an average of 14 mg/l of ammonia-ammonium ions in sewage effluents while the recommended effluent limit is 1 mg/l as nitrogen. Ammonium ions predominate up to pH 9 - 10. Since the pH of secondary effluents is usually around 8, less than 10 percent of the ammonical nitrogen is in the ammonia form (8).

Nitrite ions average 0.4 mg/l nitrogen in secondary effluents. Their recommended effluents limit is 1 mg/l as nitrogen. Nitrate ions average 2.0 mg/l in secondary effluents, while the U.S. Public Health Service limit for drinking water is 10 mg/l as nitrogen (7).

It is obvious from the preceeding paragraphs that a major source of nitrogen pollution to receiving streams is secondary waste treatment plant effluents. In fact, typical sewage treatment plant effluent discharging into a water-course would require at least a two-to-one dilution to approach the 1962 U.S.P.H.S. Standard of 10 mg/l (3).

Industrial Sources of Nitrogen

In any discussions of nitrogen, contributions from industry must not be overlooked. But because so many different industries exist and the nitrogen form and content from the different industries is so varied, it is exceedingly difficult to obtain a proper perspective of the role of industry in the nitrogen scheme. One aspect remains clear, however. Many treated industrial wastewaters have sufficiently high nitrogen concentrations to prohibit their disposal by dilution into freshwater bodies without exceeding ammonia nitrogen toxicity limits and/or nutrient concentrations associated with eutrophication (6).

Rural Sources of Nitrogenous Waste

As the demand has increased for higher production and yields of food and fiber, agricultural operations have increased in size, become industrialized, and have increased fertilizer utilization (both manure and commercial) resulting in a steadily increasing agricultural nitrogen-related pollution problem. This problem results in part from nitrogen containing surface runoff and seepage entering into surface and groundwater. This nitrogen pollution is a result of the following:

1. Animal wastes from barnyards and feedlots;
2. Excessive rates of application of commercial fertilizers and manure, particularly to frozen soil;
3. Decomposition of crop residue and soil organic matter (3);
4. Irrigation return water (9);
5. Septic tanks.

Atmospheric Sources of Nitrogen

The atmosphere surrounding the earth is considered to be the reservoir upon which the nitrogen cycle is dependent (3). In particular, approximately 80 percent of the earth's atmosphere by volume is nitrogen, amounting to about 70 million pounds of nitrogen existing above each acre of soil (10). Most of this nitrogen is in the diatomic N_s form. It was from this reservoir, presumably, that the first nitrate was produced to nourish the first plant life on this planet.

Miscellaneous Sources of Nitrogen

Nutrient nitrogen may have several other points of origin besides the ones discussed above (11).

1. Aquatic bird droppings may be a significant natural source of nitrogen.

2. Under certain situations, detritus--leaf litter, decaying aquatic plants, pollen, and soil organic matter--may be a major source of nitrogen to water regimes.

3. Depending upon the situation, evaporation and resultant concentration of salts can cause increases of nitrogen in water regimes, whereas snow-melt may act as a dilution factor (12).

4. Bottom muds or sediments may be a reservoir for both organic and inorganic nitrogen sources (13, 12).

5. Urban runoff (e.g., lawn fertilizers) may contribute significant quantities of nitrogen to receiving waters during and after periods of precipitation (14).

6. Leachate from waste disposal in dumps or sanitary landfills are a common source of nitrogen pollution.

7. Due to air pollution, the atmosphere presently harbors significant concentrations of nitrogen oxides, especially nitrous oxide, nitrogen dioxide, and nitric oxide. These oxides originate primarily from auto and industrial exhausts which form when nitrogen and oxygen react at relatively high temperatures associated with the combustion process (10).

8. There exist a few types of bacteria, fungi, actinomycetes, and a number of blue-green algae which are capable of utilizing nitrogen directly from the atmosphere and transforming it into compounds usable to other plants. This phenomena is termed nitrogen fixation.

Nitrogen Cycle

Alexander (15) states that on the basis of current knowledge, nitrogen passes through a nearly perfect cycle. The cycle is essentially identical whether it occurs in oceanic, freshwater, or terrestrial habitats (15).

Aspects of the nitrogen cycle which are of particular importance to this research are discussed in this section.

Nitrogen which exists as ammonium may be oxidized to nitrite by a group of chemoautotrophic organisms--those which utilize CO_2 as a carbon source and obtain energy from oxidation of inorganic matter--represented by Nitrosomonas bacteria and physiologically related bacteria and fungi that are common to soil, ocean bottoms, marine muds, and freshwater habitats. Subsequently, the nitrite formed may be further oxidized to nitrate by other groups of chemoautotrophs

(Nitrobacter or Nitrocystes). This two-step aerobic oxidative transformation of ammonia to nitrite to nitrate is commonly referred to as nitrification. However, in order to distinguish between the production of nitrite, nitrates, and nitrites plus nitrates, Prakasan and Loehr (16) proposed that the terms nitritification, nitratification, and nitrification be used, respectively. Nitrification is an almost universal phenomenon where ammonium is present, provided that oxygen is available, the E_h and pH are relatively high, and toxins affecting the nitrifying organisms are absent (15).

Certain nitrate-reducing bacteria are able to convert nitrates to nitrites under anaerobic conditions (17). Prakasam and Loehr (16) termed this reaction denitratification. Denitrifying bacteria may carry the process a step further and reduce the nitrite with the consequent liberation of free nitrogen (17); Prakasam and Loehr have coined the term "denitritification" to describe the reaction. This reduction of the nitrite provides the primary pathway for re-entry of nitrogen into the atmosphere (18, 19, 3). The term denitrification is commonly used in order to describe the liberation of nitrogen gas from nitrites and nitrates.

Relatively few microbial species are denitrifiers and these form nitrogen gas only under rather specific conditions (20).

In this multi-step reduction of nitrate to atmostpheric nitrogen (N_2), traces of nitrous oxide (N_2O) and nitric oxide (NO) may evolve before the nitrate is reduced further to nitrogen gas, which is both the major and the final product of the reaction sequence (15).

A process referred to as assimilatory nitrate reduction also results in the disappearance of nitrates (21). This is a reduction of the nitrate ion to the ammonia oxidation level by incorporation of the nitrogen into the bacterial cell mass (13).

Nitrites formed during the nitrification process may decompose or react with soil organic matter to yield gaseous nitrogen (22) and/or gaseous nitrogen oxide (18). These reactions have been termed chemo-denitrification as they do not involve microbial transformations directly.

In acid environments [below pH 5.5], nitrite decomposes spontaneously to nitric oxide (NO), N_2O, and N_2 (23, 15). The NO formation proceeds chemically according to the reaction represented by Equation 1.

$$3\ HNO_2 \rightarrow 2\ NO + HNO_3 + H_2O \qquad \text{(Equation 1)}$$

Nitrogen Removal Processes

Conventional wastewater treatment plants are not designed to remove significant amounts of nitrogen. The primary sedimentation process typically removes about 29 percent of the total nitrogen while secondary biological treatment removes about 14 percent for a total removal of about 43 percent (6). If the raw sludge solids containing the nitrogen are separated from the slurry by filtration and land-filled or incinerated, most of the nitrogen will be removed from the system. However, if the sludge is processed by either aerobic or anaerobic digestion, the ammonia nitrogen is released in the biological

decomposition of the sludge solids and returned to the influent of the treatment plant and passes out in the treated effluent. Therefore, depending upon the nitrogen concentration in the raw wastewater, method of treatment and sludge handling, conventional wastewater treatment will normally remove from 10 to 43 percent of the total influent nitrogen (6). Great effort has been expended to develop economical, reliable means of removing the remaining 57 to 90 percent (24, 25).

Presently, nitrogen removal facilities are being built in situations where existing or anticipated use of water has dictated the need for this form of treatment (26). In order to comply with the proposed future effluent standards, more and more wastewater treatment plants must be designed for nitrogen control (27).

Over the past few years, there have been several intensive investigations of approaches to nitrogen removal (28). Current nitrogen removal methods, however, are dependent upon the form and concentration of nitrogen compounds in the process influent, required effluent quality, other treatment processes to be employed, cost, reliability, and flexibility (14). Consideration should also be given to the operational safety of the process and the influence of climatic conditions on the process efficiency (29).

Of these processes, many have been developed with the specific purpose of transforming nitrogen compounds or removing nitrogen from the wastewater stream. Others can remove several compounds, including significant amounts of nitrogen. Still others may remove only a small amount of nitrogen or a particular form of nitrogen which is a small fraction of the total (14).

Of the processes, nitrification-denitrification is perhaps the most generally applicable because of relatively good reliability, suitability to a variety of conditions, low area requirements, and moderate cost (28, 30, 31). Figure 1 is a schematic diagram of a simple nitrification-denitrification treatment system.

The Nitrification Phenomenon

In many wastewaters, oxidizable ammonia-nitrogen is present in excess of stoichiometric requirements for microbial synthesis reactions (13).

The biological oxidation of this ammonia or ammonium ion to nitrate by these autotrophic organisms is known as nitrification (32). Nitrification is a diphasic process occurring in two distinct phases (33): ammonia is converted first to nitrite and then to nitrate. In the first stage, ammonia is aerobically oxidized to the nitrite form by autotrophic bacteria of the genus Nitrosomonas according to the following reaction (34, 35):

$$NH_4^+ + 1.5\ O_2 \xrightarrow[6e]{\text{Nitrosomonas}} NO_2^- + H_2O + 2H^+ + \text{energy} + \text{Nitrosomonas cells} \qquad \text{(Equation 2)}$$

Bergy's Manual of Determinative Bacteriology (2) lists four other genera of autotrophic nitrifying microorganisms capable of oxidizing ammonia to nitrite, namely Nitrosococcus, Nitrosospira, and Nitrocystis oceanus (36, 27, 37). Many heterotrophic bacteria and fungi can also

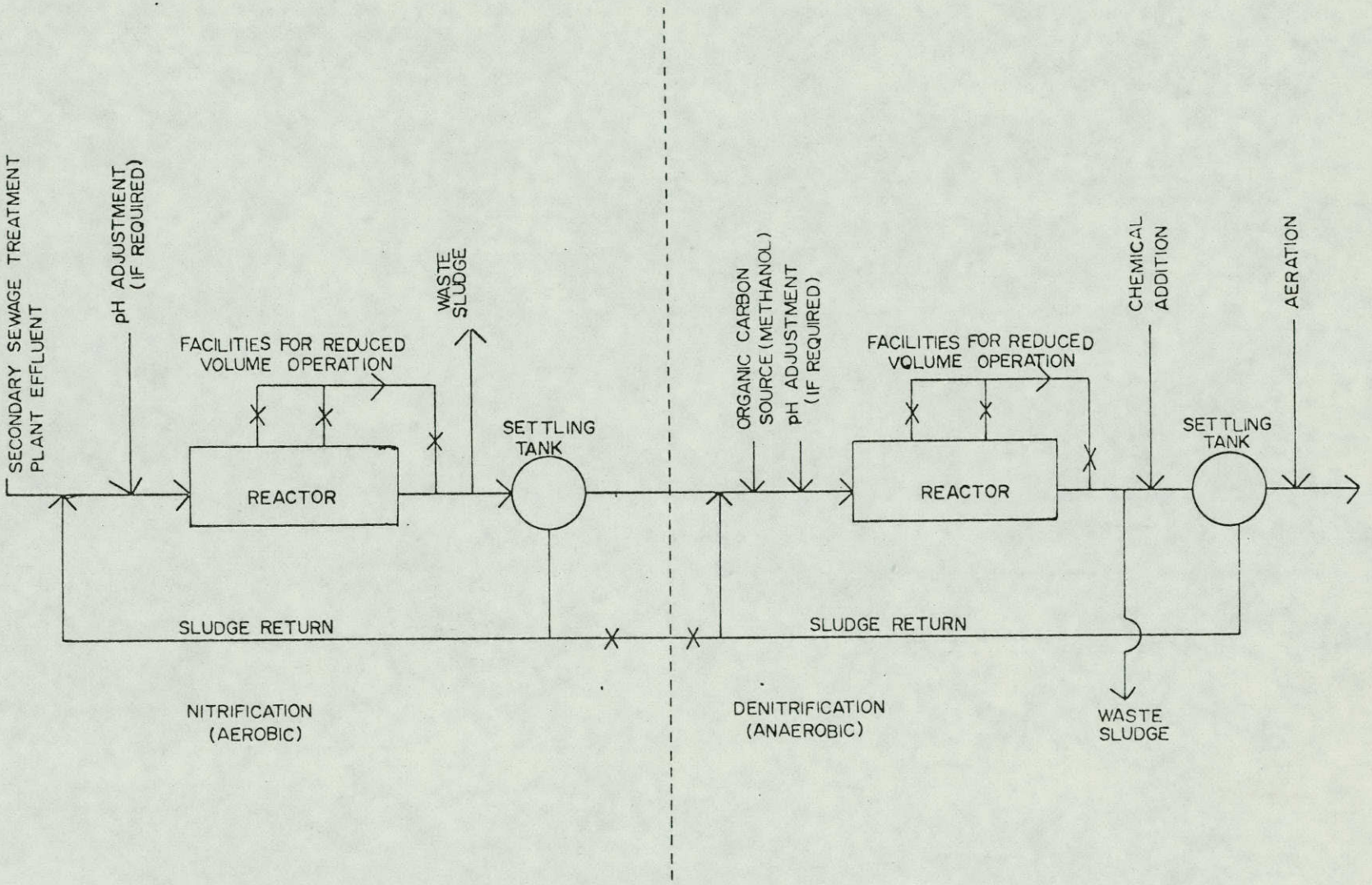


Figure 1. Flowsheet for a two-stage biological treatment process for nitrogen removal.

oxidize ammonia to nitrite (36, 35). Examples of these nitrite formers include Achromobacter, Corynebacterium, Nocardia, Agrobacterium, and Alcaligenes. The extent to which these heterotrophic microbes are involved in nitrification is not certain (10).

Although it has been documented that the preceeding microorganisms are capable of converting ammonia to nitrite, the organism of greatest significance is the Nitrosomonas species (30).

The second step, the aerobic conversion of nitrite to nitrate is accomplished largely by a collective group of autotrophic bacteria called Nitrobacter which utilize nitrite as their sole energy source. This collective group includes numerous species; species characterization is still an active field of study (32). This reaction is described in the equation below (33, 37, 38).

$$NO_2^- + 0.5\ O_2 \xrightarrow{\text{Nitrobacter}} NO_3^- + \text{energy} + \text{Nitrobacter cells} \qquad \text{(Equation 3)}$$

The autotroph Nitrocystis as well as the heterotrophic fungus, Aspergillus flavus, also possess the potential for converting nitrite to nitrate. However, the microorganism of greatest significance regarding this reaction is Nitrobacter (19).

The overall nitrification reaction is represented by the summary equation 4 (19).

$$NH_4^+ + 2\ O_2 \longrightarrow NO_3^- + 2H^+ + H_2O + \text{energy} + \text{cells} \qquad \text{(Equation 4)}$$

From this relationship, it can be discerned that 1.0 gram of ammonia-nitrogen stoichiometrically requires 4.57 grams of dissolved oxygen for complete nitrification (29, 19).

Nitrification depends upon whether or not conditions existing in the treatment system are favorable for the growth of nitrifying organisms. The critical variables are pH, temperature, detention time, carbonaceous matter, oxygen supply, and whether or not any substances specifically toxic or inhibitory to the microorganisms are present (3, 27). Also a certain acclimatization period may be required to establish the growth of the nitrifiers (5).

Therefore, the parameters which are controlled by sanitary engineers in the nitrification process are waste retention time, pH, nutrient elements, mixing, oxygen, microbial mass, and temperature. All other factors are fixed by the inherent nature of the waste and the metabolic rates of the microorganisms (39).

The following are tentative design considerations for nitrification systems based on available nitrification plant operation experience.

Oxygen Consideration

A primary prerequisite for satisfactory nitrification is an adequate oxygen supply.

It is often reported in the literature that a dissolved oxygen concentration of at least 0.5 to 1.0 mg/l is adequate to sustain the nitrification reaction (30).

Temperature Considerations

Investigators (19, 37) have demonstrated that the rate of nitrification continuously increases through the temperature range of 10 degrees to 30 degrees C. in reasonable agreement with the Van't Hoff-Arrhenius Law (40).

The reaction rate was shown to decrease markedly at reduced temperatures; lowering the temperature from 10 degrees C. to 5 degrees C. halves the rate of ammonia oxidation (6).

Temperature is a factor which is impractical to control in most nitrification systems because of economic considerations (41).

pH Considerations

The response of both Nitrosomonas and Nitrobacter in pure culture to various environmental conditions has been studied extensively. The effect of pH on the rate of ammonia oxidation by Nitrosomonas as reported by Meyerhof (6) is shown in Figure 2. The pH effect on Nitrobacter, as reported by Meyerhof (6) is shown in Figure 3. It should be noted that the rate of oxidation of nitrite begins to decline rapidly above about pH 9.5.

The production of hydrogen ions during nitrification results in destruction of alkalinity. For every 1 mg/l of NH_3-N oxidized to nitrite, 7.13 mg/l of alkalinity as $CaCO_3$ is destroyed.

Denitrification

The biochemistry and microbiology of denitrification are generally well known (13).

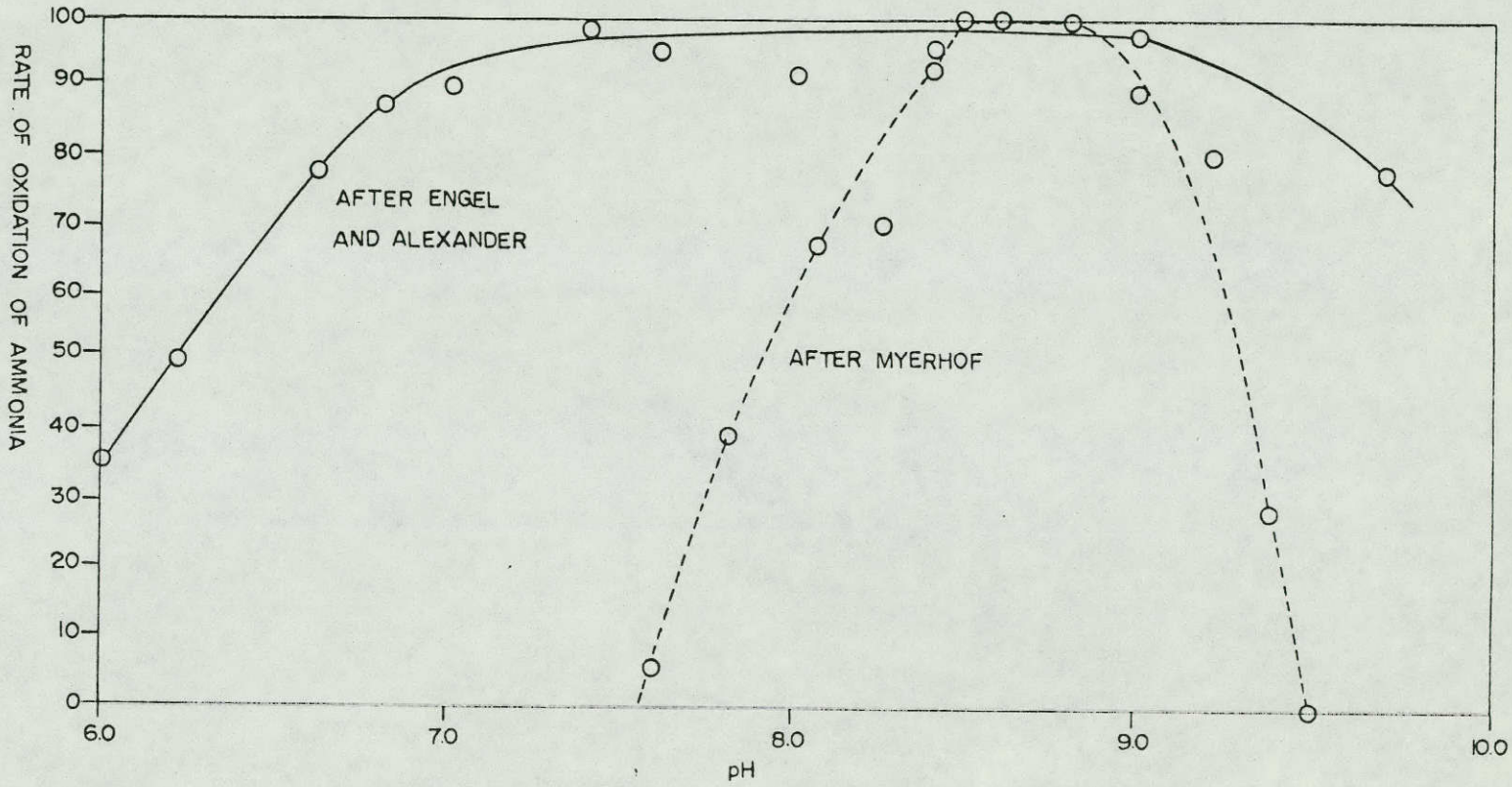


Figure 2. The effect of pH on oxidation of ammonia by Nitrosomonas (93).

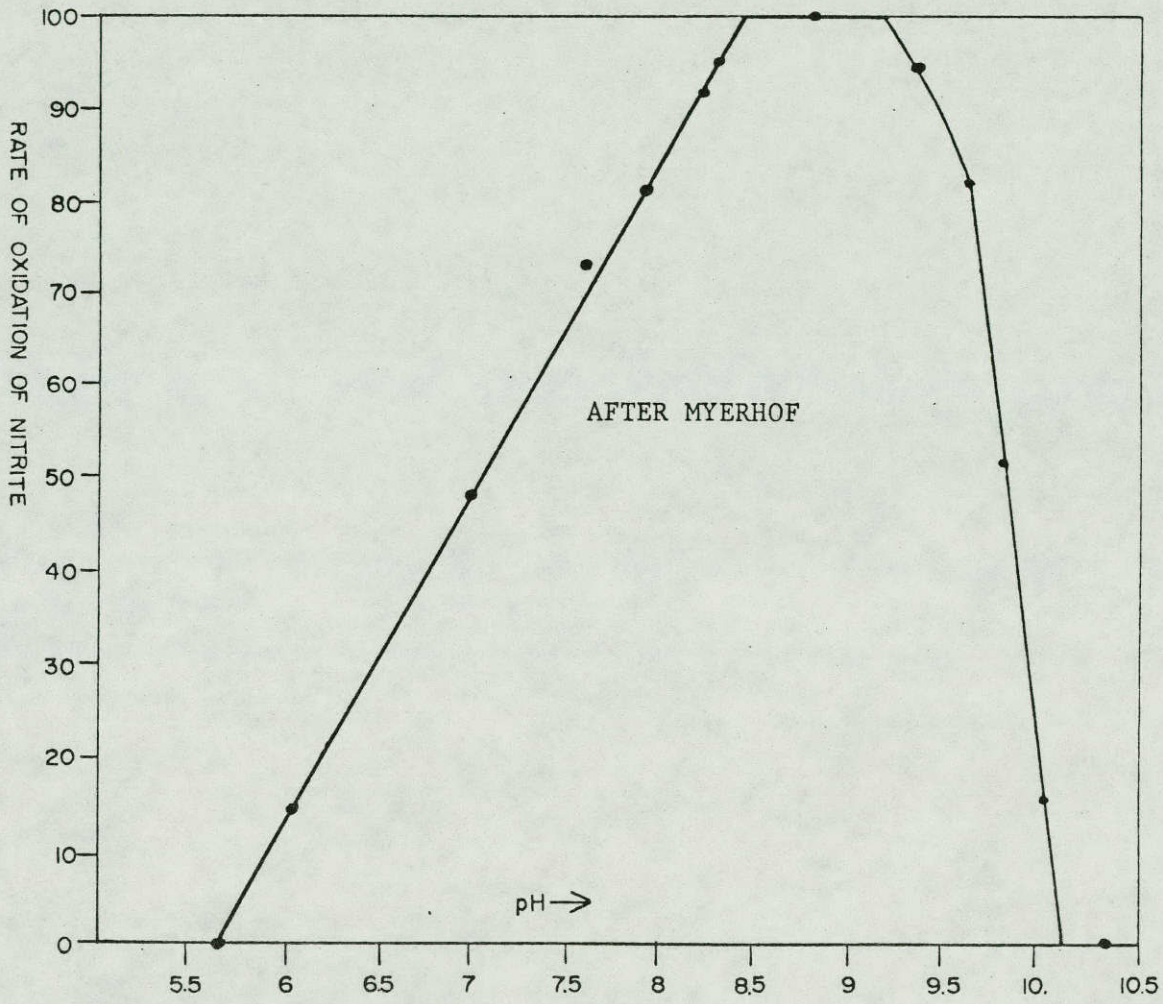


Figure 3. Rate of oxidation of nitrite by Nitrobacter (93).

Biological denitrification is an anaerobic process by which the nitrite and nitrate ion fulfills the role normally occupied by oxygen in aerobic respiration--that of hydrogen ion acceptor in the electron transport system (27). In other words, in the bacterial species that are capable of denitrifying, the presence of nitrate or nitrite ions permit the cell to maintain aerobic metabolism in the absence of free oxygen. In the process, nitrates and nitrites are reduced to nitrogen gas, nitrous oxide and nitric oxide, and the organic compounds present are oxidized (13). There are two alternatives for the reduction of nitrates or nitrites by bacteria. The process described above is dissimilatory respiratory reduction (13). Another type which may occur simultaneously is assimilative reduction. This is the process by which nitrates and nitrites are reduced to ammonia in order to supply nitrogen for cell synthesis (25). The amount of nitrate reduced by assimilation is considered negligible compared to that reduced by respiration (42). If ammonia is already present, assimilation of nitrate need not occur to satisfy cell requirements (14).

Biological reduction of nitrate may occur with or without nitrite as an intermediate (6). Equation 5 describes the combined dissimilation synthesis reactions for dentrification.

$$NO_3^{-*} + 1.08\ CH_3OH^{**} + 0.24\ H_2CO_3 \longrightarrow \text{(Equation 5)}$$

$$0.056\ C_5H_7NO_2 + 0.47\ N_2 + 1.68\ H_2O + HCO_3$$

* electron acceptor

** electron donor

From equation 5 the meaning of the terms electron acceptor and donor become evident. Nitrate gains electrons and is reduced to nitrogen gas, hence it is the electron acceptor. The carbon source, methanol in this case, loses electrons and is oxidized to carbon dioxide, hence it is the electron donor (14).

Using methanol as an electron donor and neglecting synthesis for a moment, denitrification can also be represented as a two-step process as shown in Equations 6 and 7 (14).

First step: Nitrate to nitrite.

$$NO_3^- + 0.33\ CH_3OH \longrightarrow NO_2^- + 0.33\ H_2CO_3 \quad \text{(Equation 6)}$$

Second step: Nitrite to nitrogen gas.

$$NO_2^- + 0.5\ CH_3OH + 0.5\ H_2CO_3 \longrightarrow \quad \text{(Equation 7)}$$

$$0.5\ N_2 + HCO_3^- + H_2O$$

In contrast to nitrification, the ability to bring about denitrification is characteristic of a wide variety of common heterotrophic facultative bacteria including the genera Pseudomonas, Micrococcus, Spirillum, Achromobacter, and Bacillus (42, 27).

Characteristics of Denitrified Water

Denitrified water is not dark and odorous as might be expected because of familiarity with some anaerobic waters. This is because denitrification is not an anaerobic reaction in the usual sense. It is instead similar to aerobic oxidations with nitrates serving as the

final electron acceptor. Unless excessive hydrogen donors are present, products such as indol, skatol, and hydrogen sulfide do not form. For this reason, the organic dosage must be carefully controlled (43).

Other constituents exist in denitrified water which may require removal or neutralization. The treated water may contain some nitrate, nitrite, and ammonia nitrogen; exhibit turbidity resulting from biological solids that escape the final clarifier; and be devoid of oxygen. Therefore, a reaeration facility should follow the denitrification unit.

Cost Considerations

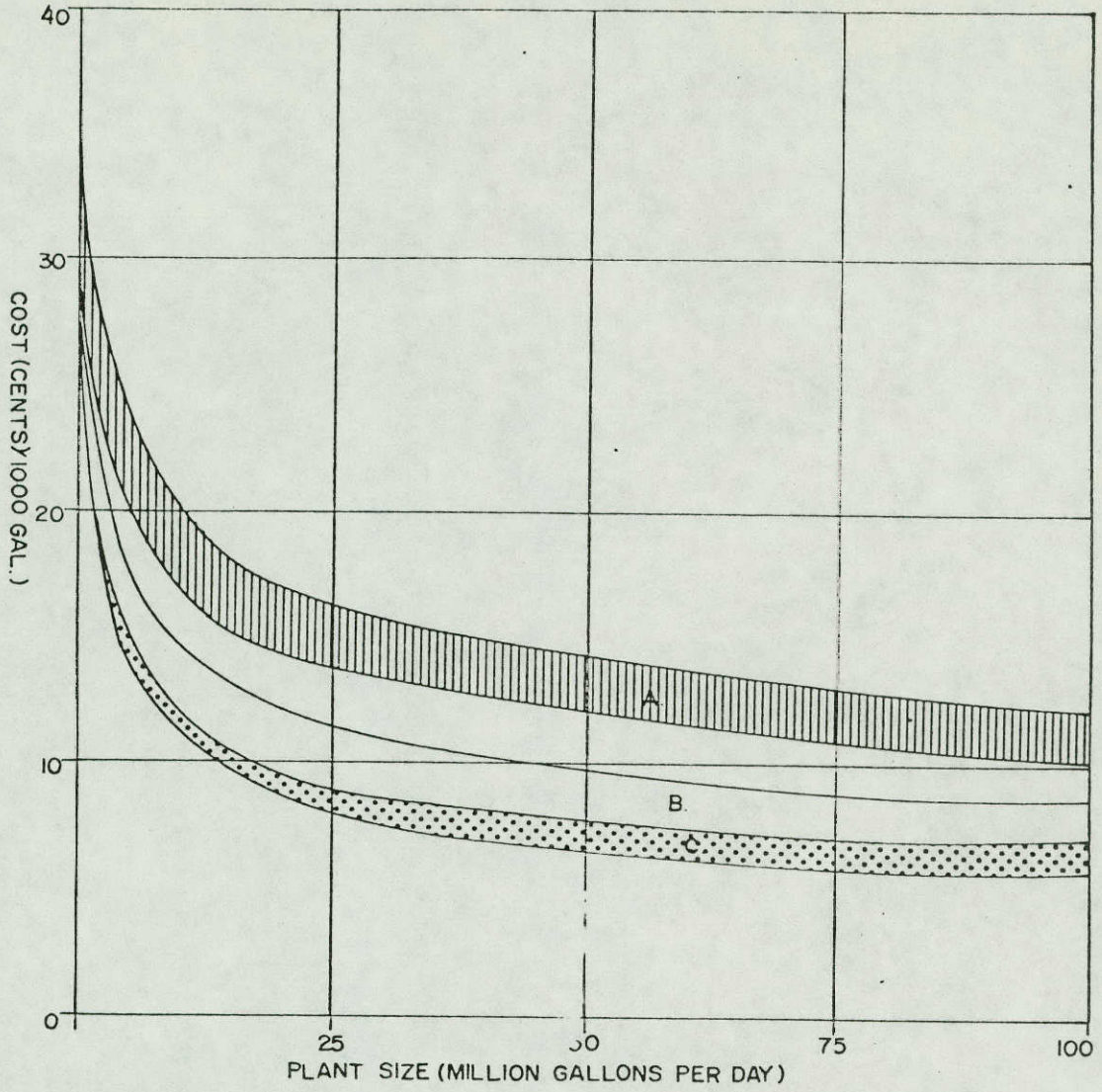
DuTort and Davies (44) found nitrification-denitrification to be an economically feasible method of removing nitrogen from wastewater in large-scale reclamation projects. Figure 4 shows the approximate national average total costs including amortization (25 years at 6 percent) operation and maintenance for biological N control methods.

Nitrite Accumulation

Previous Research

It is the purpose of this portion of the chapter to present studies by researchers which demonstrate the nitrite accumulation phenomena. This portion of the literature review will be divided into three separate sections each representing an area of particular interest:

1. Nitrite Accumulation in Soil Systems;
2. Nitrite Accumulation in Domestic Waste Treatment; and
3. Nitrite Accumulation in Natural Water Regimes.



A. CONVENTIONAL TREATMENT
PLUS NITRIFICATION
PLUS DENITRIFICATION

B. CONVENTIONAL TREATMENT
PLUS NITRIFICATION

C. CONVENTIONAL TREATMENT

Figure 4. Cost of various methods of nitrogen control (37).

The data presented in this portion of the literature review were accumulated by independent researchers. However, their respective research projects were not specifically directed toward the nitrite accumulation phenomenon.

Nitrite Accumulation in Soil Systems. Soil scientists have long been cognizant of the fact that heavy applications of nitrogenous fertilizers significantly affect the microbiological nitrification process in soil systems. Nitrite accumulation due to fertilizer addition to soils was first observed by Kelly (36) in 1916. Since then, nitrite buildup in soils proceeding excessive application of nitrogenous fertilizer has been noticed extensively.

During a study of commercial fertilizer, Martin, et al. (36) concluded that ammonia will not be converted to nitrate in alkaline soils until the pH value is reduced to a value of 7.7 ± 0.1. This research demonstrated a pronounced decrease in pH of the alkaline soil preceding nitrite or nitrate formation. A significant concentration of nitrite accumulated for extended periods of time. It was concluded that the nitrite buildup was a function of the magnitude of the pH value above 7.7.

In other research, Duisberg, et al. (36), observed nitrite accumulations preceding the application of excessive ammonia concentrations. The nitrate-nitrogen level increased only after the pH value fell to 7.67 which substantiates the pH value reported by Martin, et al.

In order to study pH effects, Morrell and Dawson (36) percolated ammonia sulfate through 55 soil samples over a 28-day period and noted, in part, the following patterns:

1. Ammonium rapidly oxidized to nitrite which accumulated over a long period before being oxidized to nitrate.

2. Ammonium and the resultant nitrite were both rapidly oxidized.

3. Ammonium slowly oxidized to nitrate without nitrite being detected.

These researchers concluded that the soil pH appeared to be the single best measurement indicative of its nitrifying capacity. Morrell (36) concluded that nitrite accumulated (Pattern 1) with pH values above 7.3. Pattern 2 occurred in most soils between pH values of 7.3 and 5.5, while Pattern 3 generally manifested itself below pH 5.7.

However, nitrite accumulation in soil regime was considered by many as not singularly due to pH effect.

For instance, Stojanovic and Alexander (36) found that accumulations of nitrite in soil was proportional to the amount of ammonium sulfate initially present. These researchers concluded that the nitrite accumulated only when ammonium nitrogen remained in the metabolite solution. It was observed that the nitrites rapidly vanished once the ammonium nitrogen had been oxidized. This led Stojanovic and Alexander to suggest that "...the buildup and persistence of this substance (nitrite) is favored by alkaline reactions and high level of applied ammonium or ammoniums forming fertilizers possibly by means of an inhibition of Nitrobacter by free-ammonia" (36).

Research by Olsen, et al. (36), seems to substantiate the hypothesis that ammonia is inhibiting to Nitrobacter. This group evaluated the effect of varying concentration of cattle manure on soil nitrification. The two highest manure application rates resulted in nitrite buildup within the first four weeks. Lower manure application rates resulted in nitrate formation without significant accumulation of nitrite.

Nitrite Accumulation in Domestic Waste Systems. Under normal circumstances, nitrite accumulation is not recognized as a common problem when aerating domestic sewage, because the relatively low ammonia levels encountered (30 to 40 mg/l as N) are usually oxidized completely to nitrate with only a transient accumulation of nitrite observed (36). However, analysis of a number of pilot plants treating nitrogenous wastewaters revealed that, in certain circumstances, nitrification in these mixed liquors is incomplete resulting in an accumulation of nitrite (16).

Nitrite Accumulation in Natural Water Regimes. After excessive nitrite concentrations were repeatedly detected in a Swedish lake, research was initiated to analyze the autotrophic oxidation of ammonium to nitrate. Water and sediment samples were taken with sterilized samplers on four occasions, and the concentration of autotrophically nitrifying bacteria were determined by indirect most-probable number procedures. It was found that Nitrosomonas were 100 to 10,000 times more abundant than Nitrobacter in the bottom water of the lake. The activity of Nitrobacter in the lake was also reduced, clearly indicating a disturbance of the oxidation of nitrite to nitrate (45).

One river was characterized by having a nitrite concentration four times greater than the ammonia concentration (46).

Theories Explaining Nitrite Accumulation

The traditional hypothesis for nitrite accumulation during nitrification processes (especially nitrifying systems receiving municipal wastes) has been based upon different growth rates or reaction rate kinetics for Nitrosomonas and Nitrobacter (36).

Pathways which delineate the nitrification-denitrification process are shown in Figure 5 (36).

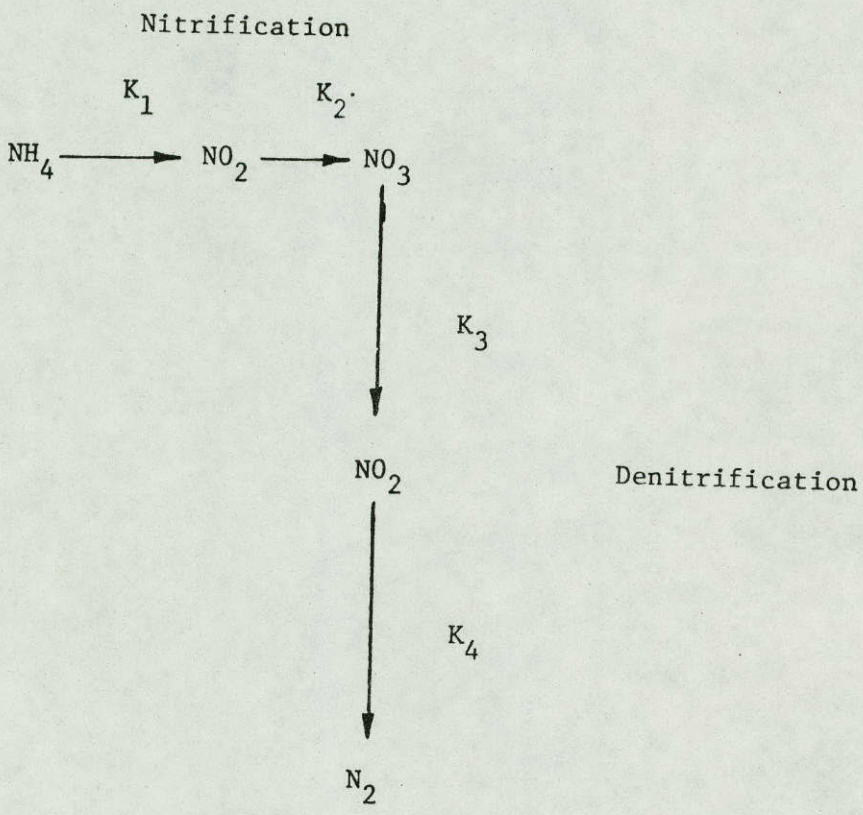


Figure 5. Simplified pathways for nitrification and denitrification.

Constants K_1 and K_2 represent the reaction rates of Nitrosomonas and Nitrobacter, respectively, while constants K_3 and K_4 represent reaction rates of denitrifying processes (36).

However, another concept, that of bacterial growth kinetics (36), associates reaction rate kinetics more directly to the microbiological processes involved (Figure 5). Thus, the concept of bacterial growth kinetics characterizes the constants K_1 and K_2 (Figure 5) as representing the growth rates for Nitrosomonas and Nitrobacter, respectively. which are proportional to the concentration of the microorganisms present and are a function of the respective substrate levels and environmental conditions (36).

These concepts are based on reaction rate kinetics, and are appropriate for the prediction of nitrogen conversion reactions in environments where rate constants K_1 and K_2 are uninhibited. Therefore, the nitrogen transformation which occur under uninhibited environments include only transient nitrite accumulations. According to the appropriate predictive equations based upon these concepts, accumulations of nitrite will not occur as ammonia is oxidized completely to nitrate.

However, research has demonstrated that nitrite accumulation under certain circumstances is a reality. For instance, nitrite, in certain microbial environments has accumulated to levels in excess of 1,000 mg/l for periods up to 200 days (36). This phenomenon does not conform to what might be expected according to the concept of reaction rate kinetics. However, this concept does serve as a basis from which one may develop a hypothesis for nitrite accumulation. This hypothesis

states that nitrite will accumulate in the nitrifying process when the rate constant K_2 is less than K_1, whatever the reason (36).

Although accumulations of nitrite have generally been explained by a relatively high pH environment, other researchers have found that free ammonia is the culprit (36). The rationale behind this theory is that certain concentrations of free ammonia will inhibit Nitrobacter but not Nitrosomonas, thus causing nitrite to accumulate (36).

More recently, research has demonstrated that unionized ammonia (FA) and unionized nitrite (FNA) are inhibitory to nitrifying organisms, and their presence cause nitrites to accumulate and persist (36). In this research, both laboratory and pilot plant tests were completed to investigate the reaction rates for the organisms Nitrosomonas and Nitrobacter. Results indicated that FA inhibition to Nitrobacter occurred at concentrations much lower than those required to inhibit Nitrosomonas. Nitrites thus accumulated without subsequent oxidation to nitrate. In liquid waste systems, accumulation of nitrite was demonstrated to be directly proportional to concentrations of FA and FNA and independent of pH, ammonia, and nitrite concentrations. FA inhibits Nitrosomonas nitrification at 10.0 to 150.0 mg/l; FA inhibits Nitrobacter at 0.1 to 1.0 mg/l; and FNA inhibits both Nitrosomonas and Nitrobacter nitrification at 0.22 to 2.6 mg/l, respectively (36).

Nitrite Reduction

This microbial reduction of nitrates and/or nitrites is accomplished by a number of species of facultative anaerobic bacteria, i.e., Pseudomonas, Achromobacter, and Bacillus (19). Most of these microbes

utilize oxygen preferentially as a hydrogen acceptor but may also use nitrates and nitrites as a substitute hydrogen (electron) acceptor.

The specific denitrifying capacity of these microorganisms may differ with some only being able to reduce nitrates to nitrites; others reducing only nitrates to molecular nitrogen; and some species achieving the reduction of both nitrates and nitrites to molecular nitrogen. Because of this, denitrification must be considered a two-step biological process (19). Nitrite is considered to be the first product of nitrate reduction, and its further reduction is facilitated by the enzyme nitrite reductase (35).

Nitrite, since it is an intermediate in the nitrification and denitrification processes, can link the nitrification and denitrification steps directly without passing through the nitrate stage. First, nitrite is formed from oxidation of ammonia by Nitrosomonas sp.; then the nitrite can be denitrified to nitrogen gas. By this route, less oxygen is required for nitrification and less organic matter (energy) is necessary for denitrification. The Process Design Manual for Nitrogen Control (14) states that this is a special case, however, and not broadly applicable to municipal wastewater treatment (47) as nitrate, not nitrite, is the normal substrate for microbial dissimilatory nitrogen reduction processes (48).

Since the research performed for this dissertation depended to a great extent upon successful acclimation of denitrifying bacteria to relatively rich concentrations of nitrite and subsequent nitrogen gas production, a significant effort was made to find bacterial strains capable of producing the necessary nitrite reducing enzyme(s). The

results of this effort appear in the following review of literature listing various studies concerned with nitrite reductase.

1. In one study, nitrite reduction was examined by utilizing a strain of Achromobacter liquefaciens. This pure culture (strain 39) was isolated from Victorian soil by Dr. Nancy Millis (49). The organism is characterized by its ability to reduce nitrite but not nitrate to nitrogen gas (50).

2. Youatt (50), using this strain of bacteria in a denitrification study found that it was capable of removing nitrite at the rate of 180 to 1,140 ppm/hr. Her results indicated that under anaerobic conditions growth was very slight, but highly active cells could be produced in good yields if a period of anaerobic growth followed initial aerobic growth. A pronounced reduction of "nitritase" activity was exhibited in media rendered deficient in iron, and the addition of 0.5 ppm of iron supplied as ferrous sulfate resulted in cells of normal activity.

3. Skerman, et al. (36, 49), performed a study which also demonstrated that the same strain of Achromobacter liquefaciens reduced nitrite but not nitrate. The nitrite reduction occurred in the presence of oxygen with the rate of nitrite reduction increased when the oxygen demand exceeded the supply.

4. Experiments by Kefauver and Allison (51) merit comments. Their results on the growth of Bacterium denitrificans demonstrated that the organism was capable of reducing nitrite to gaseous forms very rapidly. Perhaps the most significant fact established by the work with B. denitrificans was that the organism can utilize both

nitrite and oxygen simultaneously, although the latter is utilized preferentially. Regardless of oxygen level in these experiments, nitrite was reduced in cultures that were simultaneously using large quantities of oxygen.

5. Another researcher discovered that Hydrogenomonas eutropa (strain H) could anaerobically reduce nitrite. Anaerobic growth of the bacteria occurred in two phases. During the first phase, nitrate was reduced to nitrite which accumulated. The second growth phase was characterized by nitrite reduction and nitrogen evolution (28).

Further work revealed that H. eutropa contains only one nitrate reductase which is repressed by ammonia. The nitrite reductase could be synthesized by this bacterial species only under anaerobic conditions (28).

6. Pseudomonas stutzeri has been found to possess enzymes capable of catalyzing the formation of nitrogen gas from nitrate, nitrite, and N_2O. With pure microbial cultures or in soil environments, there is no nitrogen gas release from organic compounds, and denitrification specifically requires some form of oxidized nitrogen, i.e., nitrate, nitrite, or N_2O. Also, an electron or hydrogen donor is essential. Considering nitrate as the point of initiation, the disappearance of nitrate under reducing conditions is accompanied by nitrite accumulation in soil. If aerobic, nitrate and nitrite form N_2O. If anaerobic, nitrate and nitrite form nitrogen gas (52).

Further studies revealed that nitrite reductase from Ps. stutzeri required copper and iron for its reaction (52).

7. Radcliff and Nicholas (47) found a nitrite reducing enzyme in Pseudomonas denitrificans. The purified enzyme reduced nitrite stoichiometrically to nitric oxide, while suspensions of whole cells reduced nitrite to a mixture of nitric and nitrous oxide (47).

8. Ishague and Aleem (53) reported that the chemoautotrophic bacteria Thiobacillus denitrificans could catalyze the reduction of nitrate, nitrite, and nitric oxide stoichiometrically to nitrogen gas.

9. In order to further clarify the participation of denitrifying microbes in nitrite volatilization, researchers performed a series of experiments utilizing Pseudomonas aerriginosa in soil systems at a pH range of 7 to 8.3. Ps. aerugenosa reduced 100 percent of the nitrite concentration (250 ug/g). The nitrite was reduced primarily to molecular nitrogen (54).

Other efforts identified nitric oxide as a major product of bacterial denitrification by Ps. aerugenosa (48).

One scientist reported that Ps. aeruginosa grown on nitrate is rich in nitrate reducing enzume, while on nitrite, it forms much of the nitrite reducing system that yields nitrogen gas (32).

10. Many fungi can utilize nitrate and nitrite as a nitrogen source under aerobic conditions in an assimilatory process, but little is known about the anaerobic growth of fungi and the possible formation of nitrogenous gases (54).

In summary, a considerable amount of experimental evidence exists documenting the fact that denitrification beginning at the nitrate stage can reduce nitrite to either nitrogen gas or gaseous nitrogen oxides.

CHAPTER III

EXPERIMENTAL PROCEDURE

In order to accomplish this research, several separate experiments were performed. Rather than separate the studies into conventionally reported components, i.e., procedure, results, and discussion, each study will be considered independently and in its entirety. Conclusions and recommendations from the combined studies will be reported in the final chapter. This chapter will delineate the overall research approach to this study.

Objectives. The objective of this research project was to ascertain the feasibility of developing a non-conventional biological system for denitrifying sewage effluent. The research plan was to terminate the nitrification[1] process, the conversion of ammonia to nitrate, at the nitrite step; at which point denitrification would be promoted. The nitrification process was expected to accumulate nitrite without subsequent oxidation to nitrite because of purposeful manipulation of environmental conditions as described in the literature. This manipulation was designed to result in a treatment regime conducive to the metabolic activities of nitrite formers (Nitrosomonas europea) and inhibitory to nitrate-producing bacteria. There exists strong experimental evidence that Nitrobacter is more sensitive than

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[1]To distinguish between the production of nitrites, nitrates, and nitrites plus nitrates, the terms nitritification, nitratification, and nitrification will be used, respectively, throughout this dissertation.

Nitrosomonas to high pH, free-ammonia conditions (36). This differential sensitivity should result in the accumulation of a relatively high concentration of nitrite.

The next step in the experimental procedure was to reduce the resultant accumulated nitrite to nitrogen gas by utilizing strain 39 of Achromobacter liquefaciens.

Therefore, the experimentation and analyses performed throughout this research may be separated into two major sections--nitritification studies and denitrification studies.

A series of four preliminary nitritification studies were conducted to permit scrutiny of the nitrite accumulation phenomenon prior to the primary nitritification study. They were designed to gain greater familiarity and understanding of the growth trends and environmental influences affecting Nitrosomonas europea. The data accumulated in the preliminary nitritification studies proved useful for the planning phase of the primary nitritification study.

The objective of the primary nitritification study was to aggregate the conclusions reached in the preliminary studies in order to develop a biological system which, on a continuous basis, would convert ammonia-N to nitrite-N without subsequent oxidation to nitrate.

The denitrification studies were divided into a preliminary study and a primary study. The preliminary laboratory investigation was conducted prior to the primary denitrification study to permit scrutiny of the purported nitrite reducing capabilities of Achromobacter liquefaceins (strain 39). The objective of the primary denitrification study was to demonstrate the technology necessary to biologically reduce

nitrite present in a synthetic wastewater to nitrogen gas and/or volatile nitrogen oxides on a continuous basis.

Sample Preparation and Analytical Techniques. When in operation, nitritification and/or denitrification studies were monitored and sampled once a day, seven days a week.

Samples for analysis of NO_3, NO_2, NH_3, dissolved solids, soluble COD, phenolpthalein alkalinity, methyl-orange alkalinity, and pH were filtered through 0.45 micron Gelman membrane filters. The samples were analyzed within three hours of their acquisition. Unfiltered samples were immediately analyzed for organic nitrogen and optical density.

Nitrite analyses were performed by diazotization procedures and ammonia-ammonium analyses were performed by the nesslerization procedure both outlined by the Bausch and Lomb Analytical Systems Division (55) using a Bausch and Lomb Spectronic 20.

Nitrite interferes with the brucine-sulfate nitrate analysis, nitrate ion specific electrodes, and nitrate analysis by U.V. spectrophotometry. Therefore, the Hach cadmium reduction method was used for nitrate analyses after compensation for nitrite interference was made by sample pretreatment (56).

Organic nitrogen was determined by digestion of the samples after removal of free ammonia according to procedures described by Bausch and Lomb (55).

The soluble COD analyses were performed by the dichromate reflux method described in Standard Methods for the Examination of Water and Wastewater (57). Nitrite exerts a COD of 1.14 mg per mg nitrite-N.

To eliminate interference from nitrites, sulfamic acid in the amount of 10 mg for every 1 mg of nitrite-N in the refluxing flask was added to the dichromate solution.

Suspended solids determinations, and phenolpthalein and methyl-orange alkalinity analyses were performed according to procedures described in Standard Methods for the Examination of Water and Wastewater (57). Dissolved oxygen was determined either by using the Winkler titration method or a Weston and Stack, Model 330 D.O. analyzer. Dissolved solids were measured using a Myron L Deluxe Dissolved Solids Meter. After the meter became inoperational, dissolved solids analyses were performed gravimetrically. Optical density analyses were measured with a Beckman Model 24 Spectrophotometer using a wavelength of 640 nm. Throughout the investigation, frequent observation of the contents of the reactor basins were performed by microscopic examination of slide preparations stained by Gram-stain techniques.

Batch reactor experiments were carried out within a "Bio-Kulture" bench scale fermentor (SA Series) manufactured by Fermentation Design, Inc., and pictured in Figure 6. The fermentor, equipped with turbine impellers with variable speed agitator control, had a 15-liter capacity. In order to ensure proper air control to the vessel, the precision air supply pressure indicator and regulator valve was mounted on the main control panel. A panel-mounted flow meter with an integral needle control valve was also provided.

In order to ensure a supply of sterile air, the fermentor assembly was supplied with a stainless steel air filter with holding clips mounted

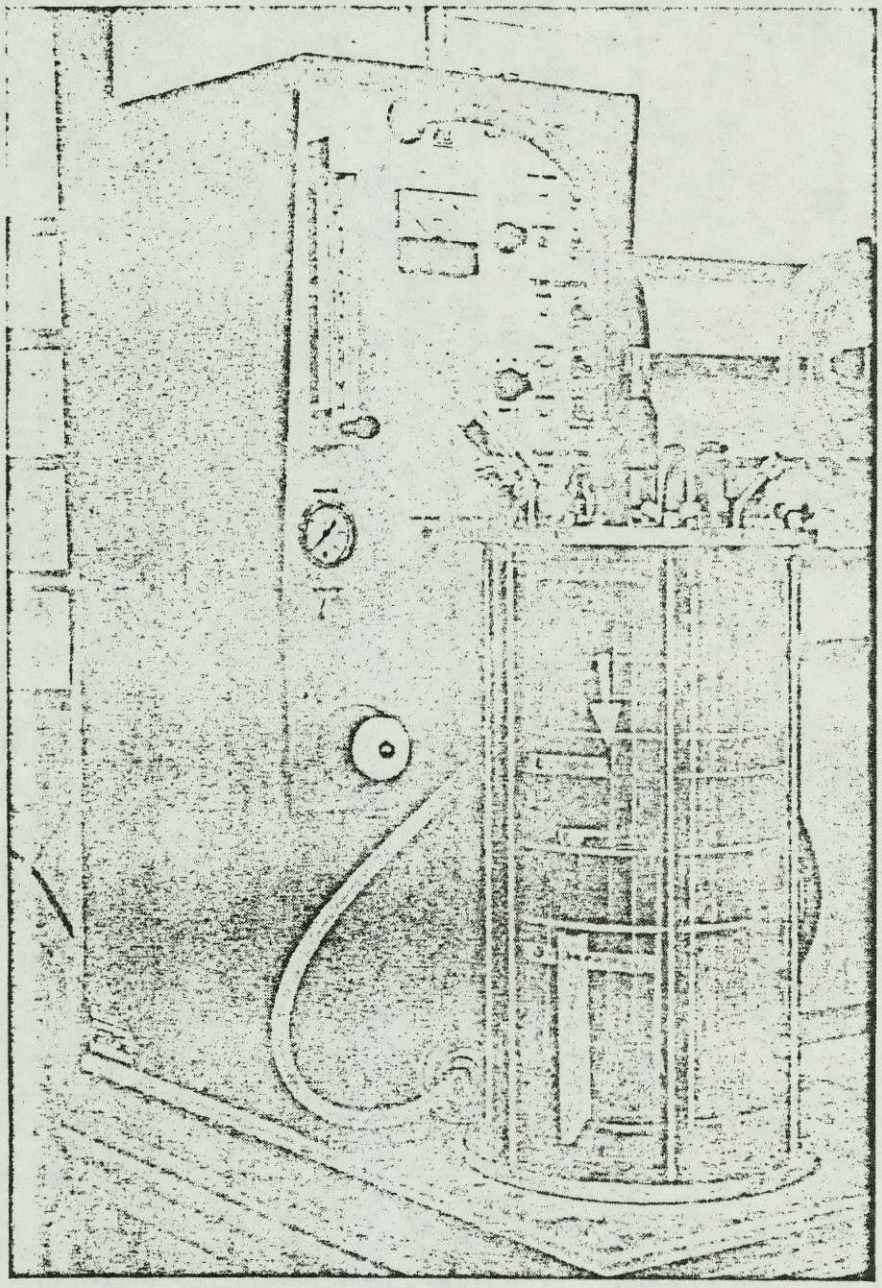

Figure 6. Photograph of the "Bio-Kulture" fermentor.

beneath the main control panel. The filter was removed for autoclaving with the culture vessel. It was packed with a specially blown resin-bonded fiberglass for effective air sterilization. This packing gave positive filtration of all bacteria and other organisms 0.5 microns or larger in size. The temperature of the contents of the fermentor vessel were controlled by continuously circulating water through a heat exchanger within the vessel. This allowed precise temperature control to within $\pm 1/4$ degrees C.

The assembly could be operated in either an aerobic or anaerobic mode. The vessel, agitators, and other accessories were autoclaved conveniently for contamination control. The vessel also included headplate ports for convenient aseptic sampling, air exhaustion, and aseptic liquid addition.

The batch bacterial cultivation process, when selected, was chosen over the continuous culture method for various portions of the research for the following reasons:

1. It is relatively easy to define a batch system (35);

2. The slow growth rates of nitrifying bacteria may preclude efficient continuous methods of cultivation (35);

3. The theoretical, mathematical, biological, physical, and chemical analyses derived from the batch culture of bacteria may be used for predicting behavior in continuous regimes.

The continuous flow mode of operation, when selected for a particular study, was chosen because it offers the opportunity of allowing a microbial population to reach a condition of equilibrium. Continuous studies also permitted the further exploration of the

nitritification process, and assisted in the identification of problems associated with the maintenance of an active bacterial population.

Data accumulated in the continuous nitritification studies were obtained utilizing a Bio-Matic Bench Scale Activated Sludge Plant (Model CF 325) manufactured by Princeton Aqua Science (New Brunswick, New Jersey). Figure 7 is a photograph of the system.

Bio-Matic system provided an 18.9-liter (5-gallon) influent reservoir tank which contained the feed solution (synthetic wastewater). The essential part of the system was the 9-liter constant overflow aeration chamber. A 2-liter settling basin was also provided.

The "synthetic sewage" feed was transferred to the aeration tank by a timer-activated positive displacement pump with air assist. The feed rate from the influent reservoir to the aeration vessel was controlled by the "Feed Rate" selector switch located on the front panel.

The rate of diffused air flow into the bottom of the aeration chamber was controlled by a flow meter located on the front panel. The flow rate could be adjusted from 0.01 to 10 cubic feet per hour (cfh).

Agitation was provided by two air sources entering the aeration basin. Diffused air entering the bottom of the tank was the major source of agitation. Also, the synthetic wastewater was transferred to the aeration tank with air assistance. The influent line may not have been placed in the aeration basin liquid. If the influent line was in the aeration basin liquid, the degree of agitation and, consequently, oxygen transfer was considerably increased. This rate of air assistance could not be controlled.

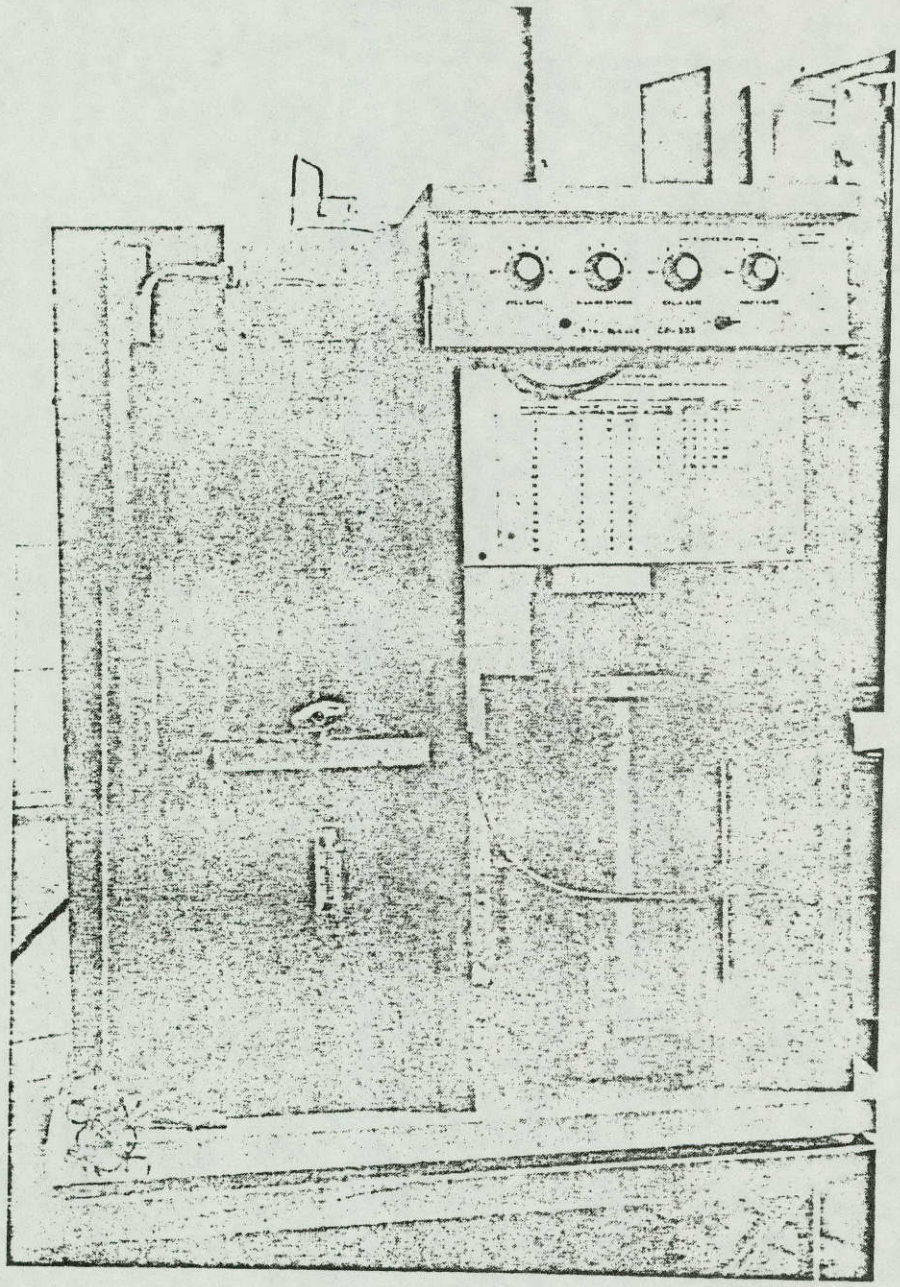

Figure 7. Photograph of the "Bio-Matic" system.

One hundred percent sludge return capability from the 2-liter settling basin was desired. Therefore, the contents of the settling basin were filtered each day through Gelman membrane filters (0.45 u). The cells accumulated on the filter paper as a paste, and were scraped back into the aeration basin. Any wasting of cells, such as for suspended solids analysis, was incidental. The filtrate was analyzed for chemical, physical, and biological attributes and discarded.

Temperature control in the aeration vessel was not available. However, due to the indoor location of the system, the range of temperature variation from the optimum for nitrifying bacteria was considered to be negligible. Since one of the goals of the continuous system studies was to define a system applicable to typical sewage treatment plant situations, it was concluded that temperature control was not necessary. Alkalinity and pH control in both the batch and the continuous nitritification and denitrification studies were obtained by daily alkalinity and pH monitoring and addition of alkalinity or acid, if necessary, in order to maintain the pH in the desired range used for the particular experiment.

Values for the parameters (e.g., pH) used to develop a system for the success of the various phases of the research were obtained from the literature.

CHAPTER IV

RESULTS AND DISCUSSION

Preliminary Nitritification Study--Phase I

This section describes the first of four preliminary nitritification experiments performed prior to conducting the primary nitritification study.

Because Nitrobacter sp readily converts nitrite-N to nitrate-N concentrations as high as 0.6 mg/l of nitrite-N are rarely found in natural surface waters (55). Recent information regarding the nitrite accumulation potential of pure culture systems of Nitrosomonas europea is scarce. Therefore, the major objective of this phase of the preliminary nitritification study was to demonstrate that a pure culture system of Nitrosomonas europea was capable of accumulating relatively high concentrations of nitrite.

The objective of this phase was to culture Nitrosomonas europea in a pure culture batch system utilizing a liquid growth medium containing a relatively high concentration of ammonia nitrogen plus other compounds essential for the autotrophic growth characteristics of N. europea. Environmental conditions (temperature, pH, etc.) conducive to the growth of the bacterial culture were controlled.

It was felt that a pure microbial culture was necessary for obtaining conclusive results in the nitritification study. Although

many laboratory incubation studies leave doubt as to whether the data obtained apply to actual waste treatment operations, it was felt that the experimental laboratory conditions were necessary if the environmental factors which control the biological reactions were to be critically evaluated.

Daily monitoring of the system was planned to result in the determination of the fate of the ammonia-N based upon the nitrogen transformations occurring within the reaction vessel. The degree of nitrite accumulation potential of N. europea was determined by use of a batch system. In order to initiate the experiment, immediately upon acquisition of an American Type Culture Collection culture of Nitrosomonas europea (ATCC No. 19718), a "mother culture" was aseptically prepared utilizing the directions furnished by ATCC. Appendix A provided instructions furnished by ATCC for medium preparation.

The "mother culture" was incubated in the dark at 30 degrees C. for several days before bacterial growth was apparent. Light exclusion during the incubation period was necessary to inhibit photonitrification.

> "Photonitrification is explained on the premise that ammonium salts in the presence of sunlight and various photosensitizers undergo oxidation to the nitrite and/or the nitrate stage. In the absence of the proper sensitizers, the oxidation is much less pronounced, or negligible. Certain sensitizers are ranked as follows:
>
> TiO_2, ZnO, CdO, $Na_2U_2O_7$, Al_2O_3, MgO, SiO_2
>
> Whether photochemical nitrification occurs and, if so, the extent to which it is important are controversial opinions. The phenomenon is attributed more commonly to lateritic soils under tropical conditions (36)."

Next, 15 l of the Nitrosomonas sp medium was prepared, autoclaved, and aseptically added to the previously sterilized Bio-Culture Bench Scale Fermentor. The quantities of ingredients required to prepare the medium are listed in Table 2.

TABLE 2

RECIPE FOR NITROSOMONAS SP. MEDIUM

Ingredient	Quantity
$(NH_4)_2\ SO_4$	45 grams
K_2HPO_4	7.5 grams
$MgSO_4$	0.75 grams
$CaCl_2$	0.06 grams
Chelated Iron*	1.5 mg Fe
Distilled Water	15 liters
Cresol red	0.75 mg

* EDTA Ferric Sodium Salt

On June 8, 1976, a ten ml inoculum from the "mother culture" which contained a profuse growth of N. europea was aseptically added to the 15 liters of sterile medium in the aerated reaction vessel. Agitation control was set at 200 rpm in order to ensure proper mixing. The temperature control was set at 30 degrees C. The aeration control for addition of filter sterilized air was set to deliver approximately 8000 cc/min.

The outside of the basin was covered to preclude light in order to prevent the growth of any photo-autotrophic contaminants and to inhibit photonitrification. The pure culture, batch nitritification system was aseptically sampled on a daily basis for 13 days (June 8 to June 20), and the samples were immediately analyzed for pH, phenolpthalein alkalinity, and total alkalinity, NH_3-N, NO_2^--N, NO_3^--N, total organic nitrogen suspended and dissolved solids, and dissolved oxygen. Data accumulated from this study are present in Table 3.

Table 4 reveals that the initial ammonia-nitrogen concentration in the 15 liters of sterile medium was 630 mg/1. However, calculations indicate that with the quantity of ammonium sulfate utilized to prepare the medium, the theoretical concentration of ammonia-nitrogen which should have been present was approximately 773 mg/1. A discrepancy of 143 mg/1 of ammonia-nitrogen existed and was thought to be the result of ammonia-N volatilization which occurred during preparatory autoclaving.

The medium chosen for this phase of the research was not intended to be representative of the typical secondary treatment plant effluent. On the contrary, the medium was chosen for the primary purpose of demonstrating that Nitrosomonas europea cells were capable of accumulating relatively high nitrite-N concentrations under environmental conditions known to be conducive to N. europea growth. Indeed, scrutiny of Table 4, which summarizes a portion of the qualitative and quantitative changes which occurred as a result of N. europea activity after the 13-day detention period of the study, indicates that the medium served its purpose. Figures 8 and 9 represent the transformation of the initial 630 mg/1 total system nitrogen during the study period.

TABLE 3.

DATA ACCUMULATED DURING THE PRELIMINARY NITRITIFICATION STUDY--PHASE I

Date	pH	Phenol, Alkalinity mg/l as $CaCO_3$	Methyl-Orange Alkalinity mg/l as $CaCO_3$	Nitrite Nitrogen mg/l	Ammonia Nitrogen mg/l	Total Organic Nitrogen mg/l	Dissolved Solids mg/l	Nitrate Nitrogen mg/l	Suspended Solids mg/l	D.O. mg/l	Volatilized** mg/l
6/8/76	8.40	90	480	N.D.	630	N.D.	4725	N.D.	I.C.	2.8	0
6/9/76	8.40	90	480	N.D.	581	--	4725	N.D.	I.C.	6.2	.49
6/10/76	8.40	90	452	0.9	542	--	--	N.D.	I.C.	5.8	38.1
6/11/76	8.39	70	422	1.0	522	N.D.	4550	N.D.	0.6	6.2	21
6/12/76	8.21	70	360	7.4	490	--	--	N.D.	1.1	6.0	21.9
6/13/76	7.6	0	280	15.0	465	0.3	--	N.D.	2	6.2	25.3
6/14/76	6.7 ↓ *	0 ↓	50 ↓	49	418	1.1	--	N.D.	--	5.4	16.6
	8.4	86	556								
6/15/76	6.74 ↓	0 ↓	110 ↓	156	298	3.0	6500	N.D.	58	6.0	10.1
	9.18	206	1240								
6/16/76	7.07 ↓	9 ↓	218 ↓	396	6.5	2.2	8000	N.D.	53	5.6	13
	8.45	74	720								
6/17/76	8.48	74	716	393	4.0	5.7	--	N.D.	63	5.4	48
6/18/76	8.45	72	716	395	2.9	5.8	--	N.D.	61	6.0	I.C.
6/19/76	8.47	72	720	393	1.2	5.0	--	N.D.	57	6.2	I.C.
6/20/76	8.45	74	716	396	1.4	4.3	--	N.D.	63	6.0	I.C. Total=227.3

N.D. = Not Detected
I.C. = Incompatible Results

* Arrows indicate addition of alkalinity

**Nitrogen not accounted for by nitrite, nitrate, organic-nitrogen, or ammonia analyses was assumed to have volatilized.

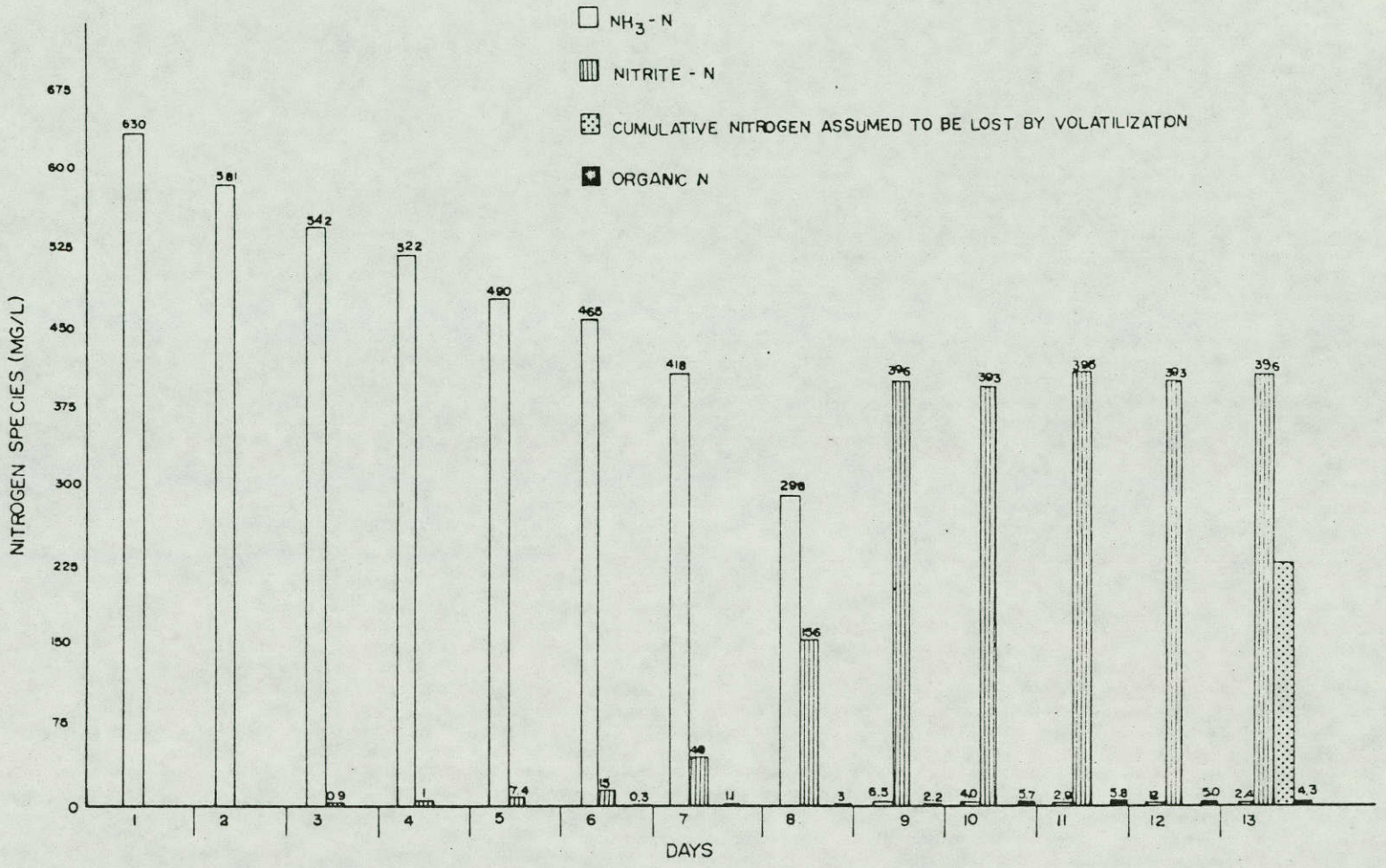

Figure 8. Nitrogen balance for preliminary nitritification study--Phase I.

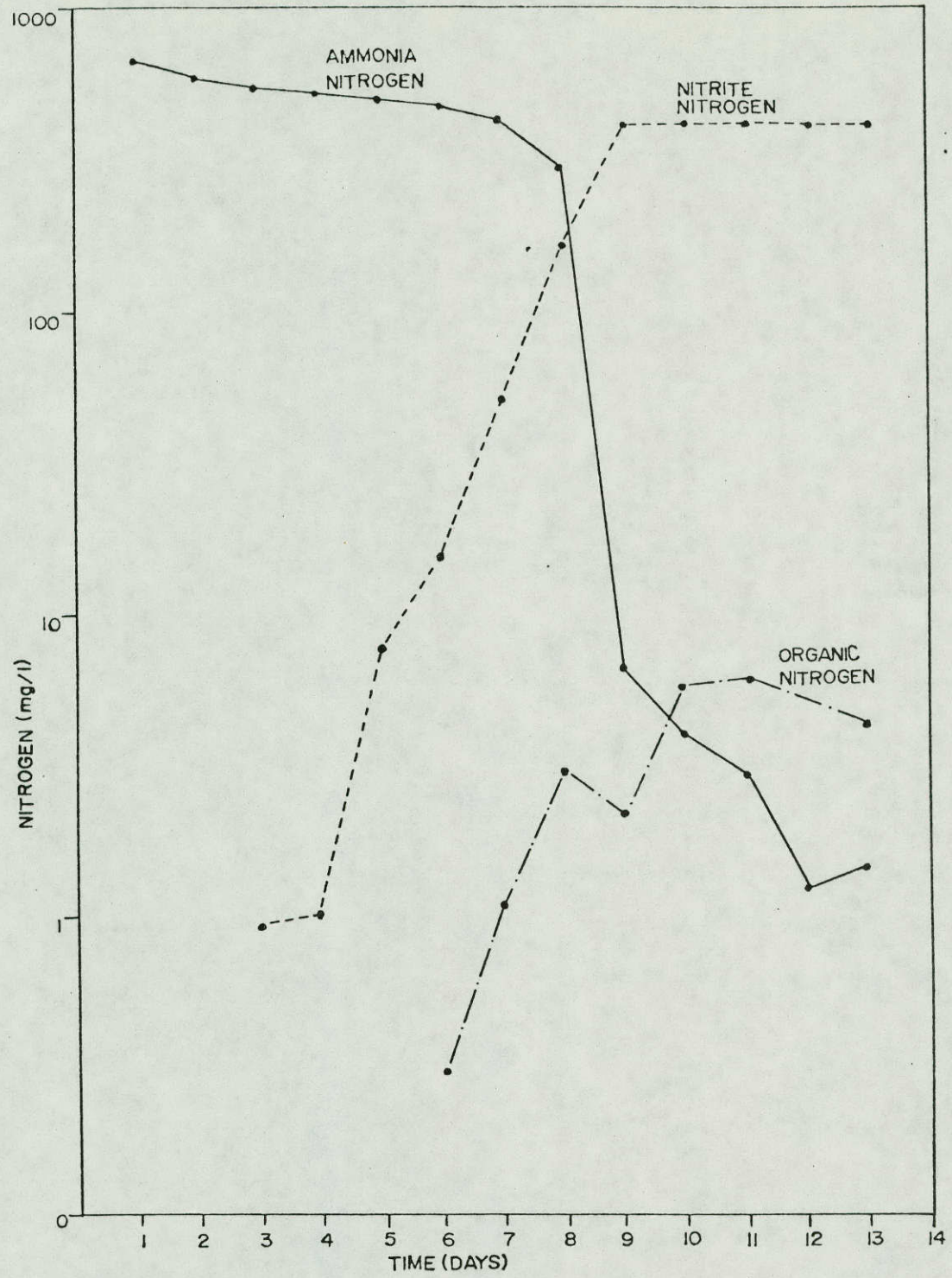

Figure 9. Nitrogen transformation for the Phase I portion of the preliminary nitritification study.

TABLE 4

QUALITATIVE AND QUANTITATIVE CHANGES OCCURRING DURING THE 13-DAY DETENTION PERIOD AS A RESULT OF NITROSOMONAS EUROPEA ACTIVITY

Parameter	Initial Analyses of Sterile Medium mg/l*	Following 13 Days of N. europea Activity mg/l
Nitrite Nitrogen	Not Detected	396
Ammonia Nitrogen	630	1.4
Nitrate Nitrogen	Not Detected	Not Detected
Organic Nitrogen	Not Detected	4.3
pH	8.40	8.45
Phenolpthalein Alkalinity	90	74
Methyl-Orange Alkalinity	480	716
Dissolved Solids	4725	8000
Suspended Solids	Not Detected	63

* pH is given in pH units.

Table 4 and Figures 8 and 9 further reveal that ammonia-N removal in excess of 99 percent was achieved. Of the 630 mg/l of ammonia-N initially present in the reactor basin, only 1.4 mg/l of ammonia-N remained after 13 days. Approximately 396 mg/l was oxidized to nitrite-N by Day 13 of the study. This represents a conversion of approximately 63 percent. Organic nitrogen analyses revealed that of the 630 mg/l ammonia-N initially present in the nitrification system, approximately 5.8 mg/l were converted to cellular mass and/or organic waste products. This represents less than one percent of the total nitrogen initially present in the system.

Summation of nitrite-N (396 mg/l), organic-N (5.8 mg/l), and residual ammonia-N (1.4 mg/l) yields 403.2 mg/l of nitrogen which was

accounted for at the termination of the batch study. Completion of the mass balance by difference yields 226.8 mg/l nitrogen which was assumed to have volatilized from the system. This represents a 36-percent loss of ammonia-N through volatilization alone. This relatively high degree of ammonia-N volatilized was not surprising when factors such as the long detention time (13 days), temperature (30 degrees C.), pH (6.7 - 9.18), agitation (200 rpm), and high degree of aeration were considered. Column 12 of Table 3 lists the assumed concentrations of ammonia-N "stripped" from the system each day.

If one assumes that the 226.8 mg/l of ammonia-N volatilized from the system was not available for cellular metabolism (energy and synthesis), then more meaningful conclusions may be reached regarding the percentage of available nitrogen converted to nitrite and assimilated by the Nitrosomonas europea cells. Calculations reveal that of the 403.2 mg/l of available nitrogen, approximately 98.2 percent was converted to nitrite, and less than 1.0 percent available nitrogen was converted to organic material.

Figure 10 is indicative of the fate of suspended solids throughout the course of the experiment. The suspended solids concentration was assumed to represent the concentration of Nitrosomonas europea cells. The rationale for this assumption was as follows:

1. A massive innoculation of Nitrosomonas europea cells was initially added to the aeration basin and a subsequent decrease in ammonia and increase in nitrite was noted. Such metabolic activity is indicative of Nitrosomonas.

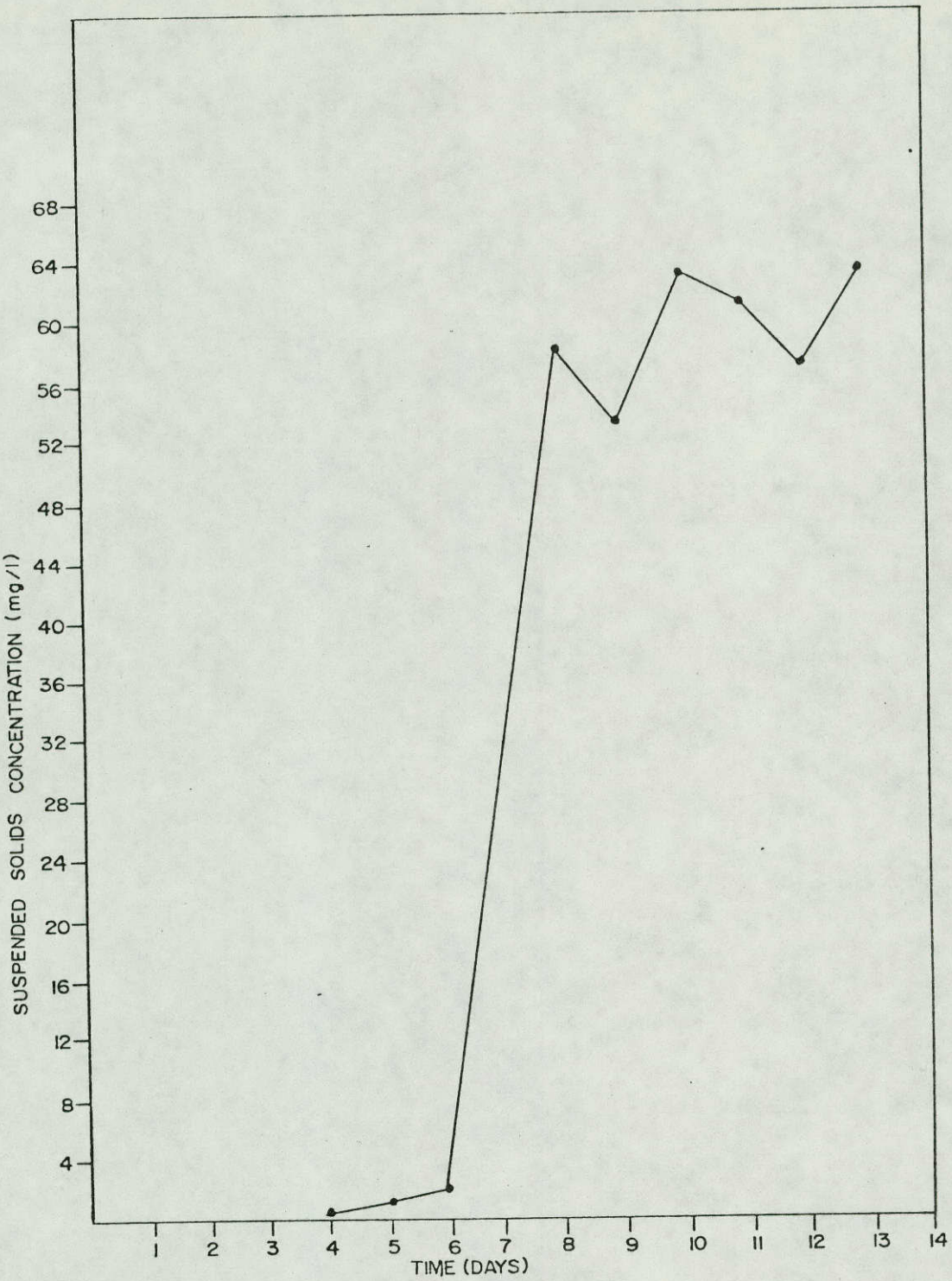

Figure 10. Suspended solids vs. time for the Phase I portion of the preliminary nitritification study.

2. Aseptic techniques were emphasized in order to maintain a pure culture system of N. europea.

3. Nitrate was not detected; therefore, Nitrobacter sp. was probably not present.

4. Slide preparations stained by Gram-stain techniques and subjected to microscopic examination were characteristic of Nitrosomonas sp.

5. Environmental conditions and the characteristics of the growth medium were selective to the growth of only a few types of organisms.

As can be seen from the data in Table 3 of the 63 mg/l of cells produced, approximately 5.2 mg/l (average of last 4 days) of organic nitrogen was detected. It can be calculated from this relationship that for 12.1 mg/l of cells produced, 1.0 mg/l of organic-N is produced. Assuming that all of the organic nitrogen is viable cellular material instead of organic by-products, this ratio indicates that organic nitrogen comprises about 8.3 percent of the organic material produced. This value compares to the 10 to 13 percent normally reported in the literature.

Figure 11 represents the fate of pH and alkalinity during the experiment. Daily decrease in both pH and alkalinity were assumed to be the result of aklalinity destruction due to the nitritification reaction. Theoretically, 7.13 mg of alkalinity as $CaCO_3$ should be destroyed for every 1 mg of NH_3-N converted to nitrite. From Day 7 to Day 10 of the experiment, 50 percent K_2CO_3 (sterile) was added as needed to the system in order to raise the pH to a range conducive to

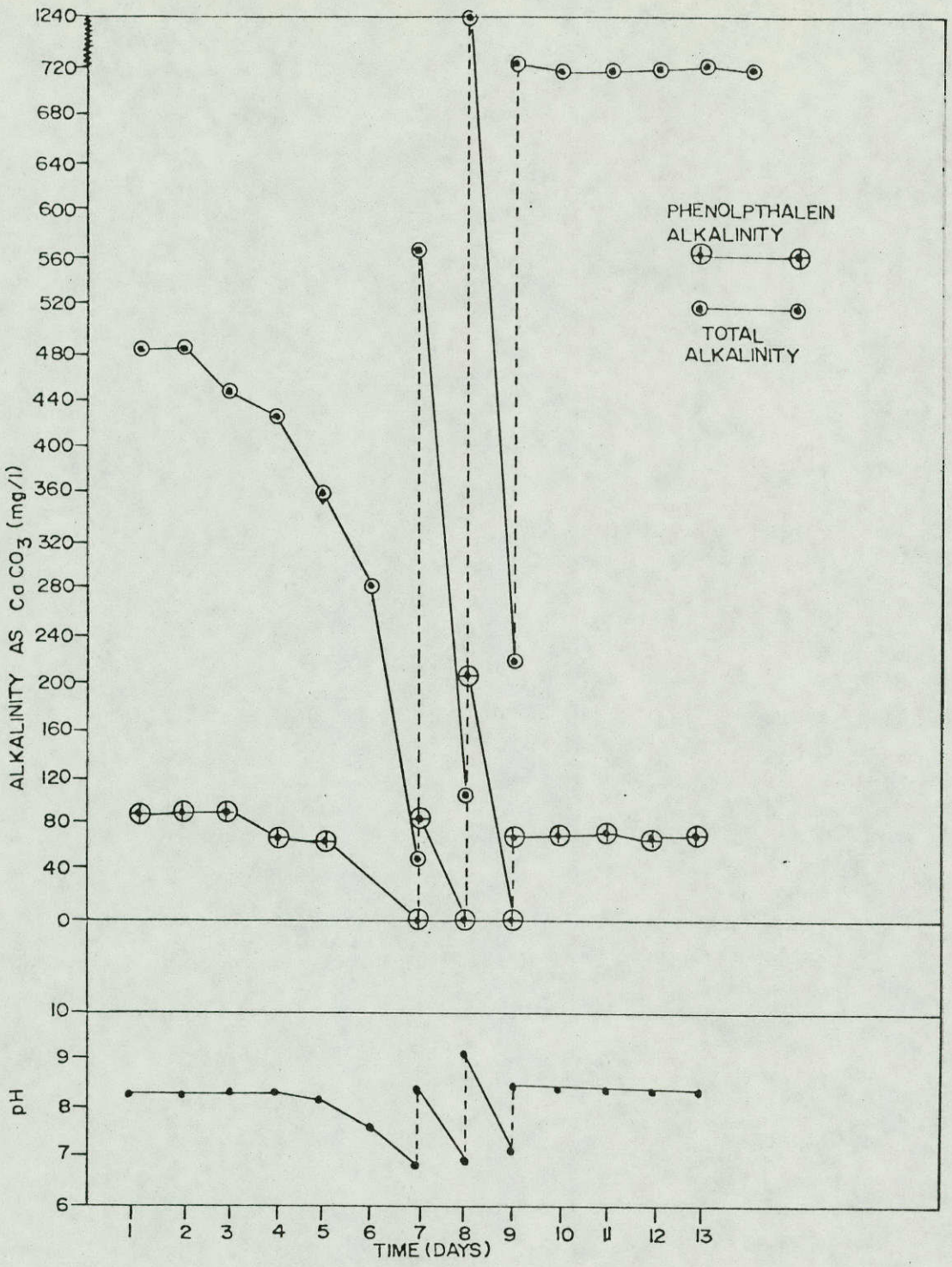

Figure 11. Alkalinity and pH vs. time in Phase I of the preliminary nitritification study.

the growth of N. europea. Consequently, dissolved solids and alkalinity increased. The dotted lines appearing on Figure 11 represent the addition of the 50 percent sterile K_2CO_3 to the system and the resultant increases in pH and alkalinity.

Conclusions. The significant conclusion reached from this study phase was that Nitrosomonas europea populations continue to metabolize at nitrite-N concentrations as high as 396 mg/1. Poduska and Andrews (58) stated in their study concerning nitrification dynamics that Engel and Alexander investigated product inhibition of Nitrosomonas by nitrite and reported that 5.6 mg nitrite-N/1 had no effect and 340 mg/1 nitrite-N/1 yielded only slight inhibition.

Preliminary Nitritification Study--Phase II

The next problem to be resolved for the nitritification study concerned the utilization of an ammonia concentration typically found in secondary treatment plant effluents. The concentration of ammonia-N resulting from the use of the medium suggested by ATCC for the growth of Nitrosomonas was approximately 773 mg/1. This amount of ammonia nitrogen is more than 25 times the ammonia-N concentration one would expect to encounter in a typical secondary sewage treatment plant effluent (e.g., 30 mg/1). Therefore, the question to be addressed was whether or not Nitrosomonas europea cells could successfully thrive in a batch type environment containing only approximately 30 mg/1 ammonia-nitrogen. In order to make this determination, methods very similar to those utilized for the Phase I study were employed.

Fifteen liters of medium were prepared according to the directions provided in Appendix A, but with the following exceptions. Only enough sterile ammonium sulfate was provided to result in an ammonia-N concentration of about 33 mg/1, and because the cresol red pH indicator caused an interference with the colorimetric analyses, its use was discontinued. Results of analyses made on the sterile medium are listed in Table 5.

Ten ml of the "mother culture" containing a profuse growth of N. europea were aseptically added to the 15 1 of sterile medium and the mixture was added to a sterile bench scale fermentor. Identical to the experimental procedures utilized in the Phase I study, environmental conditions were controlled in order to create an environmental conducive to the growth of N. europea. Table 6 lists the values at which the environmental control parameters were set.

TABLE 5

INITIAL ANALYSIS OF STERILE MEDIUM UTILIZED IN PHASE II OF THE PRELIMINARY NITRITIFICATION STUDIES

Characteristics	Value
Nitrite-N	Not Detected
Nitrate-N	Not Detected
Ammonia-N	33 mg/1
pH	8.3
Phenolpthalein Alkalinity	18 mg/1
Methyl Orange	288 mg/1
Dissolved Solids	1050 mg/1
Suspended Solids	Not Detected
Total Organic Nitrogen	Not Detected

TABLE 6

OPERATIONAL CONSTANTS UTILIZED DURING THE PHASE II PRELIMINARY NITRITIFICATION STUDY

Parameter	Value
Agitation	50 rpm
Aeration	8000 cc/min
Temperature	30 degrees C .

Again, the outside of the basin was covered to preclude light. The pure culture, batch nitritification regime was aseptically sampled (500 ml) on a daily basis during a period extending from the "startup" date (June 30, 1976) until July 12, 1976).

It was thought that optical density measurements coupled with suspended solids analysis would better monitor the fate of N. europea than would the use of suspended solids analyses alone. Therefore, optical density, in addition to all the analysis made in the first study phase, was also determined during the Phase II study.

Unresolved difficulties were encountered in attempts to measure the concentrations of ammonia volatilized from the system by using a technique employing boric acid sorption coupled with titration of the boric acid with a standardized titrant. Therefore, values for assumed volatilized or "air-stripped" ammonia-N were arrived at in a manner identical to that described in the Phase I preliminary study.

Data accumulated during the period of this study are presented in tabular form in Table 7. Figure 12 depicts, by means of bar graphs, the nitrogen mass balance for the batch nitritification system. The nitrogen transformations involved and nitrogen losses encountered are represented by this bar graph presentation of the data. Figure 13 illustrates, by means of a line graph, the nitrogen transformations monitored during the study period.

As may be seen from Figures 12 and 13, the medium initially contained 33 mg/1 of ammonia-N (June 30). Six days later (July 6), 0.46 mg/1 of ammonia-N remained (98.5 percent reduction). In an attempt to demonstrate system continuity, an additional quantity of ammonium sulfate was aseptically added to the system in order to increase the ammonia-N concentration in the batch system to 33.3 mg/1. By July 10, the ammonia-N concentration was reduced to 0.39 mg/1 (99 percent reduction). An additional dosage of ammonium sulfate (33.6 mg/1) was added aseptically into the batch system. Two days later (July 12), sample analysis revealed that the concentration of ammonia-N had been reduced to about 0.9 mg/1.

Calculation of a nitrogen mass balance for the entire experimental period from June 30, 1976, to July 12, 1976, yielded the following results. Approximately 99.9 mg/1 of ammonia-nitrogen was added to the reacter during the study period. Of this, about 42.4 mg/1 were oxidized to nitrite-nitrogen. This figure represents a 42.4 percent conversion of ammonia-N to nitrite-N. At the end of the period, 0.9 mg/1 ammonia-N remained. One mg/1 of ammonia-N was converted to

TABLE 7.

RESULTS OF PHASE II PRELIMINARY NITRITIFICATION STUDY--PHASE II

Date	Comments	pH	Phenolpthalein Alkalinity mg/l as $CaCO_3$	Methyl Orange Alkalinity mg/l as $CaCO_3$	Nitrite Nitrogen mg/l	Ammonia Nitrogen mg/l	Total Organic Nitrogen mg/l	Nitrate Nitrogen mg/l	Volatilized Ammonia-N Or Unaccounted For Nitrogen mg/l	Dissolved Solids mg/l	Suspended Solids mg/l	Dissolved Oxygen mg/l
06-30-76	System Start Up	8.32	18	288	N.D.	33.0	LT* 0.02	N.D.	--	1050	I.C.	2.3
07-01-76		8.10 ↓ 9.76	0 ↓ 108	288 ↓ 596	0.34	32.5	--	N.D.	0.16	1050	I.C.	6.0
07-02-76		9.08	76	594	0.61	32.0	LT 0.02	N.D.	0.23	1050 ↓ 1375	0.9	5.7
07-03-76		9.00	72	588	1.7	26.0	--	N.D.	4.91	1550	1.3	6.2
07-04-76		8.91	66	570	4.25	10.2	--	N.D.	13.25	1625	--	6.2
07-05-76		8.90	60	522	10.56	1.2	0.64	N.D.	2.05	1650	4.0	6.2
07-06-76		8.91	60	512	11.44	0.46	0.54	N.D.	I.C. **	1800	4.0	6.4
07-06-76	Analysis of Medium Immediately After Addition of Approx. 33 mg/l NH_3-N	8.90	62	516	11.40	33.3	--	--	--	1825	--	--
07-07-76		8.50	44	480	14.75	19.9	--	N.D.	10.05	--	5.0	6.2
07-08-76		8.27	0	390	28.5	3.2	--	N.D.	2.95	--	4.0	6.3
07-09-76		8.21	0	370	26.0	1.0	--	N.D.	I.C.	--	--	6.3
07-10-76		8.12	0	370	28.8	0.39	1.0	N.D.	I.C.	--	5	6.0
07-10-76	Analysis of Medium Immediately After Addition of Approx. 33 mg/l NH_3-N	8.14	0	380	28.6	33.6	--	--	--	--	--	--
07-11-76		7.51	0	250	42.3	2.4	0.9	N.D.	17.5	--	6.4	6.2
07-12-76		7.52	0	250	42.2	0.9	1.0	N.D.	I.C.	--	6.2	6.3

*Less Than

**Incompatible Results

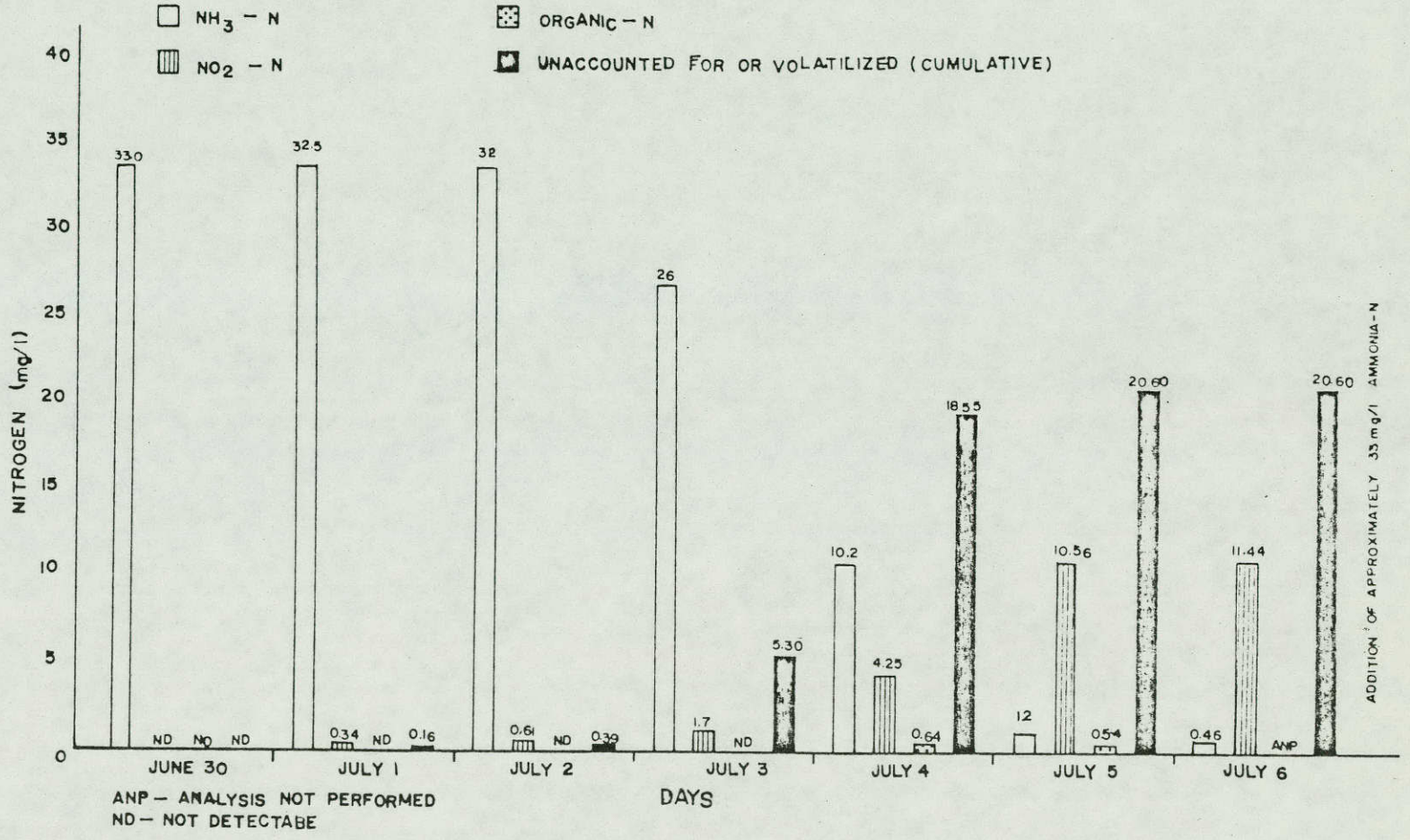

Figure 12. Nitrogen transformations vs. time in Phase II nitritification study.

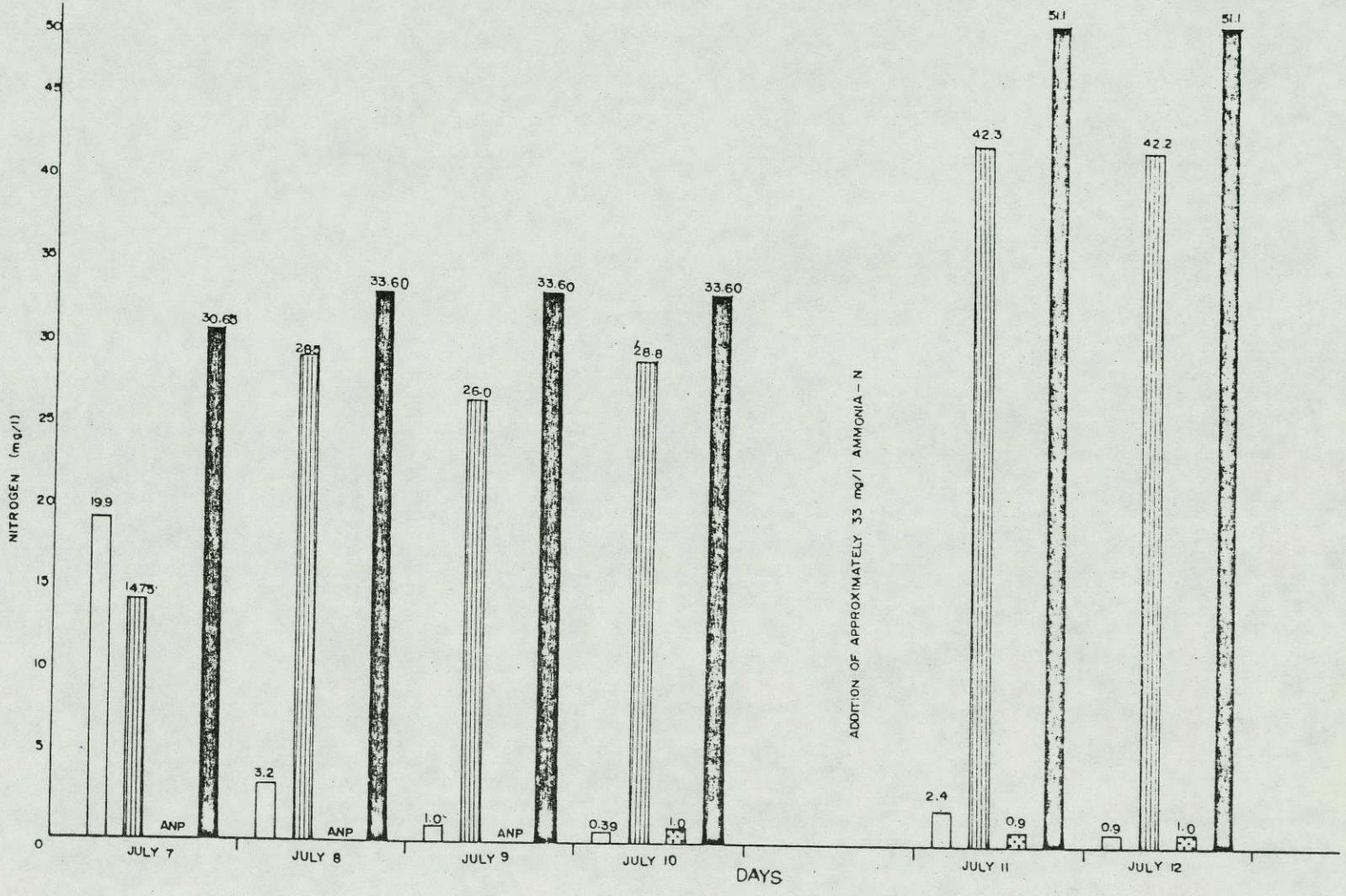

Figure 12. (cont'd)

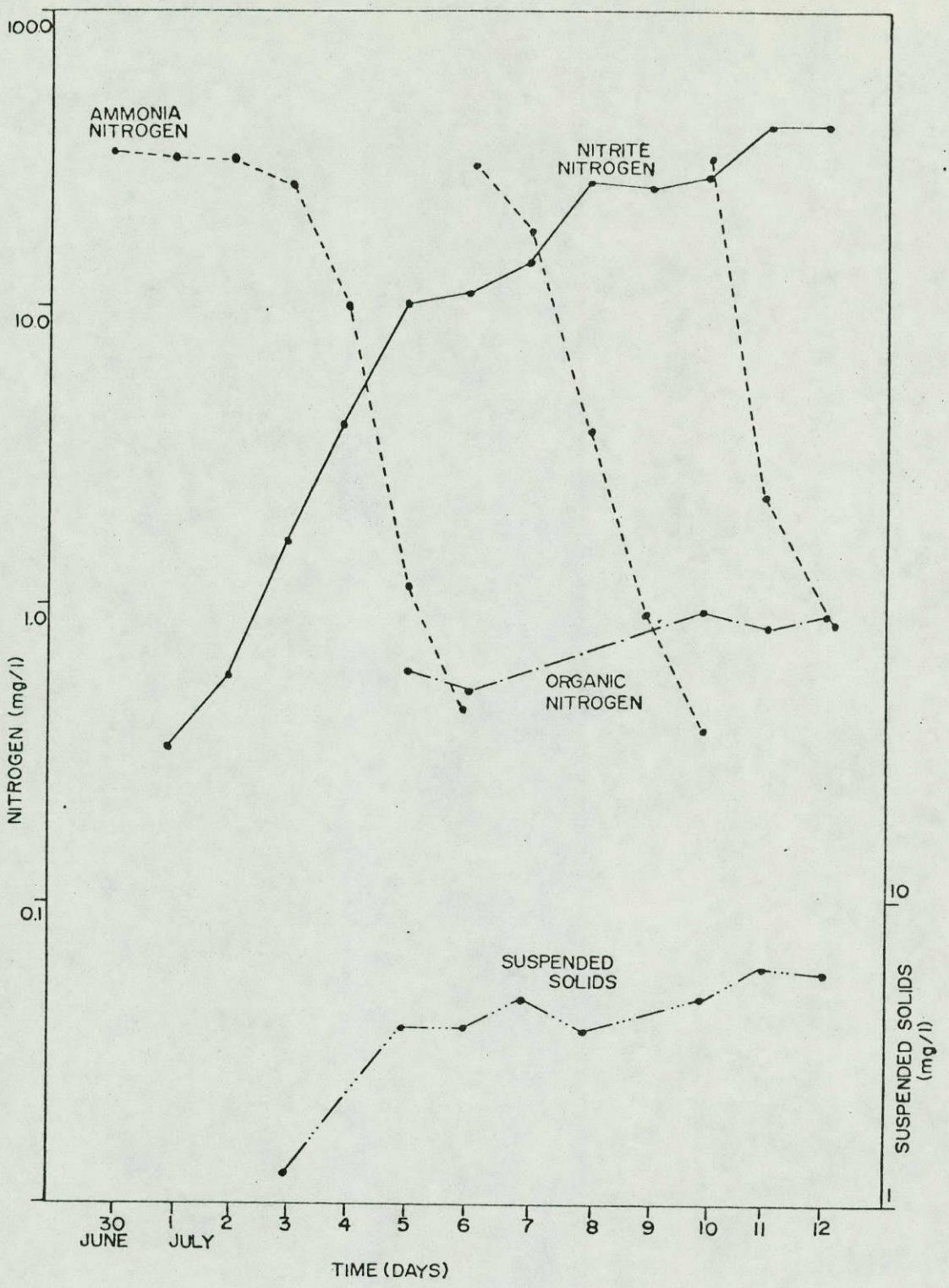

Figure 13. Nitrogen transformation and suspended solids vs. time during Phase II nitritification study.

organic-N. Therefore, of the 99.0 mg/l of ammonia-N, only 44.3 mg/l was accounted for. Calculation by difference yields 55.6 mg/l of ammonia-N which was assumed to have volatilized from the reactor via the exhaust port.

Figure 14 represents the pH and alkalinity fluctuations caused by the nitritification phenomenon. The dotted line represents addition of sterile, 50 percent K_2CO_3 in order to increase the alkalinity and incidentally increase the pH. Figure 13 illustrates the increase of suspended solids (assumed to be N. europea) during the course of the 13-day experiment. Both alkalinity destruction and suspended solids increases are characteristic of nitrification processes described in the literature.

It may be concluded from this study phase that:

1. Concentrations of ammonia-N comparable to those of secondary sewage treatment plant effluents were able to support a successful growth of N. europea under optimal environmental conditions in a batch system.

2. Ammonia removal by volatilization, oxidation to nitrite, and reduction to cellular material was significant.

3. After the second addition of 33 mg/l ammonia-N to the batch reactor, the organic nitrogen did not increase appreciably. This perhaps indicates that the system was reaching a state of equilibrium. The decrease in time required to deplete the ammonia-N concentration after the third addition of the ammonia feed tends to substantiate the hypothesis that the system was approaching equilibrium in terms of cell population which could be supported by such a low ammonia-N concentration.

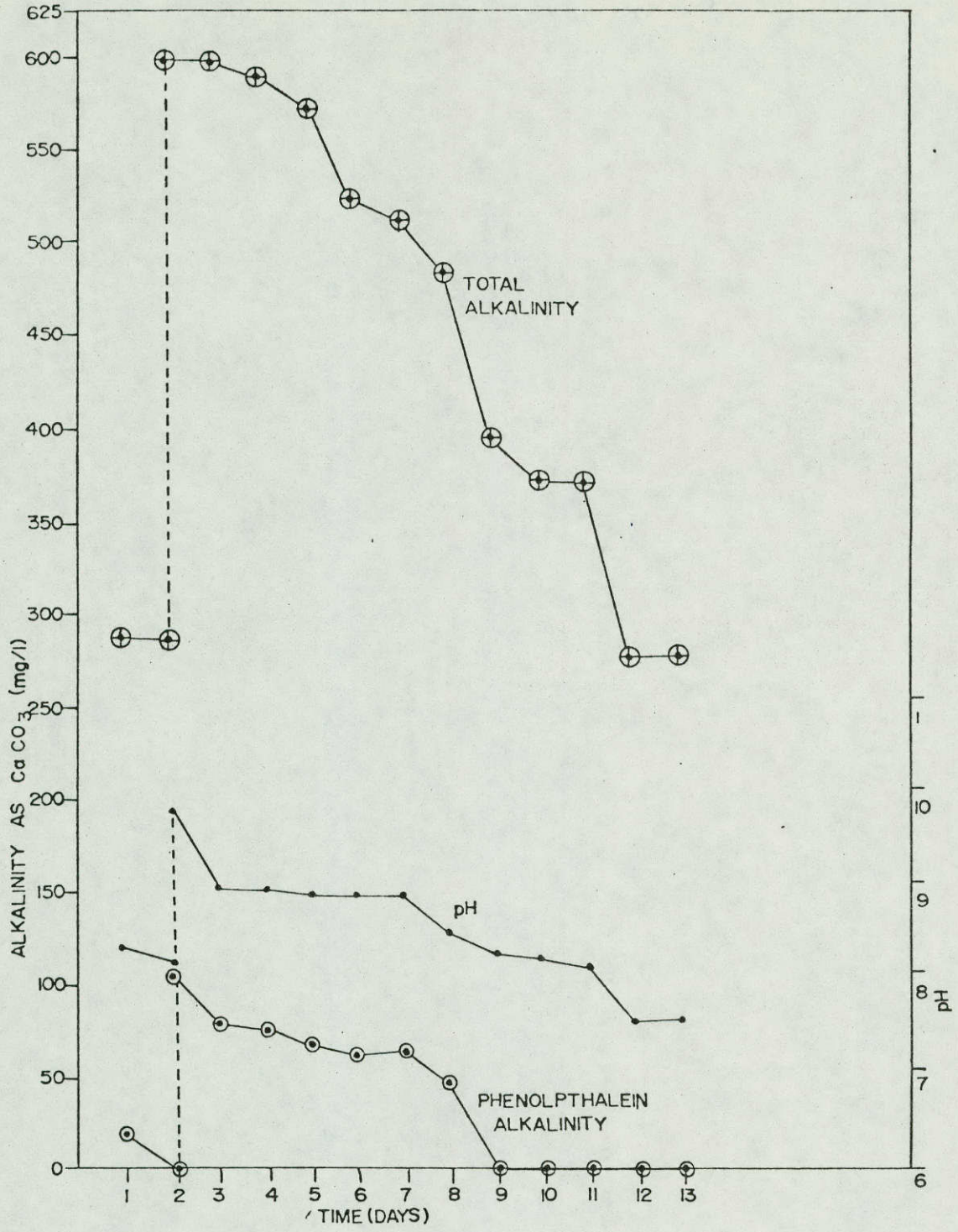

Figure 14. Alkalinity and pH vs. time during Phase II nitritification study.

It would have been interesting to continue the sequence of ammonia-N addition and subsequent monitoring of its fate. However, due to time and equipment limitations, the continuous nitritification studies were initiated.

Preliminary Nitritification Study--Phase III

Phases I and II of this research documented the accumulation of nitrite by N. europea. However, maintenance of a pure culture system of Nitrosomonas europea for the tertiary treatment of secondarily treated effluents would more than likely be an uneconomical proposition because in most natural environments and at nitrification facilities, Nitrobacter sp. is almost always found in conjunction with Nitrosomonas sp. Consequently, nitrite rarely accumulates in mixed culture environments associated with biological waste treatment.

Research by Anthonisen (36) has demonstrated that free ammonia is inhibitory to Nitrobacter sp. in relatively low concentrations (0.1 - 1.0 mg/1). The research also demonstrated that Nitrosomonas sp. is not inhibited by such low concentrations of free ammonia. Anthonisen concluded that it may be this differential which causes nitrite to accumulate without subsequent oxidation to nitrate. Other researchers attributed the nitrite accumulation phenomenon to the differential effect that pH exhibits on Nitrosomonas and Nitrobacter. It has been demonstrated that pH values above 9.5 are inhibitory to Nitrobacter but not to Nitrosomonas (40). It is interesting to note that at pH 9.5 and at 30 degrees C., the fraction of the ammonium-ammonia complex which is considered to be free ammonia is 72 percent. Other

researchers (36) attribute the nitrite accumulation phenomenon in part to the inhibitory nature of undissociated nitrous acid to Nitrobacter.

It was not within the scope of this phase of the research to resolve the mechanism to nitrite accumulation. Rather, it was the purpose of this phase to determine if the concepts just described can serve as mechanisms for nitrite accumulations in the waste treatment study. That is, the study phase was designed to demonstrate whether or not by using an artificial wastewater, environmental conditions could be managed in a manner which would be conducive to the growth to autotrophic nitrite producers (e.g., Nitrosomonas europea) and simultaneously be inhibitory to the growth of nitrate producers (e.g., Nitrobacter sp.).

Secondary objectives were to demonstrate nitrite accumulation in a continuous system without giving special emphasis to pure culture techniques. These two considerations were deemed important because it was felt any economical and reliable nitritification system must be able to function in a continuous fashion in the non-aseptic environment characteristic of sewage treatment plants.

In order to address the objectives outlined above, the experiment was initiated as follows.

The Bio-Matic bench-scale, activated-sludge assembly was chosen for use in this continuous nitritification study phase. Twenty-six liters of ammonia-containing artificial wastewater were prepared and sterilized. This experimental medium was prepared according to the recipe listed in Table 8. In view of the fact that the feed solution contained a relatively low concentration of ammonia-N (55 mg/1) plus

elements essential for the growth of nitrifying organisms, the feed was considered to be representative of certain secondary treatment plant effluents.

The volume of liquid to be maintained in the aeration vessel was chosen to be 8 liters. Therefore, for startup purposes, 8 liters of medium was placed in the aeration basin on July 22, 1976.

TABLE 8

RECIPE FOR PREPARATION OF AMMONIA-N FEED SOLUTION USED IN THE PHASE III NITRITIFICATION STUDY

Substance	Quantity
Deionized Water	18 liters
Ammonium Sulfate	9.32 grams
K_2HPO_4	9.0 grams
$MgSO_4$ - Anhydrous	0.90 grams
EDTA - Ferric Sodium Salt	1.8 mg Fe
$CaCl_2$ - Anhydrous	0.072 grams

Chemical and physical analysis of the feed solution are presented in Table 9.

Next, 10 ml of the "mother culture" containing a pure population of viable Nitrosomonas europea cells were placed in the aeration vessel. Also, the medium was inoculated with the centrifugally concentrated cells from 50 ml of a previously developed seed culture. The seed culture was developed in a manner conducive to the growth of a viable population of Nitrobacter according to the technique

TABLE 9

ANALYSES OF THE FEED SOLUTION UTILIZED FOR THE PHASE III CONTINUOUS NITRITIFICATION STUDY

Parameter	Value
Nitrite-N	Not Detected
Nitrate-N	Not Detected
Ammonia-N	55 mg/l
Organic-N	Not Detected
pH	8.11
Phenolpthalein Alkalinity	0
Methyl Orange Alkalinity	146
Dissolved Solids	775
Suspended Solids	Not Detected
Turbidity	0.0

described in Appendix B. A complete-mix experimental system was the design mode of operation and was maintained throughout the study. Agitation was caused by vigorous aeration resulting from an air supply entering near the bottom of the aeration basin and the air supply entering through the feed inlet tube.

Eighteen liters of the artificial wastewater feed were placed in the 18.9 liter stainless steel reservoir from where it was delivered to the aeration basin at the rate of 1 liter/day. This feed was sterilized and placed in the sanitized reservoir tank in order to prevent loss of NH_3-N through metabolic activities before its introduction to the reaction (aeration) vessel. Periodic chemical

analyses of the feed solution were performed in order to assure that the ammonia-N concentration remained constant. New feed medium was prepared as necessary prior to depletion of the 18-liter supply in the feed reservoir.

Figure 15 is a schematic representation of the experimental arrangement.

As the one liter/day influent was added to the 8-liter aeration basin, one liter/day of effluent overflowed into the 2-liter settling basin. This effluent was filtered through Gelman membrane filters (0.45 micron pore size) and the resultant suspended solids paste was subsequently removed (scraped) from the filter paper and placed back in the aeration basin. This procedure was performed once daily.

Data were accumulated from analyses of samples taken daily from the two-liter sedimentation basin. The period of the study lasted from July 22, 1976 until August 22, 1976. Data accumulated during this phase of the research are present in Table 10.

As can be seen in Figure 16, for the first 23 days of the study, pH values were maintained in the range 7.35 to 9.0 by the addition of 50 percent K_2CO_3 as needed. According to the literature, this range was well within the limits cited as optimal for nitrifying bacteria.

As can be seen from Figure 17, trace amounts of nitrite first appeared nine days after the initiation of the study, and by Day 15, nitrite-nitrogen had increased to 37.5 mg/1. The nitrite-N concentration remained fairly constant (34 to 37 mg/1) for the next three days. During this same 18-day period, ammonia-N content decreased

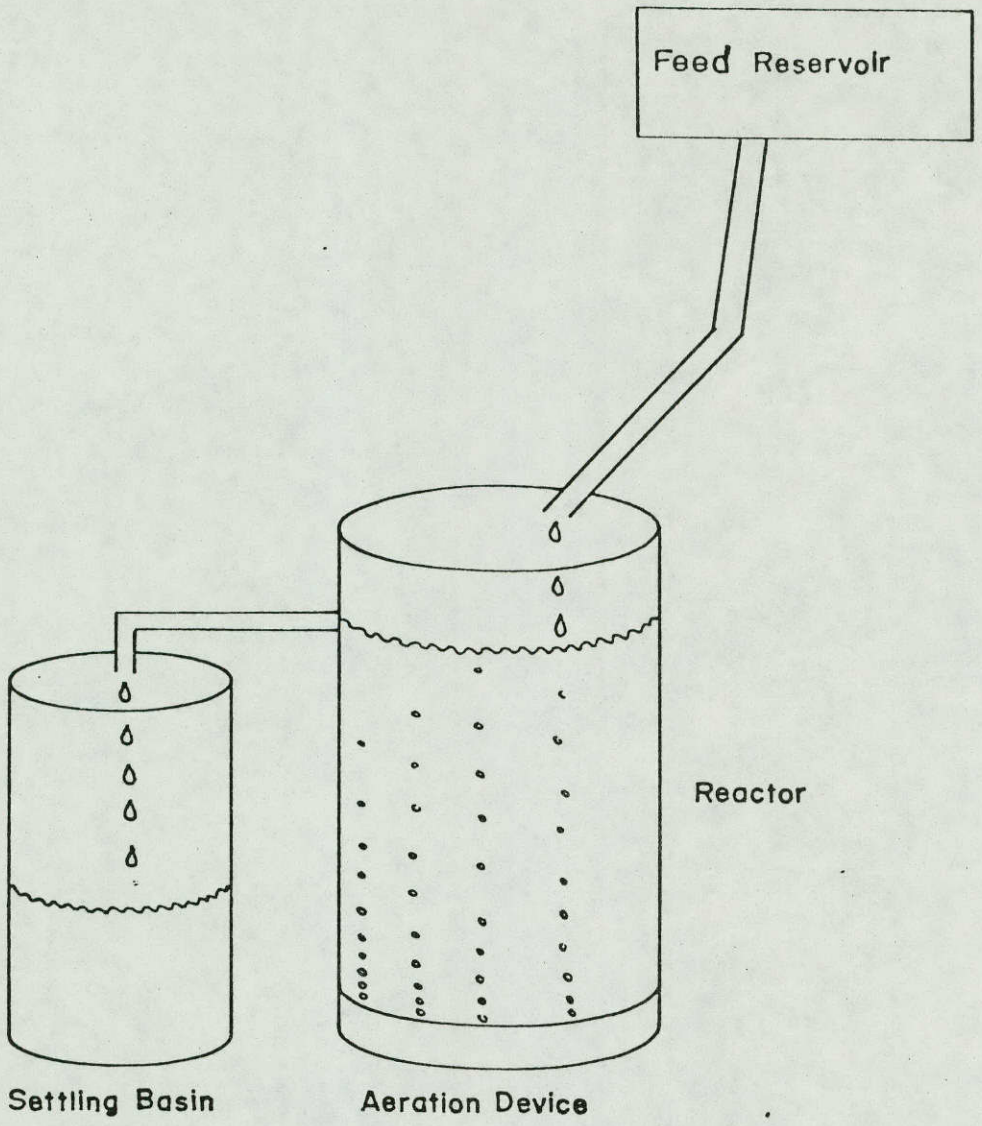

Figure 15. Experimental set-up for continuous system nitritification experiments.

TABLE 10.

DATA ACCUMULATED DURING THE PHASE III PRELIMINARY NITRITIFICATION STUDY

Date	pH	Phenol. Alkalinity mg/l as $CaCO_3$	Methyl Orange Alkalinity mg/l as $CaCO_3$	Nitrite Nitrogen mg/l	Ammonia Nitrogen mg/l	Total Organic Nitrogen mg/l	Nitrate Nitrogen mg/l	Dissolved Solids mg/l	Suspended Solids mg/l	Dissolved oxygen mg/l
07-22-76	8.11	0	146	N.D.	55	N.D.*	N.D.	775	I.C.	2.0
07-23-76	8.1	0	146	N.D.	51	N.D.	N.D.	775	I.C.	5.7
07-24-76	8.0	0	148	N.D.	52	N.D.	N.D.	775	I.C.	6.1
07-25-76	8.1	0	146	N.D.	43	N.D.	N.D.	775	I.C.	6.2
07-26-76	8.11	0	142	N.D.	44	N.D.	N.D.	750	I.C.	6.2
07-27-76	8.15	0	146	N.D.	44	N.D.	N.D.	775	I.C.	6.0
07-28-76	8.10	0	148	N.D.	40	N.D.	N.D.	775	I.C.	5.6
07-29-76	8.16	0	144	N.D.	39	N.D.	N.D.	750	I.C.	5.6
07-30-76	8.00	0	146	0.25	40	N.D.	N.D.	--	I.C.	6.0
07-31-76	7.87	0	140	0.11	39	N.D.	N.D.	--	I.C.	5.9
08-01-76	7.91	0	144	0.90	43	N.D.	N.D.	--	I.C.	6.1
08-02-76	7.85	0	140	6.55	40	N.D.	N.D.	--	0.6	6.1
08-03-76	7.35 ↓ 8.60	0 ↓ 38	12 ↓ 392	9.50	19	--	N.D.	--	I.C.	5.9
08-04-76	8.21 ↓ 9.0	0 ↓ 144	180 ↓ 600	17	1.2	0.4	N.D.	--	2.0	5.9
08-05-76	8.29	0	402	37.5	0.67	0.2	N.D.	--	7.1	5.9
08-06-76	8.32	0	398	34	0.16	--	N.D.	--	6.6	6.0
08-07-76	8.11	0	412	37	0.62	--	N.D.	--	I.C.	6.1
08-08-76	8.24 ↓ 8.81	0	400	37	1.2	0.6	N.D.	--	5.7	6.1
08-09-76	8.6	16	420	22.5	1.1	0.3	0.06	--	7.2	6.3
08-10-76	8.41	14	324	12.5	2.4	--	0.58	--	9.0	5.5
08-11-76	8.26	0	322	6.0	2.5	0.5	3.2	--	11.9	5.9

TABLE 10.

(CONTINUED)

Date	pH	Phenolphthalein Alkalinity mg/l as $CaCO_3$	Methyl Orange Alkalinity mg/l as $CACO_3$	Nitrite Nitrogen mg/l	Ammonia Nitrogen mg/l	Total Organic Nitrogen mg/l	Nitrate Nitrogen mg/l	Dissolved Solids mg/l	Suspended Solids mg/l	Dissolved Oxygen mg/l
08-12-76	8.27	0	144	2.0	4.6	--	20.6	--	12.9	6.1
08-13-76	8.15 + 9.93	--	--	0.15	3.8	--	29.0	--	13.6	6.1
08-14-76	9.71	--	--	0.45	4.6	0.4	27.3	--	15.7	6.2
08-15-76	9.73	--	--	3.5	4.2	--	23.1	--	12.8	6.2
08-16-76	9.52 + 9.80	--	--	3.8	4.0	0.7	19.6	--	11.4	5.9
08-17-76	9.58	--	--	2.9	3.7	--	15.7	--	15.0	5.8
08-18-76	9.58	--	--	8.0	6.4	0.5	11.0	--	13.9	6.1
08-19-76	9.52	--	--	8.0	5.2	--	6.6	--	11.1	6.0
08-20-76	9.55	--	--	7.8	5.2	1.2	2.1	--	12.5	6.0
08-21-76	9.52 + 9.77	80	414	8.3	9.1	--	N.D.	--	10.9	5.7
08-22-76	9.55	--	--	7.7	8.3	0.9	N.D.	--	14.3	6.3

*Not Detected

**Incompatible Results

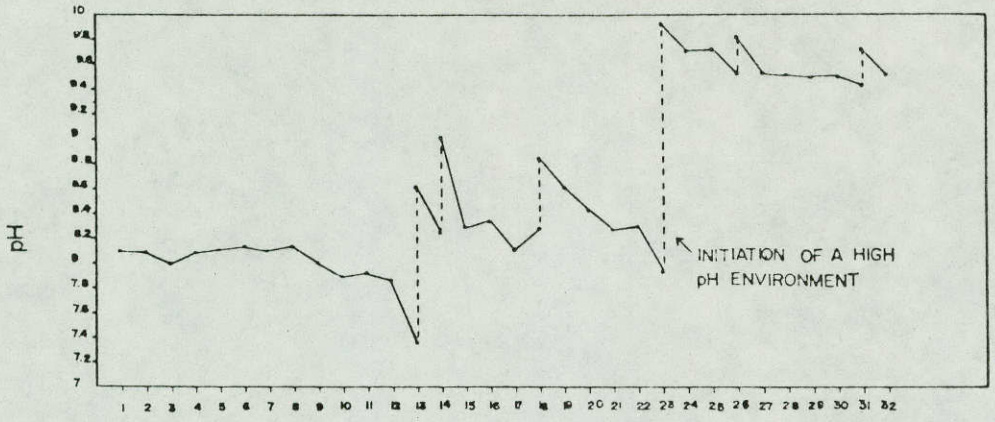

Figure 16. pH response during Phase III nitritification study.

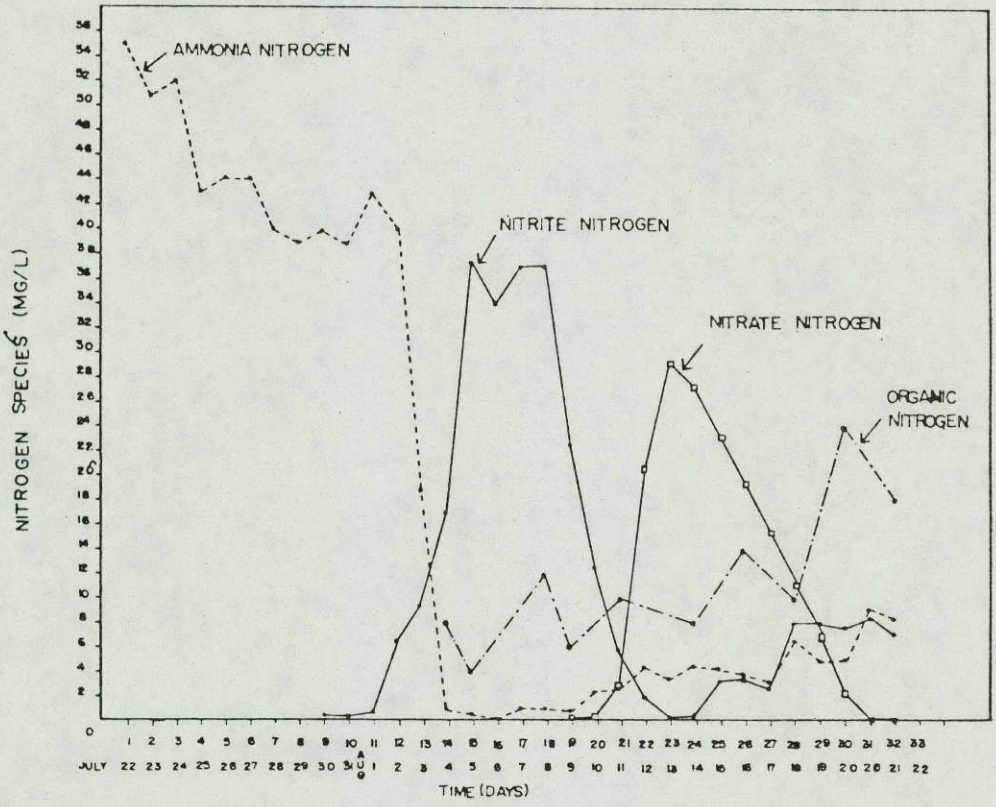

Figure 17. Nitrogen transformation during Phase III nitrification study.

from 55 mg/l to 1.2 mg/l. The presence of nitrite-nitrogen coupled with a rapid decrease in ammonia-N on Days 12 through 14 were indicative of the presence of a viable population of Nitrosomonas.

On the 19th day of the study, a trace of nitrate-N was noted. The rapid increase in nitrate-N coupled with the rapid decrease of nitrite-N during the period of the 19th through the 23rd day of the study was indicative of the presence of a recently developed viable population of Nitrobacter. On the 23rd day of the study, the nitrate concentration had reached a level of 29.0 mg/l. In an effort to demonstrate that environmental conditions could be adjusted so as to be simultaneously conducive for nitrite production but inhibitory for nitrate production, the pH was dramatically increased to 9.93 by the addition of 50 percent K_2CO_3. The pH was maintained in the range of 9.52 to 9.93 for the remainder of the study phase (10 days).

Nitrogen transformations were monitored during this period of study in order to ascertain if this control mechanism was successful. Scrutiny of the data accumulated during this period as illustrated in Figure 17 reveals that the nitrate-N decreased to a concentration not measurable by the nitrate-N analysis procedure.

During these last 10 days, nitrite-N and the associated decrease in nitrite-N was attributed either directly or indirectly to the pH increase above the 9.5 level cited in the literature as inhibitory to Nitrobacter.

The fact that nitrite-N concentration remained fairly constant during the last 5 days of the study and never reached the level

attained during the period August 4, 1976 to August 9, 1976, was explained as follows. The increase in pH may have significantly shifted the ammonium-ammonia equilibrium to the right and a greater percentage of the ammonia in the feed was volatilized. Consequently, less ammonia was available for oxidation to nitrite by Nitrosomonas sp. Scrutiny of the suspended solids (Figure 18) data and the organic nitrogen data during this last 10 days of the study revealed relatively pronounced fluctuations. One would predict an increase in organic-N and suspended solids during this period. However, this was not the case. It is thought that daily suspended solids analysis of the effluent caused a severe drain of the Nitrosomonas sp. from the system. Two hundred ml of the one liter of effluent was utilized daily for suspended solids analysis. This represented a daily cell wasting rate of 20 percent. It was reasonable to assume that the cells were being wasted more rapidly than they were being produced. Figure 18 illustrates the content of dissolved oxygen maintained in the reactor and the suspended solids concentration.

Table 11 compares the initial feed to the system with the effluent following the 32 days of operation.

Inspection of Table 11 reveals that ammonia-N was reduced by the system to 8.3 mg/1. This represents an 85 percent ammonia-N reduction. Approximately 16 percent of the 55 mg/1 of ammonia-N in the feed was converted to organic-N; 14 percent of the feed ammonia-N was oxidized to nitrite. Calculation by difference yields a value of 69.4 percent ammonia assumed volatilization. Using the data obtained in this study phase, it may be concluded that:

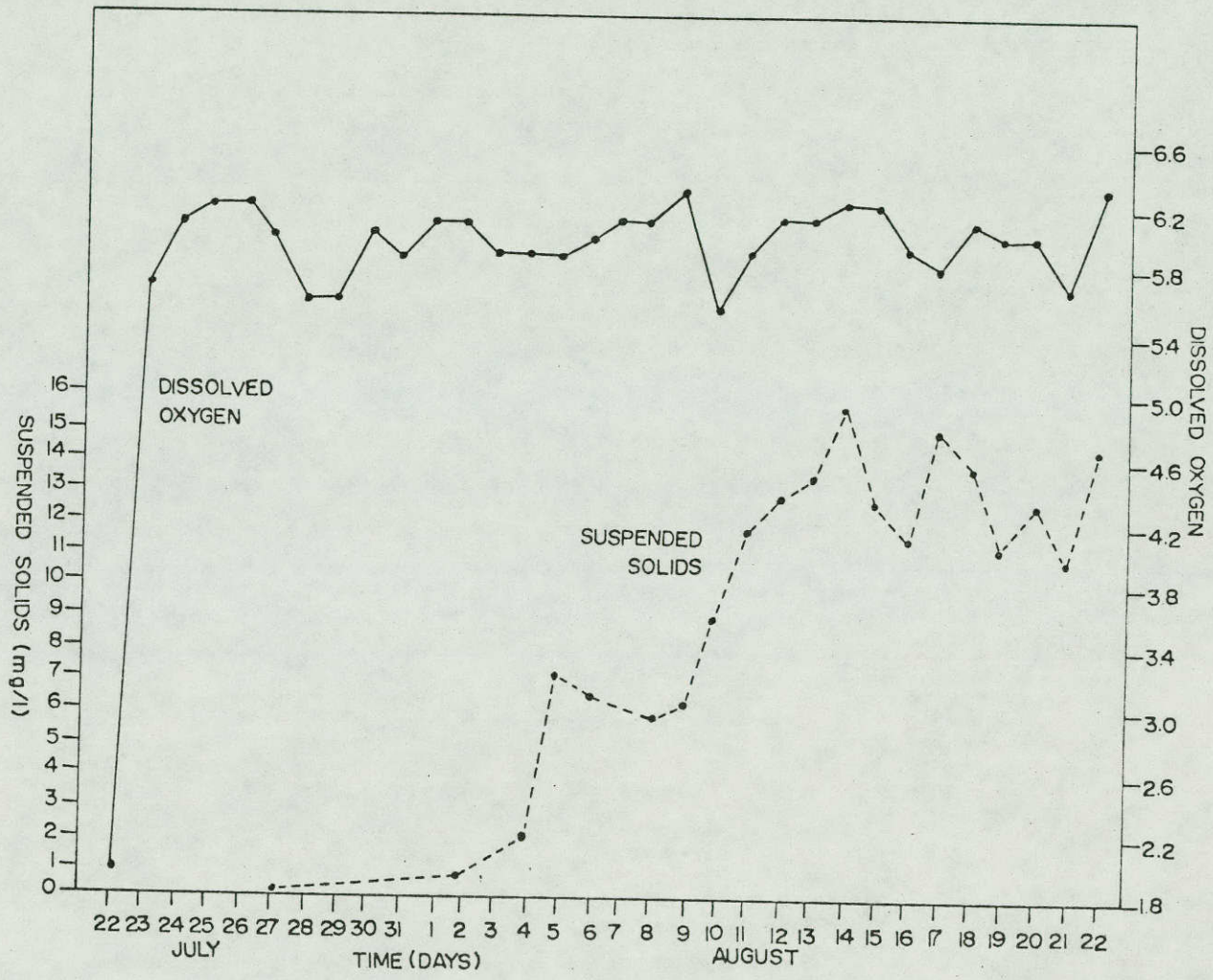

Figure 18. Suspended solids and dissolved oxygen vs. time in Phase III nitritification study.

TABLE 11

QUALITATIVE AND QUANTITATIVE COMPARISON OF AMMONIA CONTAINING FEED AND EFFLUENT FOLLOWING 32 DAYS OF OPERATION OF THE PHASE III PRELIMINARY NITRITIFICATION SYSTEM

Parameters	Ammonia Containing Feed	System Effluent
pH	8.11	9.58
Phenolpthalein Alkalinity	0	--
Methyl Orange Alkalinity	146 mg/l	--
Nitrate-N	Not Detected	Not Detected
Nitrite-N	Not Detected	7.7 mg/l
Organic-N	Not Detected	0.9 mg/l
Ammonia-N	55 mg/l	8.3 mg/l
Dissolved Solids	775 mg/l	--
Dissolved Oxygen	2.0	6.3 mg/l
Suspended Solids	Not Detected	143 mg/l

1. A significant portion of the ammonia fed into the continuous nitritification aeration vessel was lost from the system via volatilization;

2. A pH increase above 9.5 in this particular study regime was either directly or indirectly responsible for inhibition of nitrate-producing bacteria. The pH increase did not noticeably affect Nitrosomonas sp. activity. The exact cause of the inhibition could not be determined from the data obtained;

3. A continuous nitritification system appeared to exhibit nitrite accumulation potential above pH 9.5 in a non-aseptically operated, mixed culture environment;

4. For the artificial wastewater studied, the biological treatment system on the 32nd day of operation, achieved about an 85 percent reduction of ammonia-N, about 0.9 mg/l organic-N and approximately 14.3 mg/l suspended solids.

Preliminary Nitritification Study--Phase IV

In the Phase III study, a 55 mg/l ammonia-N feed resulted in an effluent containing a relatively low concentration of nitrite-N. It was thought that the addition of a feed containing a higher concentration of ammonia-N (195 mg/l) to the continuous nitritification study aeration basin would result in a system effluent containing a commensurately higher concentration of nitrite-N. The experimental plan was based upon the assumption that an increased concentration of ammonia-N entering the aeration basin via the feed tube (under environmental conditions similar to the conditions utilized in the Phase III study) should result in more available ammonia-N which could be oxidized to nitrite.

In an attempt to prove this hypothesis, experimental methods very similar to those used for the Phase III study were employed. The contents of the aeration basin from the Phase III study were utilized in order to initiate the Phase IV study for two reasons.

1. System continuity under a wide range of ammonia-N concentrations in the feed would be demonstrated if the system did not "die."

2. A large population of nitrite-producing bacteria were readily available from the Phase III study, and it was reasoned that time could be spent more widely accumulating data from an established system rather than initiating another phase with system startup.

The feed containing the ammonia-N was prepared in 18-liter volumes and placed in the 18.9-liter feed reservoir form where it was delivered at the rate of 1 liter/day to the aeration basin containing the 8 liters of mixed liquor present at the termination of the Phase III study. The feed contained 195 mg/1 ammonia-N.

The constituents and quantities of the chemicals utilized to prepare the feed solution were identical to those listed in Table 8, with one exception. A greater quantity (33.09 grams) of ammonium sulfate was added.

After medium sterilization and addition to the feed reservoir, the feed was analyzed. The results of the chemical and physical analyses of the feed are presented in Table 12.

TABLE 12

ANALYSES OF THE FEED SOLUTION UTILIZED FOR THE PHASE IV CONTINUOUS NITRITIFICATION STUDY

Parameter	Value
Nitrite-N	Not Detected
Nitrate-N	Not Detected
Ammonia-N	195 mg/1
Organic-N	Not Detected
pH	7.99
Phenolpthalein Alkalinity*	0 mg/1
Methyl Orange Alkalinity*	162 mg/1
Dissolved Solids	1875 mg/1
Suspended Solids	Not Detected
Optical Density (nm)	0

* as $CaCO_3$

An effort was made to maintain a pH level in the aeration basin equal to or greater than 9.5 by the addition of 50 percent K_2CO_3 or 50 percent Na_2CO_3 as needed.

Agitation and cellular recycle techniques were identical to the methods used in the Phase III study. Experimental data were accumulated from analyses of samples taken daily from the two-liter sedimentation basin.

In particular, nitrogen transformations were monitored during the period of the study in order to determine the degree of nitrite-N accumulation potential under the environmental regime characteristics (e.g., ammonia-N concentration, pH range, temperature, degree of aeration, etc.)

The period of the study lasted from August 23, 1976 until September 24, 1976. As in the Phase III study, no special effort was made to maintain aseptic conditions during the research period. In fact, the filtered cells from 50 ml of the "seed culture" which contained nitrate-producing bacteria (Nitrobacter), was added daily to the aeration basin.

Data accumulated as a result of the Phase IV preliminary nitrification study are presented in tabular form in Table 13.

In view of the fact that the feed solution used in the study contained a relatively high concentration of ammonia-N, the feed was not considered to be representative of secondary sewage treatment plant effluent. However, the concentration of ammonia-N contained in the feed (195 mg/l) may not be atypical of the ammonia-N content characteristic of certain industrial waste effluents.

TABLE 13.

DATA ACCUMULATED DURING PRELIMINARY NITRITIFICATION STUDY--PHASE IV

Date	Comments	pH	Phenol. Alkalinity mg/l as $CaCO_3$	Methyl Orange Alkalinity mg/l as $CaCO_3$	Nitrite Nitrogen mg/l	Ammonia Nitrogen mg/l	Total Organic Nitrogen mg/l	Nitrate Nitrogen mg/l	Optical Density	Dissolved Solids mg/l	Suspended Solids mg/l	Dissolved Oxygen mg/l
08-23-76	Vigorous Aeration NH_3-N, Feed 195 mg/l	9.56	72	590	6.0	12.1	1.6	N.D.	0.89	2150	16.1	6.0
08-24-76	"	8.9	30	530	5.5	13.0	--	--	0.84	1975	15.8	6.0
08-25-76	"	9.06	42	630	5.5	2.4	--	N.D.	0.84	2150	--	6.3
08-26-76	"	8.39	12	456	6.8	1.12	--	N.D.	0.87	2000	--	5.8
08-27-76	"	9.11	48	596	5.15	1.8	--	N.D.	0.75	1850	--	6.3
08-28-76	"	9.6	--	--	4.46	1.9	--	--	0.89	1700	35	6.2
08-29-76	"	8.8	24	428	8.35	0.93	--	N.D.	0.101	2100	--	5.9
08-30-76	"	8.91	36	494	8.92	0.86	--	N.D.	0.115	2450	--	5.4
08-31-76	"	9.7	92	702	7.23	1.0	--	N.D.	0.137	2375	--	6.0
09-01-76	"	8.86	48	580	6.86	0.59	2.3	--	0.125	--	30	6.1
09-02-76	"	--	--	--	8.23	0.82	--	N.D.	0.143	2700	--	6.1
09-03-76	"	9.8	--	--	17.75	1.01	--	N.D.	0.140	2975	--	5.9
09-04-76	"	9.56	--	--	17.43	2.0	--	N.D.	0.168	3300	--	5.9
09-05-76	"	--	--	--	--	--	--	--	--	--	--	--
09-06-76	"	8.89	88	792	8.87	0.93	--	N.D.	0.181	3700	42	6.3
09-07-76	"	9.22	124	990	6.60	0.85	--	N.D.	0.175	--	--	6.2
09-08-76	"	8.78	80	614	15.81	0.8	4.7	--	0.189	4150	38	6.2
09-09-76	"	9.21	92	780	--	1.22	--	N.D.	0.180	4375	--	6.2

The experimental design for this phase of the research dictated that pH levels were to be sustained at a level equal to or greater than 9.5 in the aeration basin. This pH range was desired because the literature and the results of the Phase III study indicated that inhibition of nitrate formers and the subsequent autotrophic accumulation of nitrite were either directly or indirectly contingent upon system pH perpetuation at 9.5 or greater. However, due to unresolved technical difficulties, the pH range was not perpetuated continuously in the target range. Figure 19 illustrates the pH range sustained during the study period (8.38 to 9.98). The pH level during the 32-day study period was above 9.5 for 14 days. During the remaining 18 days, the pH was relatively close to 9.5. It was expected that nitrate-N would become evident under this pH regime. However, nitrate was not detected during the investigation. One possible explanation for the absence of nitrate-N was that the sporadic pH level was adequate to either directly or indirectly inhibit the metabolic activity of nitrate-forming microorganisms present in the system.

As previously mentioned, a feed containing 195 mg/l ammonia-N was added at the rate of 1 liter/day for 32 days to the aeration basin used in the Phase IV nitritification study. Figure 20 represents the nitrogen transformations which occurred during the month long experiment.

Table 14 compares the feed to the system with the effluent following the 32 days of operation. The assumption was made that

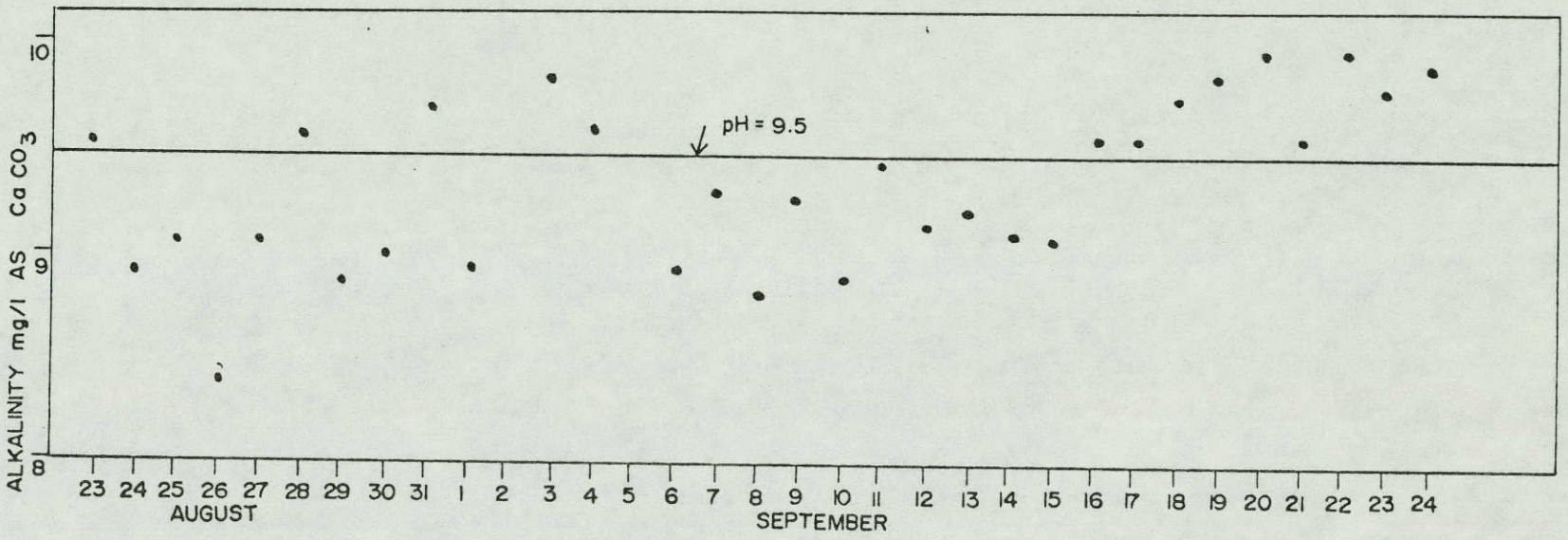

Figure 19. pH regime in the Phase IV nitritification study.

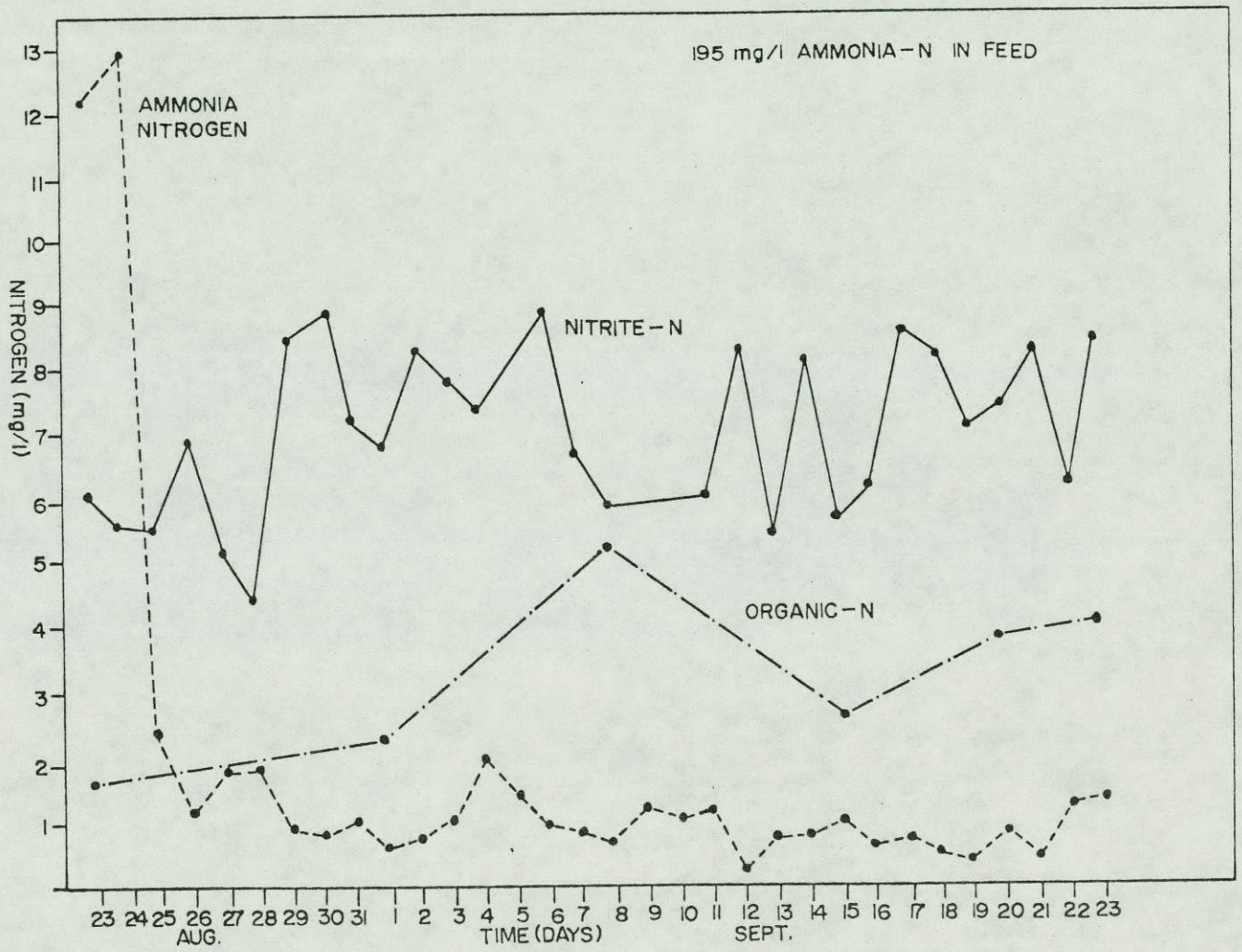

Figure 20. Effluent nitrogen from the Phase IV nitritification study.

TABLE 14

QUANTITATIVE AND QUALITATIVE COMPARISON OF FEED VS. EFFLUENT
FROM THE PHASE IV PRELIMINARY NITRITIFICATION STUDY

Parameter	Feed	System Effluent		
		Average	Minimum	Maximum
pH	7.99	--	8.39	9.98
Nitrite-N	ND*	7.0 mg/1	5.15 mg/1	8.92 mg/1
Ammonia-N	195 mg/1	1.79 mg/1	0.25 mg/1	13.0 mg/1
Nitrate-N	ND	ND	ND	ND
Dissolved Oxygen	--	6.1 mg/1	5.8 mg/1	6.4 mg/1
Dissolved Solids	1875 mg/1	3247 mg/1	1700	4890 mg/1
Phenopthalein Alkalinity	0	159	12	990
Methyl Orange Alkalinity	162	--	348	3110

* ND--Not Detected.

the effluent quality was identical in quality to the contents of the aeration basin. This assumption was considered valid because the system was a complete mix regime characterized by the fact that particulates in the tank and substances leaving the tank exist in proportion to their statistical population. Periodic chemical and physical comparisons of the aeration basin contents and the effluent from the aeration basin substantiated this assumption.

Figure 20 presents in line graph form the nitrogen transformations which occurred during the same 32-day period. The nitrite-N concentration during the 32-day experiment ranged from 5.15 to 8.92 mg/l, averaging 7.0 mg/l. It is evident after comparison of this value to the values obtained on the last day of the Phase III study (7.7 mg/l) that nitrite-N concentration did not increase as anticipated.

Scrutiny of the ammonia-N and organic-N analyses provides a clue for the reason that nitrite did not accumulate to the level expected. During the 32-day study period, effluent residual ammonia-N concentration ranged from 0.25 mg/l to 13 mg/l (averaging 1.79 mg/l). Organic-N concentration ranged from 2.6 to 4.7 mg/l during the same experimental period. The average organic-N concentration was only 2.7 mg/l. Nitrate-N remained undetected during the entire study. Analysis of the nitrogen balance during the experiment indicates that of the 195 mg/l/day of ammonia-N added each day for 32 days, a major portion of it remained "unaccounted for." The nitrogen was assumed to have volatilized as a result of the relatively high pH level maintained, coupled with the vigorous aeration rate. It is

noteworthy that organoleptic analysis of the aeration basin yielded a distinct ammonia-like odor for the entire 32-day period of the experiment.

Review of the literature seems to substantiate the large ammonia losses through volatilization assumed in this study. Studies of Culp (59) at Lake Tahoe, California, on ammonia removal as function of pH have indicated that up to 92 percent of the ammonia-N can be removed at pH 9.7.

Suspended solids analyses were only performed nine times during the study period. In order to maintain a relatively large cellular concentration, S.S. analyses were not performed more frequently. Figure 21 traces the general trend of cellular development as the S.S. increased from 16.1 mg/1 to 42 mg/1 during the study period. Dissolved oxygen concentration in the effluent ranged from 5.8 to 6.4 mg/1 (average = 6.1 mg/1).

It may be concluded from this study phase that:

1. Inability of the system to demonstrate a high nitrite-N accumulation potential seemed to the result of large losses of ammonia through volatilization.

2. Sporadic pH values approximating 9.5 seemed to be adequate in this experiment to completely inhibit nitrate-forming bacteria, while allowing nitrite producers to metabolize.

3. System continuity through a wide range of ammonia-N feed values was demonstrated by the successful transition noted from the Phase III study to the Phase IV study. The system which had been

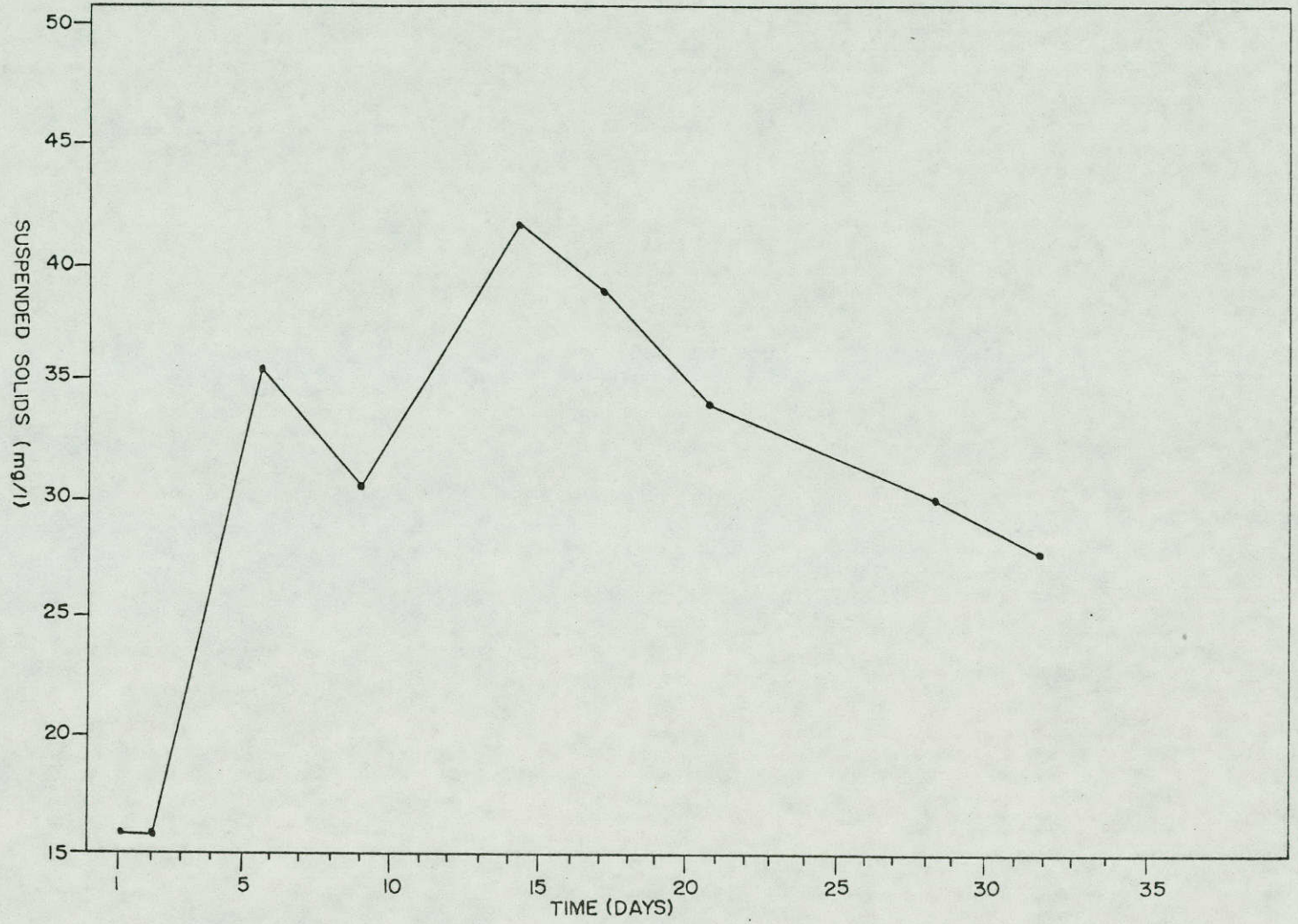

Figure 21. Suspended solids vs. time in Phase IV nitritification study.

successfully accumulating nitrite with a 55 mg/l ammonia-N feed was successfully acclimated to nitrite accumulation using a feed containing 195 mg/l ammonia-N.

4. For the artificial wastewater studies having a design total ammonia-N concentration of 195 mg/l, the biological treatment system achieved about a 97 percent reduction of ammonia-N and produced an effluent quality characterized by having an average 1.8 mg/l ammonia-N, 7.0 mg/l nitrite-N, about 4.1 mg/l organic-N, and about 29 mg/l suspended solids.

Primary Nitritification Study

Because of the environmental conditions (e.g., high pH, excessive aeration rate, temperature) imposed in the Phase IV research, a significant percentage of the influent ammonia-N was volatilized from the nitritification system. Consequently, only a relatively small fraction of the influent ammonia-N remained available to be oxidized to nitrite-N.

Demonstration that relatively large concentrations of nitrite-N could be induced to accumulate was requisite to perfecting any system capable of treating ammonia-N concentrations typical of secondary sewage treatment plant effluents. Therefore, the following alternatives, which would possibly lead to the accumulation of relatively large nitrite-N accumulations, were evaluated:

1. Decrease the temperature. Ammonia-N solubility in water is inversely proportional to temperature. Unfortunately, autotrophic microorganisms are notorious for their decreased metabolic activities as temperature falls. Therefore, this alternative was rejected.

2. Decrease in pH. Ammonia ions in water exist in equilibrium with ammonium as shown below.

$$NH_4^+ + OH^- \rightleftarrows NH_3 + H_2O \qquad \text{(Equation 8)}$$

As the pH of the water is decreased below 7, the equilibrium is shifted to the left. Thus, decreasing the pH was rejected as a viable operational alternative because, as indicated in the literature and by the results of the Phase III and IV studies, nitrite-N accumulation without interference from nitrate formers is contingent upon the pH being held near or above 9.5.

3. Increase the ammonia-N concentration in the influent. At a given pH, temperature, and aeration rate, a particular percentage of ammonia-N will be volatilized. Consequently, maintaining the identical pH, temperature, and aeration rate while simultaneously adding more ammonia-N should result in a greater concentration of ammonia-N available for the metabolic activities of nitrite formers.

4. Decrease the aeration rate. Ammonia volatilization at a given temperature and pH is proportional to the air-to-liquid ratio. Consequently, a decrease in the aeration rate should result in a proportionately greater concentration of residual ammonia available for subsequent oxidation to nitrite-N.

Since pH and temperature decreases would tend to decrease nitrite accumulation without nitrate production, decreasing the aeration rate and increasing the ammonia-N concentration in the effluent to 550 mg/l were implemented during this part of the research in order to accumulate greater concentrations of nitrite.

More specifically, a continuous electrical propeller-type stirring device was substituted for the air supply used for mixing in Phases III and IV. This arrangement allowed more precise aeration control without unnecessary agitation. Otherwise, the experimental procedure was very similar to that utilized in the Phase IV preliminary nitritification study.

The contents of the Phase IV study's aeration vessel were chosen to initiate this experiment for two reasons:

1. If the experiment were successful, system continuity under a wide range of ammonia-N concentrations in the feed would again be demonstrated.

2. A large population of nitrite-producing bacteria were readily available from the Phase IV study.

In order to initiate the experiment, 18 liters of artificial wastewater feed containing 550 mg/l ammonia-N was prepared and placed in the 18.9 feed reservoir from where it was continuously delivered to the aeration basin at a rate of 1 liter per day. The constituents and quantities of the chemicals utilized to prepare the feed solution were identical to those listed in Table 8, with one exception. A greater quantity (93.3 grams) of ammonium sulfate was added.

The sterile feed solution was analyzed immediately after it was added to the 18.9 liter reservoir tank. The results of the chemical and physical analyses of the feed are presented in Table 15.

As in the Phase IV study, an effort was made to maintain the pH in the aeration vessel equal to or greater than 9.5 by the addition of 50 percent Na_2CO_3 or 50 percent NaOH, as required.

TABLE 15

RESULTS OF THE CHEMICAL AND PHYSICAL ANALYSES OF THE FEED SOLUTION UTILIZED FOR THE PRIMARY NITRITIFICATION STUDY

Parameter	Value
Nitrite-N	Not Detected
Nitrate-N	Not Detected
Ammonia-N	550 mg/1
Organic-N	Not Detected
pH	7.48
Phenolpthalein Alkalinity	0 mg/1
Methyl Orange Alkalinity	146 mg/1
Dissolved Solids	3500 mg/1
Suspended Solids	Not Detected
Optical Density (nm)	0

Cellular recycle techniques were identical to those used in the Phase III and IV studies.

The period of the study lasted from September 26, 1976 until October 27, 1976. As in the Phase III and IV studies, no special effort was made to maintain aseptic conditions during the research period, and the filtered cells from 50 ml of seed culture containing Nitrobacter were added to the system daily.

Nitrogen, suspended solids, dissolved solids, alkalinity, dissolved oxygen, and optical density were monitored daily during the experiment in order to determine the degree of nitrite accumulation potential under this environmental regime. Data accumulated are presented in Table 16.

TABLE 16. DATA ACCUMULATED DURING THE PRIMARY NITRITIFICATION STUDY

Date	pH	Phenolpthalein Alkalinity	Methyl-Orange Alkalinity	Nitrite Nitrogen	Ammonia Nitrogen	Temperature	Total Organic Nitrogen	Nitrate Nitrogen	Optical Density	Dissolved Solids	Suspended Solids	Dissolved Oxygen
						°C						
09-26-76	9.92	848	3280	8.41	13.5	26.5	3.8	N.D.**	0.200	--	567	6.0
09-27-76	9.88	904	3530	18.4	23.5	24	--	N.D.	0.191	--	--	5.3
09-28-76	9.72	869	3390	32.5	27.2	25.5	5.2	N.D.	0.210	10,438	65.0	4.5
09-29-76	9.53	654	3240	24.5	35.4	25	--	N.D.	0.193	--	--	3.9
09-30-76	9.32	760	3840	39	41.7	24	--	N.D.	0.226	--	--	3.4
10-01-76	9.2	810	4200	132.5	49.3	24.5	--	N.D.	0.258	--	--	2.7
10-02-76	9.15	846	4520	192.5	56.8	24.5	--	N.D.	0.271	--	--	3.1
10-03-76	9.30	1440	6000	160	65.8	25	7.3	N.D.	0.263	--	101.3	3.0
10-04-76	9.53	2000	7260	275	73	24.0	--	N.D.	0.278	--	--	2.5
10-05-76	9.63	2812	7830	245	58	24.0	--	N.D.	0.284	21,000	--	2.5
10-06-76	9.74	3520	10,420	230	87	24.5	--	N.D.	0.296	24,000	--	3.0
10-07-76	9.79	4340	13,600	230	90	24.0	--	N.D.	0.303	--	--	2.7
10-08-76	9.73	4400	12,300	172.5	55	24.0	16.1	N.D.	0.306	--	154.5	2.8
10-09-76	--	--	--	--	--	--	--	--	--	--	--	--
10-10-76	--	--	--	--	--	--	--	--	--	--	--	--
10-11-76	--	--	--	--	--	--	--	--	--	--	--	--
10-12-76	9.38	1690	6680	223	75	24.5	15.7	N.D.	0.296	15,023	146	3.0
10-13-76	9.52	2400	8000	230	68	24.5	--	N.D.	0.311	--	--	2.9
10-14-76	9.48	1920	8160	225	70	24	--	N.D.	0.318	13,000	--	3.1
10-15-76	--	--	--	--	--	--	--	--	--	--	--	--
10-16-76	--	--	--	--	--	--	--	--	--	--	--	--
10-17-76	9.57	2400	8000	221	50	24.5	--	N.D.	0.300	23,000	--	2.4
10-18-76	9.76	3980	11,260	221	57	24	--	N.D.	0.306	23,000	--	2.3
10-19-76	9.95	5700	14,440	229	45	24	L.A.*	N.D.	0.316	26,000	150.5	3.0
10-20-76	9.92	5900	14,520	230	60	25	--	N.D.	0.321	--	--	2.5
10-21-76	10.09	8000	18,460	225	56.5	24	--	N.D.	0.307	23,500	--	2.5
10-22-76	10.39	8000	16,800	220	61	24	--	N.D.	0.307	--	--	3.1
10-23-76	9.09	6800	14,600	218	63	24.5	--	N.D.	0.302	--	--	3.0
10-24-76	9.61	--	--	226	59	24	13.1	N.D.	0.312	--	144.5	2.6
10-25-76	9.42	--	--	225	54	24	--	N.D.	0.324	--	--	2.1
10-26-76	9.53	--	--	230	62	24	--	N.D.	0.316	--	--	2.9
10-27-76	9.39	--	--	227	66	24	14.5	N.D.	0.305	--	146.8	3.0

*Lab Accident

**Not Detected

Note: All data reported in mg/l except temperature, pH, and optical density

Table 16 shows in part that the feed was characterized by a relatively high ammonia-N concentration (550 mg/l). Although this concentration of ammonia-N is atypical of secondary sewage treatment plant effluents, it could be representative of the inorganic nitrogen concentration contained in certain industrial waste effluents.

Any effort to evaluate the efficiency of a particular waste treatment process dictates that a comparison be made concerning characteristics between the system influent and effluent. Table 17 presents a summary of this comparison. The data used for this comparison were taken from the results accumulated after the first eight days of the study.

Since the feed rate was one liter/day and the detention time in the aeration basin was eight days, it would, therefore, require eight days for the aeration basin to no longer contain the feed from the Phase IV investigation. It was felt that this data would be particularly meaningful with respect to the typical system effluent quality.

As seen from Figure 22, the ammonia-N concentration increased dramatically from 1.3 mg/l on Day Zero* to 65.8 mg/l on Day 8. The series of small peaks superimposed on the general increase of ammonia-N concentration during the first eight days of the experiment as depicted in Figure 22 probably represented a real phenomenon since the data points were well outside the range of experimental error for

---

* Day Zero refers to the last day of the Phase IV research (Sept. 24, 1976).

TABLE 17

QUANTITATIVE AND QUALITATIVE COMPARISON OF FEED VS. EFFLUENT FROM THE PRIMARY NITRITIFICATION STUDY FOLLOWING AN EIGHT-DAY ADJUSTMENT PERIOD

Parameter	Feed Solution (Influent)	Effluent After An Eight-Day Adjustment Period		
		Average	Minimum	Maximum
pH	7.48	--	9.15	10.39
Nitrite-N (mg/1)	ND*	226.5	172.5	275
Ammonia-N (mg/1)	550	70	53	90
Organic-N (mg/1)	ND	14.9	13.1	16.1
Suspended Solids (mg/1)	ND	149	144	155
Dissolved Solids (mg/1)	3500	--	--	--
Dissolved Oxygen (mg/1)	1.9	2.7	2.1	3.1

* ND--Not Detected.

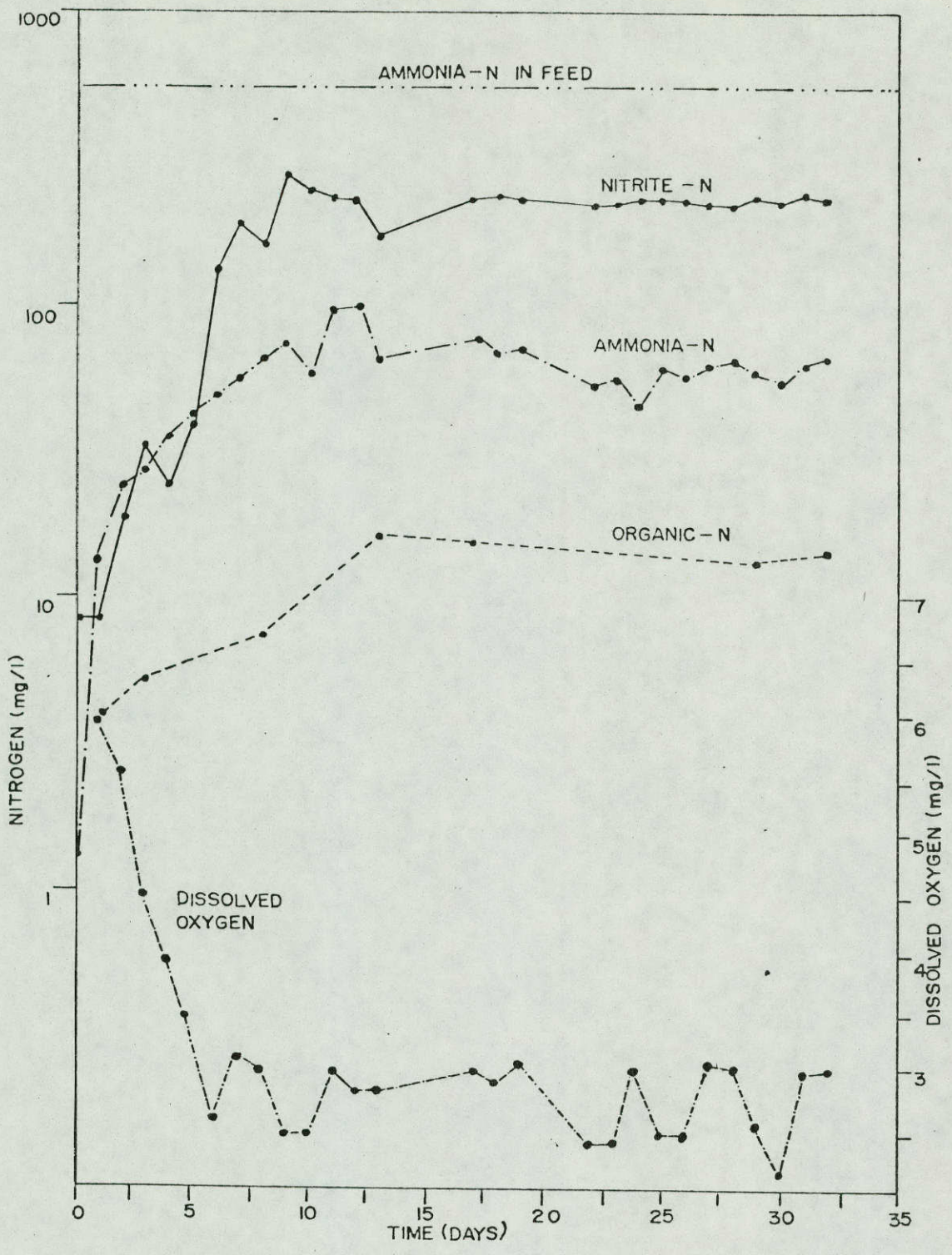

Figure 22. Nitrogen and dissolved oxygen vs. time in the primary nitrification study.

the analytical method used to determine the concentration of ammonia-N.

The ammonia-N concentration for the duration of the study fluctuated between 53 and 90 mg/l. The average ammonia-N concentration during the post-eight day acclimation period was about 80 mg/l. In fact, the ammonia-N concentration after the initial eight-day startup period seemed to be in a state equilibrium. Considering that the feed contained 550 mg/l of ammonia-N, and assuming an ammonia residual 70 mg/l, the ammonia-N removal efficiency was computed to be 87 percent.

Nitrite-N concentration increased dramatically from Day Zero (8.44 mg/l) to Day 8 (160.0 mg/l). The nitrite-N concentration seemed to level off after Day 8 of the study. The fluctuations of nitrite-N documented during this period ranged from 172.5 mg/l to 275 mg/l with an average concentration of 226.5 mg/l. Using this value to calculate the efficiency for converting the 550 mg/l of ammonia-N in the feed to nitrite yields a value of 41 percent.

The ratio of nitrite accumulated in this study to the quantity accumulated in the Phase IV study was 32:1. The increase in nitrite accumulation potential as compared to the Phase IV study is attributed to two causes.

1. Because the air-to-liquid ratio in the aeration basin was decreased significantly from that in the Phase IV study, a smaller fraction of the ammonia-N was volatilized and, therefore, more ammonia-N was available for the matabolic activities of nitrite-forming bacteria. The volatilization phenomenon will be discussed in depth later in this chapter.

2. The increased concentration of ammonia-N (550 mg/l) in the feed used in this study as compared to the ammonia-N concentration utilized in the Phase IV (195 mg/l) resulted in a greater concentration of residual ammonia-N available in the aeration basin for the metabolic activities of nitrite-forming microorganisms.

Scrutiny of the data seemed to substantiate the hypothesis that nitrite accumulated to a relatively high level because of increased metabolic activities associated with nitrite-forming bacteria. In fact, it is apparent after examination of the organic-N, suspended solids, and optical density data acquired during the first eight days of the study that cellular material increased along with the nitrite-N level concentration. Thus, it was assumed that the cellular material was composed almost entirely, if not completely, of nitrite-forming organisms. This assumption was made because the aeration basin liquor was theoretically exclusively selective for nitrite formers. In particular, nitrate-producing bacteria were not detected during the study period. Although it was not within the scope of this research to ascertain the factors which inhibit nitrate production, the results of the study have incidentally seemed to substantiate the research of others who have performed non-applied bacteriological studies concerning the inhibition of nitrate formation resulting in the accumulation of nitrite. For instance, Prakasam and Loehr (60) stated that pure culture studies of nitrifying organisms have shown that free ammonia and undisassociated nitrous acid (HNO_2) were more inhibitory than ammonium or the nitrite ion (NO_2^-).

A higher percentage of the ammonium-ammonia complex is in the form of ammonia at higher pH level, while undisassociated nitrous acid (NHO_2) decreases in favor of the nitrite ion.

The pH level maintained in this study (Figure 23) was relatively close to the 9.5 value considered by some experts to be inhibitory to Nitrobacter. During the 32-day investigation, the level was known to be above 9.5 for 17 days. The high pH level maintained (9.15 to 10.39) during the 32-day study suggests that Nitrobacter inhibition was more likely due to the inhibitory effect of free ammonia than of unionized nitrous acid (NHO_2). Free ammonia inhibition is a reasonable explanation because this high pH level is compatible for free ammonia formation but not for HNO_2 formation. It remains possible, however, that HNO_2 does cause inhibition of nitrate formers. Anthonisen (36) concludes from his research that HNO_2 inhibits Nitrobacter in the range of 0.22 to 2.6 mg/1. Because of the high concentration of ionized nitrite, perhaps the HNO_2 concentration in this nitritification study was high enough to inhibit Nitrobacter.

It was unlikely that the HNO_2 concentration was high enough to inhibit Nitrobacter in the Phase IV study because of the high pH and the low nitrite-N concentration. Because of the results obtained in the Phase III and IV studies, it is reasonable to suggest that Nitrobacter inhibition was caused by the relatively high pH level maintained in this investigation as suggested in the literature. Or a combination of pH level above 9.5, HNO_2, and free ammonia may have inhibited nitrate formers by the sum of their individual effects

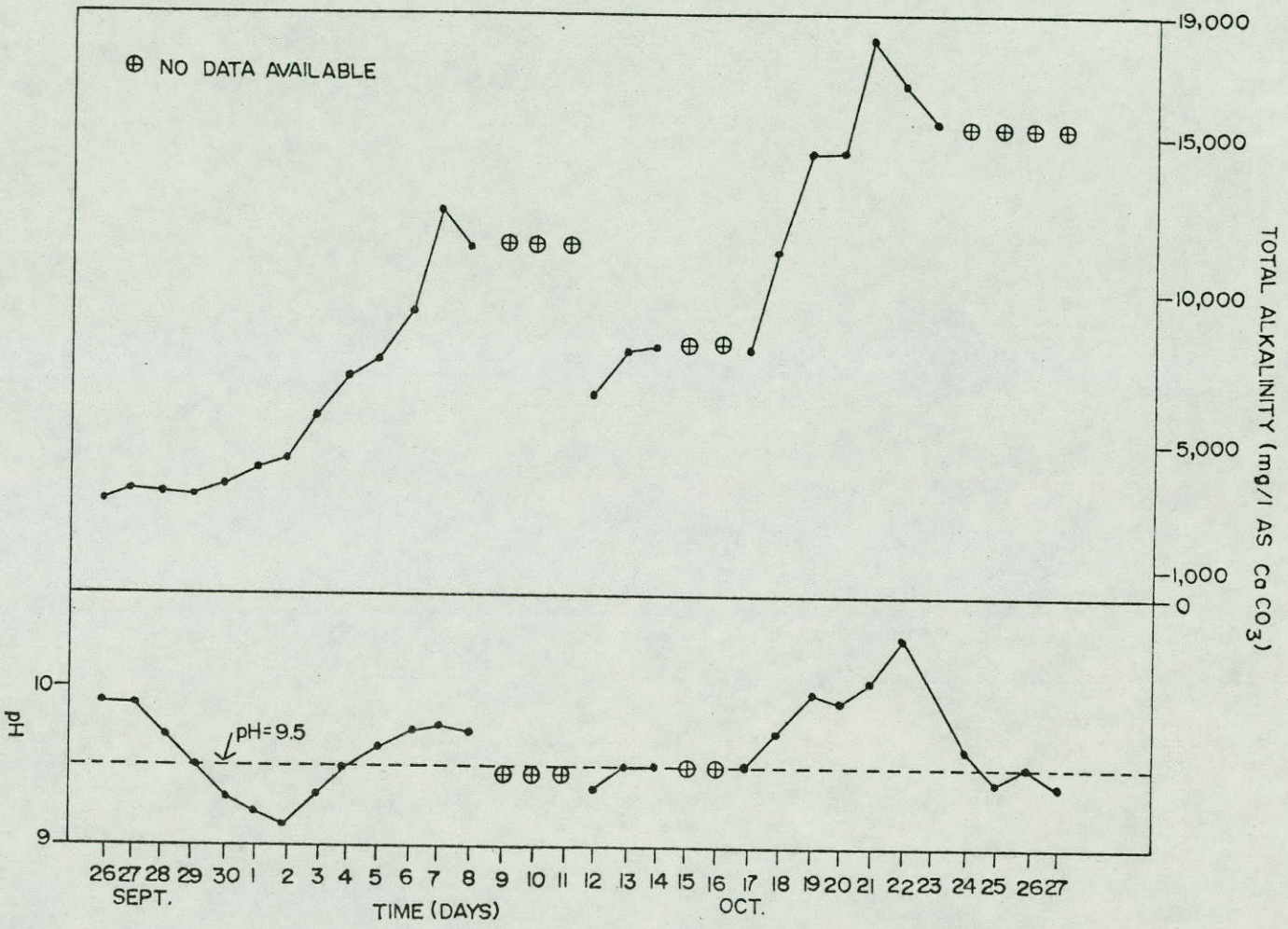

Figure 23. Alkalinity and pH relationships during the primary nitritification study.

as a type of antagonism. In any event, it was very clear that nitrite did accumulate to a relatively high level and was maintained at that level for several days without nitrate production.

Organic-N increased from 3.8 mg/l on Day 1 to 7.3 mg/l on Day 8, after which it seemed to reach an equilibrium ranging from 13.1 to 16.1 mg/l. The average organic-N concentration during the post eight-day acclimation period was 14.9 mg/l. If this average is used to calculate the percentage of ammonia-N converted to organic-N, the computations yield about three percent.

Nitrogen Balance

When all the above information is evaluated, the nitrogen balance was computed as follows:

Organic-N = 3 percent

Nitrite-N = 41 percent

NH_3-N = 13 percent

57 percent N accounted for

43 percent N not accounted for

This 43 percent not accounted for was assumed to be lost through volatilization. It was interesting to note that in the Phase IV study 95 percent was assumed to have volatilized. The significant decrease in volatilization was attributed to the decrease in the air-to-liquid ratio in the aeration basin because of less vigorous aeration.

Less vigorous aeration was also at least partially responsible for a drop in the average D.O. level (2.7 mg/l) as compared to the

average level in the Phase IV study (6.1 mg/1). Figure 22 shows that during the first eight days of the study, the D.O. decreased dramatically from 6.0 mg/1 to 3.9 mg/1. The feed contained 1.9 mg/1, so the decrease in D.O. during this period was attributed to two causes.

1. The one-liter-per-day feed containing 1.9 mg/1 caused a "dilution effect."

2. The low-aeration rate coupled with the aerobic bacterial metabolic activities combined to result in a relatively low D.O. concentration.

For the duration of the study, the D.O. level fluctuated between 2.1 and 3.1 mg/1 (average = 2.7 mg/1). According to the literature, the D.O. requirement for nitrifying bacteria is a residual D.O. above one mg/1. Therefore, the range of D.O. maintained in this investigation should have been sufficient for successful aerobic metabolism.

As previously mentioned, pH maintenance close to but preferably above 9.5 is essential. If not controlled, the pH level would decrease below the desired level because of (1) biological ammonia oxidation, (2) the dilution effect caused by the relatively low pH characteristic of the feed, and (3) ammonia volatilization.

During the investigation, both total and phenolpthalein alkalinity increased from 3,286 to 14,600 mg/1 and from 848 to 6,800 mg/1, respectively (Figure 23). The alkalinity increase was the net result of the difference of the alkalinity added for pH control and the alkalinity lost through the three methods described above. Dissolved solids also increased attendant with the alkalinity increase. It was

felt that the high alkalinity and dissolved solids concentrations will only be a temporary problem in plant-scale studies since alkalinity can readily be removed by the addition of CO_2 and/or mineral acids.

Because many nitritification-operating parameters which describe the process depend upon the concentration of nitritifiers present in the system, as well as the individual characteristics of the bacteria, it is essential to be able to measure a reasonably constant number of bacteria throughout the investigation or to have some indication of the relative bacterial population from day to day.

It is logical to assume that the number of bacteria in the system will change during the course of the study. However, because of time and equipment limitations, techniques involving isolation and identification of bacteria were not employed. It was felt that a realistic appraisal of nitritification kinetics would be obtained if a large, mixed population of nitrifying bacteria were maintained under a defined set of conditions (e.g., pH, temperature, aeration, etc.) for a relatively long period of time. Thus, suspended solids, organic-N, and optical density analyses were relied upon to give an indication of the nitrite-forming population in the aeration basin.

Unfortunately, the slow growth rate characteristic of nitrifying bacteria dictated that suspended solids and organic-N analyses not be performed on a daily basis. Therefore, the optical density analyses were heavily relied upon to give an indication of the suspended solids. Because of the nature of the experiment, all suspended solids were assumed to be indicative of nitrite formers. Figure 24 graphically represents relationships between optical density, suspended solids, and organic-N.

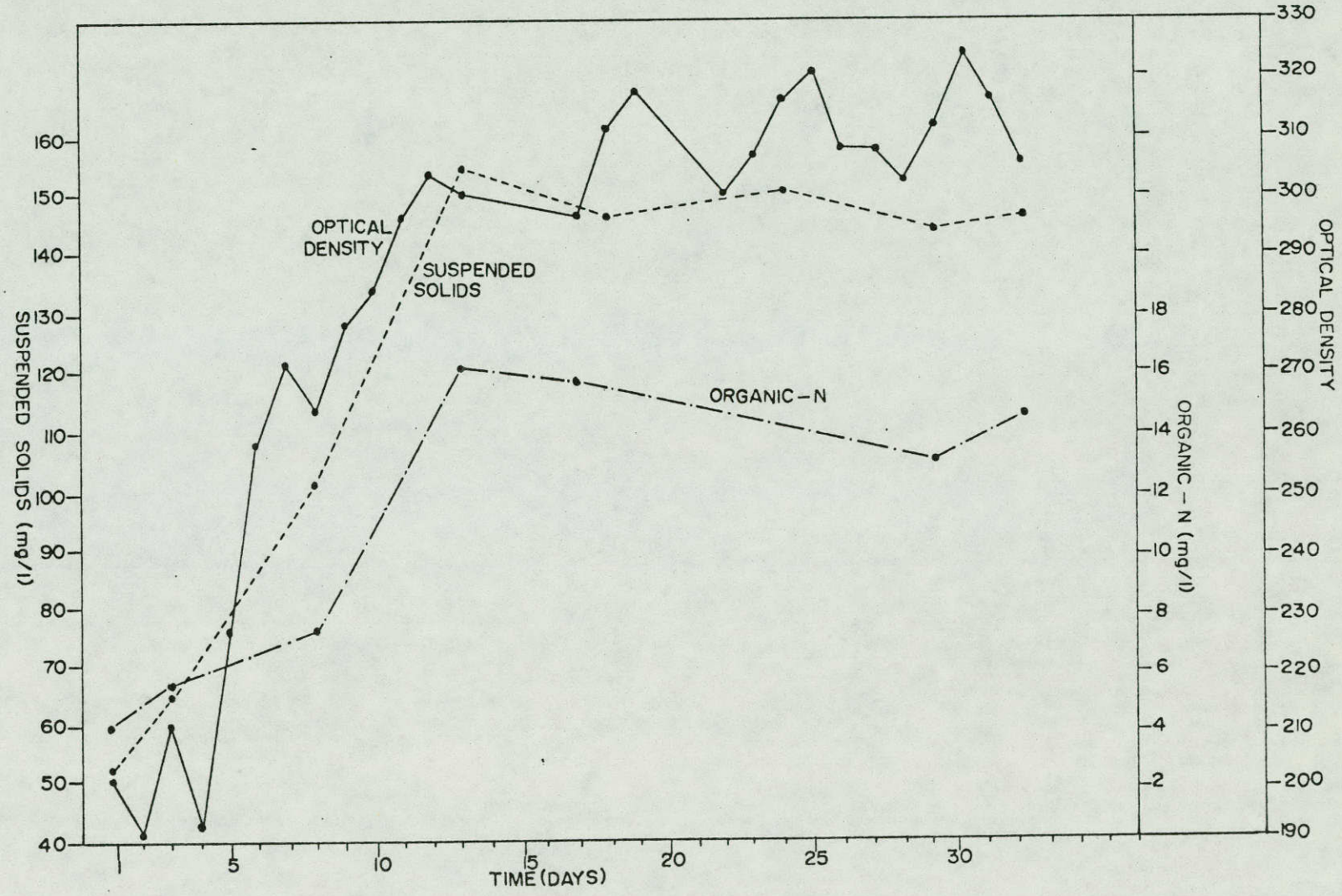

Figure 24. Organic-N, suspended solids, and optical density vs. time in the primary nitritification study.

Mean Cell Residence Time

The mean cell residence time for this study was defined as the weight of solids under aeration divided by the weight of solids lost in the effluent and wasted per day.

It was not within the scope of this research to evaluate the effects of various mean cell residence times upon system efficiency.

In fact, due to the desire for 100-percent cell recirculation, the mean cell residence time would be difficult to calculate.

However, considering that the amount of solids under aeration at the end of the study was approximately 1,192 mg/8 liters, one can estimate the mean cell residence time. One can determine by examination of Figure 24 that, over the last 25 days of the study, only about 179 mg of cells were wasted by periodic suspended solids analysis. This is equivalent to 7.2 mg/day wasted. Calculation of this ratio yields a mean cell residence time of approximately 166 days. It is noteworthy to mention that recommended mean cell residence values listed in the literature for complete mix nitrification systems vary from 12 to 40 days.

Suspended Solids

The suspended solids concentration characteristics of conventional nitrification reactors ranges from 3,000 to 4,000 mg/1. The suspended solids concentration for this study during the equilibrium period was about 149 mg/1. It, thus, becomes obvious that this nitrification system exhibited very low solids production characteristics. Sludge handling is a major cost element in waste treatment. The low sludge

production exhibited by this non-conventional nitrification system makes it an increasingly attractive treatment alternative.

Food-to-Microbe Ratio

The food-to-microorganisms ratio (U) is often utilized as a controlling variable for conventional nitrification facilities. Therefore, it was felt that it might be worthwhile to compare the U value calculated for this experiment to the U value utilized for conventional nitrification facilities (e.g., 0.1 mg N/day per mg MLVSS). This loading factor is often used as the nitrification facility design criterion for treatment of a secondary sewage treatment plant effluent containing 25 mg/l or more of ammonia-N.

The feed rate for this experiment was 550 mg ammonia-N per day. The average suspended solids concentration near the end of the experiment was 149 mg/l. (Suspended solids was assumed equivalent to MLVSS.) Using these figures to compute the food-to-microorganism ratio yields a value of 0.46.

Settling Characteristics

Although the suspended solids concentration was relatively low, the biomass in the two-liter clarifier exhibited excellent settling characteristics throughout this study.

It may be concluded from this study that:

1. For the artificial wastewater containing a total ammonia-N concentration of 550 mg/l, the biological treatment system achieved about an 87-percent reduction of ammonia-N and produced an effluent quality having an average of 70 mg/l ammonia-N, zero nitrate-N, 226.5 mg/l nitrite-N, 14.9 mg/l organic-N, and 149 mg/l suspended solids.

2. Large, stable, reproducible populations of nitrite-forming bacteria may be maintained by a daily input of ammonium sulfate feed solution to the complete mix nitritification regime.

3. A relatively large concentration of nitrite was induced to accumulate without a simultaneous appearance of nitrate.

4. The percentage of ammonia-N volatilization decreased significantly as compared to the Phase IV study. This decrease was attributed to addition of a mechanical stirring device coupled with a concurrent decrease in the aeration rate. Although this research was designed to develop a system that accumulates relatively high nitrite-N concentrations, the incidental loss of ammonia-N through volatilization was considered to be an asset. Control of pH and aeration rates may be a practical method for maintaining relatively constant conditions in the nitritification reactor.

5. The microorganism population in the reactor seemed to thrive well under the environmental conditions provided.

6. The rheological properties associated with the system effluent indicate that the "sludge" settling characteristics are satisfactory.

7. The results obtained from the operation of the systems seemed to substantiate the assumption that aseptic operational procedures were not required to accumulate nitrite. Perpetuation of system pH levels near 9.5 inhibited nitrate production.

Preliminary Denitrification Study

The nitritification research discussions which preceded this chapter documented the phenomenon of induced nitrite accumulation.

The next phase was to develop a system capable of reducing nitrite-N to diatomic gaseous nitrogen or volatile nitrogen oxides.

A search of the literature was performed in an attempt to find investigations which considered the reduction of nitrite to nitrogen gas. Unexpectedly, several research papers were found which outlined chemical, physical, and biological techniques for nitrite reduction.

Of the biological techniques, the most promising utilized a strain (strain 39) of Achromobacter liquefaciens. It is relevant to note that the scope of the investigation utilizing this strain was non-applied bacteriological research and not related to waste treatment. In an effort to obtain this particular bacterial strain, a request was made to American Type Culture Collection proprietors. The American Type Culture Collection curators informed the writer that their bacterial stock did not include strain 39 of A. liquefaciens. Consequently, written requests were made concerning the acquisition of this bacterial culture to two Australian scientists who had performed research utilizing this particular strain of bacteria. Both scientists kindly responded by sending lyophilized cultures of A. liquefaciens (strain 39).

One of the scientists, Dr. Jean Youatt (Monash Univ., Dept. of Chem., Victoria, Australia), described the culture as non-pathogenic to humans, plants, and animals. In a personal communication, she explained one method of isolating this culture.

> "When I was working with these organisms, I decided at one stage to see how many were around our University site. I went out and collected soil from all the places I knew remained habitually damp. I transferred them to a culture medium containing nitrite and plated out all the cultures which produced a lot of gas. All the muddy spots produced well." (61)

The other scientist, Dr. V. B. D. Skerman (Univ. of Queensland, Dept. of Micro-biology, Brishbane, Australia), explained in a personal communication (62) that A. liquefaciens (strain 39)

> ". . . is a very unusual strain in that it is unable to reduce nitrate but tolerates and reduces nitrite at levels well over 1,000 ppm, at which level the nitrite is completely toxic to the oxygen utilization system so that we have organisms that can reduce nitrite in the presence of oxygen."

Bergey's Manual of Determinative Bacteriology (2), although not listing strain 39, did describe A. liquefaciens as being a Gram-negative rod not producing nitrites from nitrates and being waterborne.

Following the literature search, an attempt was made to determine the nitrite-reducing potential of A. liquefaciens (strain 39).

On August 20, 1976, sterile medium for the growth of A. liquefaciens was prepared according to instructions provided by V. B. D. Skerman. Appendix C describes the directions for the medium preparation. Appendix D is a copy of the instructions provided by Skerman for reviving the lyophilized cultures.

As per the directions provided in Appendix D, the lyophilized culture of A. liquefaciens (strain 39) was aseptically transferred to a test tube containing the recommended broth. The contents of the test tube were next incubated at the recommended temperature (28 degrees C.) until growth was visually apparent. The "test-tube culture" was refrigerated at 4 degrees C. until August 28, 1976. On this date, the cells in the test tube were subcultured to a sterile one-liter flask containing the autoclaved recommended medium. The medium was subsequently incubated at 28 degrees C. Profuse

growth was apparent in the flask on September 1, 1976. The contents of this flask were referred to as the "mother culture."

The conditions postulated to be particularly conducive to the denitrification process are the presence of relatively large amounts of nitrite, an abundant supply of organic carbon, and anaerobic conditions.

The medium recommended for the general aerobic growth requirements for A. liquefaciens is listed in Appendix C. It consisted only of water, peptone, NaCl, and yeast extract.

In order to demonstrate nitrite reduction by A. liquefaciens, it became necessary to supplement the medium with a nitrite-N source (final electron acceptor) and a hydrogen donor (glucose). Inorganic nutrients were also added in order to more closely approximate a synthetic wastewater.

The Bio-Kulture bench-scale fermentor assembly was chosen for use in this study due to the necessity of maintaining a mono- or pure-culture bacterial regime consisting of strain 39 of A. liquefaciens.

On September 1, 1976, the reaction vessel was aseptically filled to the 14-liter mark with a medium consisting of the ingredients listed in Table 18.

Table 19 lists the results of chemical and physical analyses of the 14 liters of sterile medium.

On September 1, 1976, the sterile medium in the "fermentor" was inoculated with 10 ml of the "mother culture." Temperature control was selected to maintain the temperature regime of the

TABLE 18

CONSTITUENTS OF MEDIUM UTILIZED IN THE PRELIMINARY DENITRITIFICATION STUDY

Ingredients	Quantity
Peptone	140 grams
NaCl	70 grams
Yeast Extract	70 grams
Glucose	140 grams
KNO_2	28 grams
$CaCl_2$	1.4 grams
$FeCl_3$	0.14
$MgSO_4$	1.4
Deionized Water	14 liter

TABLE 19

RESULTS OF CHEMICAL AND PHYSICAL ANALYSES OF THE MEDIUM UTILIZED IN THE PRELIMINARY DENITRITIFICATION STUDY

Parameter	Value
pH	7.66
DS (mg/1)	9500
Phenolpthalein Alkalinity (mg/1)	0
Total Alkalinity (mg/1)	282
Nitrate-N (mg/1)	0
Nitrite-N (mg/1)	310
Ammonia-N (mg/1)	--
Organic-N (mg/1)	--
Optical Density	0.020
Suspended Solids	0

reactor contents at 28 degrees C. In order to maintain complete-mix conditions, the agitation control was set at 50 rpm.

Daily, from September 1, 1976 to September 7, 1976, a 500 ml sample was aseptically taken from the reactor basin. Within three hours of sample acquisition, but before the sample was pretreated by filtration through Gelman membrane filters (0.45 u), optical density, suspended solids, organic-N, and dissolved oxygen analyses were performed. Alkalinity, pH, nitrite-N, ammonia-N, nitrate-N, and dissolved solids analyses were performed on the filtrate.

The data accumulated during the period of this investigation are presented in tabular form in Table 20.

It was evident from the graphical representation (Figure 25) of the nitrite-N data accumulated during the seven-day experiment that the nitrite-N concentration did not decrease from Day One to Day Two. This may be attributed to one or both of the following reasons.

1. The bacteria must become acclimated to the new environmental regime and did not develop to a level where nitrite reduction was noticeable in two days.

2. Another explanation was that dissolved oxygen suppressed denitritification. In fact, post Day-Two oxygen levels were indicative of anaerobic conditions, and the nitrite-N concentration decreased dramatically for the duration of the study. During the seven-day experiment, the nitrite-N concentration decreased from 310 mg/l to less than 0.1 mg/l.

TABLE 20.

DATA ACCUMULATED DURING THE PRELIMINARY DENITRIFICATION STUDY

Date	pH	Phenolpthalein Alkalinity	Methyl Orange Alkalinity	Nitrite-N	Optical Density	NO_3	NH_3	Dissolved Solids	Suspended Solids	D.O.	Organic-N
Initial Analysis 09-01-76	7.66	0	282	310	0.020	--	--	9500	I.C.*	2.2	--
09-02-76	7.74	0	286	314	0.063	--	--	9800	I.C.	<0.1	I.C.
09-03-76	7.86	0	328	288	0.117	N.D.**	--	9400	15	<0.1	I.C.
09-04-76	7.91	0	360	242	0.173	--	--	9500	94	<0.1	7.0
09-05-76	8.51	22	532	228	0.252	N.D.	--	9500	123	<0.1	13.8
09-06-76	8.55 ↓ 7.42	34 ↓ 0	898 ↓ 600	68	0.484	--	--	11,500	331	<0.1	28.9
09-07-76	7.88	0	786	<0.1	0.600	N.D.	N.D.	10,000	406	<0.1	42.5

* Incompatible Results.

**Not Detected

Note: All data reported as mg/l except pH and optical density.

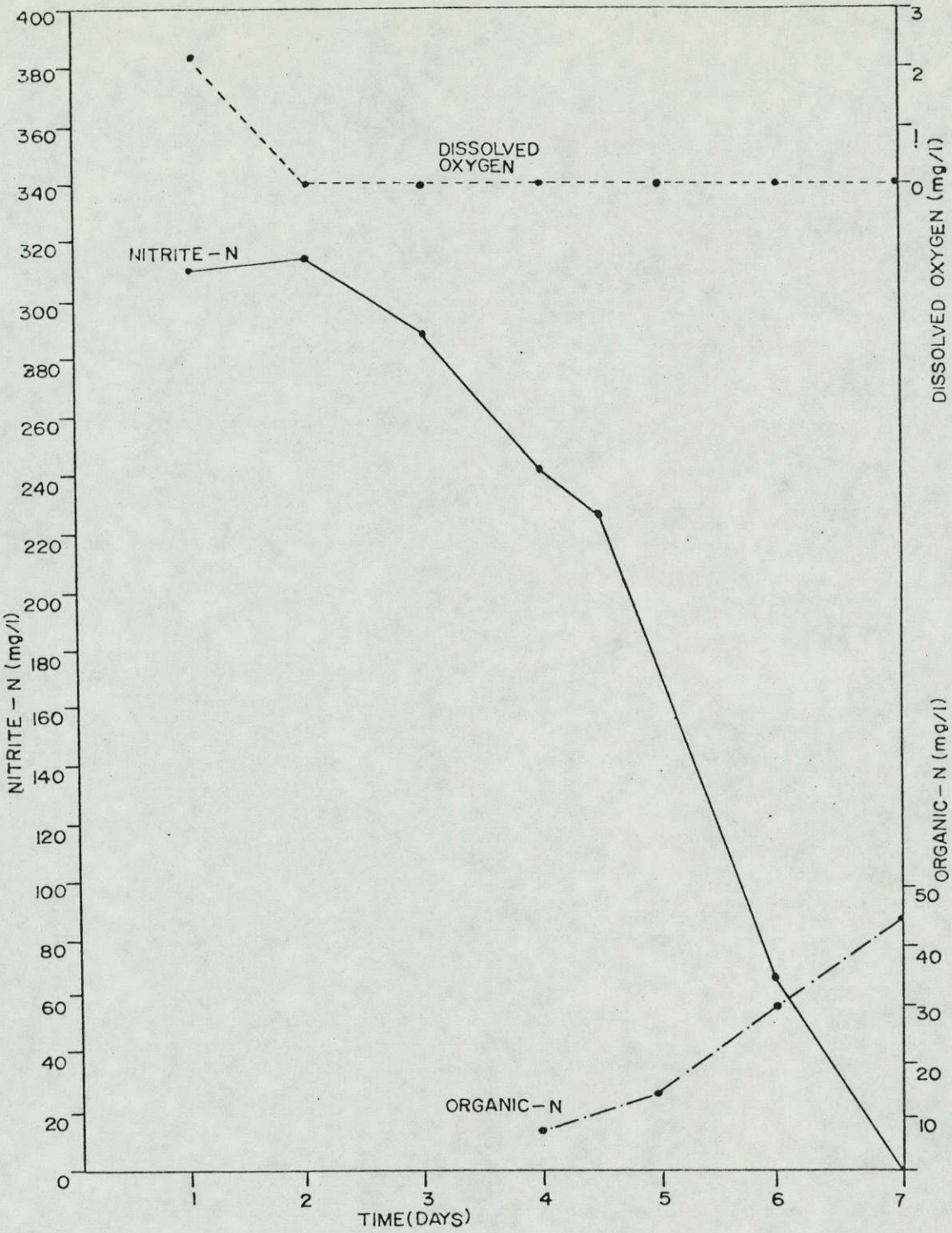

Figure 25. Nitrite-N, organic-N and dissolved oxygen vs. time in the preliminary denitrification study.

Youatt (50) who has extensively studied strain 39 of A. liquefaciens found that highly active cells could be produced in good yields if a period of anaerobic growth followed an initial period of aerobic growth. Figure 25 indicates that the A. liquefaciens cells were highly active following an initial aerobic period. Figure 26 indicates that the cells were produced in good yields as indicated by organic-N, optical density, and suspended solids analyses.

Estimation of cell production is important for design purposes. Because nitrite reduction is a biological process, new cell material was produced. Organic-N, suspended solids, and optical density were all indicative of cellular material variations. Figure 26 illustrates the commensurate increase in the three indices. The increase in cellular material was assumed to be due totally to increases in A. liquefaciens. This assumption should be valid because of the efforts made to maintain a pure culture system. Although precise predictions cannot be made of growth with respect to time, the data presented in Figure 26 concerning cell yield may be used to give order to magnitude values.

From the second day of the study until the last day, numerous tiny gas bubbles were generated in the reactor indicating the formation of nitrogen gas or volatile oxides of nitrogen. The gas bubbles were considered indicative of nitrite reduction for several reasons.

1. Nitrite-N decreased during this period.

2. It is highly improbable due to the anaerobic conditions that the nitrite was oxidized to nitrate. Sample analyses on day seven did not reveal the presence of nitrate-N.

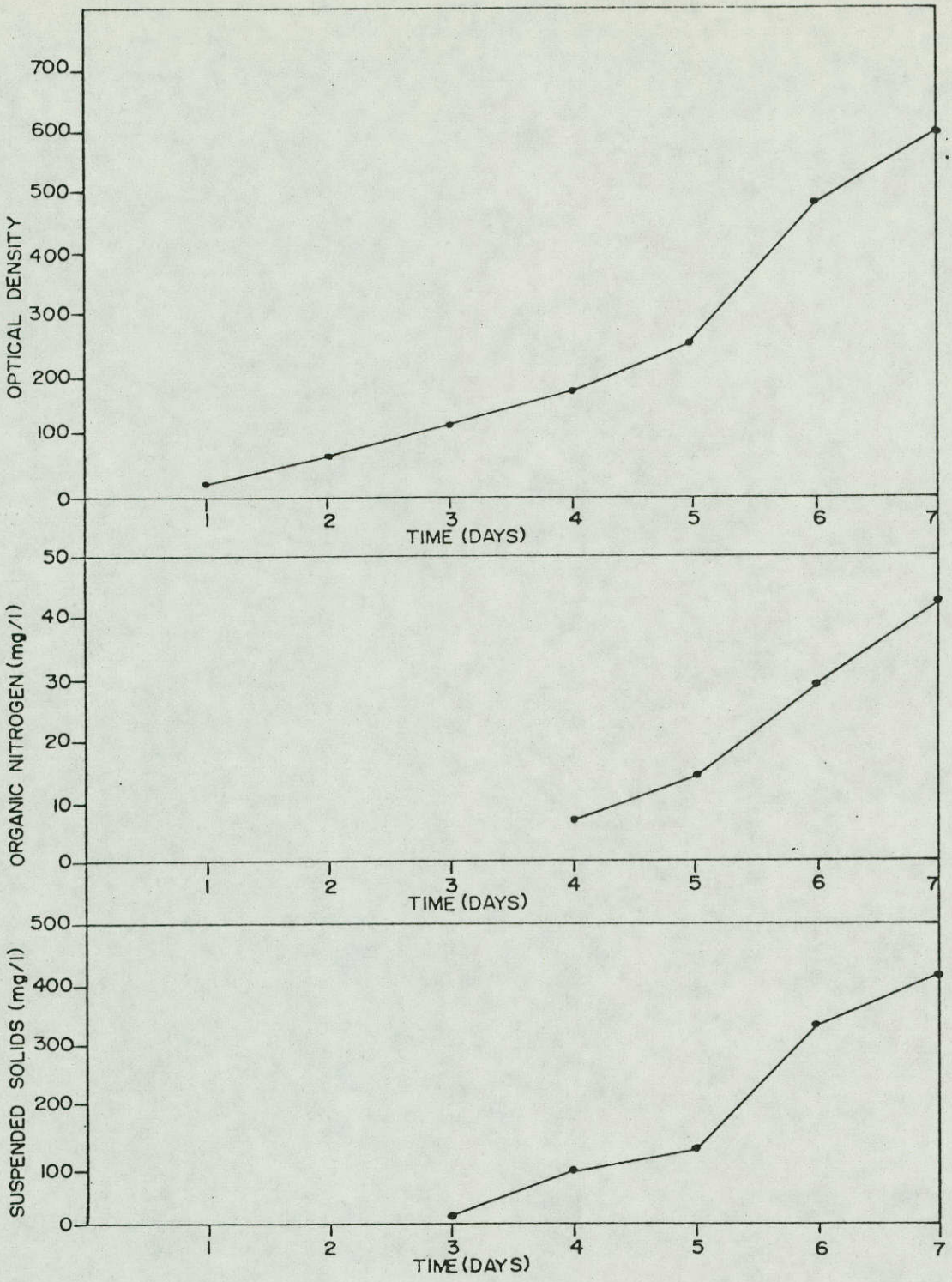

Figure 26. S.S., organic-N, and optical density vs. time in the preliminary denitrification study.

3. Ammonia-N analyses performed on day three, five, and seven were negative. It is true that ammonia could have volatilized even though the pH was not excessively high. It also seems unlikely that 310 mg/l of nitrite-N could have been transformed to ammonia and volatilized without detection of ammonia-N.

4. Organic-N was measured as 47.5 mg/l on day seven. This does not account for the fact of all the 310 mg/l of nitrogen-N present on day one (September 1, 1976).

5. Alkalinity and pH increases were indicative of nitrite reduction.

6. Youatt (50) states that this strain of Achromobacter liquefaciens reduces nitrite to nitrogen gas.

As seen in Figure 27, during day one through day six, the pH increased from 7.66 to 8.55. During this same period, phenolpthalein alkalinity increased from zero to 34 mg/l and total alkalinity increased from 282 to 898 mg/l. On day six of the study, the pH and alkalinity were decreased by the purposeful addition of HCl. Twenty-four hours later, the pH and alkalinity rose again and nitrite decreased from 68 mg/l to less than 0.1 mg/l. These substantial increases in pH and alkalinity were further evidence that nitrite was reduced in a biological denitrification.

When all the above information is evaluated, it can be concluded that, for the synthetic wastewater studied having a design nitrite-N concentration of 310 mg/l, a batch system containing strain 39 of Achomobacter liquefaciens achieved about 86 percent reduction of nitrite-N and produced an end product having less than 0.1 mg/l of

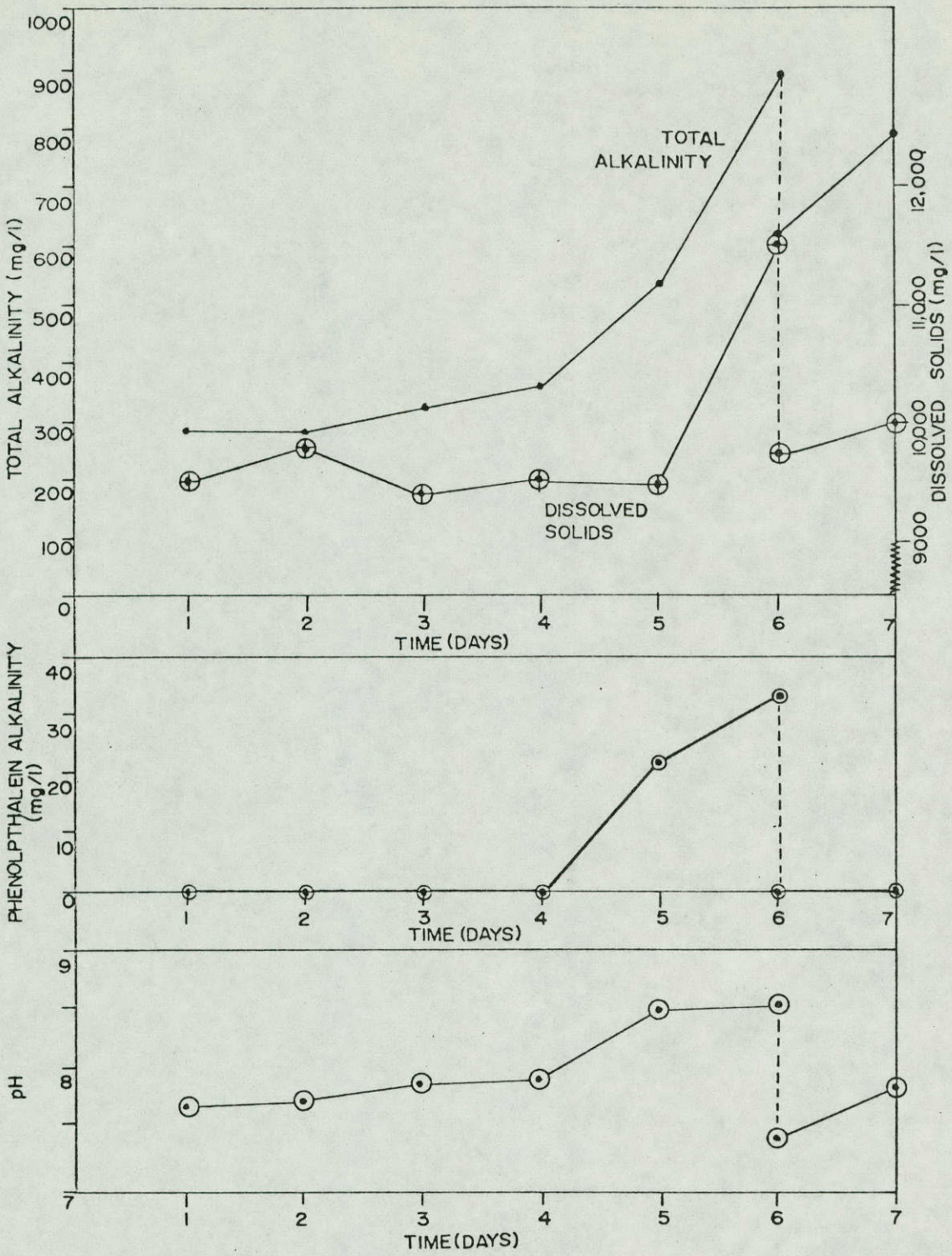

Figure 27. Alkalinity, pH, dissolved solids vs. time in the preliminary denitrification study.

nitrite-N and 42.5 mg/l organic-N. It was hypothesized that strain 39 of A. liquefaciens accomplished nitrite reduction by nitrite dissimilation whereby nitrite replaced oxygen in the respiratory processes of the organism under anoxic conditions.

Primary Denitrification Study

The conclusions reached in the preliminary phase of the denitrification batch study validated the fact that strain 39 of Achromobacter liquefaciens was capable of reducing nitrite-nitrogen to a gaseous form. The next logical step was to design an experimental procedure utilizing this bacterial strain which would demonstrate the technology necessary to reduce nitrite-nitrogen on a continuing basis for a prolonged, indefinite time frame.

Experimental Plan

Laboratory studies were directed at determining the feasibility of biologically treating a nitrite-nitrogen bearing artificial wastewater on a continuous basis. The scheme was to maintain environmental conditions conducive of the propagation of strain 39 of Achromobacter liquefaciens.

Fourteen liters of medium were prepared and sterilized on November 8, 1976. Table 21 lists the quantity of the various constituents used for medium preparation.

The medium was purposefully prepared to contain nitrite-nitrogen and C.O.D. concentrations of 305 mg/l and 3150 mg/l, respectively.

TABLE 21

CONTENTS OF MEDIUM USED IN THE DENITRIFICATION STUDY

Constituent	Quantity
Deionized Water	14.0 liters
Peptone	7.0 grams
Yeast Extract	14.0 grams
Dextrose	14.0 grams
Potassium Nitrite	25.532 grams
Alkalinity	*
Sodium Chloride	70.0 grams

* Enough 50 percent NaOH was added to bring the pH to 6.8

The sterile medium was aseptically placed in the "Bio-Culture" reaction vessel. The temperature control was set at the level (28 degrees C.) recommended by Bergey's Manual of Determinative Bacteriology (2) for the optimum growth of Achromobacter liquefaciens. In order to maintain complete mix conditions, the agitation control was set at 50 rpm.

The medium was immediately analyzed for the various chemical and physical attributes listed below:

1. Soluble COD
2. Nitrite-Nitrogen
3. Organic-Nitrogen (Total)
4. Nitrate-Nitrogen
5. Ammonia-Nitrogen
6. Dissolved Solids

7. Suspended Solids
8. Phenolpthalein Alkalinity
9. Methyl Orange Alkalinity
10. pH
11. CO_2
12. Dissolved Oxygen

On November 19 the sterile medium in the reaction vessel was aseptically inoculated with a 10 ml portion of the viable "mother culture" of Achromobacter liquefaciens (strain 39).

The experimental design system used for this study was a "draw-and-fill" operation. The "draw-and-fill" method was essentially a semi-continuous type of operation. The unit was operated on a once-a-day feed, i.e., the Bio-Kulture Unit was operated on a once-a-day waste and feed cycle which consisted of replacing two liters of the mixed liquor per day with fresh medium with incidental bacterial cell wasting. By daily wasting two liters of the mixed liquor from the reactor, the microorganisms were kept in a rapid state of growth. Before the mixed liquor was discarded, it was analyzed for the attributes listed above.

Fluctuations of any of the daily measured parameters were indicative of system activity. More specifically, a decrease in nitrite-nitrogen and C.O.D. with an attendant increase in alkalinity and pH was indicative of the biological denitrification phenomenon. The collected data were used to determine the degree of nitrite reduction, C.O.D. utilized per gram of nitrite reduced, and other pertinent information necessary for process evaluation. This data

also could be utilized to develop a scheme for future studies of the economic feasibility of utilizing strain 39 of Achromobacter liquefaciens for biological denitrification.

Immediately following analysis of the sample, an equivalent quantity of feed medium, freshly prepared by a specific set of procedures, was sterilized and aseptically added to the reaction vessel in order to replenish the contents of the reactor to its original volume (14 liter). The feed medium was added on a daily basis in order to replenish the total system C.O.D. concentration to the 3,150 mg/l level initially present on day one of the study. This mode of operation throughout the study period resulted in a C.O.D.* level which was constantly in excess of stoichiometric requirements for denitrification. By decanting two liters of reactor contents daily and by the subsequent addition of freshly prepared feed medium, it was possible to evaluate the extent of daily C.O.D. consumption attributable to the denitrification process.

This feed medium also contained an amount of nitrite-nitrogen equivalent to that measured in the two-liter volume which was withdrawn. For example, if analysis of the two liters which were aseptically withdrawn from the reaction vessel indicated that the nitrite-nitrogen concentration in the reaction vessel was 107.8 mg/l for that particular day, the two liters of freshly prepared feed medium was prepared so that upon its addition to the reaction vessel

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* The system C.O.D. resulted from the presence of yeast extract, peptone, and dextrose present in the ratio of 2:1:2 by weight. The nitrite-nitrogen present did not exert a chemical oxygen demand because sulfate acid pretreatment oxidized any nitrite-related chemical oxygen demand.

the entire 14-liter reactor contents would contain 107.8 mg/l of nitrite-nitrogen. Since the primary purpose of the experiment was to monitor daily decreases in nitrite-nitrogen, this procedure enabled nitrite monitoring without using a dilution factor to compensate for daily dilution.

On occasion, when nitrite-nitrogen analysis revealed that the nitrite-nitrogen concentration was nearly depleted, the two liters of feed medium was prepared so that upon its addition the contents of the reactor again contained 305 mg/l nitrite-nitrogen (the concentration present on Day One of the experiment). This procedure is described by the term "spiked addition" in the results section.

Five grams of NaCl were dissolved in each liter of feed medium added to the reactor. This practice resulted in a maintenance of a constant 5000 mg/l concentration of NaCl. Concentrated hydrochloric acid and 50 percent NaOH were also added as required to maintain the pH between 6 and 8, which is the desired range for the growth of strain 39 of Achromobacter liquefaciens.

The procedures described above were repeated daily throughout the study period with the exception of those days identified in the results section.

Control

Potassium nitrite, a strong oxidizing agent, was utilized in the medium as a nitrite-nitrogen source throughout the study period. Therefore, it was assumed that system C.O.D. might be oxidized as a result of spontaneous potassium nitrite reduction. In order to

determine the extent of spontaneous potassium nitrite reduction and C.O.D. oxidation, a control system was used. Fourteen liters of medium identical to that prepared for the primary denitrification study was prepared, sterilized, and placed in the Bio-Culture reaction vessel (sterile). Temperature, oxygen, and agitation regimes were identical to those employed in the primary denitrification study. Periodic analysis of the reactor's contents did not detect a significant system C.O.D. decrease nor a nitrite-nitrogen decrease.

Results and Discussion

A portion of the data accumulated during the period November 14 to December 6 are presented in Table 22.

Discussion of these results is presented in two phases. The first phase is a general overview aimed as an aid for the reader orientation. This synopsis outlines system startup, difficulties encountered which caused system failure, mitigation techniques used to overcome these difficulties, revitalization of the bacterial population, and finally the attainment of system equilibrium or steady state conditions.

The second phase discusses the research results in much greater detail with special emphasis on the day-to-day fluctuations and interactions of system parameters.

Phase I - General Overview or Synopsis Describing the Experimental Work

Figure 28 is a diagram which presents the system status in terms of operational parameters utilized and system activity noticed during the course of the experiment.

TABLE 22

DATA ACCUMULATED DURING THE PRIMARY DENITRIFICATION STUDY

		Nitrite-Nitrogen	Organic-Nitrogen	Ammonia Nitrogen	Nitrate-Nitrogen	Temperature (degrees C.)
11/18/76	Day 1	305	8.40	3.2	NO	28
11/19/76	2	307	7.0	2.8	NO	28
11/20/76	3	300 - 308	9.9	2.9 - 2.4	NO	28
11/21/76	4	245 - 245	-	9.5 - 7.1	--	28
11/22/76	5	155 - 155	-	11.5 - 7.6	NO	28
11/23/76	6	80 - 80	30	12.6 - 8.1	--	28
11/24/76	7	80 - 308	-	12.5 - 9.6	--	28
11/25/76	8	308 - 308	-	11.9 - 8	NO	28
11/26/76	9	305 - 305	17	9.6 - 6.4	--	28
11/27/76	10	230 - 230	-	9.5 - 6.0	--	28
11/28/76	11	108 - 108	-	19.5 - 13.0	--	28
11/29/76	12	3.6 - 305	-	24.5 - 14.3	NO	28
11/30/76	13	76 - 300	79	22.5 - 15.0	--	28
12/ 1/76	14	< 0.1 - 320	-	165 - 11.1	--	28
12/ 2/76	15	0.64 - 299	84	15.7 - 10.2	--	28
12/ 3/76	16	0.92 - 301	-	17.0 - 11.8	NO	28
12/ 4/76	17	0.69 - 290	-	15.8 - 10.9	--	28
12/ 5/76	18	< 0.1 - 295	-	16.5 - 10.6	--	28
12/ 6/76	19	< 0.1	93	16.8	NO	28

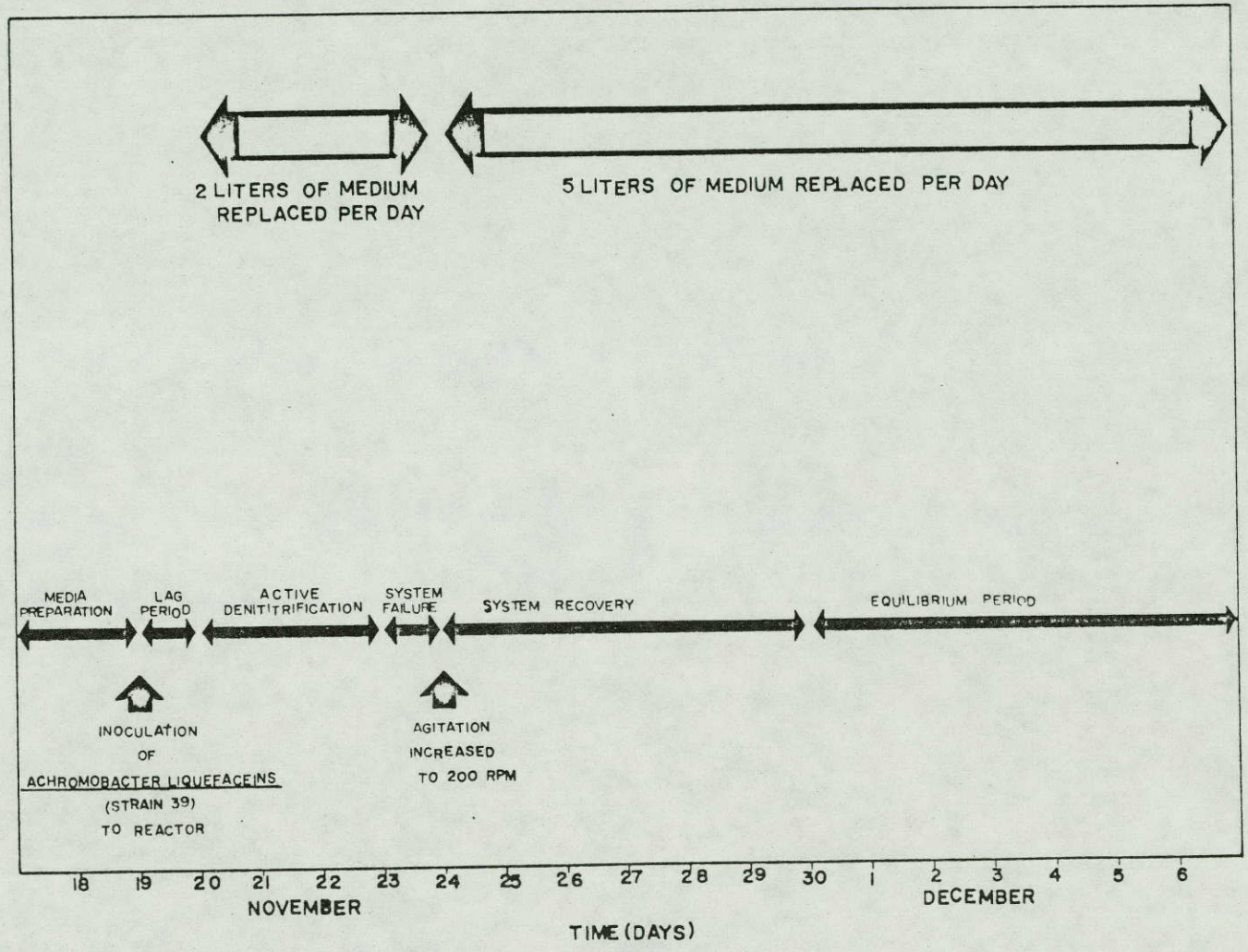

Figure 28. Generalized flowchart of system characteristics which describe the status of the primary denitrification study.

On November 18, 1976, the experimental medium (nitrite-containing artificial wastewater) was prepared, added to the anaerobic reactor basin, and analyzed to determine a portion of its chemical and physical characteristics. Table 23 lists the results of the analyses.

On November 19, 1976, the sterile medium was aseptically inoculated with strain 39 of Achromobacter liquefaciens. On the following day, the experimental routine, daily removal of two liters of medium and replacement with two liters of fresh medium, was initiated.

TABLE 23

CHEMICAL AND PHYSICAL CHARACTERISTICS OF THE STERILE MEDIUM UTILIZED FOR THE INITIATION OF THE PRIMARY DENITRIFICATION STUDY

Parameter	Value
pH	6.86
Dissolved Solids (mg/l)	8,100
Suspended Solids (mg/l)	Not Detected
Phenolpthalein Alkalinity (mg/l)	0
Methyl Orange Alkalinity (mg/l)	100
Nitrite-Nitrogen (mg/l)	305
Nitrate-Nitrogen (mg/l)	Not Detected
Ammonia-Nitrogen (mg/l)	3.2
Organic-Nitrogen (mg/l)	8.4
C.O.D.* (mg/l)	3,150
Temperature	28 degrees C. (82.4 degrees F.)
Dissolved Oxygen (mg/l)	Not Detected

* Nitrite normally causes a chemical oxygen demand in the C.O.D. analysis. However, the sample was pretreated as described in the experimental procedure so that nitrite would not cause a chemical oxygen demand.

System behavior as evaluated by daily chemical, physical and biological analyses indicated that the bacterial population in the reactor was in a lag or acclimation stage during the 24-hour period from November 19 to November 20.

By November 21, it was apparent that the system was actively denitrifying biologically. This conclusion was evidenced by the following system characteristics:

1. The nitrite concentration significantly decreased with an attendant decrease in C.O.D. concentration.

2. Alkalinity increased with an attendant increase in pH.

3. Suspended solids concentration significantly increased with an attendant increase in organic-nitrogen concentration.

4. A very apparent increase in the volume of the foam layer floating on the liquid medium was noted.

5. Significant concentrations of ammonia-nitrogen were not detected. This indicated that nitrite losses resulted predominantly from respiratory nitrite reduction instead of assimilatory reduction.

6. Nitrates were not detected. Therefore, nitrite losses from chemical or biological oxidation of nitrites to nitrates was not a system reality.

The period of active biological denitrification continued until November 23. On November 24, results of the laboratory analyses indicated that the system had failed for unknown reasons. System failure was accompanied by the following perturbations:

1. The nitrite concentration did not decrease.

2. Alkalinity increased with an attendant increase in pH.

3. Suspended solids concentration significantly increased with an attendant increase in organic-nitrogen concentration.

4. A very apparent increase in the volume of the foam layer floating on the liquid medium was noted.

5. Significant concentrations of ammonia-nitrogen were not detected. This indicated that nitrite losses resulted predominantly from respiratory nitrite reduction instead of assimilatory reduction.

6. Nitrates were not detected. Therefore, nitrite losses from chemical or biological oxidation of nitrites to nitrates was not a system reality.

The period of active biological denitrification continued until November 23. On November 24, results of the laboratory analyses indicated that the system had failed for unknown reasons. System failure was accompanied by the following perturbations:

1. The nitrite concentration did not decrease.

2. Alkalinity and pH did not increase and, in fact, decreased.

3. Suspended solids concentration dramatically decreased. It was concluded that a suspended solids decrease could only be the result of lysis of a significant portion of the bacterial population present in the reactor.

4. The foam layer disappeared.

In an attempt to determine the cause of system failure, an effort was made to analyze the system contents by additional laboratory procedures (e.g., CO_2 and volatile acids determinations). The CO_2

analysis revealed that the mixed liquor contained 270 mg/l of dissolved carbon dioxide. This relatively high CO_2 concentration was explained by the fact that the denitrification reaction results in the formation of CO_2 and nitrogen gas. Both are relatively soluble in water. Therefore, it was surmised that, due to the absence of vigorous mixing afforded by the 50 rpm agitation speed, the mixed liquor in the reaction tank became supersaturated with CO_2 (270 mg/l) which lowered the pH to a level that was either directly or indirectly inhibitory to strain 39 of Achromobacter liquefaciens.

In an effort to revive or revitalize the bacterial population, two procedural modifications were instigated on November 24. First, the agitation rate in the reactor was increased from 50 rpm to 200 rpm. This allowed "excess" CO_2 to escape to the atmosphere. Subsequent dissolved oxygen analyses revealed that the increased agitation rate did not cause a detectable degree of reaeration.

Second, the possibility also existed that accumulated toxic or inhibitory metabolic end-products caused the system failure. The increased agitation rate would probably not assist in the removal of these substances unless they were highly volatile. Therefore, in order to ensure that toxic or inhibitory compounds would not accumulate to lethal concentration, the hydraulic detention time was decreased from seven days to 2.8 days. This was accomplished by increasing the rate of medium withdrawal and replenishment from two liters per day to five liters per day. Thus, 35.7 percent of the system volume was replaced each day instead of only 14 percent.

These two procedural mitigation techniques were successful as evidenced by the fact that the system slowly began to exhibit signs of recovery during the period from November 25 to November 30. By December 1, the system was actively denitrifying again. In fact, the system reached steady-state conditions or a state of equilibrium. Steady-state conditions were evidenced by the fact that the daily "spike" additions of approximately 305 mg/1 of nitrite were reduced essentially to zero by the end of each 24-hour period. This phenomenon continued for the duration of the study (through December 6).

Phase II--Detailed Discussion of Research Results

This section discusses in detail the results of the experimental study in terms of the various physical, chemical, and biological analyses performed.

CO_2, Suspended Solids, and Organic-Nitrogen. Because this study was a pure culture system, it was reasonable to assume that measured suspended solids concentrations were equal to the quantity of Achromobacter liquefaciens in the reactor.

Figure 29 shows that, after system inoculation with A. liquefaciens, the period from November 20 to November 23 was characterized by a dramatic day-to-day increase in suspended solids concentration. On November 23, however, the system failed as evidenced by a significant decrease in suspended solids. This decrease was presumably due to the direct or indirect effect on A. liquefaciens of the high CO_2 concentrations (270 mg/1) and resultant low pH.

Figure 29 shows that the two mitigation techniques instigated on November 24, 1976, in order to revive the system were successful.

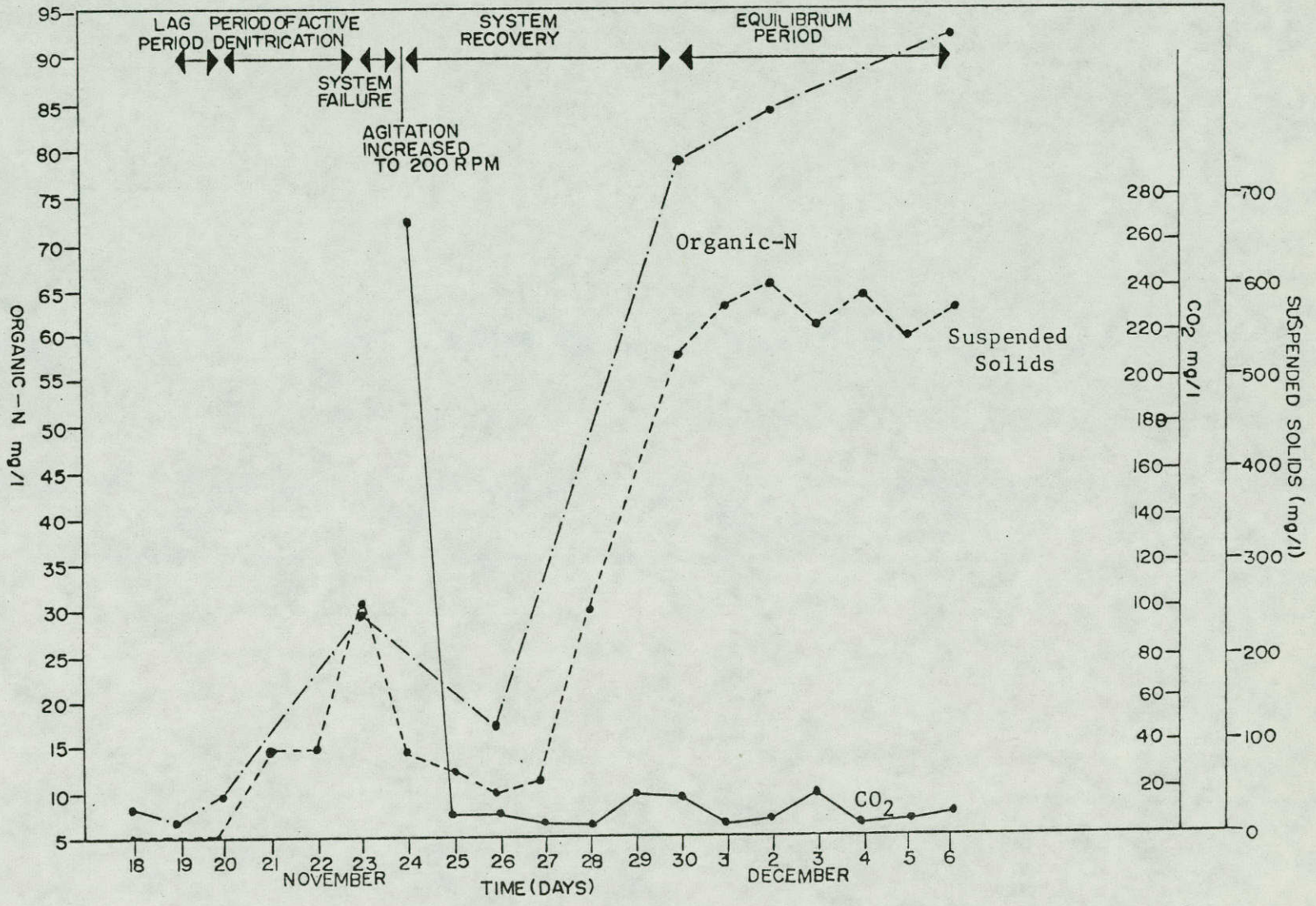

Figure 29. Organic-N, suspended solids, and CO_2 vs. time during the primary denitrification study.

The CO_2 concentration was reduced almost 30 fold the following day. This resultant relatively low CO_2 level was maintained for the duration of the study. Presumably, due to the system CO_2 decrease, the suspended solids concentration increased significantly and seemed to be in a state of equilibrium from November 30 until the end of the study period.

An average value for suspended solids concentration found in a typical conventional denitrification reaction basin is about 2,400 mg/l. Utilizing the suspended solids concentrations measured during the study equilibrium period (November 30 to December 6), an average suspended solids concentration was calculated to be 574.6 mg/l. It, thus, becomes obvious that this pure culture system exhibited low solids production characteristics.

The organic-nitrogen content in the sterile medium, 8.4 mg/l, was contributed by organic matter used to prepare the media (yeast extract and peptone). Fluctuations in the organic-nitrogen concentration generally mirrored or corresponded to variations with the suspended solids concentrations.

C.O.D. and Nitrite-Nitrogen

Denitrification systems inherently require an adequate quantity of a readily usable source of carbon as a carbon and energy source (electron donor). The preliminary denitrification system demonstrated that a combination of yeast extract, peptone, and dextrose was a source of usable carbon for Achromobacter liquefaciens (strain 39). This carbon source for this study was easily monitored by the dichromate chemical oxygen demand analysis (C.O.D.).

In order to ensure that carbon was not limiting in this experiment, the organic carbon was added in a quantity above the stoichiometric requirement for the denitrification reaction. The sterile medium added to the reactor on November 18, contained 3150 mg/l C.O.D. As described in the section explaining the experimental procedure, every 24 hours the system was "spiked" with a fresh supply of C.O.D. (in the form of dextrose, yeast extract, and peptone) in order to replenish the quantity of C.O.D. utilized during the previous 24-hour period. Figure 30 clearly shows the degree of carbon oxidation occurring during each phase of the study period. The lag phase was characterized by negligible C.O.D. utilization. The active denitrification phase (November 20 to November 23) was characterized by a much more pronounced degree of C.O.D. utilization. The period cited as system failure and the first three days of the system recovery period were characterized by a noticeable decrease in the quantity of C.O.D. utilized. The last days of the system recovery period and the equilibrium period were characterized by a substantial degree of C.O.D. utilization.

Figure 31 shows the nitrite-N relationship during the study period. Comparison of this graph with the graph (Figure 30) representing the daily fate of C.O.D. indicates that the degree of nitrite reduction was directly related to the degree of C.O.D. utilized and vice versa. In particular, the lag phase was characterized by an absence of nitrite loss through conversion to nitrogen gas. The active denitrification phase was characterized by a much more pronounced degree of nitrite utilization. The period sited as system

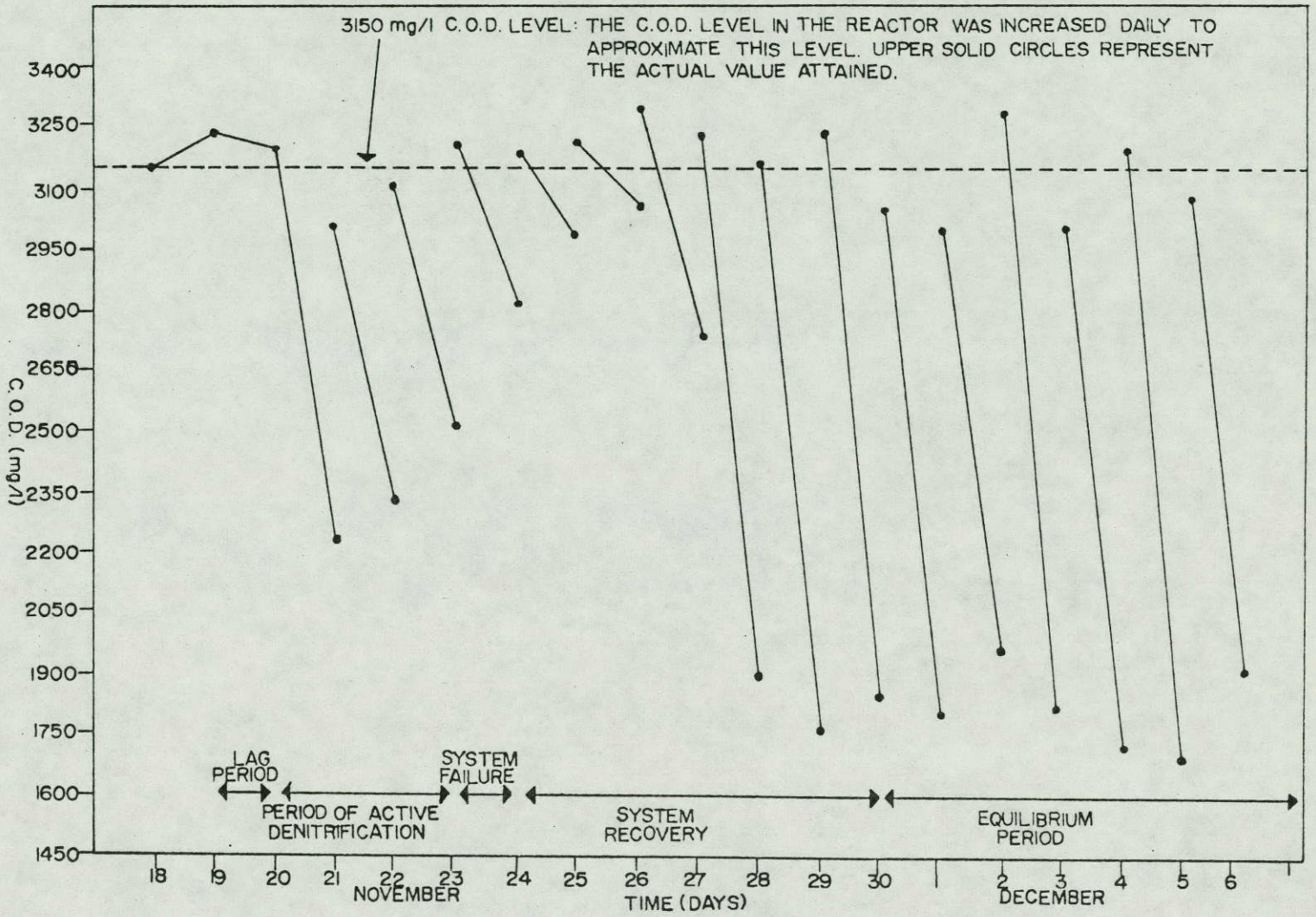

Figure 30. COD concentration during the primary denitrification study.

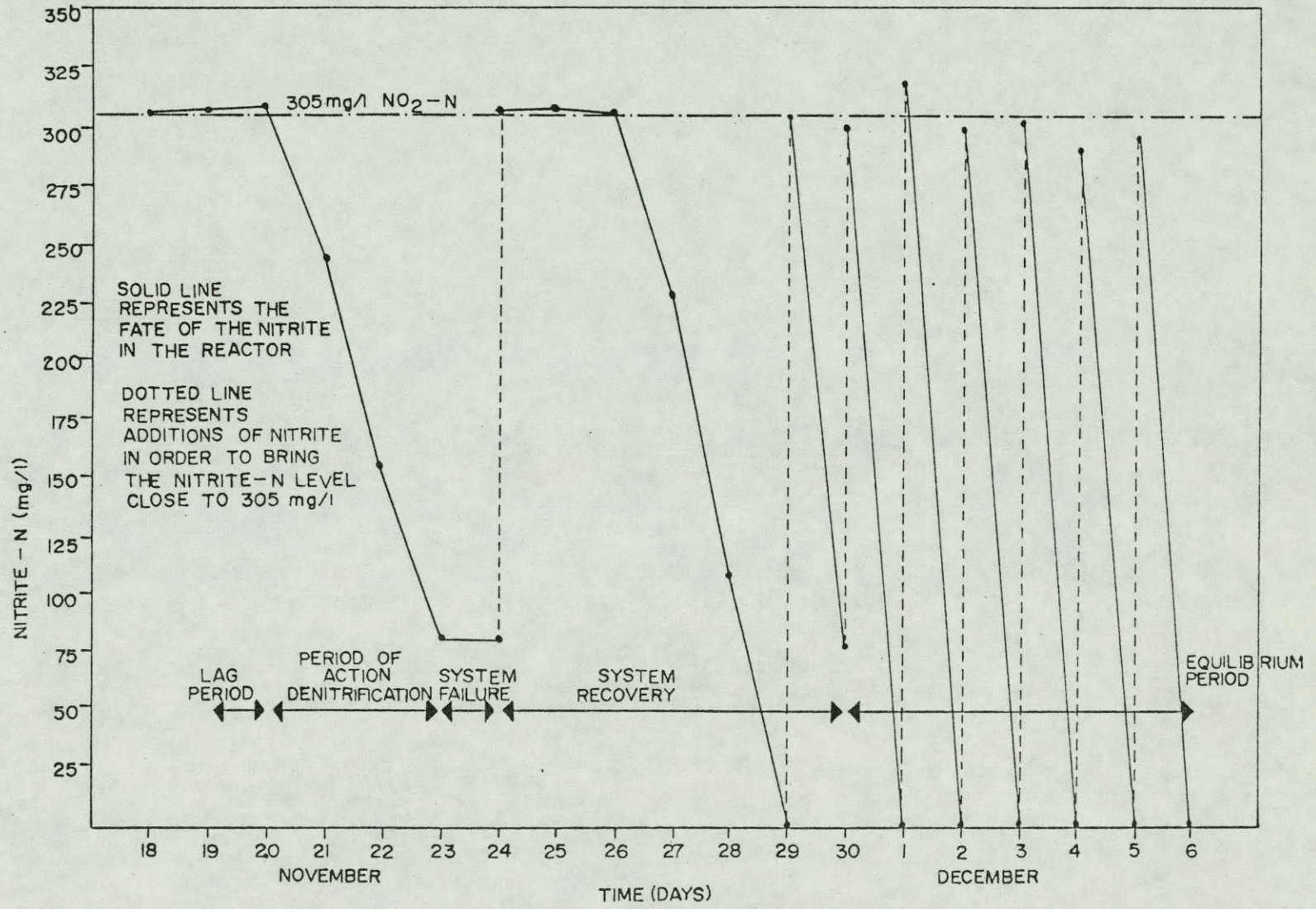

Figure 31. Nitrite-N vs. time relationships during the primary denitrification study.

failure was characterized by a cessation of nitrite reduction. System recovery was characterized by an apparent revitalization of A. liquefaciens as was evidenced by the fact that nitrite was again utilized as a final electron acceptor. The equilibrium period was characterized by the almost total utilization of nitrite on a daily basis.

This study demonstrated that a particular quantity of C.O.D. was utilized per unit of nitrite-nitrogen reduced. For conventional denitrification studies, this ratio is usually reported in the literature as mg methanol/1 to mg nitrate-N/1. The methanol requirement were nitrate-N, nitrite-N, and dissolved oxygen are present can be computed using the following empirically derived equation (4).

$$Cm = 2.47\ N_o + 1.53\ N_1 + 0.87\ D_o \qquad \text{(Equation 9)}$$

where Cm = required methanol concentration, mg/1

N_o = initial nitrate-nitrogen concentration, mg/1

N_1 = initial nitrite-nitrogen concentration, mg/1

D_o = initial dissolved oxygen concentration, mg/1

If one ignores nitrate and dissolved oxygen, the ratio of methanol required for reduction of nitrite-nitrogen is simply 1.53 N_1. Denitrification facilities which use other forms of organic carbon as the terminal electron acceptor reported C.O.D. removed per mg nitrate-nitrogen removed. Typical values for the ratio ranged from 2.8 to 3.20.

Table 24 is a summary of the values measured during the equilibrium phase of the study. Column One shows the quantity of

C.O.D. oxidized daily. Column Two shows the amount of nitrite-nitrogen reduced per day, and Column Three presents the computed ratio for the C.O.D. oxidized to nitrite-nitrogen reduced for a particular day.

TABLE 24

C.O.D., NITRITE-NITROGEN RELATIONSHIP
DURING THE EQUILIBRIUM PERIOD

24-Hour Period	C.O.D. Oxidized	Nitrite-N Reduced	C.O.D./ Nitrite-N
11/30/76 to 12/01/76	1302 mg/1	229 mg/1	5.69
12/01/76 to 12/02/76	1049	320	3.28
12/02/76 to 12/03/76	1462	298	4.91
12/03/76 to 12/04/76	1263	301	4.19
12/04/76 to 12/05/76	1486	289	5.14
12/05/76 to 12/06/76	1174	295	3.98

Using the data in Column Three in Table 24, the average ratio of C.O.D., oxidized per unit of nitrite-N reduced may be calculated for the equilibrium period of the study. This computation yields a value of 4.52.

Ammonia

Figure 32 illustrates the fluctuation of ammonia-nitrogen during the study. Analysis of the sterile medium indicates a soluble ammonia-nitrogen concentration of 3.2 mg/1. This soluble ammonia-nitrogen resulted exclusively from the carbon (C.O.D.) source (yeast extract and pepton) added to the denitrification unit. It is apparent after

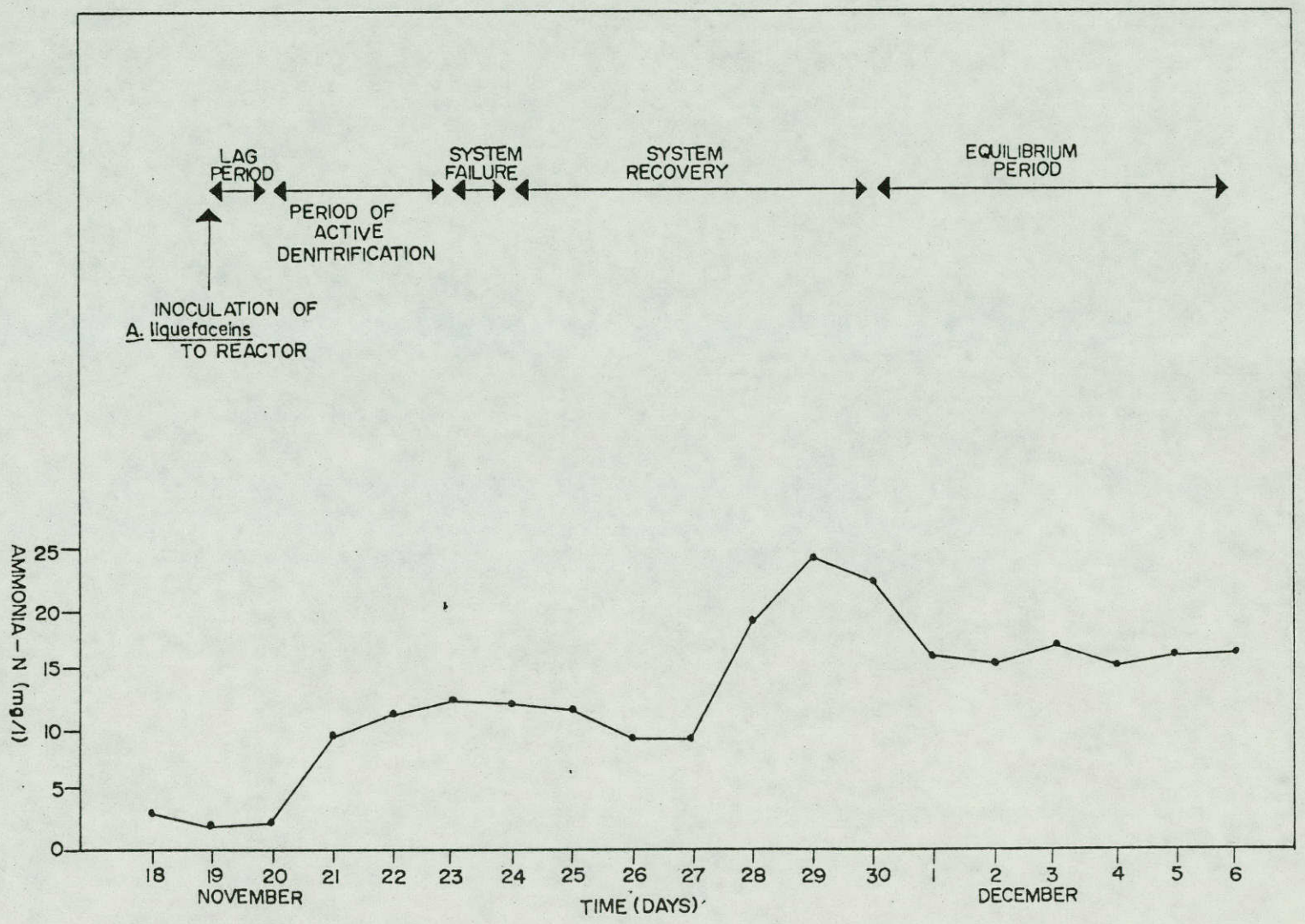

Figure 32. Ammonia-N vs. time during the primary denitrification study.

scrutinizing the data presented in Figure 32 that the ammonia-nitrogen increased slightly during the course of the study. This increase was probably due to assimilatory reduction. It is possible that a significant percentage of the nitrite-N was converted to ammonia-N and subsequently volatilized. This possibility is highly unlikely, however, since the pH in the reactor was not high enough to result in a loss of 305 mg/l per day of nitrite-nitrogen by conversion to ammonia and subsequent volatilization. Also, other experimental evidence (simultaneous decrease in nitrite and C.O.D., increase in alkalinity and pH, noticeable production of gas) indicates that biological denitrification was the predominant system reaction.

Alkalinity and pH Relationships

Figures 33 and 34 show phenolpthalein alkalinity and methyl orange alkalinity (total) relationships, respectively. Figure 35 presents the pH fluctuations which occurred as a result of alkalinity changes.

Denitrification is characterized by a net gain in alkalinity. In general, it may be concluded that pH and alkalinity relationships during this study were typical of conventional denitrification studies. That is, total alkalinity and pH increased as nitrite was reduced. Figure 35 should be compared to Figure 31. Scrutiny of the two graphs show that the pH level substantially increased after two mitigation techniques were employed in order to decrease the CO_2 concentration.

Any attempt to evaluate the effectiveness of the non-conventional denitrification system dictates that a comparison be made of

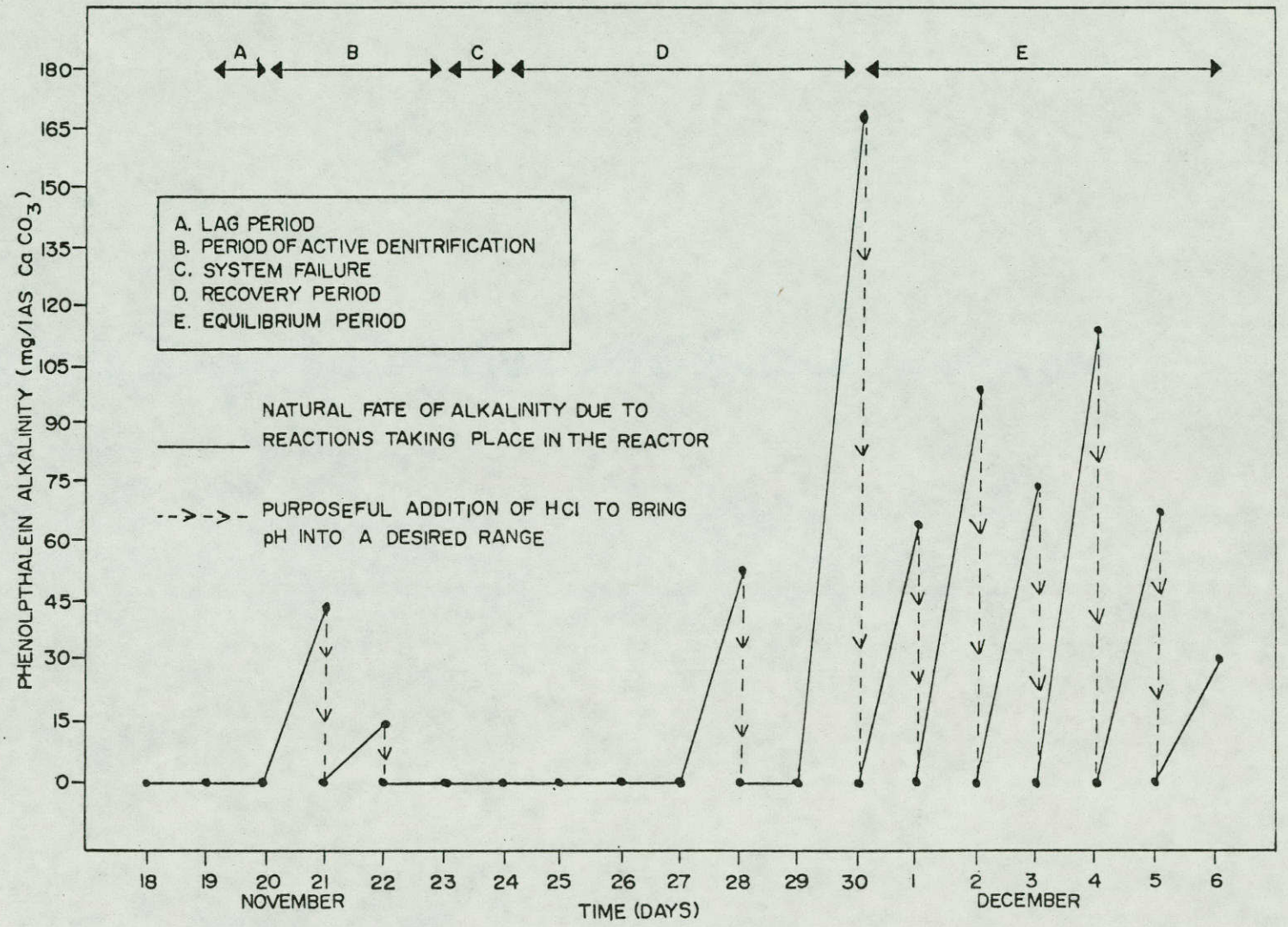

Figure 33. Phenolpthalein alkalinity relationships during denitrification study.

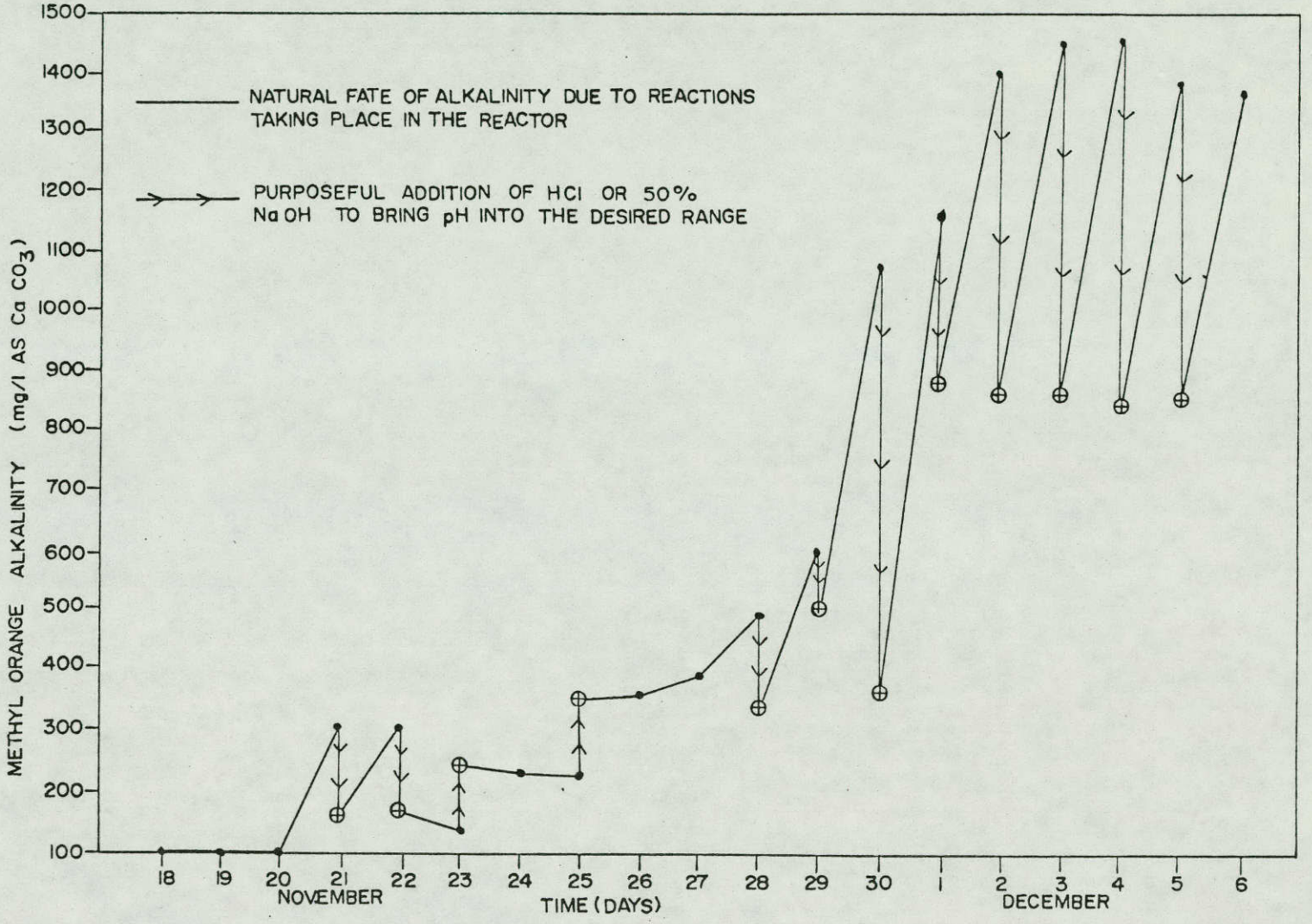

Figure 34. Total alkalinity relationships during the primary denitrification study.

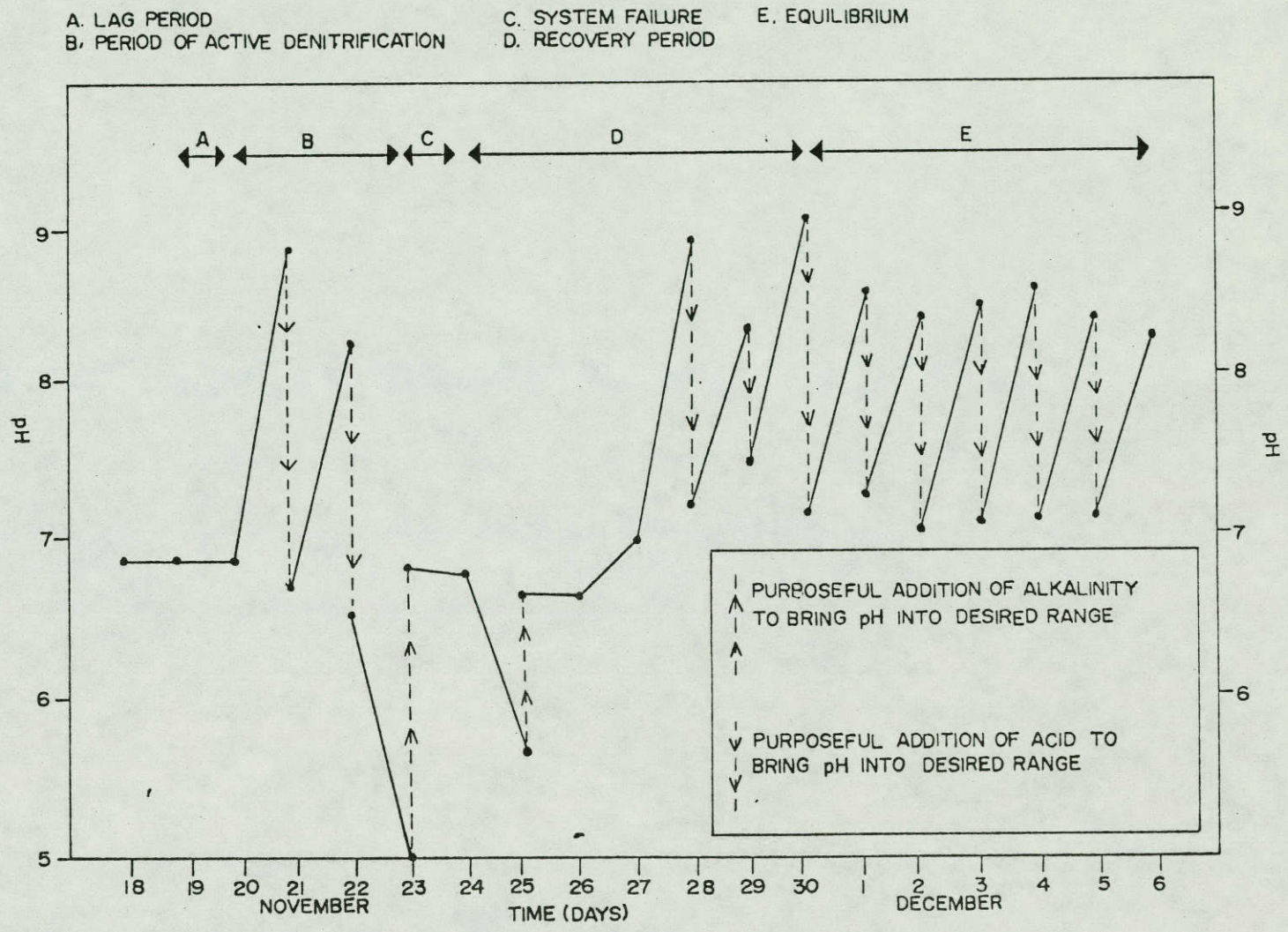

Figure 35. Time vs. pH during the primary denitrification study.

the system influent and effluent. The values found in Table 25 are a summary of the data accumulated during the influent and effluent quality characteristics. In this discussion, the term "influent quality" is not used in the usual sense, but refers to the characteristics of the medium in the reactor (14 liter) immediately after the daily addition of 5 liters of freshly prepared medium.

Table 25 highlights the fact that, of the 300.8 mg/1 nitrite-nitrogen (average value during the equilibrium period) added as a daily "spike" only 0.43 mg/1 remained after a 24-hour period. Calculations using this result and an average ammonia-nitrogen concentration of 16.4 mg/1, and assuming that organic nitrogen was removed by sedimentation, yield a nitrite-nitrogen removal efficiency of approximately 94 percent.

Conclusions

1. The bench scale study results demonstrated that closed nitrogen balances can be performed on a continuously stirred reactor operated by a "draw-and-fill" type regime to account for the bacterial conversion of nitrite to nitrogen gas.

2. At 28 degrees C., daily "spike" additions of artificial wastewater which resulted in a system nitrite-nitrogen concentration of about 305 mg/1 were completely denitrified on a continuing basis in a stirred tank reactor utilizing a pure culture of Achromobacter liquefaciens (strain 39).

TABLE 25

SUMMARY OF DATA ACCUMULATED DURING THE EQUILIBRIUM PERIOD OF THE PRIMARY DENITRIFICATION STUDY (LAST 6 DAYS)

Parameter	5 l/day addition of medium results in the reactor's contents having this quality			Daily Effluent Quality (5 l/day)		
	Min.	Max.	Avg.	Min.	Max.	Avg.
Nitrite-N mg/l	290	320	300.8	L.T.0.1	0.92	L.T.0.43
Soluble C.O.D. mg/l	3280	2991	3103	1683	1951	1811
pH	7.0	7.22	--	8.2	8.51	--
Organic-N mg/l	--	--	--	84	93	88.5
Ammonia-N mg/l	10.2	11.8	10.9	15.7	17.0	16.4
Phenolpthalein Alkalinity mg/l	0	0	0	66	116	76.7
Methyl Orange Alkalinity mg/l	848	892	864	1160	1456	1370
Nitrite-N mg/l	0	0	0	0	0	0
Dissolved O_2 mg/l	0	0	0	0	0	0
CO_2 mg/l	--	--	--	6	20	10.3
Suspended Solids mg/l	--	--	--	547	602	574.6

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

The principal objective of the experimental work previously discussed was to demonstrate on a bench-scale basis a non-conventional nitrification-denitrification system. In interpreting all of the experimental results obtained in the study, it was apparent that the technology necessary to define such a system was demonstrated. In particular, suitable data were obtained for the development of future experimental designs to more adequately define the feasibility, effectiveness, and operational parameters of such a system.

Emphasis must be placed on the fact that a number of operational problems arose during the study. In any event, the major problems were resolved resulting in operational success of the experimental system.

After evaluation of the complete study results, the following general conclusions were reached:

1. The data obtained from the Phase I preliminary nitritification study indicated that a pure culture of N. europea cultivated in a batch system were capable of accumulating high levels of nitrite nitrogen (396 mg/l).

2. The data obtained from the Phase II preliminary nitrification study indicated the concentrations of ammonia-N comparable to those of

secondary sewage treatment plant effluents were able to support a successful growth of a pure culture of N. europea under optimal environmental conditions in a batch-type system.

3. The data accumulated from the Phase III preliminary nitritification investigation demonstrated that it was possible to accumulate nitrite without subsequent oxidation to nitrate in a non-aseptically operated, mixed-culture environment by utilizing operating procedures that consider the differential inhibitory effects of pH to Nitrosomonas and Nitrobacter.

4. Data obtained from the final preliminary nitritification study demonstrated successful system continuity through a wide range of feed ammonia-N concentrations. Again it was shown that it was possible to control the nitritification pattern that occurred during the oxidation of ammonia to nitrite by maintaining a relatively high pH level (9.5 or above).

5. The primary nitritification study showed that a large, stable, reproducible population of nitrite-forming bacteria may be cultured and maintained by a daily input of ammonium sulfate feed solution to a complete-mix treatment regime. Good process control (pH) was required to ensure the biological transformation of ammonia-N to nitrite without simultaneous production of nitrate-N. Biological nitritification by this system was a reliable process for accumulating relatively high concentrations of nitrite.

6. The preliminary and primary denitrification studies validated the fact that strain 39 of Achromobacter liquefaciens was capable of

reducing relatively high concentrations of nitrite-N. The draw-and-fill mode of continuous operation in the primary study indicated that this form of non-conventional denitritification was a reliable process for removing nitrites from wastewater.

The conclusions arrived at in this research indicate the nitritification-denitrification concept developed in this study may eventually evolve into an economical and reliable system. A hypothetical flow diagram for such a retrofit system might follow a design sequence as shown in Figure 36 and described below.

Lime would be added to secondary sewage treatment plant effluent in the lime reactor. The reactor will not only serve as a flocculation basin, but will also function as a preaeration basin. Polymer and/or ferric chloride may also be added at this point. The purpose of the lime addition is to remove residual particulate organic-carbon prior to the nitritification stage, thus allowing stable oxidation of ammonia to nitrite and to raise the system pH to the range 9.5 to 11.5. Lime also removes heavy metals potentially toxic to the microbial populations used in the nitritification reactor. Lime, polymer, and ferric chlorides are all proven phosphate removers. Since phosphorus is also a plant nutrient, this scheme allows phosphorus to be a limiting factor for eutrophication potential. However, care must be exercised to provide adequate phosphorus for the nitritification and denitritification reactions.

The effluent from the lime reactor would next enter the primary clarification basin. The sludge could be pumped to sludge processing

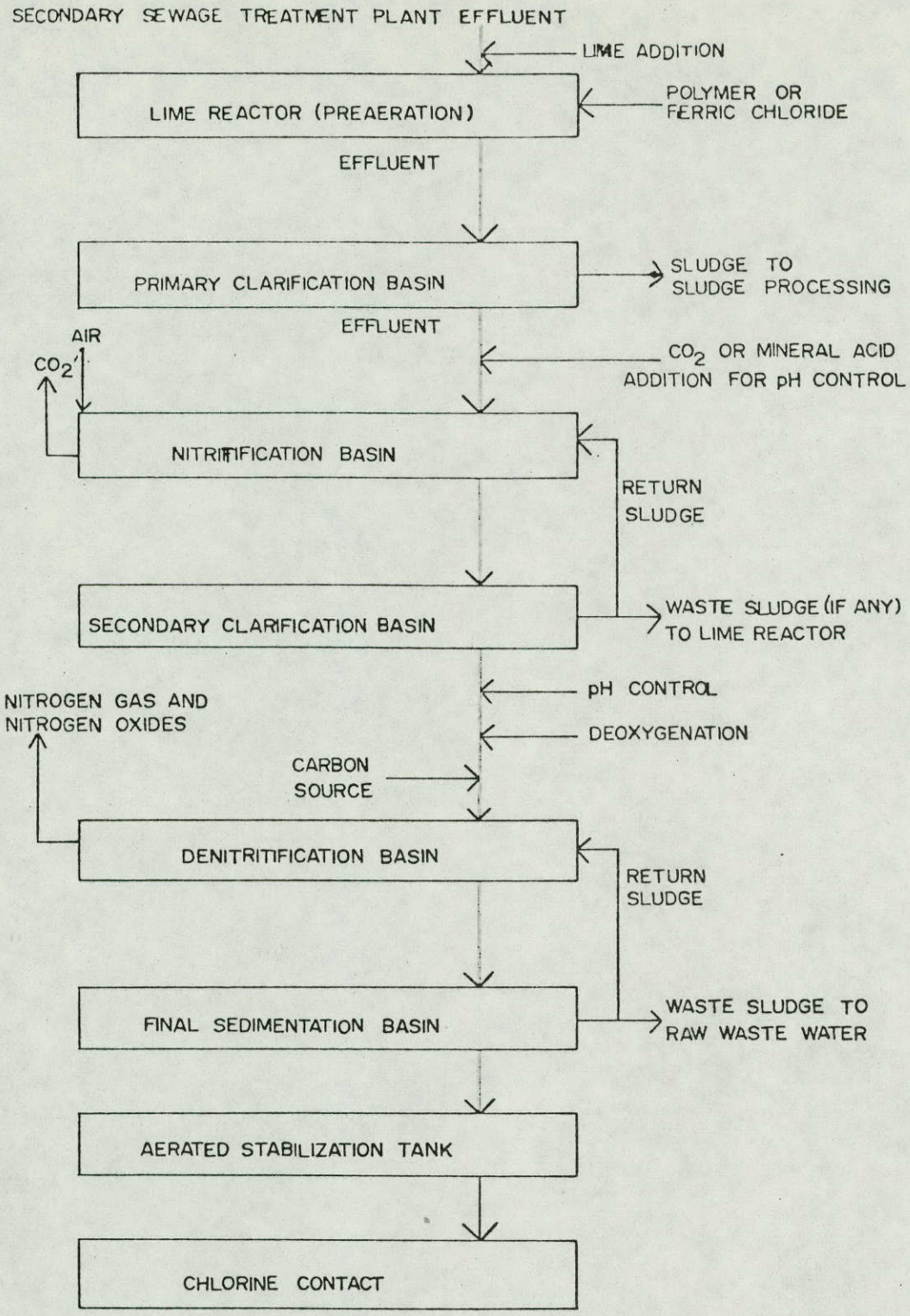

Figure 36. Flow chart for proposed sequence for a non-conventional nitrification-denitrification facility.

while the clarified liquid would be treated with CO_2 or mineral acid in order to decrease the pH to a range conducive for the growth of nitrite-forming bacteria, but inhibitory for nitrate-forming bacteria prior to introduction into the aerated nitritification reactor (complete-mix, continuous-aeration basin).

The effluent from the nitritification basin would next enter the secondary clarifier where sludge would either be wasted to the lime reactor or be returned to the nitritification reactor.

The effluent from the secondary clarifier would be deoxygenated before addition to the anoxic denitrification reactor containing strain 39 of Achromobacter liquefaciens. A carbon source would be added as a hydrogen donor and the pH regime would be monitored to ensure the maintenance of the proper pH level.

Overflow from the denitrification basin would enter the final sedimentation basin. The sludge would leave the clarification basin via the return sludge stream and/or the waste sludge stream. Clarified effluent would then be stabilized, chlorinated, and released.

Lime treatment may possibly provide an interesting alternative to the waste treatment sequence described above. It is a well-known fact that high pH levels resulting from lime treatment may produce sterile waste streams. If sterile conditions could be maintained until the stream reaches the nitritification reactor, the pH level need not be maintained at a level inhibitory to nitrate-forming bacteria. Thus, less ammonia-N would be lost from the system through volatilization. In addition, not having to maintain conditions inhibitory to nitrate production should simplify operational techniques considerably.

Although this non-conventional nitritification-denitrification system seems to be a potential solution toward the problem of eliminating unwanted nitrogen from wastewater, the process operating considerations remain frequency and inadequate. The data reported in this research represent a contribution to the problem of development of a non-conventional nitritification-denitrification process, but considerably more research is needed in order to be able to utilize the basic study findings in actual wastewater treatment situations. In conjunction with the results of this research and the conclusions drawn, the following recommendations are made relative to the anticipated development of such a non-conventional nitritification-denitrification system.

1. Other mechanisms for the selective inhibition of the second step of nitrification resulting in the accumulation of nitrite should be investigated.

2. Additional research on the role Achromobacter liquefaciens (strain 39) and other bacteria capable of reducing nitrite should be undertaken. Considerably more basic knowledge on such organisms is required if one is interested in monitoring their activities under various environmental conditions.

3. Nutritional requirements which may be selective for the growth and activity of nitrite reducers should receive special considerations.

4. Nitrite is toxic to many microorganisms; yet it is not known to what extent it may inhibit the denitrification process. An experimental method should be devised to make this determination.

5. Certain wastewaters contain secondary and tertiary amines. The accumulation of large concentrations of nitrite may create the potential for the formation of nitrosamines (a proven carcinogen). A determination of this potential should be attempted.

6. The ultimate goal should be to develop a more cost-effective denitrification treatment technology similar to the one outlined in Figure 36 which is capable of treating secondary sewage treatment plant effluent. Different research approaches should be used in order to find effective means for optimizing or stimulating conditions necessary for such a system.

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APPENDIX

A. *Nitrosomonas sp.* Medium Preparation Instructions

B. Procedure Utilized to Develop a Biological "Seed Culture" Capable of Oxidizing Nitrite-N to Nitrate-N

C. Peptone Yeast Extract Medium

D. Reviving Lyophilized Cultures

APPENDIX A: NITROSOMONAS MEDIUM PREPARATION INSTRUCTIONS

$(NH_4)_2SO_4$	3.0 g
K_2HPO_4	0.5 g
$MgSO_4$	0.05 g
$CaCl_2$	0.004 g
Chelated iron (Chel-138-HFe, Geigy Chem. Co.)	0.1 mg Fe
Cresol red	0.05 mg
Distilled water	1.0 L

Autoclave calcium and magnesium salts separately to avoid precipitation. Maintain pH during growth with sterile 50% K_2CO_3. pH should be maintained at 8.2-8.4.

Transfer culture to fresh medium upon arrival. It is imperative that the medium be prepared according to directions in clean glassware. As the cells multiply, indicator in the medium will change from pink to yellow. The pH of the medium should be maintained by neutraolizing with a drop or two of 50% K_2CO_3 (sterile). Abundant growth should be evident in static culture in 7 to 10 days. Rate of growth can be accelerated appreciably by incubation in a reciprocal shaker. Growth should be evident in 3 to 5 days under these conditions.

APPENDIX B: PROCEDURE UTILIZED TO DEVELOP A BIOLOGICAL "SEED CULTURE" CAPABLE OF OXIDIZING NITRITE-N TO NITRATE-N

One liter of elective medium was prepared as described in Appendix A. The sterile medium was placed in a two-liter florence flask. Ten ml of activated sludge was collected from a local secondary sewage treatment plant and added to the medium. Ten grams of soil was collected from a local sewage oxidation pond and added to the medium. The flask contents were gently but continuously aerated. The pH level was maintained in the range from 6.0 to 8.0.

After 17 days, nitrates were detected. It was, therefore, assumed that the "seed culture" contained nitrifying bacteria (i.e., Nitrosomonas and Nitrobacter).

In order to maintain a viable "seed culture" of nitrate producers, the "seed culture" was subcultured to new media periodically. The seed culture was not utilized as a source of Nitrobacter unless nitrate had been freshly detected.

APPENDIX C: PEPTONE YEAST EXTRACT MEDIUM

Ingredient	Quantity
Peptone	10g
Yeast Extract	5g
NaCl	5g
Distilled Water	1,000 ml

Dissolve and adjust the pH to 7.2. Sterile at 15 lbs/20 min.

APPENDIX D: REVIVING LYOPHILIZED CULTURES

To open the ampoule, make a file cut across the tube at the mid-point of the cotton wool plug and break by pressure or by touching the cut with a red-hot glass rod of small diameter.

To transfer the culture, add aspetically to the dried culture a few drops of appropriate broth using a Pasteur pipette; mix well and transfer the total mixture to a test tube of the same medium. Incubate at the appropriate temperature and, after allowing sufficient time for growth to occur, sub-culture as required. Allow at least one week for incubation of the lyophilized material before discarding as non-viable.

Ampoules not opened soon after receipt should be stored in the dark, preferably in a cool place.