BATCH STUDIES

ON THE

BIOLOGICAL DENITRIFICATION OF WASTEWATER.

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BY

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ABSTRACT :

This dissertation examines the influence of the process variables, organic carbon concentration, nitrate concentration, pH, organism concentration, and temperature, on the rate of denitrification of dominant batch cultures of Pseudomonas denitrificans. The aim of the work was to determine which of the first four variables were important in controlling the rate, and whether significant interactions existed between the variables. As well, the ability of these typical bacterial denitrifiers to operate at low temperature conditions was to be ascertained.

The experimental procedure indicated that pH and carbon concentration are the major influences on the unit denitrification rate as well as the overriding factor temperature which profoundly influences any bacterial process. Organic carbon concentration controls the rate up to the point where the stoichiometric requirements for nitrate reduction

and the carbon needs for new cell growth are satisfied. A broad pH optimum within the normal range experienced in most wastewater treatment conditions was demonstrated. The temperature dependancy of the unit denitrification rate was shown to follow an Arrhenius relationship between 3°C and 27°C. As well, for the simplified system investigated the unit rate was independent of nitrate concentration.

The dominant culture was related to mixed cultures of activated sludge to provide an estimate of the denitrifying rate of activated sludge on a similar simple batch system.

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Introduction.

Removal of nutrient materials necessary for growth from wastewater is a recognized method of limiting artificial eutrophication of surface waters. Nitrogen and phosphorous in addition to carbon are required in relatively large quantities by aquatic fauna and flora, and are cited most frequently as the growth limiting parameters. Existing conventional activated sludge waste treatment plants are designed primarily for organic carbon removal, and achieve only 30% to 50% reduction of the inflowing nitrogen and phosphorous. Chemical precipitation of phosphorous has been developed to the stage where phosphorous in wastewater effluents can be practically and economically controlled. However, the problem of nitrogen reduction is under active investigation as nitrogen has been determined to be the controlling nutrient in several cases, and the combined effect of nitrogen and phosphorous is suspected in others.

Biological nitrification and denitrification appears to be the most feasible method of nitrogen reduction. Nitrification, i.e. the conversion of ammonia through nitrite to nitrate through the action of two strains of autotrophic bacteria Nitrosomonas and Nitrobacter has been practised at activated sludge plants for many years because of the adverse

effect of ammonia on the receiving water. When ammonia is discharged to a receiving stream these two types of nitrifying bacteria thrive by aerobically metabalising ammonia using the bicarbonates as a carbon source. As a result, dissolved oxygen is removed from the stream in addition to that required to satisfy the residual carbon requirement of the effluent. Many pollution control agencies require that nitrification of wastewater be practised. Consequently, this phase of the nitrification-denitrification sequence has been the subject of considerable research. However, denitrification, or the reduction of nitrate to elemental nitrogen gas by the activities of certain heterotrophic bacteria such as Pseudomonas denitrificans has received relatively little attention.

Most of the recent work on denitrification has concentrated on mixed cultures of activated sludge operating on complex wastes to demonstrate the possibility of nitrification-denitrification. The variety of organic materials involved, and the many organisms interfering with one another obscure the fundamental processes operating and, therefore, create difficulty in the determination of design data. Laboratory reactors operating on single carbon sources upon which a single micro-organism is grown in batch culture with all other nutrients in excess have been successful in elucidating carbon removal kinetics useful in conventional

waste treatment design. This work extends the principle to the examination of factors affecting anaerobic nitrate metabolism involved in bacterial nitrogen removal from wastewater.

In cold temperature regions, it will be necessary for the denitrification process to operate at temperatures approaching 5°C. Therefore, emphasis is placed on evaluating the effect of temperature on unit denitrification rate.

Scope of the Work.

Batch laboratory investigations of pure cultures of Pseudomonas denitrificans utilizing a defined media were undertaken to study the factors affecting denitrification rate.

Inititally the reactors were run at 27°C in a completely anaerobic state since temperature and dissolved oxygen were considered major variables which would mask the effect of pH, carbon to nitrate ratio, nitrate concentration, and organism concentration. A full factorial experimental design of 16 experiments was run on these four variables to screen their relative effects on denitrification rate. Once these influences were established separate experimental series were undertaken to determine the optimum pH range and the variation of denitrification rate with carbon concentration.

The temperature dependency of the specific denitrification rate was then established by running duplicate experiments at 5°C, 10°C, 15°C, 20°C, and 27°C at the optimum conditions of pH and carbon concentration derived from prior experiments.

The dominant culture studies were then related to mixed cultures by a series of batch experiments utilizing varying proportions of Pseudomonas denitrificans to activated

sludge metabolizing the same defined media.

Nitrogen balances were carried out on all the closed system batch reactors as a check on the accuracy of the analyses. As well, the organism growth during the reaction was monitored both by total suspended solids measurements, and by organism colony counts.

Literature Review.

Justification for Nitrogen Removal.

Nitrogen and phosphorous have been implicated as the nutrient materials responsible for accelerating artificial eutrophication of natural waters (Tarzwell, 1953), (Lackey, 1958), (Sawyer, 1967), (Wuhrmann, 1969), (Fruh, 1969). Together their presence stimulates the growth and reproduction of aquatic micro-organisms and plants to such an extent that originally oligotrophic lakes have changed to eutrophic water bodies within a matter of years. Nitrogen is present in domestic wastewater in the range of 15 to 35 mg/l as total nitrogen, while phosphorous ranges from 6 to 12 mg/l as total phosphorous (Fruh, 1969). Many authors including Wuhrmann, (1969) have demonstrated that domestic and industrial wastes contribute significantly greater quantities of these elements as compared to runoff from agricultural areas. In most cases phosphorous has been cited as the critical material which if removed would control eutrophication. Accordingly, most of the research has been directed towards phosphorous removal. Chemical precipitation methods are now available to remove phosphorous to levels which will not damage the aquatic environment. However, in other cases nitrogen has been determined as the controlling nutrient (Fruh 1969) partially because the amount required for cell synthesis is approximately 15 times the phosphorous requirement.

The discharge of nitrogen as ammonia in wastewater effluents creates a vigorous nitrifying flora of Nitrosomonas sp and Nitrobacter sp which convert the ammonia to nitrite and nitrate respectively and result in an oxygen demand in addition to that of any residual carbonaceous material (Delwiche, 1956). Many wastewater authorities having recognized this fact, require that bacterial nitrification, or conversion of NH_3 to NO_3, be carried out in the treatment plant to avoid further oxygen depletion due to nitrogen compounds. However, it is well documented that nitrate is used preferentially to ammonia by many species of algae as a nitrogen source, so that the fertilization problem remains unless the nitrogen is converted to a form which is not directly available to algae.

It has been argued that nitrogen removal is not a sensible method of controlling surface water fertilization since many algal species are capable of fixing nitrogen from the atmosphere sufficient for their needs. However, it is conceivable that denitrification occurs at a similar rate to fixation under natural conditions and, therefore, problems of rapid lake fertilization occur only when nitrogen added from wastewater and agricultural activities upset this delicate balance of nitrogen transformations. Delwiche (1970) is particularly alarmed at this imbalance and the possible consequences of a nitrate build-up in the environment.

Probably the strongest argument in favour of

investigating nitrogen removal methods is the fact that wastewater authorities have made nitrogen removal mandatory in many areas in which nitrogen or the interaction between nitrogen and phosphorous are suspected as the major causes of surface water eutrophication.

Nitrogen Chemistry

The chemistry of nitrogen is complex because of the several valence states that nitrogen can assume and because changes in valence can be brought about by living organisms. Nine oxidation states are known with a 10th contested as shown in Table 1 (Fewson 1961a). The compounds which can be formed by biological reactions are marked with an asterisk. The biological transformations occur under a variety of conditions, sometimes resulting in a valence change and sometimes not.

The main biological processes involving nitrogen can be summarized as shown in Figure 1. (Painter 1970).

In some cases such as nitrification, the object for the organism is to gain energy from the transformation, while in others, such as assimilation, the purpose is to make nitrogen available for incorporation into cellular material such as protein. These processes occur on a world wide scale, and are the phenomena responsible for the maintenance of all living organisms. Biological transformations carry out most of the changes in this so-called nitrogen cycle pictured in Figure 2.

TABLE 1.

NITROGEN COMPOUNDS INVOLVED IN BIOLOGICAL TRANSFORMATIONS

After Fewson (1961a).

Oxidation State	Formula	Name
+ 6	# NO_3	Nitrogen Trioxide
+ 5	* NO_3^-	Nitrate
+ 4	NO_2	Nitrogen Dioxide
+ 3	* NO_2^-	Nitrite
+ 2	* NO	Nitric Oxide
	$N_2O_3^=$	Nitro-Hydroxylamate
+ 1	(NOH)	Nitroxyl
	* N_2O	Nitrous Oxide
	$N_2O_2^=$	Hyponitrite
	$NO_2{:}NH_2$	Nitramide
	$NH(OH)_2$	Dihydroxy Ammonia
	* N_2	Nitrogen
	HONH NHOH	Dihydroxy Hydrazine
- 1	NH_2OH	Hydroxylamine
- 2	NH_2 NH_2	Hydrazine
- 3	* NH_3	Ammonia

Valence State Contested.

* Compound Formed by Biological Reaction.

FIGURE NO. 1

BIOLOGICAL TRANSFORMATIONS OF NITROGEN

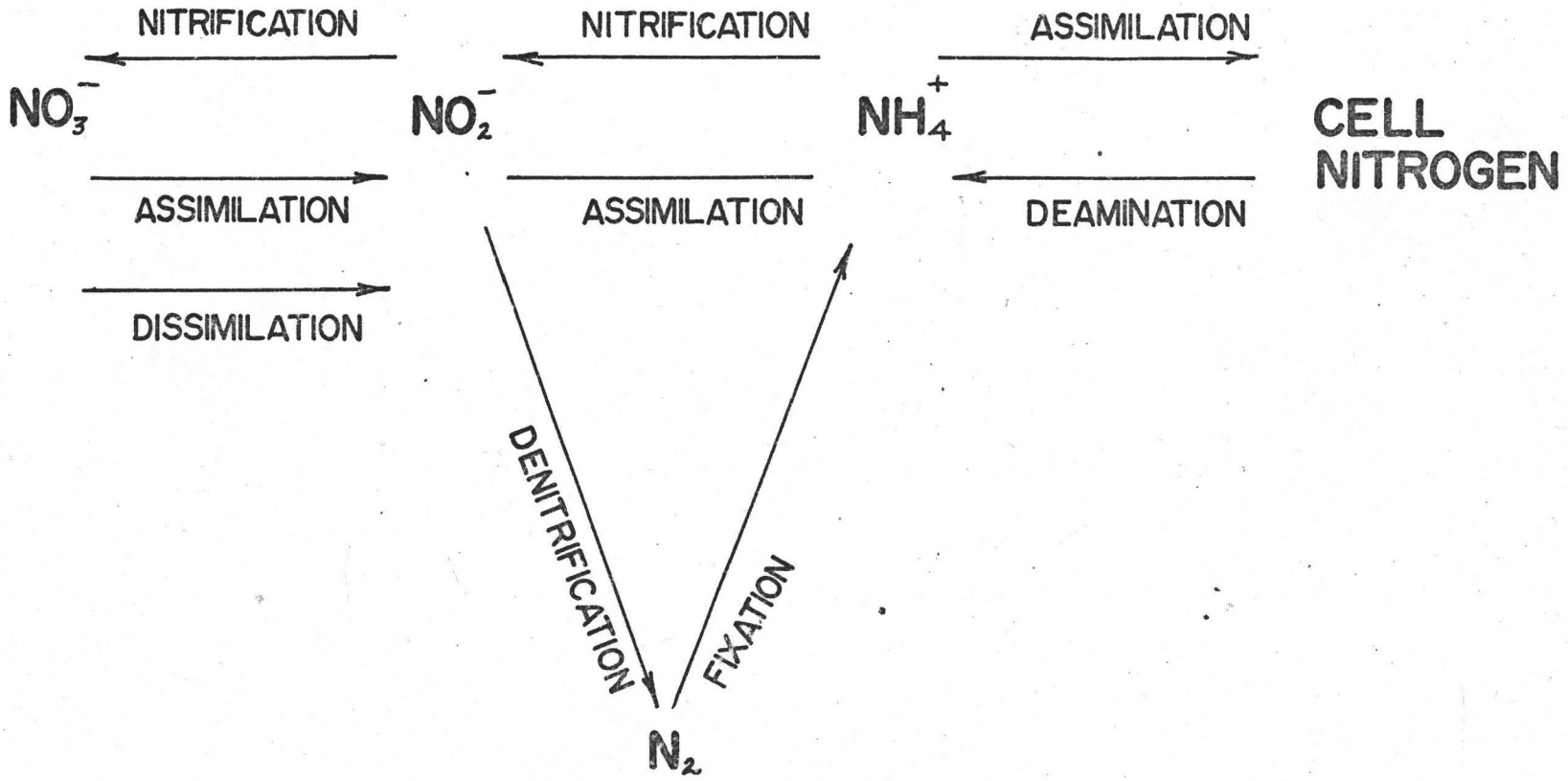
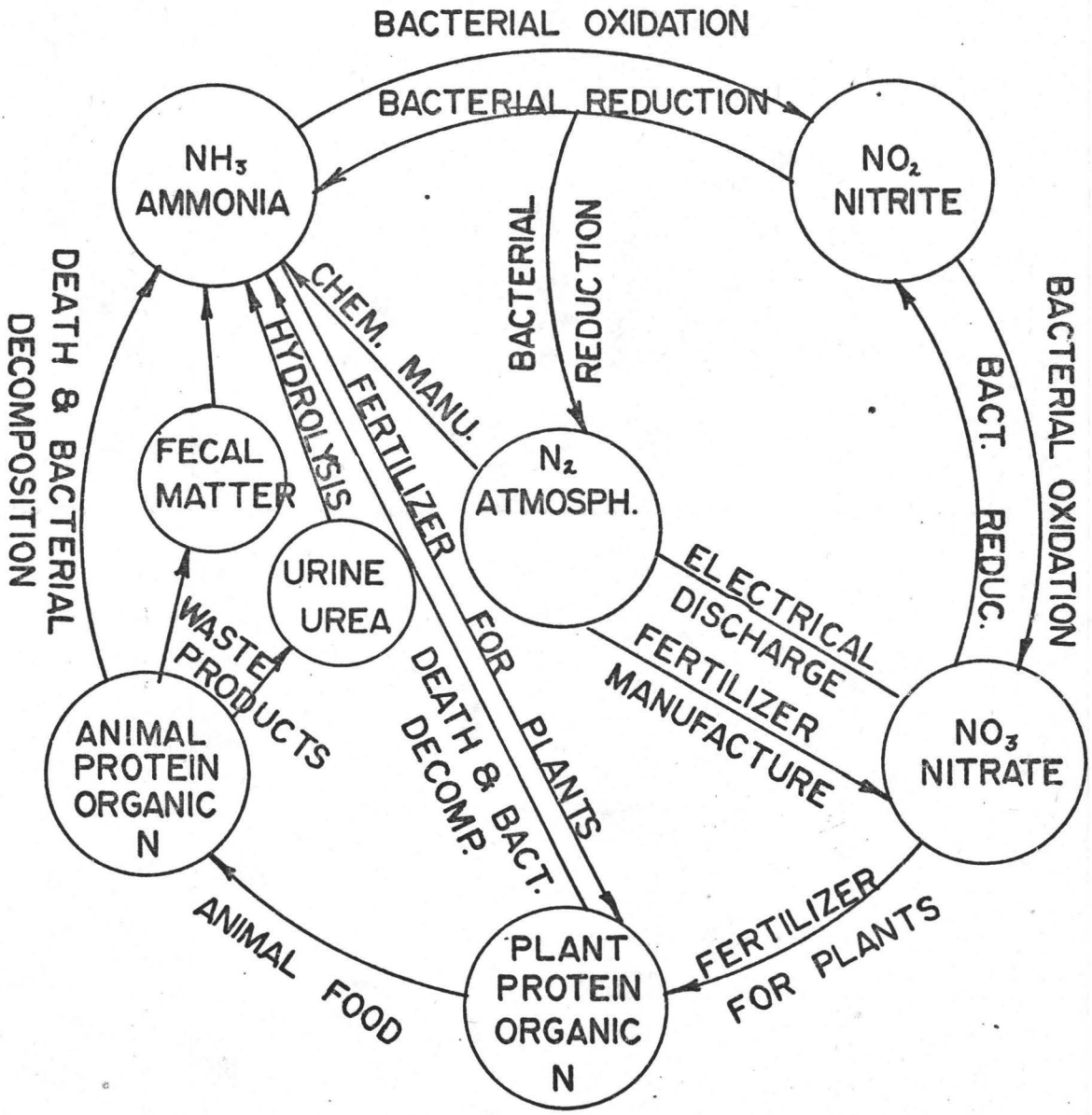


FIGURE NO. 2

NITROGEN CYCLE - MACROSCALE

(SAWYER, 1960)



The atmosphere acts as a reservoir from which nitrogen can enter or leave the cycle, and most of these processes achieve some sort of equilibrium. However, the activities of modern man have considerably disrupted this cycle by the extensive use of fertilizers for agriculture, and by the discharge of considerable quantities of domestic and industrial waste. Delwiche 1970 has expressed concern that the process of denitrification is not occurring as rapidly as the input processes and fears an excessive build-up of nitrates in the environment to toxic levels.

Nitrogen Transformations in Wastewater Treatment.

Most of the nitrogen compounds compounds comprising the 15 to 35 mg/l of total nitrogen in domestic wastewater are in the form of ammonia or are bound up in organic compounds such as urea which are readily decomposable to ammonia through the activities of bacteria. Wuhrmann (1969) estimated that nitrogen in settled domestic wastewater is present as follows.

NH_4 - N	55 - 60%
Organic N	40 - 45%
Oxidized Forms	0 - 5%

In industrial wastewater the total nitrogen concentration can be much greater than domestic wastewater and could be mostly composed of nitrate compounds depending upon the industrial activity. Present day wastewater treatment plants are designed primarily for the removal of 90-95% of the

inflowing carbonaceous material employing some modification of the activated sludge process. Essentially the process is a series of physical separations, biological and chemical reactions designed to convert soluble organic material into biological cells which are separated from the liquid phase for ultimate disposal. Nitrogen removal is not a specific aim of present day waste treatment. Any nitrogen content reduction is simply complementary to the removal of organic carbon compounds.

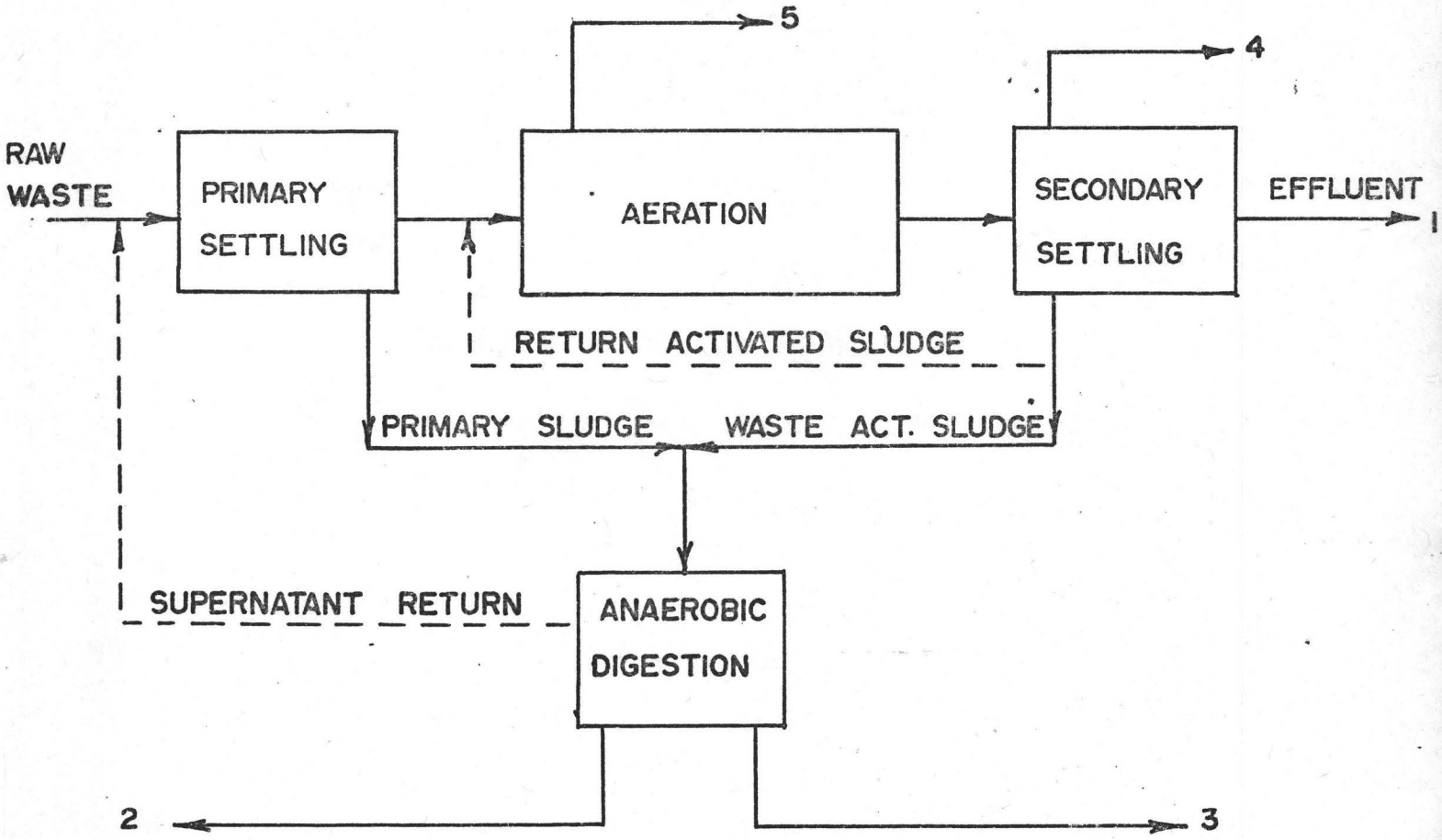
A schematic diagram of the processes involved in a typical conventional wastewater treatment plant is shown in Figure 3. The conversions of nitrogen compounds during wastewater treatment can best be traced by following the flow pattern through each of the units generally used in activated sludge plants.

Primary settling of 1-2 hours removes approximately 60% of the inflowing suspended solids containing 30% of the organic carbon. Solids accumulating in the form of sludge with a solids content of 6-9% are mechanically removed and transported to an anaerobic digester for further treatment. Since municipal waste water and many other industrial organic wastes contain dead cellular material and proteins, nitrogen from 15 to 20% (Johnson 1969) of the inflowing load will be removed in this sludge. However, a portion of this nitrogen will be returned to the plant flow as soluble nitrogen

FIGURE NO. 3

CONVENTIONAL ACTIVATED SLUDGE WASTE TREATMENT SCHEMATIC SHOWING NITROGEN LOSSES

(LUDZACK, 1962)



NITROGEN LOSSES

1 Effluent dissolved inorgnic nitrogen
suspended solids organic nitrogen

2 Digested Sludge organic nitrogen
ammonia

3 Digester Gas nitrogen gas

4 Denitrification in secondary sludge

5 Denitrification within sludge flocs

compounds in the supernatant discharge from the digesters, i.e. sludge treatment and dewatering.

In the aeration section, the waste flow is contacted with a preconditioned mass of mixed micro-organisms chiefly bacterial flocs - called activated sludge. These organisms utilize the soluble organic carbon compounds as a source of food, oxidizing them for energy and using carbon and amino acids as building blocks for new cells. These bacteria form flocs which further entrap any suspended material in the waste flow. The chief reactions occurring are similar to those proposed by Eckenfelder and O'Connor (1961).

1. $C_xH_yO_2 + O_2 \xrightarrow{\text{Cells}} CO_2 + H_2O \ {}^{-}\Delta H$

Respiration or oxidation of organics

2. $C_xH_yO_2 + NH_3 + O_2 \xrightarrow{\text{Cells}} \text{cells} + CO_2 + H_2O \ {}^{-}\Delta H$

Sythesis of cells

3. $\text{cells} + O_2 \xrightarrow{\text{Cells}} CO_2 + H_2O + NH_3 \ {}^{-}\Delta H$

Degredation of cellular material (endogenous respiration).

The latter reaction occurs when cells die or when food becomes scarce and cells oxidize stored cellular material. Proteolytic bacteria such as Pseudomonas sp, Proteus sp, and Bacillus sp attack organic nitrogen compounds and break these down to peptides, amino acids, purines and pyrimidines, (Delwiche 1956).

Some of the amino acids are available for direct incorporation into bacterial cell material while the remainder

undergo deamination to ammonia and simpler organic carbon compounds.

e.g. oxidative deamination of alanine

$$CH_3CHNH_2COOH + \tfrac{1}{2}O_2 \xrightarrow{\text{Alanine deaminase}} CH_3COCOOH + NH_3$$

alanine — pyruvic acid — ammonia

Sawyer (1960)

The amount of nitrogen taken up by the growth of activated sludge depends upon the relative amounts of carbon oxidized for energy and carbon assimilated to cells. For many bacteria the ratio of change in carbon concentration to change in organism concentration is approximately 0.5 Hoover and Porges (1952) have proposed an approximate formula for activated sludge organisms growing on milk wastes of $C_5H_7NO_2$ indicating that 12% of activated sludge consists of nitrogen. A ratio of carbon to nitrogen to phosphorous in sludge organisms of 40:5:1 has been substantiated by several authors (Eckenfelder, 1961), (Helmers 1951). Nitrogen removal from wastewater in the aeration tank in the form of sludge solids, therefore, can be readily calculated. However, other nitrogen conversions also occur simultaneously in the aeration section. The autotrophic bacteria Nitrosomonas sp and Nitrobacter sp respectively convert ammonia to nitrite, and nitrite to nitrate. If the aeration period is sufficiently long - 6.0 hrs, the carbon loading sufficiently low 0.3 to 0.4 lb BOD/lb MLSS, and the solids residence time - 3 to 4

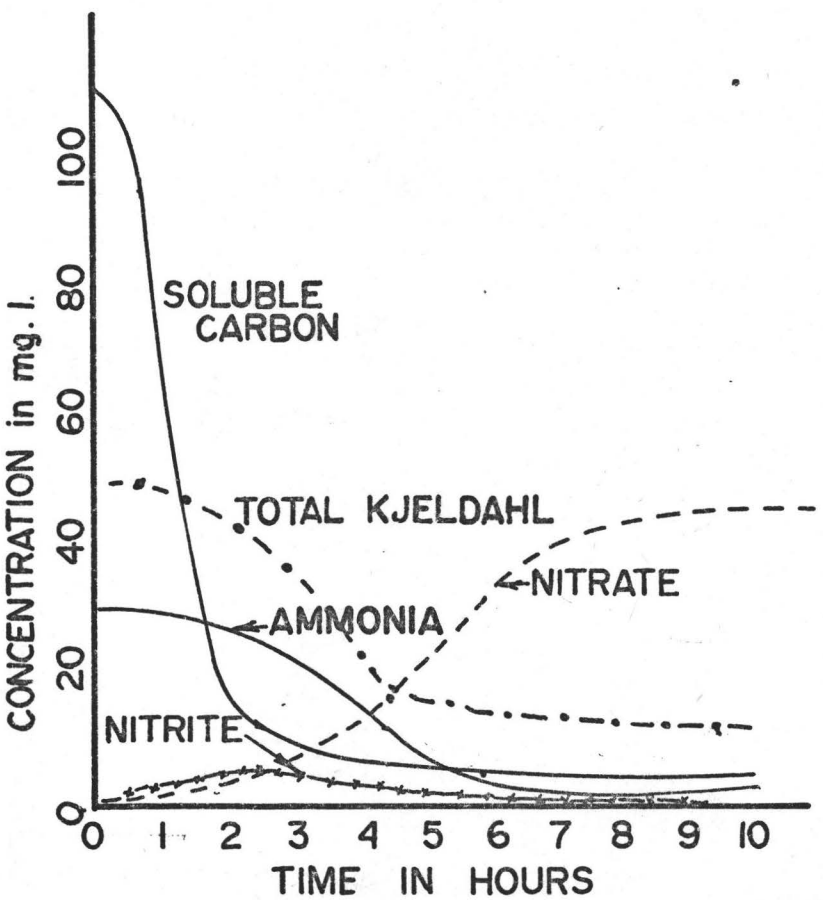
days, the nitrifying bacteria population will reach a sufficient concentration for all the ammonia in excess of cell assimilation requirements to be converted to nitrate, (Balakrishnan, 1969). Since nitrifiers are relatively slow growers in comparison to heterotrophic bacteria, the sludge wasting necessary for conventional plants to achieve 90% carbon removal does not allow nitrifying bacteria to reach sufficient numbers to effect ammonia conversion. In extended aeration plants with detention times of 24 hours conversion to NO_3 is common.

Denitrification of nitrates formed through the action of nitrifying bacteria has been hypothesized to occur in the aeration section within the bacterial flocs where dissolved oxygen levels may drop to zero. The extent of this reaction along with any nitrogen fixation due to bacterial action is probably small. Results from a typical batch experiment on domestic wastewater showing the changes in nitrogen compounds with aeration period are outlined in Figure 4.

In the final clarifier of a waste treatment plant the activated sludge solids are separated from the liquid effluent within a 1 to 2 hour detention period. Depending upon the process modification 25 to 100% of the total plant flow might be recirculated to the aeration section to seed the incoming waste. The remainder of the sludge would be wasted to remove carbon from the process and to control the solids in the aeration section. Denitrification of nitrates to elemental

FIGURE NO. 4

TYPICAL BATCH CONVERSION OF NITROGEN COMPOUNDS BY ACTIVATED SLUDGE



nitrogen gas can occur in the sludge solids if they are retained too long in the final tank. A typical liquid effluent from the final clarifier would contain 8 - 25 mg/l of total nitrogen of which 80% would be nitrate or ammonia nitrogen depending upon the aerator operation. The rest of the total nitrogen would be organic nitrogen bound up in sludge solids not separated from the process. High dissolved nitrogen concentrations in the effluent are usually due to the return of soluble nitrogen in the form of ammonia from digestion of sludge solids. Digestion of primary and secondary sludge in anaerobic reactors at 90-95°F reduces sludge volumes by liquifaction and gasification so that the digested sludge is thickened, easily dewatered and non-putrecsible. Supernatant liquor from this process is normally returned to the aeration section for further aerobic treatment along with 2/3 of the nitrogen in the original sludge solids in the form of ammonia. Some nitrogen is vented to the atmosphere as elemental nitrogen gas from the digestion process.

A schematic diagram proposed by Ludzack (1962) Figure 3 shows the process responsible and location of nitrogen losses from a conventional activated sludge waste treatment plant.

Alternative Methods of Nitrogen Removal from Wastewater.

Nitrogen removal methods can be classified into three

categories; biological, chemical, and physical processes as listed below according to Elliason (1968).

1. Biological Processes.
   - (a) Algae stripping
   - (b) Aerobic nitrification followed by Anaerobic Denitrification.
   - (c) Aerobic process modifications.

2. Chemical Processes.
   - (a) Ammonia Stripping.
   - (b) Ion exchange.
   - (c) Electro dialysis
   - (d) Electrochemical treatment.

3. Physical Processes.
   - (a) Land application.
   - (b) Reverse Osmosis
   - (c) Distillation.

Each of these methods could be applicable in particular cases, but most are too expensive for general use at municipal and industrial waste treatment facilities.

1(a) Algae Stripping - Algae cultured in both stabilization ponds or activated algae reactors are capable of utilizing large quantities of nitrogen in both nitrate and ammonia forms. The nitrogen incorporated into their bodies can be removed by some harvesting technique - usually centrifugation or froth flotation. Johnson (1969) reports that 50% of inflowing nitrogen is removed in a single pond and 98% in a

series of ponds when harvesting techniques are employed. Unless a suitable market can be found for the high protein content of sewage grown algae to defray the cost of harvesting and ultimate disposal this process is usually economically not feasible.

1(b) Aerobic Nitrification followed by Anaerobic Denitrification.

Bacterial nitrification and denitrification is recognized as a feasible method for removal of nitrogen from wastewater (Eckenfelder 1968), (McCarty, 1969), (Wuhrmann 1969), (Johnson, 1964).

Nitrification refers to the conversion of ammonia to nitrite and nitrate through the action of autotrophic bacteria Nitrosomonas sp and Nitrobacter sp. These organisms gain their energy by oxidizing ammonia and nitrite as shown in the following reactions proposed by Godelewski (1895), and Meyerhof (1957).

Nitrosomonas sp

$$NH_4 + 1.5O_2 \longrightarrow 2H^+ + H_2O + NO_2 \qquad -84 \text{ K cal.}$$

Nitrobacter sp

$$NO_2^- + 0.5\ O_2 \longrightarrow NO_3^- \qquad -17 \text{ K cal.}$$

The bacteria utilize bicarbonates as a carbon source to build cellular material and the reaction is carried out under aerobic conditions. In normal wastewater treatment practise these organisms grow in the aeration section of a

conventional activated sludge plant. Usually total conversion of NH_3 to NO_3 can be achieved if aeration periods in excess of 6.0 hrs, dissolved oxygen levels >3.0 mg/l organic loadings less than 0.35 lb BOD/lb MLSS and a solids residence time of 3 days are maintained. The process is very sensitive to dissolved oxygen concentration, temperature, pH, carbon concentration etc., but considerable research has been carried out and the effects of these parameters well documented. Since the autotrophic bacteria grow much more slowly than the heterotrophic bacteria responsible for carbon removal the two processes are not compatible. In the carbon removal process the theory is to convert soluble carbon into cellular material, and waste these cells rapidly. Nitrifiers, therefore, never achieve great numbers in normal activated sludge operation. Several researchers (Barth 1968),(Mulbarger 1970), have, therefore, separated carbon removal into separate reactors in series with separate sludge separation and recycling systems so that both carbon removal and nitrification can be carried out as rapidly as possible.

Denitrification is the bacterial reduction of nitrate to gaseous end products - chiefly elemental nitrogen gas. The process is carried out by facultative anaerobes which utilize the energy present in an organic carbon substrate by successive dehydrogenations. The hydrogen ions and electrons removed are transferred by means of a series of enzymes to the final acceptor - nitrate, which is reduced. Phosphorylation

occurs during electron transfer with two moles of ATP formed per mole of nitrate (Spangler 1966). These high energy phosphate compounds are then ready for use in growth and reproduction of the bacteria. A high percentage of activated sludge organisms are capable of denitrification if anaerobic conditions prevail and many workers have demonstrated this ability in pilot and laboratory scale reactors (Johnson 1964), (Bringman 1959, 1961, 1960), (Wuhrmann, 1954, 1960a, 1960b, 1962, 1963, 1964, 1969a, 1969B), (Christianson 1956). Since nitrogen removal is a relatively recent consideration, the denitrification stage has received little attention in comparison to nitrification.

1(c) Aerobic Process Modifications - Sawyer (1969) reports that removal of nitrogen compounds is possible by binding them into cellular material. He proposes that the carbon: nitrogen:phosphorous ratio be controlled carefully by the addition of carbohydrate to the activated sludge reactors to convert all of these compounds to cells. No data is provided to substantiate the claims or estimate the cost.

2(a) Ammonia Stripping. Ammonia contained in wastewater can be removed as a gas in a stripping tower by raising the pH to approximately 11.0 by addition of lime (Slechta 1967). As this high pH ammonia is insoluble and the gas can be liberated from the liquid by forced air (3000 litres/litre of wastewater). Approximately 98% of the ammonia can be removed by this method either prior to or following biological

treatment. In either case considerable nitrogen remains in the effluent either as nitrate or as ammonia resulting from biological breakdown of complex nitrogenous organics. Scaling problems in aeration towers have been experienced due to deposition of calcium carbonate produced during pH adjustment. As well, the ammonia released to the atmosphere is readily soluble in the natural water vapour content and usually returns to the aquatic environment dissolved in rainfall. The efficiency of the process is also drastically reduced at low temperatures. The cold air contacting the wastewater quickly cools the water, and since NH_3 solubility is higher in cold water than warm, the air requirements to remove it are greater. As well, when the ambient temperature falls to $0^{\circ}C$ evaporative cooling freezes the wastewater at the air inlets rapidly making the towers inoperable (Farrell, 1970).

2(b) Ion Exchange Water reuse studies indicate that strong anion exchange resins remove organic nitrogen and nitrate while cation resins effectively reduce ammonia in aqueous solution. 80-92% removal (Eliasson, 1967). Problems with this process include a high degree of pretreatment to remove particulates and prevent clogging of the medium and the disposal of regeneration liquid - approximately 6% of total inflow. The process also awaits the development of an exchange resin selective to nitrate.

2(c) Electrodialysis Membranes which are selectively permeable to certain ions are set parallel to wastewater flow.

When a current is induced across the membrane ions migrate through the membrane. In a single pass unit approximately 50% of the selected ion can be removed. Problems include chemical precipitation of calcium carbonate and clogging of the membranes with organics and colloidal matter.

2(d) Electro-Chemical. Ammonia nitrogen can be effectively removed by the Föyn process (80-85%). Nitrogen and phosphorous are precipitated at a cathode set in a mixture of seawater and wastewater. Production of hydrogen gas lifts sludge to the surface while chlorine produced at the anode can be used for disinfection. Application to fresh water has not been attempted but would be possible if sufficient magnesium ion is provided.

3(a) Land Application - Spray and broad irrigation of waste water on permeable land with a suitable cover crop results in an uptake of nitrogen by plants but considerable quantities reach ground water and reappear as surface runoff. This procedure is limited to regions with a year round warm climate.

3(b) Reverse Osmosis. - Removal of nitrogen from municipal wastewater previously treated by conventional methods by reverse osmosis using a cellulose acetate membrane can achieve rejection of 60 to 70% of nitrate nitrogen, and 80% to 90% of the ammonia nitrogen (Smith 1970b). However, fouling of the membranes with organic compounds is a major factor in reducing membrane life. The cost of nitrogen removal by this method will probably preclude this use.

3(c) Distillation - The cost of distillation of wastewater effluents usually prohibits this method of nitrogen removal. However, where water is scarce it certainly provides a reliable removal method.

Factors Affecting Denitrification.

Organisms Responsible - Dissimilatory metabolism of nitrate is attributed to a group of facultative, anaerobic, chemo-organotrophic bacteria, which are relatively ubiquitous. Typical denitrifying genera are Pseudomonas sp, Micrococcus sp, Denitrobacillus sp, Spirillum sp, Bacillus sp, Achromobacter sp, (Painter 1970). Under anaerobic conditions these organisms will utilize nitrate as their hydrogen acceptor while metabolizing an organic substrate, but when oxygen is introduced into their growth media they quickly convert to normal aerobic oxidation of carbon. Wuhrmann and Mechsner (1963) isolated and studied six strains of denitrifiers from activated sludge including Pseudomonas sp, Spirillum sp, Micrococcus sp, and Xanthomonas sp. Similarly Smith et al (1970a)reported that 15 to 51% of the organisms scraped from a column actively denitrifying municipal wastewater were species of Pseudomonas sp, Achromobacter sp, and Bacillus sp, capable of reducing nitrate to nitrogen gas.

Biochemistry - Reduction of nitrate by certain chemo-organotrophs occurs through a series of complicated enzyme catalyzed reactions which can follow either the assimilatory or dissimilatory route. During assimilation, NO_3^- is reduced

and incorporated into cellular nitrogenous material by an aerobic process. However, in the dissimilatory route nitrate serves as the alternative hydrogen acceptor to oxygen. Three microbial reactions have been postulated by Alexander (1964).

(1) Complete reduction to ammonium, with transitory appearance of nitrite.

(2) Incomplete reduction of nitrate and accumulation of nitrite in the medium.

(3) Reduction to nitrite followed by evolution of gaseous compounds or denitrification.

In wastewater treatment we are mainly concerned with denitrification to produce N_2, N_2O or a mixture of these two gases. All micro-organisms using nitrate as a nitrogen source can carry out the first reaction whereas cultures incapable of complete reduction must be supplied with ammonium or other reduced nitrogen compounds for growth to proceed (Doelle 1969).

Fewson (1961a) proposed the biochemical pathway shown in Figure 5. This pathway assumes a sequence of two electron changes from the +5 oxidation state of nitrate to the -3 of ammonia, a shift of eight electrons. Isolated enzyme systems have been followed to determine the end products of the reactions with the responsible enzymes termed the "nitrate", "nitrite", "hyponitrite", and "hydroxylamine", reductases. The complexity of the enzyme systems involved in the alternate pathways available for Pseudomonas aeruginosa is illustrated in Figure 6.

Note that the aerobic pathway to oxygen and the nitrate reduction pathway differ only in the final enzymes, i.e. cytochrome oxidase versus nitrate reductase. Many other pathways have been proposed for other organisms utilizing nitrate as a hydrogen acceptor (Doelle, 1969), but all take the general form of the above simplified diagram.

Dissolved Oxygen. The sensitivity of denitrification to dissolved oxygen levels in a growth media is the most important environmental factor influencing the application of denitrification. It is generally accepted that denitrification is an anaerobic process occurring in pure cultures only in the complete absence of dissolved oxygen (Delwiche, 1956). Skerman and MacRae (1957a, 1957b, 1961) monitored dissolved oxygen levels in pure culture reactors and found that adapted cells of Pseudomonas denitrificans, (i.e. already metabolizing nitrate anaerobically) converted to normal aerobic oxidation of organics at dissolved oxygen levels as low as 0.2 mg/l. Similar results were reported by Sacks and Barker (1949) Assimilatory nitrate metabolism by Micrococcus denitrificans occurs under both aerobic and anaerobic conditions, but the presence of oxygen affects the dissimilatory activity in three ways according to Chang and Morris (1962).

(1) prevents adaptive formation of necessary enzymes.

(2) partially represses further dissimilatory enzyme syntheses.

(3) completely inhibits activity of preformed enzymes.

FIGURE NO. 5
BIOCHEMICAL PATHWAY FOR NITRATE REDUCTION (FEWSON, 1961a)

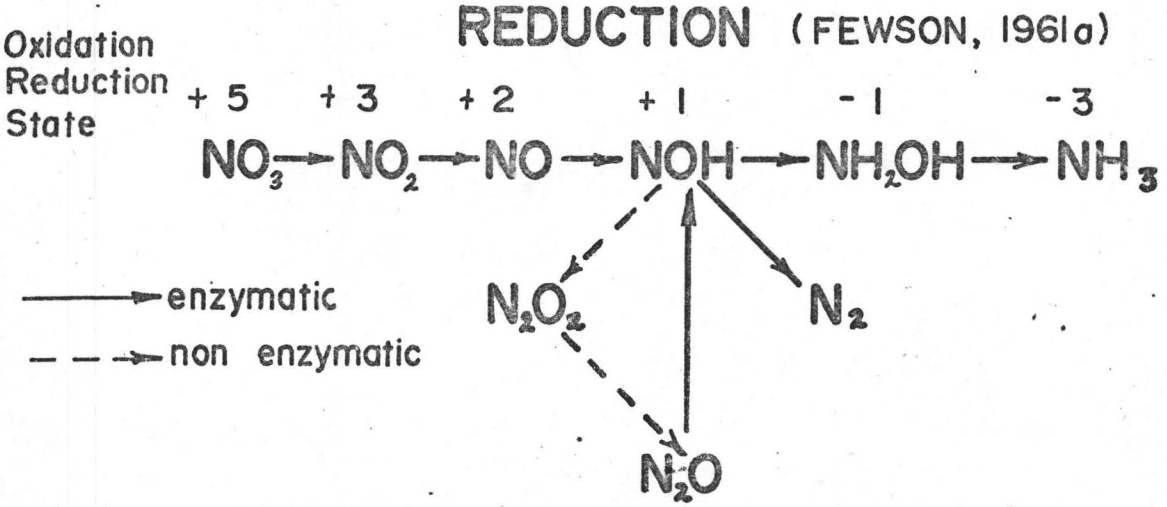
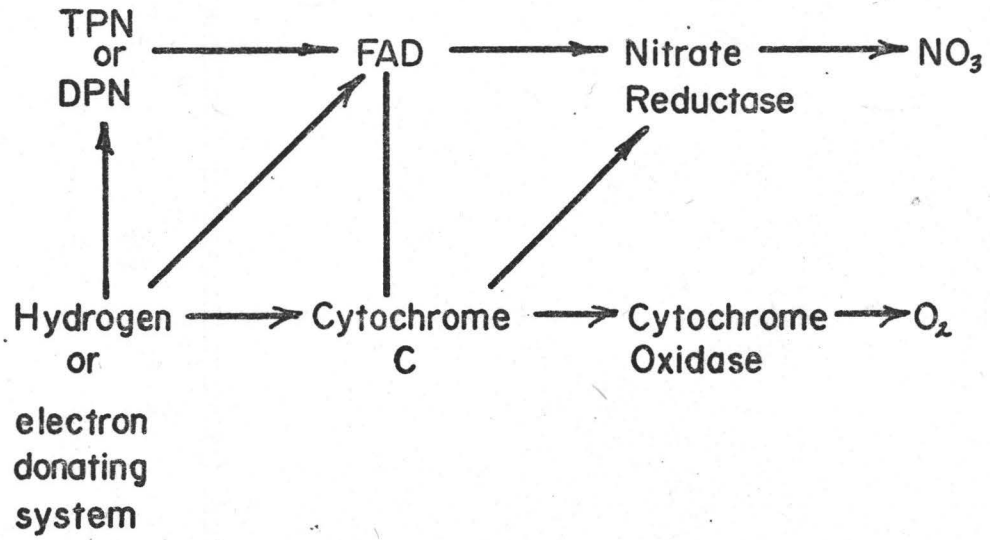


FIGURE NO. 6
ELECTRON TRANSPORT SCHEME FOR PSEUDOMONAS AERUGINOSA (FEWSON, 1961 b)



Other workers (Myers 1955), (Schmidt, 1962), show that denitrification definitely occurs under aerobic conditions in Warburg apparatus experiments. Skerman and MacRae (1957a, 1957b) suggest that denitrification in the presence of dissolved oxygen occurs because of a dissolved oxygen gradient across bacterial flocs with active denitrification occurring due to the activities of bacteria not directly exposed to dissolved oxygen.

In mixed culture batch experiments on sewage, Wheatland, (1959) showed that nitrate was reduced at oxygen levels of approximately 2.0 mg/l, but only at 10% of the rate obtained under strictly anaerobic conditions. In continuous tests as little as 0.1 mg/l of dissolved oxygen reduced the denitrification rate to 1/3 of the anaerobic rate while at 0.8 mg/l nitrification occurred and denitrification essentially ceased. When dissolved oxygen was present most of the nitrate was reduced only to the nitrite state. Pseudomonas stutzeri were isolated from the experimental reactors.

Wuhrmann and Mechsner (1963) reduced nitrate to nitrite in aerobic reactors at various rates dependant on the organism isolated and suggested that denitrification rates at a pH below 6.0 are practically independent of oxygen concentration.

McCarty (1969) denitrified agricultural drainage water containing significant quantities of dissolved oxygen by providing sufficient methanol to satisfy the nitrate

reduction demands and also utilize the dissolved oxygen by the normal aerobic degredation of carbon. Similarly, Smith (1970) denitrified municipal wastewater (12 mg/l NO_3 as N) on columns with approximately $\frac{1}{2}$ hr retention at influent dissolved oxygen levels as high as 6.0 mg/l with an effluent containing 1.0 to 1.5 mg/l of dissolved oxygen.

Temperature - Any bacterial process operates within a defined temperature range and the rate of bacterial activity is severely curtailed at both high and low temperature levels. In cold climate regions the ability of denitrifying organisms to operature at a useable rate at temperatures approaching 5°C will determine the practical application of the denitrification process for wastewater treatment. Most of the studies to date have been performed at 27°C+, close to the optimum temperature for most heterotrophs or at laboratory temperature of 20°C, possibly typical of summertime wastewater temperatures.

Wheatland (1959) performed batch tests on domestic wastewater with nitrate added up to 40 mg/l at temperatures of 5°C, 12°C, 18°C, and 25°C. At 5°C, he determined that the rate of denitrification was 1/3 of that occurring at 25°C.

In a series of continuous temperature runs at a 200,000 gpd pilot plant Mulbarger(1970) provided data on denitrification of nitrified municipal wastewater (37 mg/l NO_3 as N) using anaerobic activated sludge. Denitrification rates varied from 0.0016 mg NO_3 as N/mg of MLVSS-hr to 0.028 mg NO_3 as N/mg of MLVSS-hr at 8°C to 12°C and 0.013

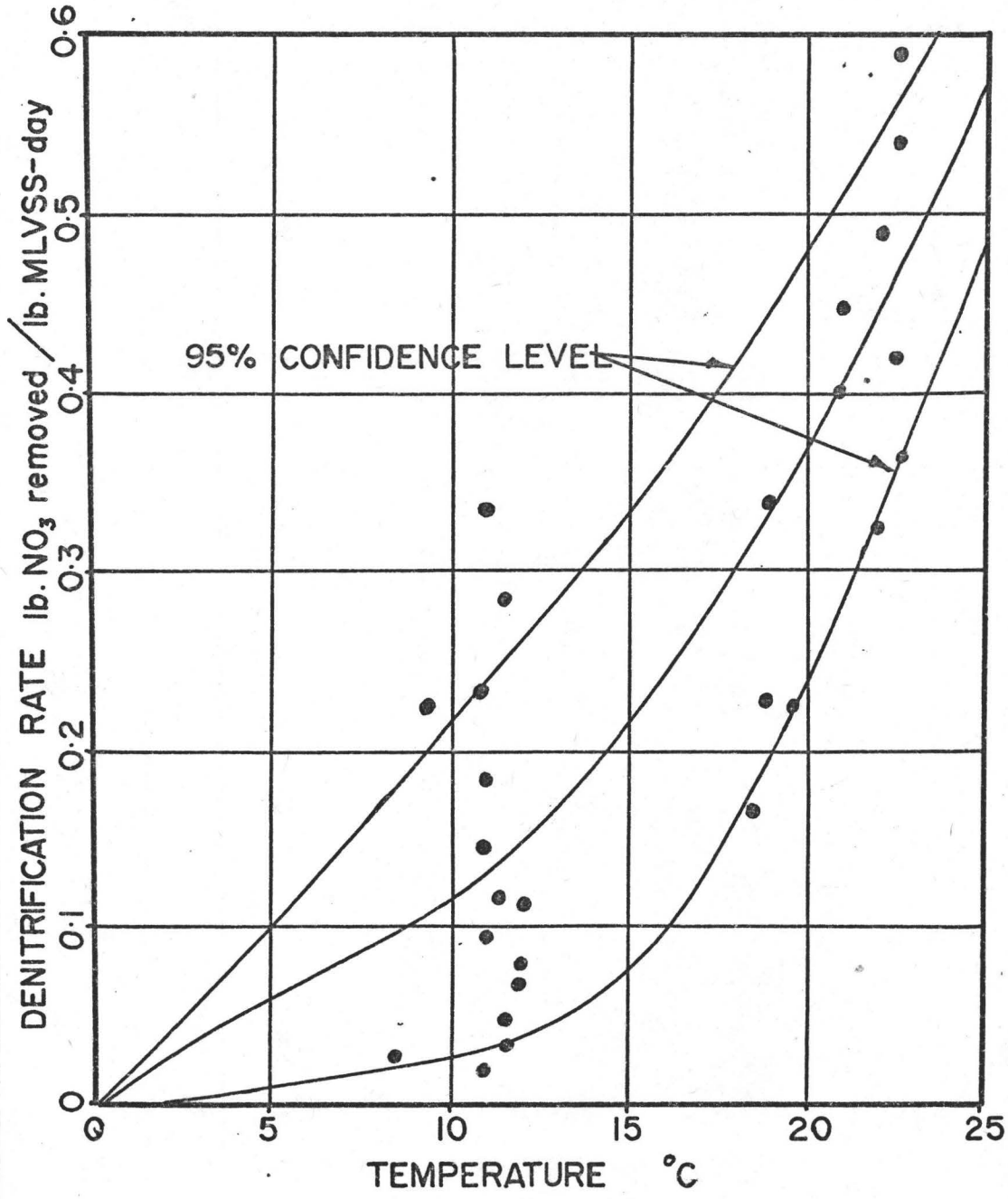
mg NO_3 as N/mg MLVSS-hr to 0.05 mg NO_3 as N/mg MLVSS-hr at 18° to 23°C. This temperature data is shown more clearly in Figure 7. Insufficient data was presented to prepare a relationship between unit denitrification rate and temperature which would be useful for design of wastewater treatment plants.

Hydrogen Ion Concentration(pH) The optimum pH for denitrification depends upon type of organisms, age of culture, and the concentration of nitrate (Delwiche 1956). Pure cultures of Pseudomonas aeruginosa denitrify between pH 5.8 to 9.2 with an optimum denitrification rate occurring between 7.0 and 8.2 Denitrifying organisms favour N_2O production at alkaline and neutral pH with reabsorption and production of N_2 above 7.3 (Wijler 1954). Below this pH level reabsorption decreases with a simultaneous increase in NO production. Isolated nitrate reducing enzymes from Pseudomonas aeruginosa showed a definite maximum activity at pH 7.4 with a reduction to 10% of the maximum rate at pH 6.0 and 50% of the maximum at pH 10.0 (Fewson and Nicholas, 1961). Nitrate reductase isolated from Spirillum itersonii by Gauthier(1970) demonstrated maximum nitrate reducing activity at a pH of 6.4 to 7.4 again with a rapid drop in activity on the acid pH. Wuhrmann (1964) claimed that the pH of the medium is the most important factor influencing the inhibiting effect of oxygen on denitrification. At pH's near neutrality usually encountered in activated sludge the oxygen inhibition of denitrification is a maximum. In

FIGURE NO. 7

DENITRIFICATION RATE VERSUS TEMPERATURE

(MULBARGER, 1970)



contrast denitrification rates below pH 6.0 are nearly independent of oxygen concentration.

Carbon Type and Concentration - Heterotrophs require the presence of a readily useable form of carbon (Lamanna and Mallette 1965). Research on pure cultures has not been concerned with the effect of carbon concentration on denitrification. The pure culture studies have always supplied carbon in the form of peptone, glucose, or glycerol (Chang and Morris, 1962, Skerman and MacRae, 1957a, 1957b) in excess of organism requirements for nitrate reduction and organism growth, e.g. 4000 mg/l.

In wastewater treatment the rate of denitrification must be maximized to minimize reactor sizes in the plant. Denitrification usually will follow carbon removal and nitrification processes, and therefore, the residual carbon concentration will vary from 5 to 20 mg/l. This carbon may or may not be in a readily useable form for further bacterial action.

Wuhrmann (1960b) in anaerobic Warburg experiments showed that denitrification is essentially a linear reaction occurring even with washed activated sludge operating on secondary waste treatment effluent with no hydrogen donor other than residual organics. However, in laboratory scale continuous activated sludge nitrification-denitrification experiments Wuhrmann (1962, 1954) reported that the addition of glucose as a hydrogen donor increased the denitrification

rate by approximately 30%. Most of the pilot plant and laboratory studies following this work utilized a bypass arrangement of raw sewage into the denitrification unit to provide a hydrogen donor. Johnson (1964) used a ratio of 1:5 raw sewage to mixed liquor, 4,000 mg/l MLSS to achieve 75% removal of 20 mg/l NO_3 as N in 3 hours at 20°C in his pilot plant and laboratory scale experiments. However, the raw waste bypass resulted in high concentrations of ammonia and organic nitrogen in the final effluent. Slechta and Culp(1967) carried out batch denitrifications studies on nitrified domestic waste using activated sludge of 2500 to 3700 mg/l MLSS and determined that as the percentage of raw sewage added to their batches was raised from 0 to 40% the percent nitrate removal increased from 60 to 100%. Balakrishnan (1969) in batch studies of denitrification of domestic wastes (20-70 mg/l NO_3 as N) with activated sludge indicated that unit denitrification rate increased with increasing COD concentration up to a maximum rate at which further increase in COD addition did not increase the rate. These experiments were again run with settled domestic sewage as the hydrogen donor. Rates with equivalent amounts of glucose as the hydrogen donor were greater than those achieved with domestic sewage as the donor. To overcome the problem of increased ammonia concentrations in the effluent several investigators (Christianson 1956), (Barth,1968) used methanol as an inexpensive organic carbon source to stimulate the denitrification reaction. The latter

found that at a weight ratio of 4 parts of methanol to 1 part of nitrate by weight essentially complete nitrate removal could be achieved on a nitrified effluent containing 12 mg/l NO_3 as N in a 3 hour detention time anaerobic reactor with a MLSS of 2,000 mg/l. McCarty (1969) studied the effect of acetone, acetic acid, methanol, and ethanol as hydrogen donors on batch denitrification of agricultural wastewater and showed that carbon type definitely affected the rate of denitrification and the amount of nitrite formed as an intermediate (See Figure 8). Acetic acid and ethanol resulted in faster denitrification rates in batch tests but methanol was considerably cheaper, and would probably be used in practice.

Based on his observation of carbon used and nitrite and nitrate utilized he presented equations for the reduction of nitrate and cellular synthesis using these compounds. For example with methanol as a hydrogen donor the equations were as follows:

Nitrate Removal.

$$\frac{1}{6}\,CH_3OH + \frac{1}{5}\,NO_3^- \longrightarrow \frac{1}{10}\,N_2 + \frac{1}{6}\,CO_2 + \frac{1}{5}\,OH^- + \frac{7}{30}\,H_2O$$

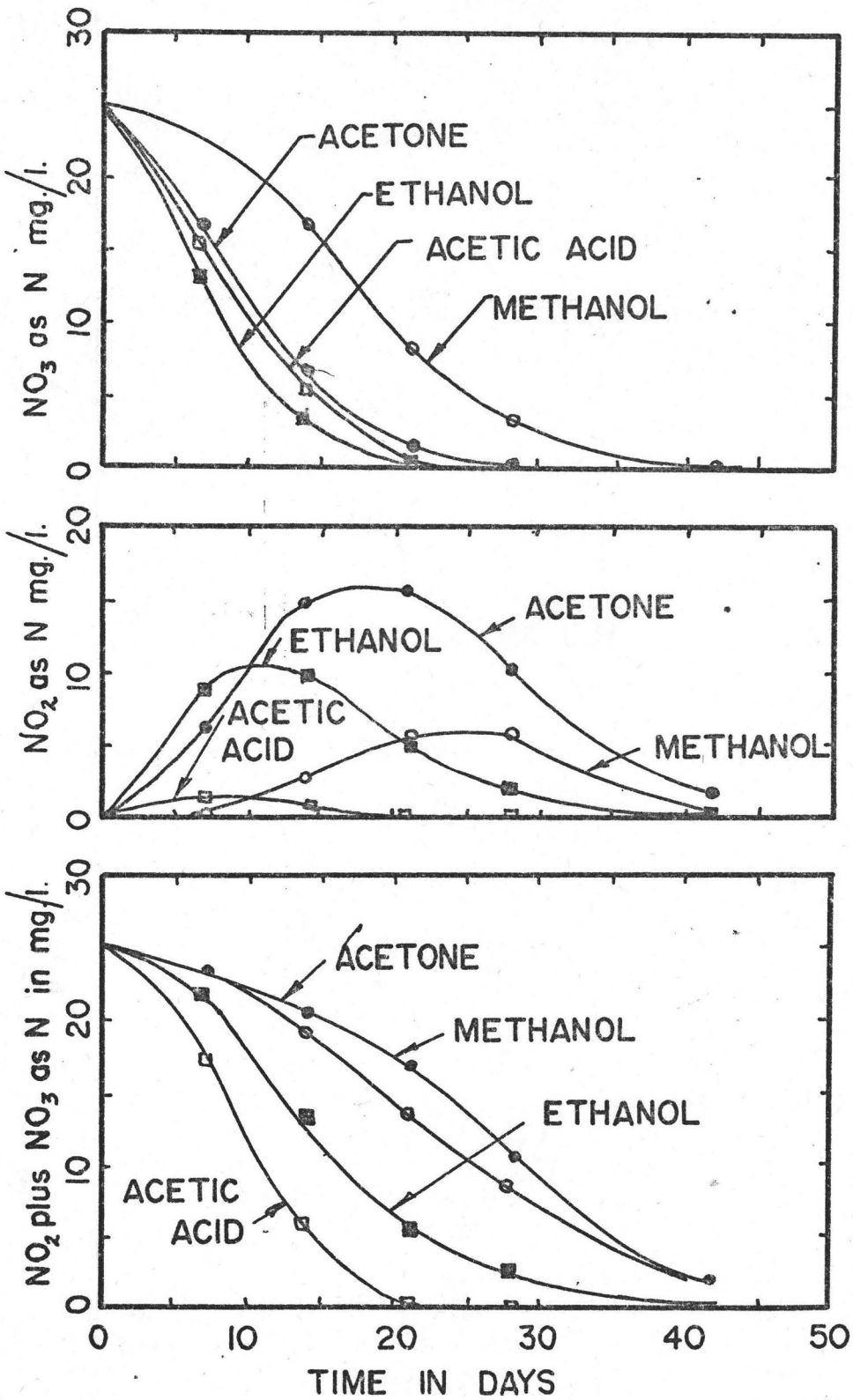
Synthesis.

$$\frac{1}{6}\,CH_3OH + \frac{1}{84}\,CO_2 + \frac{1}{28}\,NO_3^- + \frac{1}{28}\,H^+ \longrightarrow \frac{1}{28}\,C_5H_7O_2N + \frac{19}{84}\,H_2O$$

Overall Nitrate Removal.

$$NO_3^- + 1.08\,CH_3OH + H^+ \longrightarrow 0.065C_5H_7O_2N + 0.47N_2 + 0.76CO_2 + 2.44H_2O$$

FIGURE NO. 8



BATCH DENITRIFICATION DATA WITH DIFFERENT ORGANIC CARBON SOURCES

(McCARTY, 1969)

Overall Nitrite Removal.

$$NO_2^- + 0.67CH_3OH + H^+ \longrightarrow 0.04C_5H_7O_2N + 0.48N_2 + 0.47CO_2 + 1.7\ H_2O$$

The carbon requirements for denitrification with each of these compounds was compared using a "consumptive ratio", i.e the amount of soluble carbon used per unit weight of nitrate reduced as shown in Table 2.

The variation in carbon used could be related to the difference in metabolic pathway followed by each of the compounds and to the amount of solids produced during the nitrate reduction reaction. The amount of carbon used for bacterial synthesis can be calculated by using this consumptive ratio and the theoretical equation for nitrate reduction.

Organism Concentration. In biological reactors the overall rate of a particular reaction carried out by the organisms usually increases proportionally with population increase until the organism concentration reaches a saturation point. At this level the rate levels out or tends to decrease - probably due to build up of toxic intermediate compounds. In normal waste water treatment reactors this critical level is never reached as the organism concentration which can be handled in the units is limited by the physical separation processes and the aeration capacity. However, the influence of organism concentration on the denitrification reaction has not been investigated thoroughly.

TABLE 2.

CONSUMPTIVE RATIOS OF ORGANIC CARBON COMPOUNDS USED IN DENITRIFICATION
McCarty (1969).

Compound	Consumptive Ratio.
Sugar $C_{12}H_{22}O_{11}$	1.69
Acetate CH_3COOH	1.31
Methanol CH_3OH	1.3 approx.
Acetone CH_3COCH_3	1.22
Ethanol CH_3CH_2OH	1.47

Batch studies of denitrification, using typical activated sludge concentrations, by Eckenfelder (1968) showed that the overall denitrification rate increased as the solids concentration increased from 3,700 mg/l to 4,600 mg/l. Nitrate reductions of 83% and 98% were achieved in 3 and 2 hours respectively at these two solids levels from an original nitrate concentration of 22 mg/l The percentage of denitrifying organisms in the activated sludge was not estimated during the reactions. Similar results were reported by Slechta and Culp (1967).

The effect of organism concentration on denitrification rate has not been investigated in pure culture.

All of the studies, both in pure and mixed cultures, have utilized suspended solids levels as the measurement of organism concentrations. No correlation between actual viable organism population and suspended solids concentration has been attempted. Microscopic identification of organisms responsible for denitrification has been carried out in relatively few studies Smith (1970) and Wuhrmann (1963).

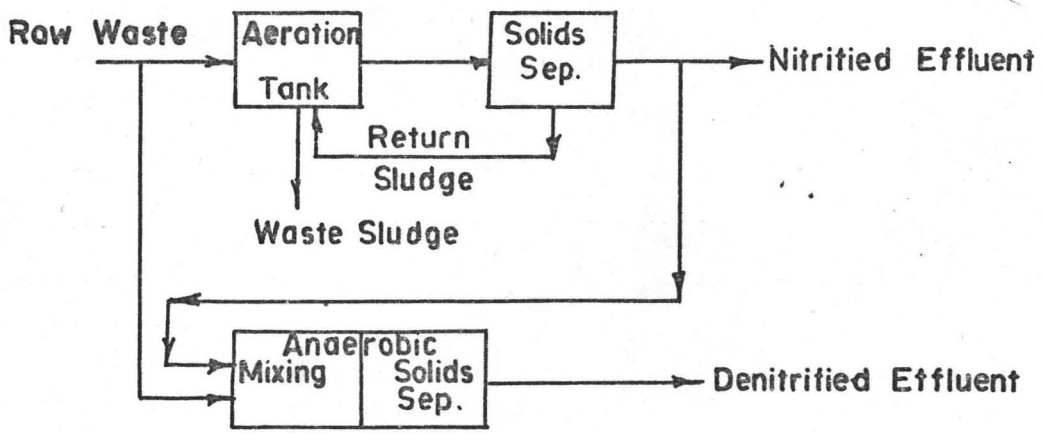
Denitrification as a Waste Water Treatment Process. Bacteria isolated from sewage treatment plants by Hulme (1914) and from natural aquatic environments Harvey (1955) produced nitrogen gas when grown anaerobically on nitrate bearing substances. Snell (1943) reported loss of nitrogen by gas evolution from nitrate bearing sludges. The phenomena of rising sludge in secondary clarifiers was attributed to

bacterial denitrification occurring in anaerobic sludge accumulated on the tank bottom according to Sawyer and Bradney (1945) and Brandon and Grindley (1944). Analysis of gas produced by a sludge utilizing nitrate and sewage as a substrate (Wheatland 1959) indicated a nitrogen content of 98%. Based on these observations workers proceeded to demonstrate the applicability of bacterial denitrification to nitrogen removal from wastewater by means of laboratory scale and pilot plant studies. In most cases these investigations were concerned with both the nitrification and denitrification processes. Wuhrmann (1962, 1960) carried out continuous flow, laboratory scale nitrification-denitrification tests with anaerobic activated sludge operating on domestic sewage to demonstrate that 90% of the influent nitrogen (20-25 mg/l NO_3 as N) could be removed with detention times of 140 to 180 minutes depending upon organism concentration and initial nitrate concentrations. In several of his tests he demonstrated that the addition of glucose as an organic carbon source improved the denitrification rate.

Bringmann (1961) successfully removed 64% of the nitrate content of digester supernatant (58 mg/l was reduced to 21 mg/l), within 45 minutes using a domestic waste as a source of carbon. Additions were controlled by the redox potential of the reactor contents. Ludzak and Ettinger (1962) utilized a modified activated sludge aerator to carry out denitrification and nitrification in the same unit. In

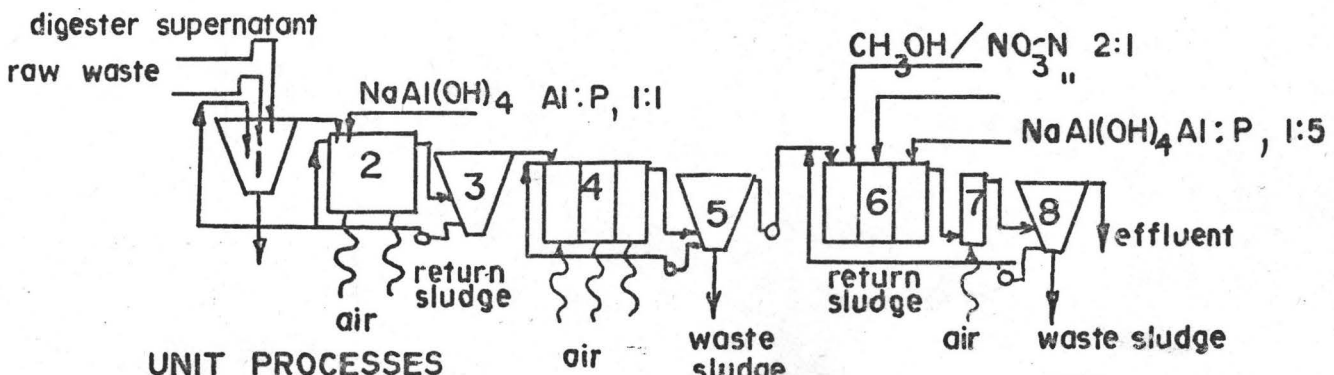
FIGURE NO. 9

SCHEMATIC DIAGRAMS OF LABORATORY ACTIVATED SLUDGE UNITS USED FOR NITRIFICATION DENITRIFICATION



CONTINUOUS NITRIFICATION DENITRIFICATION PLANT WITH RAW WASTE USED AS THE ORGANIC CARBON SOURCE (JOHNSON, 1964)

185 US. GPD. (700 L. DAY) PILOT PLANT INCORPORATING NITROGEN & PHOSPHOROUS REMOVAL (BARTH, 1968)



UNIT PROCESSES

1 Primary Clarification 70 min
2 High Rate Carbon Removal 120 min
3 Secondary Clarification 70 min
4 Nitrification 180 min
5 Intermediate Clarification 70 min
6 Denitrification 180 min
7 Flash Aeration 25 min
8 Final Clarification 180 min

FLOW RATES

Raw 500 ml./min
Return 300 ml./min

the first mechanically stirred compartment of their unit recycled mixed liquor suspended solids were contacted with raw domestic sewage and acted as a hydrogen acceptor for organic carbon. In the second section aeration was increased to the point where nitrification of ammonia compounds to nitrate could occur. Batch experiments on domestic sewage using anaerobic activated sludge by Slechta and Culp (1967) showed that the denitrification rate was greatly affected by organism concentration and the amount of settled sewage used as a hydrogen donor.

The authors of the preceeding waste treatment experiments carried out open nitrogen balances on their systems and assumed that losses of nitrogen were due to evolution of nitrogen gas. However, Johnson (1964) in a pilot plant study of the biological nitrification denitrification sequence, collected evolved gases and analyzed them utilizing a mass spectrometer. Nitrogen balances were closed within 4% and the evolved gas from the anaerobic denitrification unit (approximately 98% nitrogen) accounted for the reduction in nitrate through the unit. Since raw waste containing significant quantities of ammonia was used as a hydrogen donor the overall nitrogen removal was only 75%. A schematic diagram of his continuous tests is shown in Figure 9. Johnson (1964) utilized a synthetic waste water composed of skim milk solids as the carbon source and ammonium sulphate to act as the nitrogen content. The influent

nitrate concentrations to the denitrification reactor were 18 to 22 mg/l and the reactor solids concentrations were maintained at 4000 mg/l. Detention times were varied from 1 to 5 hours. Raw waste was added in the proportion of 1:5 raw waste to mixed liquor.

Wuhrmann (1964, 1962, 1960b, 1954) has widely published results on his laboratory and pilot plant activated sludge studies and has claimed that sufficient carbon is available as residual carbon in secondary treatment plant effluents to stimulate anaerobic nitrate reduction. In his anaerobic Warburg experiments on secondary effluent, nitrate reduction appeared as a linear reaction with respect to time even with no added hydrogen donor. However, addition of carbon increased the rate significantly.

Christianson et al (1956) compared glucose and methanol as chemical electron donors to accelerate denitrification in both batch and continuous laboratory units operating on industrial wastewater.

Barth et al (1968) conceived a modular type wastewater treatment plant combining chemical precipitation of phosphates, with biological removal of carbon, and bacterial nitrification and denitrification. Each of the biological processes was equipped with separate sludge separation and recycle systems to minimize interference between processes and achieve greater stability. As shown in Figure 9, methanol was also added as

a hydrogen donor in the ratio of 3:1, methanol:nitrate. As a result nitrification and denitrification rates were increased through the stimulation of dominant cultures and nitrogen removal efficiency was improved for the addition of methanol did not add ammonia as previously experienced with raw waste injection. In a 700 l/day pilot plant study approximately 90% of the 11.5 mg/l NO_3 concentration was removed in a 3 hour anaerobic detention period when the solids concentration was maintained at 2,000 mg/l.

Mulbarger (1970) has reported on a 200,000 US gpd demonstration pilot plant utilizing Barth's scheme of three separate recycled sludge reactors providing biological and chemical nitrogen and phosphorous removal in domestic wastewater. Complete denitrification from approximately 37 mg/l NO_3 as N to less than 2 mg/l was achieved with a sludge concentration of approximately 2,000 mg/l in 2 hours detention when 3 to 3.5 parts of methanol were added per part of nitrate nitrogen. Further operation is planned to obtain more fundamental design data.

McCarty (1969) performed laboratory scale batch and semi-continuous reactor studies of the denitrification of nitrate bearing agricultural drainage water seeded with settled domestic sewage. The effect of the type of added carbon (acetone, methanol, ethanol, acetic acid) on the denitrification rate was determined, and the types of intermediates and end products were investigated. Methanol

provided the most economical carbon source along with easily degradable nitrite as an intermediate.

The theoretical equation proposed for nitrate reduction with methanol as the hydrogen donor follows -

$$NO_3^- + 5/6\ CH_3OH \rightarrow \tfrac{1}{2}\ N_2 + 5/6\ CO_2 + 7/6\ H_2O + OH^-$$

Similar equations were presented for nitrite reduction and aerobic degredation of methanol resulting in theoretical equations for methanol requirements as follows -

$$Cm = 2.47\ N_0 + 1.53\ N_1 + 0.87\ D_0$$

where Cm is the methanol concentration mg/l

N_0 is the inititial NO_3 concentration in mg/l

N_1 is the initial NO_2 concentration in mg/l

D_0 is the inititial dissolved oxygen concentration in mg/l

The equation for predicted organism production follows -

$$C_b = 0.53\ N_0 + 0.32\ N_1 + 0.19\ D_0$$

where C_b is the solids production in mg/l

In a later article McCarty (1969) described the denitrification of agricultural waste water on upwards flow packed column denitrification units. At temperatures of 20°C 90% removal of nitrate was achieved from 20 mg/l NO_3 as N with detention times of 0.5 to 2.0 hours utilizing methanol as the hydrogen donor. Application rates varied from 0.6 to .15 US gpm/ft^2 of filter surface at a ratio of 3:1, methanol to nitrate concentration. Smith (1970) operated a 2,500 gpd columnar denitrification pilot plant following a

nitrification stage receiving secondary effluent from a conventional activated sludge domestic wastewater treatment plant. See Figure 10. Three column packings were utilized varying in media particle size from 3.4 mm to 14.5 mm max diameter. At column loading rates of 7.0 US gpd/ft^2 (0.000051b NO_3 as N per ft^2 of surface areaper min). 90% denitrification was achieved with a methanol:nitrate ratio of 2.5:1.0 at a column depth of approximately 10 feet and an actual contact time of 5 to 10 minutes. The rapid removal rates were attributed to the large surface area available for organism growth which provided an organism growth equivalent to a stirred tank containing 21,000 mg/l of solids. Operating temperatures were maintained at 27°C. Methanol requirements and organism growth observed were in agreement with the equations presented by McCarty (1969).

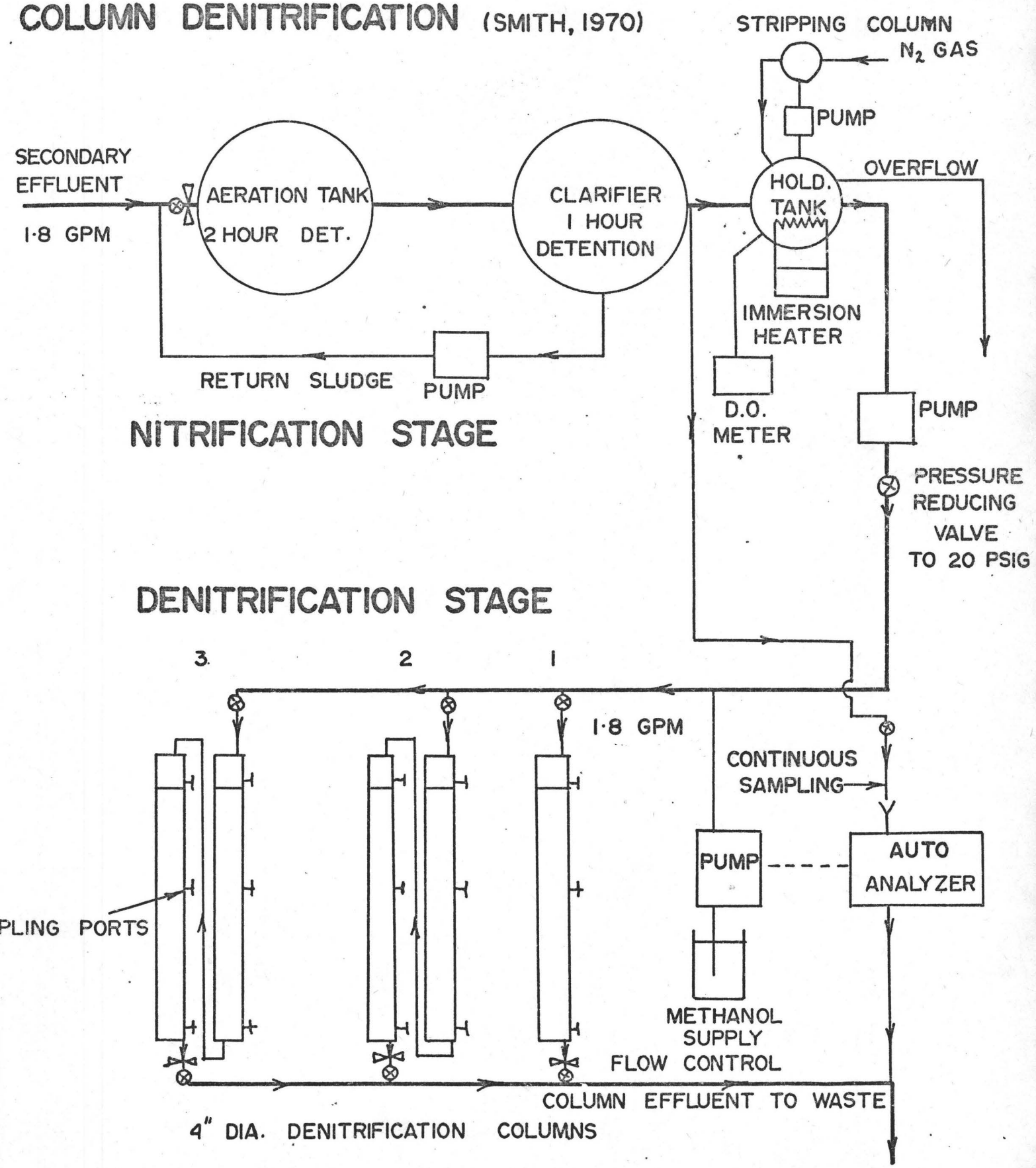
Kinetics - Little work has been done to establish the kinetic theory of denitrification. From pure culture and enzyme experiments, we know that denitrification must occur as a series of enzyme catalyzed reactions in which nitric oxide, nitroxyl, hyponitrite, and nitrous oxide have been proposed as intermediates (Fewson 1961a). In a series of reactions hypothesized by Bremner and Shaw (1958) nitramide appears as a key intermediate.

$$NHO_3 + 2H \longrightarrow HNO_2 + H_2O$$

$$HNO_2 + 2H \longrightarrow HNO + H_2O$$

FIGURE NO. 10

FLOW DIAGRAM OF 2500 GPD PILOT PLANT FOR COLUMN DENITRIFICATION (SMITH, 1970)



$$2HNO \longrightarrow H_2N_2O_2$$

$$H_2N_2O_2 \longrightarrow N_2O + H_2O$$

$$H_2N_2O_2 \longrightarrow N_2 + H_2O$$

$$N_2O + 2H \longrightarrow N_2 + H_2O$$

$$HNO_2 + H \longrightarrow NO + H_2O$$

Establishing a kinetic expression from such a complicated series of reactions is difficult and is probably not necessary since one of the enzyme catalyzed reactions probably controls the whole chain. With this in mind many of the early investigators concentrated on studying nitrate reductase isolated from different organisms as the rate controlling step (Fewson 1961b), (Nason, 1958). The rate was considered to follow a Michaelis Menton type of kinetics in which the rate per unit weight of enzyme increased with increasing nitrate concentration up to a limiting nitrate concentration above which the rate was a constant. Data from this work was quoted in terms of Michaelis constants, i.e. the concentration of nitrate at which the observed rate is one half the maximum rate, e.g. nitrate reductase from Pseudomonas aeruginosa 22 mg/l NO_3 as N @ 30°C (Fewson 1961b).

For whole cells in pure culture most authors have quoted the unit denitrification rate in terms of micro-litres of gas produced per mg of cells per unit time. This can be easily converted to mg/l of nitrate as N per mg/l of cells per hour as shown in Table No. 3. Most of the investigators experimenting with activated sludge and other

mixed cultures operating on complex wastewater have quoted unit rates in terms of NO_3 as N disappearing during reaction per weight of cells per hour. It is interesting to note that the pure culture rates shown in Table No. 3 are approximately 6 times as great as the activated sludge rate. The higher temperature 32°C of the pure culture as opposed to 20°C for the activated sludge doubltess contributes to this difference as well as the large portion of activated sludge which consists of organisms which cannot denitrify.

Both Johnson (1964) and Balakrishnan (1969) indicate that denitrification rate depends upon both carbon concentration and nitrate concentration. However, Muhrmann (1960b) has shown in anaerobic Warburg respirometer studies that denitrification of nitrified municipal waste is a linear reaction with respect to time when residual carbon compounds are used as hydrogen donors. A similar phenomenon was recently reported by Mechales (1970) in continuous flow experiments on nitrified municipal wastewater using packed columns and no added carbon source. These latter two studies indicate that denitrification is probably a zero order reaction, i.e. independent of nitrate concentration. Zero order kinetics with respect to nitrate would be compatible with carbon removal kinetics which Wilson (1967) demonstrated as independent of substrate concentration for single carbon compounds. Batch denitrification data reported by Johnson (1964) on anaerobic denitrification of nitrified artificial

TABLE 3.

UNIT DENITRIFICATION RATE DATA
LITERATURE REVIEW

Source	Organism	Temperature	Unit Denitrification Rate mg/l NO_3 as N/mg/l cells-hr
Chang & Morris (1962)	Micrococcus denitrificans	32°C	0.056 *
Pichinoty & D'Ornano (1961)	"	32°C	0.059 *
Eckenfelder & Balakrishnan (1968)	Activated Sludge	20°C	0.0007 to 0.011 **
Johnson & Schroepner (1964)	"	20°C	0.0005 to 0.009
Wuhrmann & Meschner (1963)	Mixture of 6 Bacterial Species	25°C	0.120
Moore & Schroeder (1970)	Activated Sludge	20°C	0.0355
Mulbarger (1970)	"	8°C	0.0013
		11°C	0.0008 to 0.014
		18°C	0.009 to 0.014
		25°C	0.0136 to 0.024
Barth (1968)	"	20°C	0.0017

* calculated from gas production
** calculated from experimental data.

wastewater with raw waste as a hydrogen donor support rate independence of nitrate concentration.

Carbon concentration controls the extent of reaction. Carbon is required for reduction of the nitrate according to a definite stoichiometric relationship, e.g. nitrate reduced to nitrogen gas with glucose as the hydrogen donor.

$$5\ C_6H_{12}O_6 + 24\ KNO_3 \longrightarrow 12N_2 + 24\ KHCO_3 + 6CO_2 + 18H_2O - 645.5\ K\ cal$$ (Engel 1958)

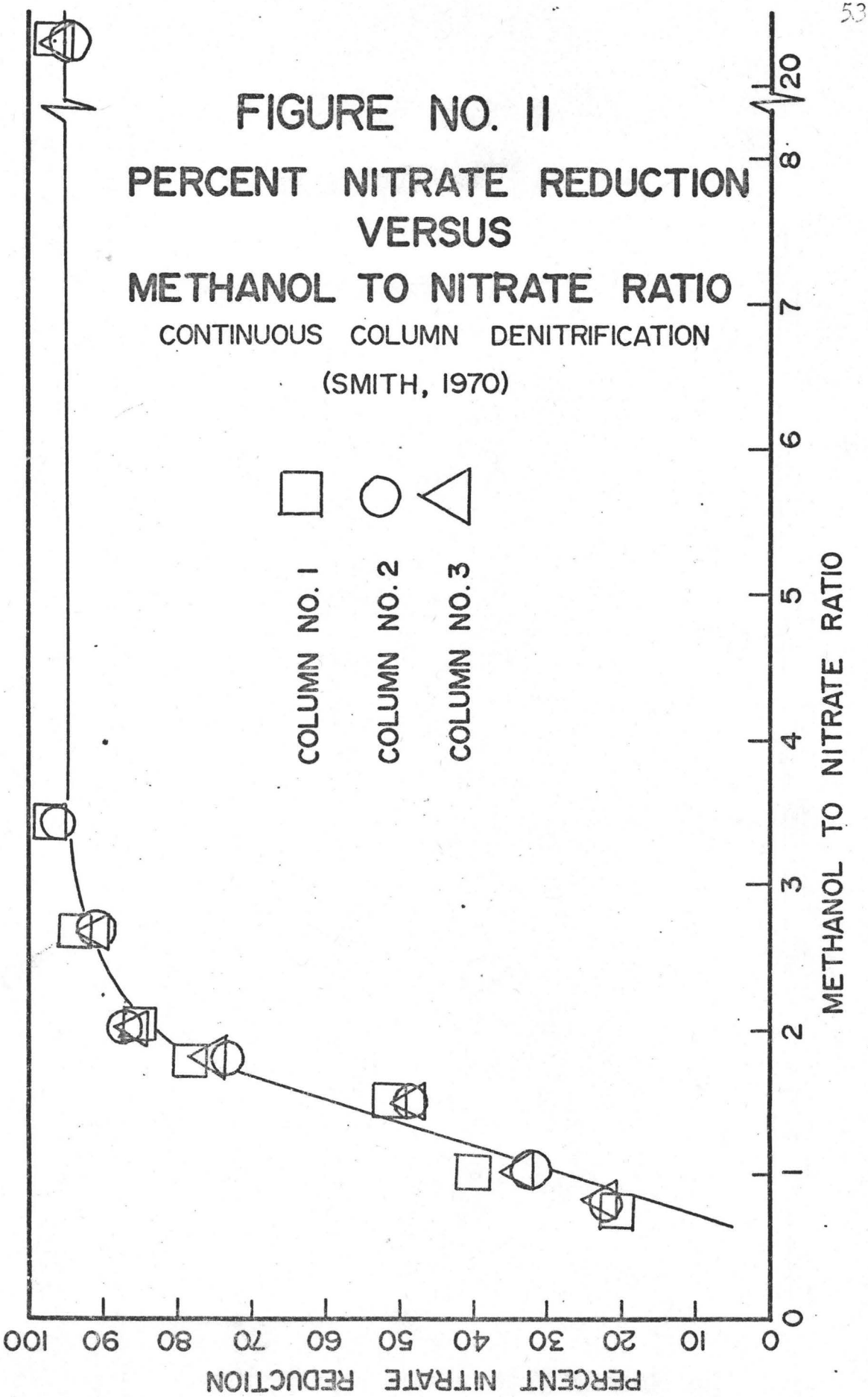
In addition, carbon is required for cellular carbon synthesis. If sufficient carbon is available to meet these two requirements all of the nitrate will be converted to gaseous nitrogen compounds and a very small amount of cellular nitrogen. Several authors have shown that carbon concentration influences the rate of denitrification, Eckenfelder (1968), Smith (1970). Smith's data appears to indicate that denitrification rate is independent of carbon concentration above the theoretical requirements for nitrate reduction and organism growth. Figure 11.

FIGURE NO. II

PERCENT NITRATE REDUCTION VERSUS METHANOL TO NITRATE RATIO

CONTINUOUS COLUMN DENITRIFICATION

(SMITH, 1970)



Experimental Description.

Apparatus.

Batch denitrification tests were carried out in 5.0 litre three-necked boiling flasks, spherical in shape. The flasks were set on a laboratory frame which could accommodate 4 to 6 simultaneous experiments. Each of the necks was fitted with a bored rubber stopper to enable insertion of a combination electrode pH probe, polarographic dissolved oxygen probe, sampling tube and/or 1/32" diameter steel gas collection tube. During the experiments the flasks were only partially filled resulting in a 100 ml to 250 ml gas space above the liquid contents. The gas collection tube was fitted with a 1/32 inch inside diameter plastic tubing connected to the top of a 500 ml glass cylinder filled with light mineral oil. This in turn was connected to a graduated glass cylinder which was open to the atmosphere. As gases were evolved in the reactor, mineral oil was displaced from the first cylinder into the second. By equalizing the oil to air interface of the two cylinders, the amount of gas collected at atmospheric pressure could be determined directly from the tube graduations. The pressure on the system was allowed to diverge from atmospheric pressure by only 1 inch of mineral oil. As no loss in pressure could be observed over extended periods of time (1 - 2 days) any gas losses from the collection system were assumed to be insignificant.

Variable speed laboratory magnetic mixers were placed beneath each flask with a 1/8 inch air space between the stirrer and flask to insulate the contents from

heat generated by the electric motor. Teflon coated steel magnetic stirring bars approximately 3/8 inch by 1 inch long were placed inside the flasks to mix the contents

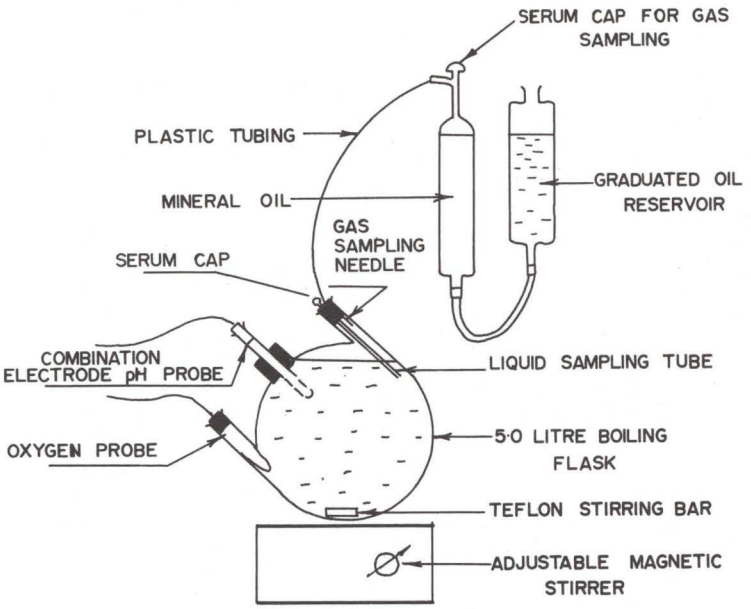
A typical experimental set up is shown in Figure 12.

Procedure

Preparation of Artificial Wastewater The artificial wastewater or defined medium for each experiment was prepared from analytical grade chemicals. In each case potassium nitrate (KNO_3) was used as the nitrogen source while sodium citrate ($Na_3C_6H_5O_7.2H_2O$) constituted the carbon source or hydrogen donor to drive the reaction. These chemicals were dissolved in tap water along with suitable quantities of potassium hydrogen, phosphate (monobasic) (KH_2PO_4) ferric chloride ($FeCl_3.6H_2O$) and magnesium sulphate ($MgSO_4.7H_2O$) to provide essential nutrient materials for organism growth. The ratios of phosphorous, iron, and magnesium, to carbon were derived from a formula developed by McLean (1968) for aerobic degradation of carbon by mixed cultures of bacteria. It was assumed that assimilatory utilization of nitrate would occur simultaneously with dissimilatory nitrate metabolism to provide the nitrogen for organism growth. Other trace elements were assumed to be present in sufficient quantity in the tap water. Prior to use in the experiments the medium was autoclaved at atmospheric pressure at 212°F for 30 minutes and allowed to cool in cotton plugged bottles. A typical recipe for experiment No. 12 is shown in

FIGURE NO. 12

ASSEMBLED LABORATORY BATCH REACTOR



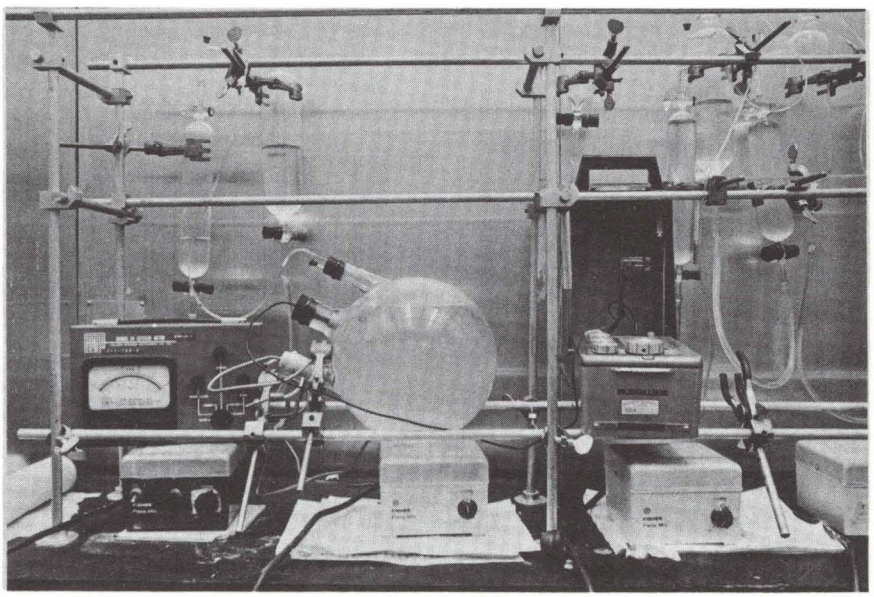


Table 4.

Preparation of Organism Seed.

Pseudomonas denitrificans in the lyophilized form were obtained from American Type culture collection. These were revived by growth at 27°C on nutrient agar slants (Bio Cert J-1087-C Fisher Scientific Co. Ltd.). Additional slants were inoculated and grown and then stored at 7°C. Streak plates were then inoculated and organisms harvested from these were then grown aerobically at 27°C on a shaker bath for 48 hours in approximately 75 ml of nutrient broth in 125 ml Erlenmeyer flasks. The full contents of these flasks were introduced into medium "A" described in Table 4, and grown anaerobically at 27°C until approximately 175 ml of gas were collected. The above quantity of gas was indicative of mid reaction since a total of 350 ml of gas was produced from a 5 litre reactor after all the nitrates were exhausted. A portion of this growth after settling and decantation, was used as the organism seed for individual experiments, since the concentration of Pseudomonas sp. had by this time reached approximately 250 mg/l. It was assumed that the organisms were in an active growth condition for use in the experiments. At each stage standard microbiological laboratory procedures were followed to insure that no contaminating organisms reached the growth medium.

After addition of the organism seed to the autoclaved reactors, the contents were scrubbed with pure nitrogen gas for approximately

TABLE NO. 4.

Artificial Wastewater and Growth Media Used in Batch Studies.

Component	Media A	Expt.No.12	Expt.No.29
KNO_3 mg/l as N	111	120	120
$Na_3C_6H_5O_7.2H_2O$ mg/l as C	245	600	300
$MgSO_4.7H_2O$ mg/l	24	180	90
$FeCl_3.6H_2O$ mg/l	0.6	3.0	1.5
KH_2PO_4 mg/l	60	450	225

5 minutes until all of the dissolved oxygen was forced from solution and the dissolved nitrogen content was at saturation. At the start of an experimental run the gas space in the reactor contained air. At the same time the pH was adjusted to the experimental condition by addition of 40% sodium hydroxide or concentrated sulphuric acid. Subsequent pH corrections were made whenever the pH as monitored by the pH probe in the reactor deviated from the experimental pH by more than 0.1 units.

Sampling. Following mixing of organism seed and artificial wastewater for 5 minutes, a 25 ml sample was taken in a 10 ml syringe by inserting the needle through a serum cap fitted to a glass tube on one of the side arms. The flask was tilted so that the magnetic mixer created a definite flow past the sample point. A sample from the gas space was also taken in order to provide a complete initial nitrogen balance. This procedure was repeated throughout the course of the batch reaction whenever 30 to 50 ml of gas had collected in the oil reservoir. A 20 ml portion of this sample was immediately filtered through a 0.47 micron Sartorius membrane filter to remove bacteria from the filtrate and for solids determinations. The filtrate was immediately analyzed for its nitrogen and soluble carbon content, frozen, or kept at 5°C for later analysis. The remaining 5 ml was used for total organic nitrogen analysis including cellular organic nitrogen. Samples for dissolved nitrogen gas analysis (0.5 ml) were periodically withdrawn in the same manner and immediately injected into the gas liquid chromatograph.

Gas analysis samples (0.5 ml) were withdrawn by means of a gas tight syringe through the serum cap in the

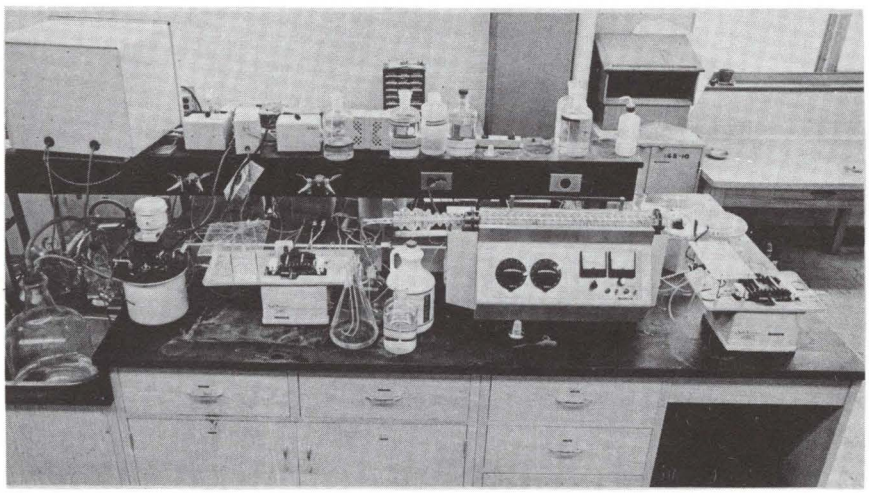
top of the gas collection reservoir.

Analyses. All of the nitrogen analyses on the liquid samples with the exception of the dissolved N_2 analysis were carried out by wet chemical methods adaptable to spectrophotometric techniques adaptable to the Technicon Autoanalyzer. This unit consists of a rotating sampling device which alternatively dips a sampling needle into a sample or distilled water for a specified period; a peristaltic pump which accurately pumps reagents at a specific rate in small batches separated by air bubbles through mixing coils; heating baths to allow retention of the liquid reactants for colour development at a specific temperature; a spectrophotometer which develops an electronic signal recorded on a strip chart as a peak enclosing an area proportional to the sample concentration of a particular component. The equipment required for simultaneous nitrite and nitrate analysis is shown in the photograph in Figure 13.

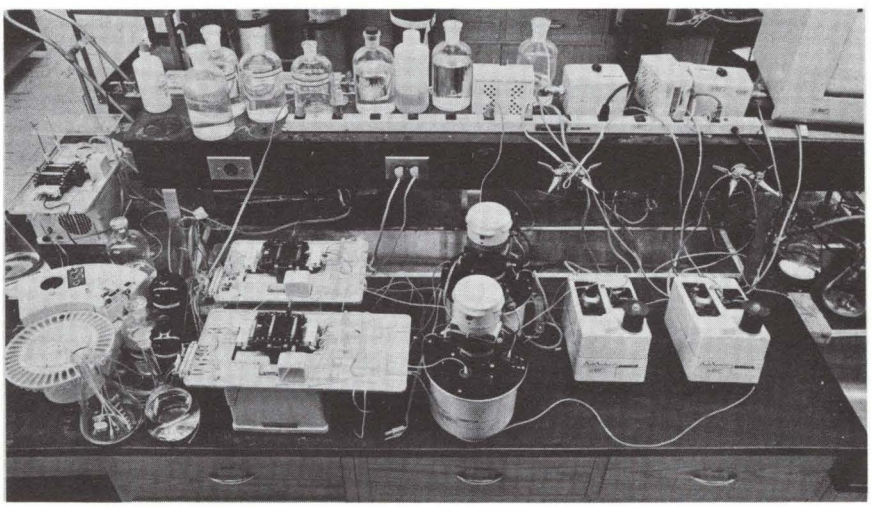
Nitrite. This automated procedure for the determination of nitrite is an adaptation of the diazolization method of "Standard Methods" (1965) proposed by Kamphake (1967). Nitrite ion under acidic conditions reacts with sulfanilimide to yield a diazo compound which couples with a n-1 naphthalethy lenediamine dihydrochloride to form a soluble dye which is measured colourimetrically. The schematic flow chart for this analysis as outlined by Technicon Corporation Industrial

FIGURE NO. 13

AUTOMATED ANALYSES



TOTAL KJELDAHL



NITRATE FOREGROUND
NITRITE BACKGROUND

Method 35-69 W is shown in Figure 2 in Appendix C Samples taken from the reactor were diluted 1/100 with distilled water prior to analysis to bring their contentration within the 0 to 1.3 mg/l analysis range.

Nitrate. Nitrate contained in the samples is reduced to nitrite by an alkaline solution of hydrazine sulphate containing a copper catalyst. The remainder of the procedure is identical to the automated nitrite analysis above. The schematic flow chart for this reaction is shown in Figure 6 in Appendix C. The nitrite concentration previously determined must be subtracted from the results of this test, since it measured both nitrite and nitrate. The samples were diluted 1/50 with distilled water to bring them within the range of the test. A range of potassium nitrate standards (0-3 mg/l) was run along with each group of analyses to establish a calibration curve.

Ammonia. Ammonia was determined using the Berthelot Reaction in which the formation of a blue coloured compound believed to be closely related to indophenol occurs when the solution of ammonium salt is added to sodium phenoxide followed by the addition of sodium hypochlorite. A solution of potassium sodium tartrate (Rochelle Salts) is added to the sample stream to eliminate the precipitation of the hydroxides of heavy metals. Filtered samples were tested undiluted, and were within the 0-10 mg/l analysis range, and each set of samples was compared to a series of calibration standards.

The flow sheet recommended by Technicon Corporation industrial method 18-69W was used as shown in Figure 8 Appendix C

Total Kjeldahl Nitrogen. The measurements of nitrogen contained in organic carbon compounds such as cellular material requires the breakdown of these compounds and release of the nitrogen content as ammonia. In the automated analysis, this is achieved by digestion of samples in a mixture of concentrated sulphuric acid and perchloric acid with selenium dioxide as a catalyst in a continuous rotating digester operated at approximately 400°C, according to the method developed by Ferrari (1960). The quantity of ammonia produced is measured by the Berthelot reaction. The Schematic flow diagram for total Kjeldahl analysis within the range 0-40 mg/l is outlined in Technicon Corporation industrial method 30-69A as shown in Figure 4 of Appendix C. Total Kjeldahl tests were done on both filtered and unfiltered samples as a measure of cellular organic nitrogen and dissolved organic nitrogen respectively. The dissolved nitrogen was found to be mostly ammonia.

Soluble Carbon. The 2 ml liquid samples which had been through the 0.47 micron membrane filters were mixed with 1 or 2 drops of concentrated hydrochloric acid to obtain a pH of 2.0. The carbon dioxide released from bicarbonates out of solution was then scrubbed from the samples by bubbling nitrogen gas through the sample for three minutes, thereby eliminating inorganic carbon. After acidification and CO_2 scrubbing, the samples could then be preserved for up to 30

days at 5°C without any deterioration of organic carbon compounds. Samples of 20 micro-litres were then withdrawn with a syringe and injected into a Beckman infrared carbon analyzer. The resulting peaks were compared to peaks produced from carbon standards prepared from sodium oxalate. A typical calibration curve within the range of samples analyzed is shown in Figure 1 of Appendix C.

Suspended Solids. The suspended solids determination was used as a measure of organism growth. A 10 or 20 ml portion of reactor contents was drawn through a 0.47 micron Sartorius membrane filter which had previously been washed with 100 ml of distilled water, dried at 103°C for 1 hour, and weighed. The same drying procedure was followed with the filters plus collected solids and the increase in weight assumed to be micro-organism solids. Igniting the filters plus sample at 600°C indicated that the organism solids were 95% volatile for cultures employing Pseudomonas sp. The volatile fraction of the activated sludge was approximately 60%. Filtration was accomplished by placing the filters on a scintered glass support and then applying a vacuum of approximately 10 psi.

Gas Analysis. A gas chromatograph was used to separate the mixture of collected gases. The fisher gas partitioner Model 25V utilizes a two column, two detector system to separate and analyse gaseous mixtures. Using a helium carrier gas at 50 millilitres per minute the gas mixture in a 0.5 ml sample was swept through a 42" silica gel column. Carbon dioxide and nitrous oxide is reversibly adsorbed on

the particles of the gel, while oxygen and nitrogen pass through without being absorbed so that the first thermal conductivity cell produces a composite peak of N_2 and O_2, and a carbon dioxide peak on the recorder chart. The sample then passes through the second column consisting of 13 feet of molecular sieve 13X which reversibly absorbs nitrogen and oxygen at different rates, and permanently absorbs carbon dioxide at room temperature. The second thermal conductivity detector, therefore, produces separate peaks for nitrogen and oxygen. At the helium flow rate of 50 ml per minute excellent separation of composite, oxygen, carbon dioxide, nitrous oxide, and nitrogen peaks was obtained. Known mixtures of air, nitrogen, carbon dioxide, and nitrous oxide were used to provide calibration of the instrument. As shown in Figure 12 Appendix C the response of the thermal conductivity cell was linear with respect to increasing gas concentrations. Machine calibration was checked several times through the course of the experiments with 0.5 ml air samples injected during each run as control samples.

The absorption columns were maintained at room temperature, and the bridge current operated at 6 volts. A recorder setting of 100 mv full scale, with a chart speed of an inch per minute was found suitable for gas analysis. When the control samples indicated that the machine was not properly calibrated, the molecular sieve columns were regenerated by heating overnight at 400°C to drive off absorbed CO_2 and

water vapour.

Dissolved Oxygen. Polarographic oxygen probes were used to measure the dissolved oxygen concentration in the reactor. A Yellowsprings Instrument Co.Ltd. polarographic probe was used with a Model 54 oxygen meter as well as several Precision Scientific Corp. Ltd. galvanic cell oxygen probes connected to micro-ammeters.

Experimental Design.

As stated previously, the aim of this investigation was to establish which are the major variables affecting the denitrification rate in pure batch culture, and then relate this to mixed culture data. A search of the literature revealed that the variables influencing denitrification are -

(1) Dissolved oxygen concentration,

(2) Temperature,

(3) Carbon concentration (organic),

(4) Organic carbon (type),

(5) pH,

(6) Organism concentration,

(7) Nitrate concentration.

Most of the literature confirms that denitrification is an anaerobic bacterial process completely inhibited by the presence of dissolved oxygen in concentration as low as 0.2 mg/l. Several mixed culture studies claiming denitrification occurs when dissolved oxygen is present have been attributed to anaerobic conditions in the interior of the floc. In this study, several preliminary experiments at dissolved oxygen concentration as low as 0.5 mg/l indicated that presence of dissolved oxygen completely inhibits denitrification. Therefore, this variable was ommitted from further experimental designs, and all tests were carried out under completely anaerobic conditions.

Temperature is a major variable influencing the reaction rate in any biological process with a definite optimum temperature range for growth of each bacterial species. Most bacteria exhibit an optimum temperature range of 20°C to 30°C. As a rule of thumb, a drop in temperature of 10°C below this optimum results in a rate reduction of 50%. The rate reduction has been attributed to physical changes of the cell protoplasm such as viscosity which, thereby, reduces diffusion rates of enzymes and substrates. In addition, low temperatures may cause accumulation of toxic metabolic intermediates. Temperature is, therefore, a major variable affecting denitrification rate which would mask the effect of other variables. Therefore, the effect of temperature variation was evaluated separately from the other variables, and its influence given particular emphasis since any application of denitrification to waste treatment would require successful operation at temperatures as low as 5°C in cold climate regions. See Table No. 5. To evaluate the remainder of the variables, a temperature of 27°C was chosen since this was the temperature recommended for growth of the supplied culture of Pseudomonas denitrificans by American Type Culture Collection.

Although organic carbon type has certainly been demonstrated to affect the rate of denitrification and the amount and type of metabolic intermediates produced, it was decided that evaluation of the various organic carbon sources

TABLE 5.

TYPICAL WASTEWATER REACTOR TEMPERATURES IN COLD CLIMATE REGIONS (AERATION TANK TEMP.)

	Preston, Ontario. 1970			Elmira, Ontario. 1970			Chicago, Ill. North Side STP. 1967		
	Avg.	Max.	Min.	Avg.	Max.	Min.	Avg.	Max.	Min.
January	6.7	10.0	3.3	6.9	11.0	4.0	8.9	10.0	6.6
February	5.9	9.5	3.3	6.9	9.0	4.0	9.4	10.0	8.9
March	6.6	8.9	4.4	8.6	10.0	7.0	10.0	11.1	6.6
April	10.3	13.9	7.8	9.6	12.0	7.0	11.1	13.3	9.4
May	13.1	15.5	10.5	14.0	16.0	13.0	14.4	16.7	13.3
June	16.0	17.8	11.7	17.9	22.0	15.0	16.7	19.4	13.3
July	18.1	20.1	16.1	19.9	23.0	17.0	20.0	22.8	17.8
August	18.9	21.2	16.7	19.7	23.0	18.0	22.8	23.9	21.6
September	17.7	20.1	15.0	17.2	21.0	14.0	21.6	23.4	20.0
October	15.3	16.7	12.8	14.4	18.0	12.0	17.2	21.1	16.1
November	12.6	15.0	7.8	12.1	14.0	8.0	15.0	16.7	12.8
December	10.3	13.9	7.8	7.6	12.0	6.0	13.3	13.9	11.7

would be beyond the scope of this work. Sodium citrate was chosen as an organic carbon, since it is an intermediate in the Krebs cycle of aerobic oxidation of carbohydrates, and also because it was recommended by American Type Culture Collection for revival of the supplied organism.

Screening Experiments The remaining four variables, pH, carbon to nitrate ratio, nitrate concentration, and organism concentration were then put through a screening procedure to determine whether any of these had a significant effect on denitrification rate. A full factorial experiment was then drawn up to evaluate the effect of the four variables at two levels of each variable. The levels of each of the variables were as follows -

Factor	Level of Factor +1	-1
pH	8.5	6.5
NO_3 Concentration	120 mg/l	40 mg/l
Carbon to Nitrate Ratio	5:1	1:1
Organism Concentration	100 mg/l	50 mg/l

The pH levels are within the normal hydrogen ion concentrations usually experienced in wastewater treatment. The nitrate concentration of 40 mg/l is a reasonable level for concentrations often contained in nitrified effluents from municipal wastewater plants, while the upper level is typical of an industrial waste water. The two carbon concentration limits were chosen so that the upper level would be above the theoretical requirements for both nitrate

reduction and organism growth, while the lower level would fall well below these requirements. In other words, it was expected that complete conversion of all the nitrate would occur at the higher carbon level with only partial conversion at the lower level. The organism concentrations chosen were 1/10 to 1/100 of those normally utilized at existing activated sludge waste water treatment plants. However, the pure culture levels were roughly estimated to contain close to the equivalent Pseudomonas sp content of actual activated sludge plants. As well, the lower organism levels were considered more suitable for colony count determinations, since flocculation should be less at these lower bacterial populations. The experimental design follows (Table 6).

As indicated by Davies (1967) all of the effects of the major factors and their interactions on the denitrification rate can be evaluated for a 2^4 factorial design of this type. It should be noted that both the NO_3 concentration and the pH could be set exactly according to the experimental prescription, However, the initial organism concentration could not be established as closely. The reason for this was that the extent of reaction in preparation of the seed organisms could not be gauged accurately, and both the added carbon and added organism might vary 10% from the specified level. The experiments were initially randomized, but this was somewhat qualified by performing two or three reactions simultaneously to save time.

TABLE 6.

EXPERIMENTAL DESIGN OF SCREENING EXPERIMENTS.

Experiment No.	NO_3	C:NO_3	pH	Organisms
1	-	-	-	-
2	+	-	-	-
3	-	+	-	-
4	+	+	-	-
5	-	-	+	-
6	+	-	+	-
7	-	+	+	-
8	+	+	+	-
9	-	-	-	+
10	+	-	-	+
11	-	+	-	+
12	+	+	-	+
13	-	-	+	+
14	+	-	+	+
15	-	+	+	+
16	+	+	+	+

Results

Discussion of Individual Experiments. Typical changes in nitrogen form and content, soluble carbon, suspended solids, gas volume collected etc., as a function of time are shown in Figure 14 through 21. The results of analyses for all of the 16 screening experiments are shown in Appendix A. In general, the batch experiments were characterized by a lag period in which the nitrate concentration decreased between 5 and 20% and a rapid linear nitrate removal with respect to time. As shown in Figure 14 and 15, the lag period could be identified in the carbon removal, nitrate and gas volume collected curves. No dissolved oxygen could be detected in the reactors during this phase. It was unfortunate that the closed system allowed sampling only after collection of from 25 to 50 ml of gas to avoid back flow of oil from the reservoir, therefore, the actual extent of the lag period could not be evaluated. The slow denitrification period is assumed to be an acclimation period similar to that experienced in McCarty's (1969) batch experiments, with methanol as the hydrogen donor. During the lag period an accumulation of nitrite as an intermediate compound was experienced. The nitrite increased to a maximum concentration and then decreased at a rapid rate along with the nitrate.

In the experiments where the carbon to nitrate ratio was 5:1, the nitrate removal was essentially complete, i.e. 120 mg/l to < 1.0 mg/l or 40 mg/l to 0.5 mg/l, while the carbon removal occurred until all of the nitrate and nitrite

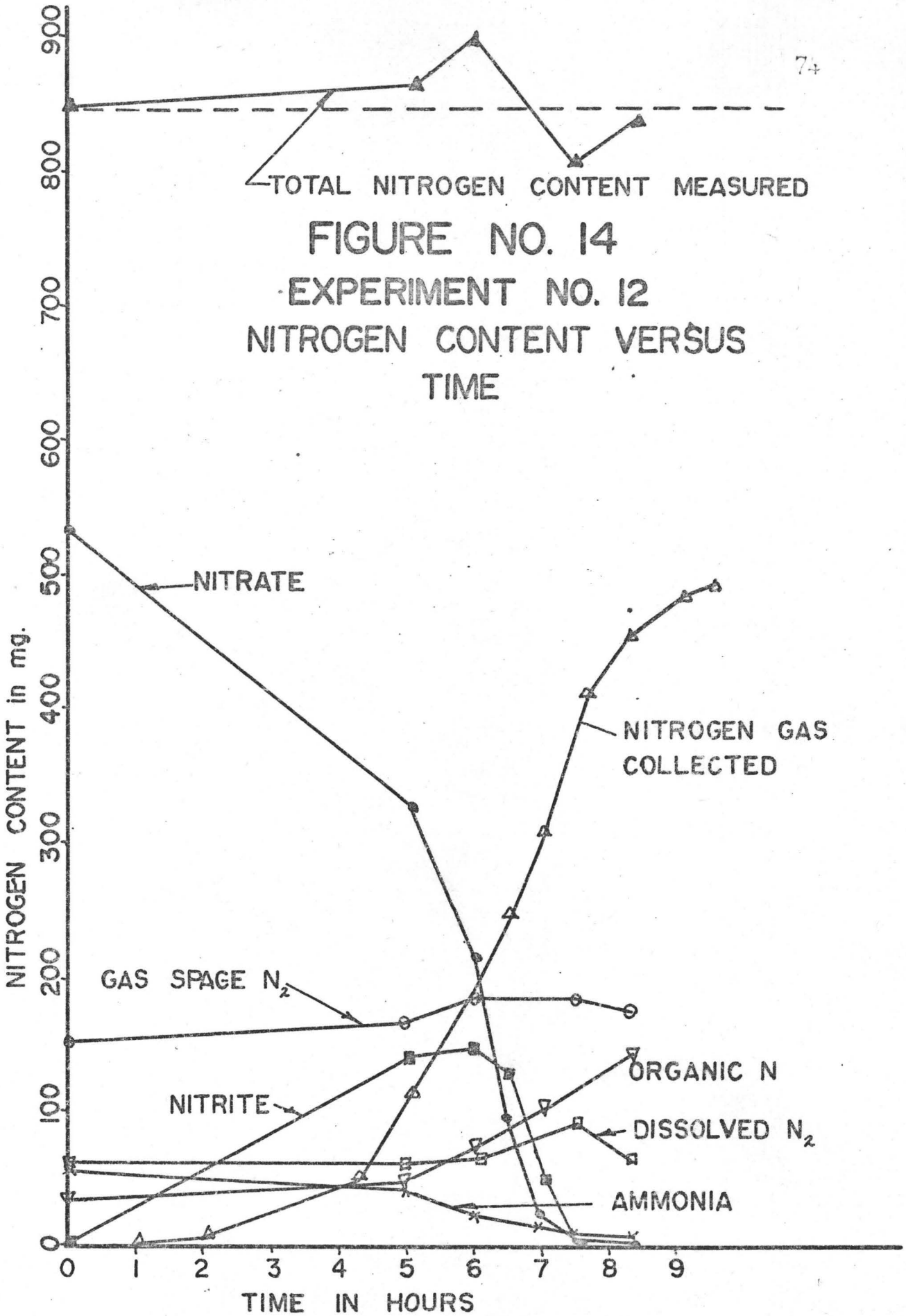
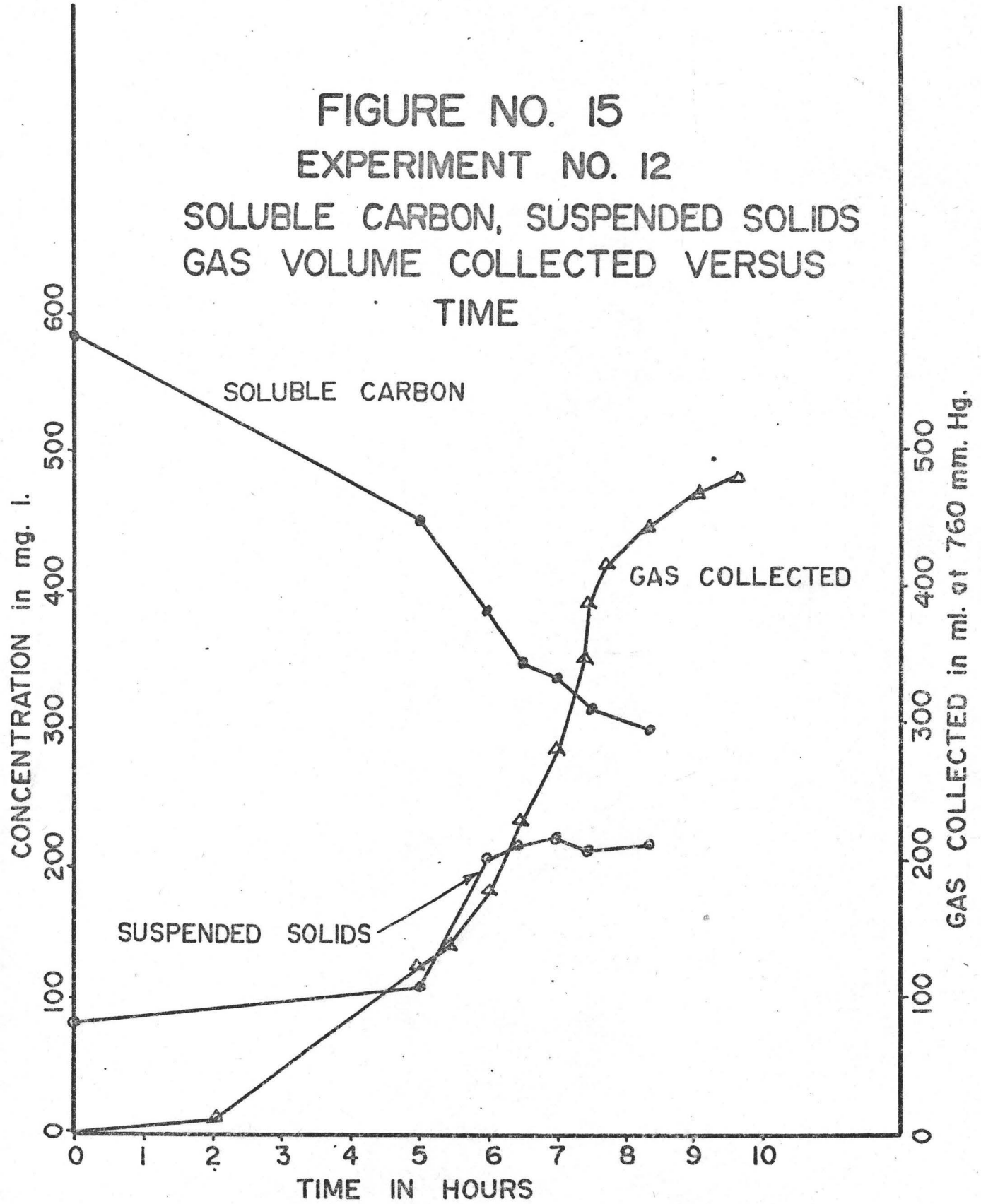


FIGURE NO. 14

EXPERIMENT NO. 12

NITROGEN CONTENT VERSUS TIME

FIGURE NO. 15
EXPERIMENT NO. 12
SOLUBLE CARBON, SUSPENDED SOLIDS GAS VOLUME COLLECTED VERSUS TIME



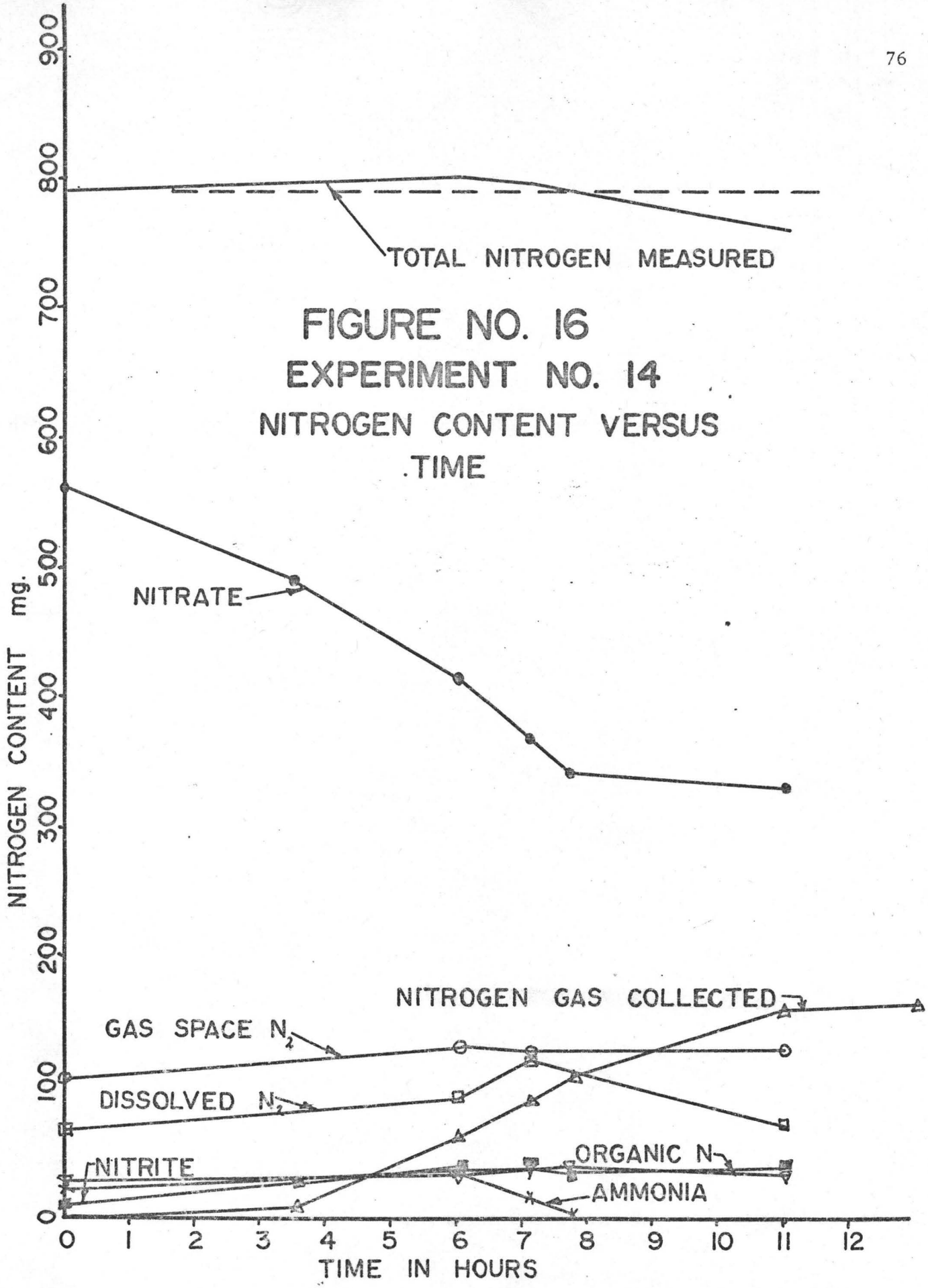
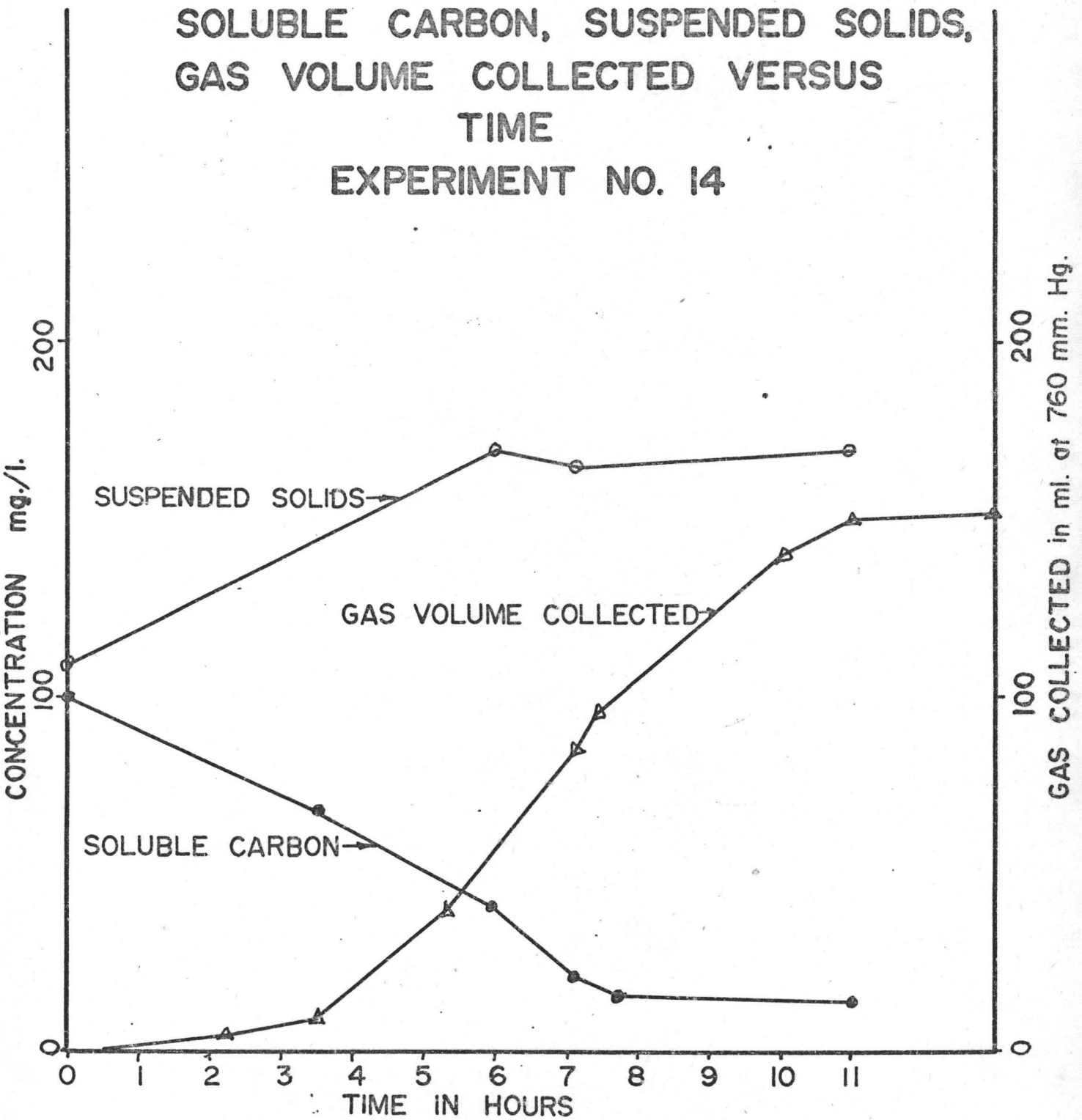


FIGURE NO. 16
EXPERIMENT NO. 14
NITROGEN CONTENT VERSUS
TIME

FIGURE NO. 17

SOLUBLE CARBON, SUSPENDED SOLIDS, GAS VOLUME COLLECTED VERSUS TIME EXPERIMENT NO. 14



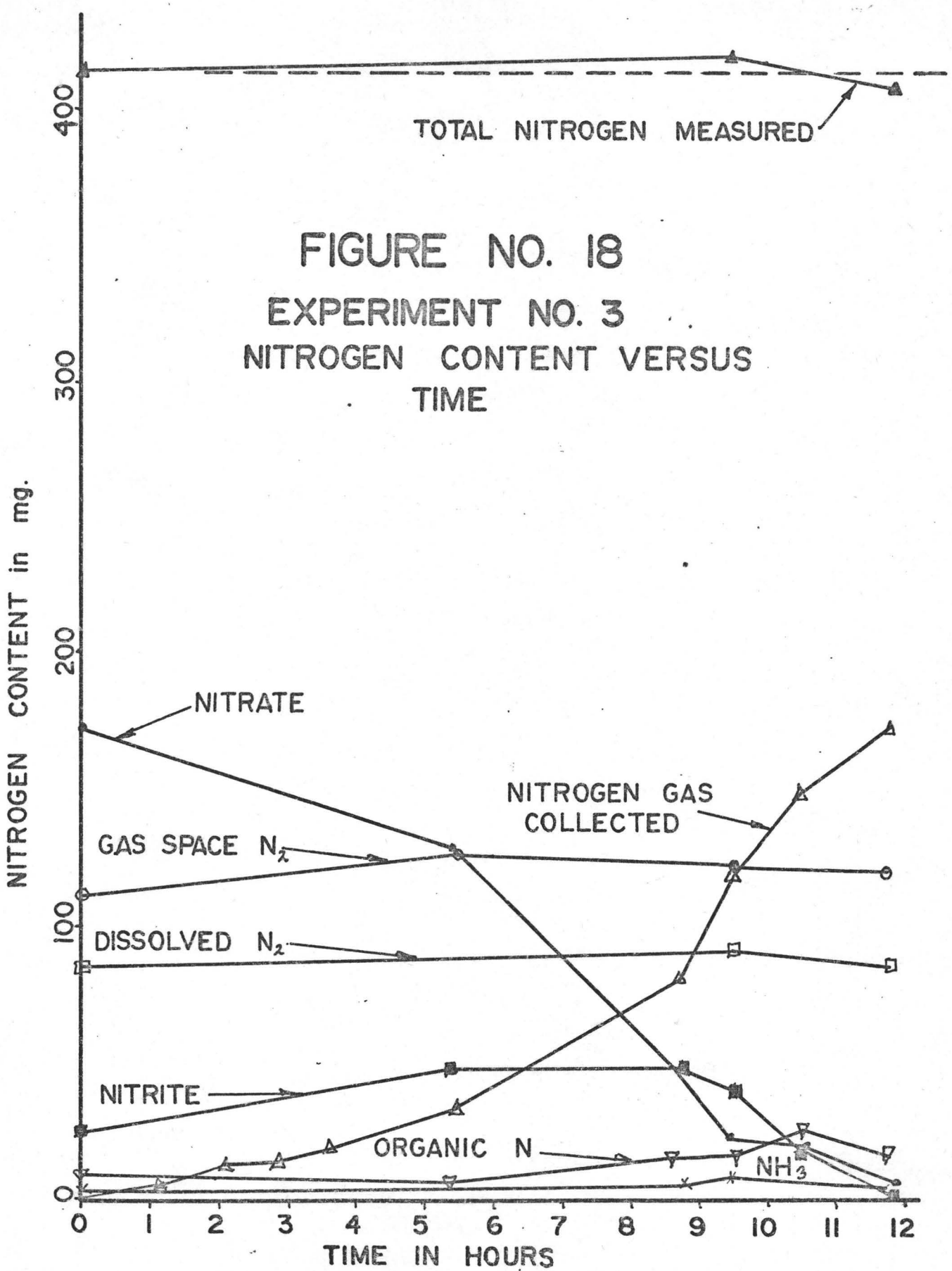


FIGURE NO. 18

EXPERIMENT NO. 3

NITROGEN CONTENT VERSUS TIME

had been reduced and then essentially ceased. Similarly, organism growth commenced following the lag period and reached a maximum level at the same time as denitrification stopped and either levelled off or the population started to decrease.

As shown in Figure 16 and Figure 17 for an experiment where the carbon to nitrate ratio was 1:1, the nitrate removal is incomplete. Carbon removal occurs to a low level (10 mg/l) where the organisms appear unable to utilize it further. In many of the experiments (Experiment No. 5, Figure 20) it was difficult to determine whether a lag period occurred or not from the nitrogen and soluble carbon data, but the gas production figures were useful to establish its existence.

As the plots of carbon and nitrate concentration were linear with respect to time after the acclimation period, it could be concluded that carbon removal and nitrate removal are independent of their respective concentrations, i.e. the reactions were zero order with respect to carbon and nitrate concentration.

The other forms of nitrogen monitored were dissolved ammonia concentration, dissolved nitrogen concentration, gaseous nitrogen concentration, and Total Kjeldahl (organic nitrogen concentration). Originally, the ammonia concentrations were fairly low, usually in the range of 5 to 10 mg/l, and reflected the NH_3 content of the organism seed solution. The ammonia rapidly disappeared during the reaction as it was

FIGURE NO. 19
EXPERIMENT NO. 3
SOLUBLE CARBON, SUSPENDED SOLIDS, GAS VOLUME COLLECTED VERSUS TIME

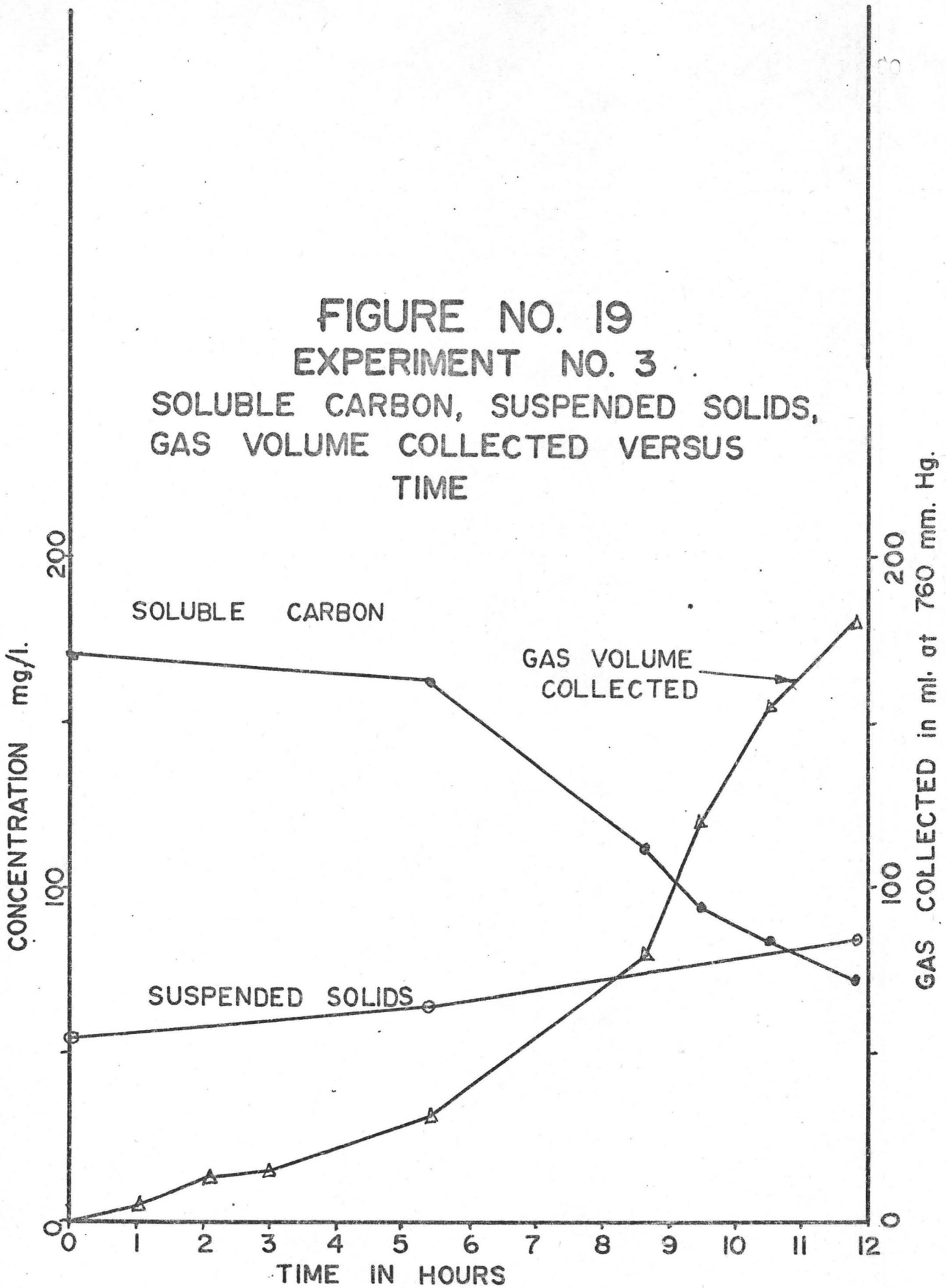


FIGURE NO. 20

EXPERIMENT NO. 5

NITROGEN CONTENT VERSUS TIME

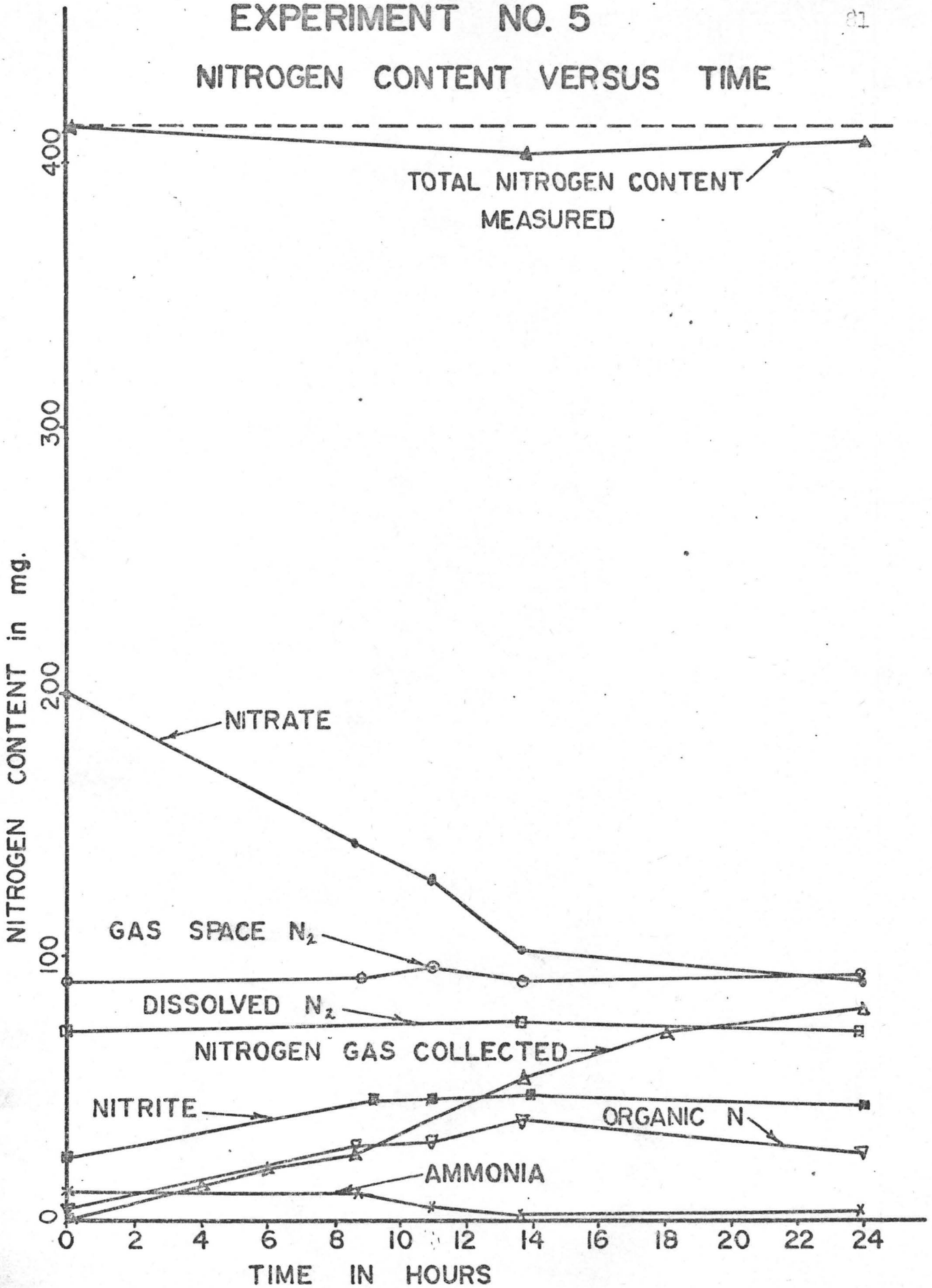
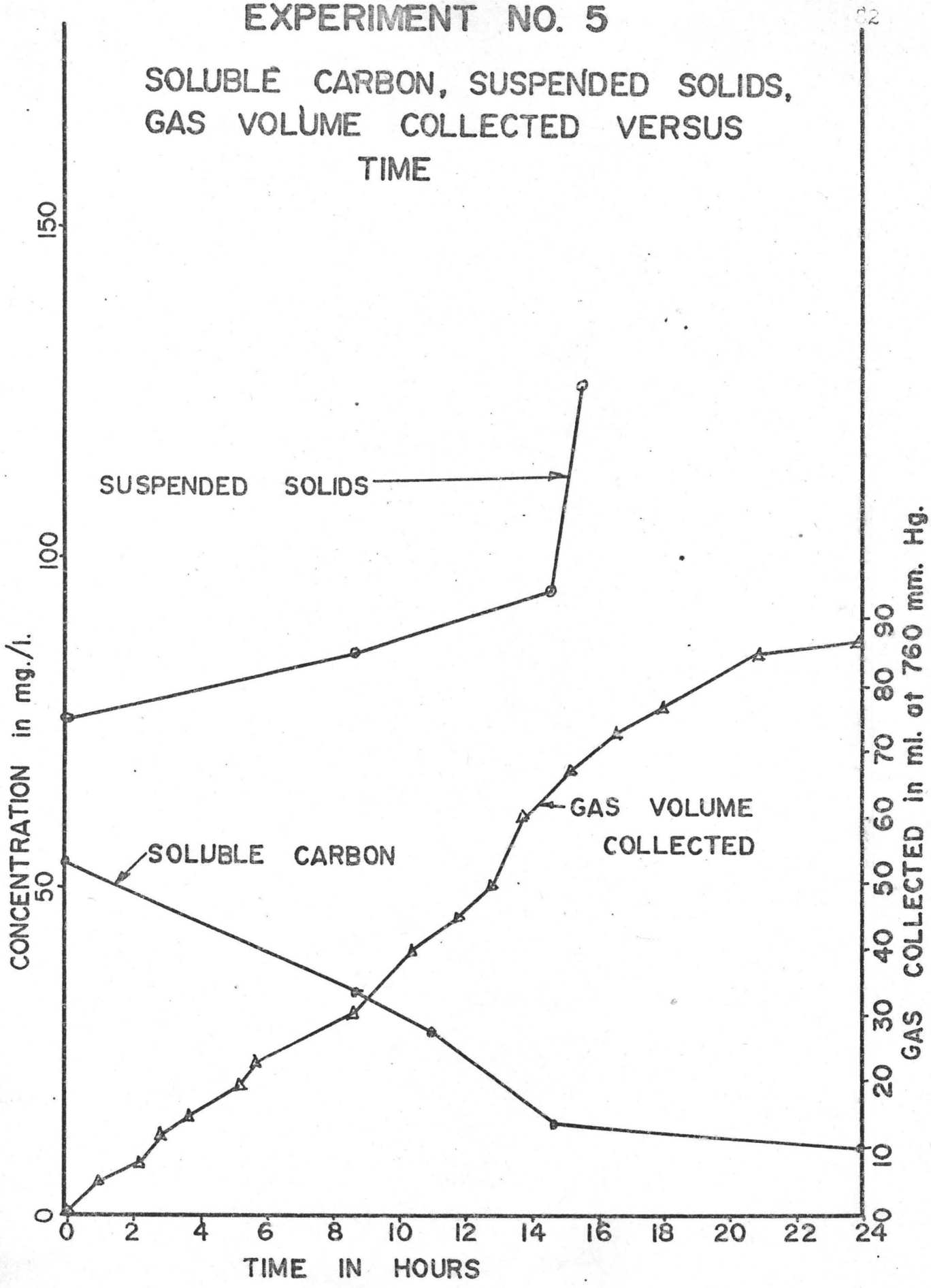


FIGURE NO. 21

EXPERIMENT NO. 5

SOLUBLE CARBON, SUSPENDED SOLIDS, GAS VOLUME COLLECTED VERSUS TIME



assimilated into cellular nitrogen compounds. This was confirmed by the fact that although the ammonia concentration dropped the total Kjeldahl concentration in the unfiltered samples usually increased or remained relatively constant throughout the reaction. The ammonia concentrations and filtered total Kjeldahl analyses agreed closely. The difference between the unfiltered and filtered total Kjeldahl was assumed to be a measure of cellular organic carbon. Since these Kjeldahl results rangedfrom 4 to 6% of the suspended solids concentrations the cellular nitrogen levels were lower than the 8 to 12% predicted as a nitrogen content by the equations for organisms of Hoover (1952), and Helmers (1951).

The dissolved nitrogen concentrations at the beginning of the batch experiments were usually close to the nitrogen saturation levels at 27°C, i.e. 16.9 mg/l since the reactor contents had been scrubbed of oxygen with nitrogen gas prior to each run. During the course of the reaction, the liquid was supersaturated with dissolved nitrogen as the rate of N_2 production exceeded the diffusion rate out of solution into the gas space.

At pH 6.5, the dissolved carbon dioxide levels gradually increased throughout the reaction, while at pH 8.5 CO_2 did not appear as a dissolved gas component. Presumably at the high pH the CO_2 produced immediately entered into the carbonate buffer equilibrium.

Chromatographic analysis of the collected product gases showed a gradual increase in the nitrogen concentration from approximately 79% at the beginning of the experiments for the reactions at pH 8.5. At the same time a decrease in oxygen concentration was noted from 21% to less than 1%. For the reactions at pH 6.5 both nitrogen and carbon dioxide concentration generally increased as the reactions progressed with a corresponding gradual decrease of oxygen to 0%.

Calculation of Denitrification Rate. For comparison of the experimental results from the 16 screening experiments a characteristic denitrification rate was established. Most engineering design work for biological waste treatment processes is based on the unit reaction rate or in this case unit denitrification rate. This can be defined as the concentration change of substrate per concentration change of organisms per unit time, i.e. for denitrification Δ mg/l NO_3 as N/Δ mg/l organisms - hr. This characteristic unit rate was calculated from the experimental data by fitting the nitrate versus time data for the linear removal period with a least squares straight line; determining the slope of this straight line, and then dividing the slope by the organism concentration at mid reaction time, (determined from least squares straight line fit of the solids data). The least squares straight lines were calculated using Program 1 in Appendix B, and the typical results are shown in Figure 22 and Figure 23 for Experiment No.16.

FIGURE NO. 22
EXPERIMENT NO. 16
LEAST SQUARES FIT OF NITRATE DATA

UNIT RATE CALCULATION

NO = 91·5 - 3·0 = 88·5 mg. l.

T = 10·7 - 4·5 = 6·2 hr.

Cell conc. at 7·6 hr. from Fig. 23 = 202 mg. l.

Unit rate = $\frac{88.5}{6.2\ 202}$ = 0·071 mg. l. NO as N / mg. l. cells - hr

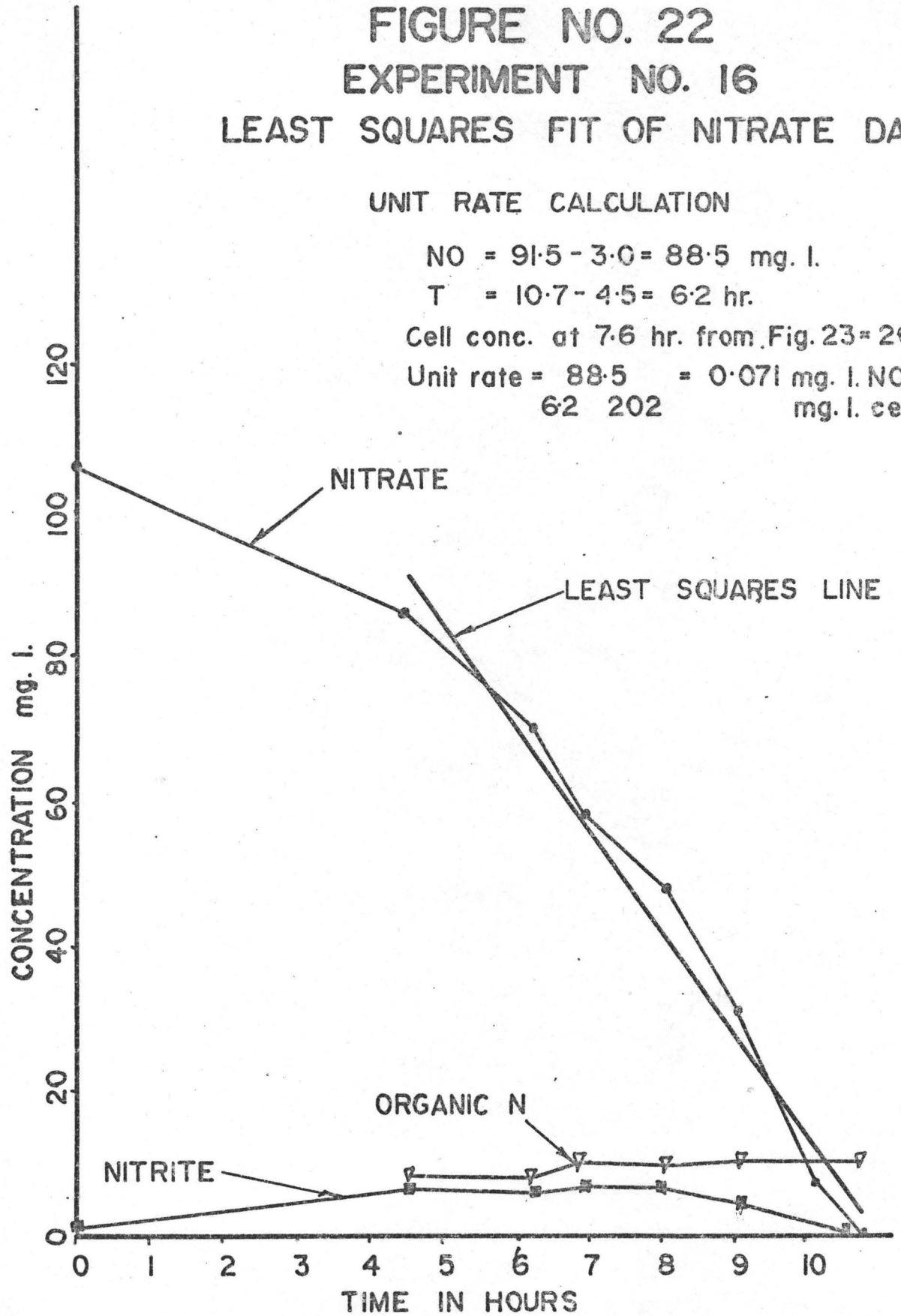
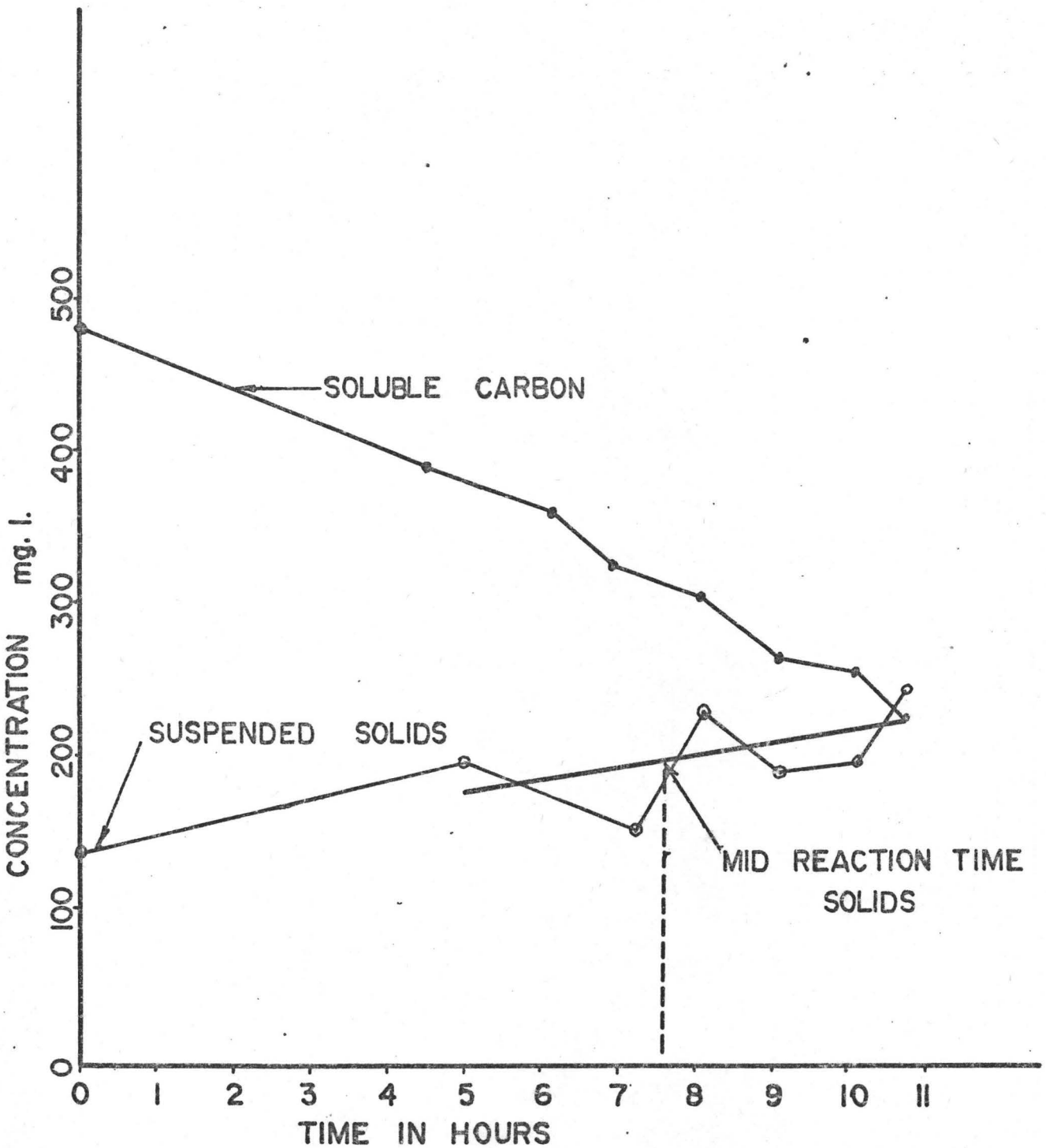


FIGURE NO. 23
EXPERIMENT NO. 16
LEAST SQUARES FIT OF SOLIDS DATA



The unit denitrification rates determined for these 16 experiments are shown in Table No.7.

Effect of Experimental Variables on Unit Denitrification Rate. The calculation of the average effect of each of the factors and interactions between the factors is shown in Appendix C, and the results are shown in Table No.8.

The unit denitrification rate was, therefore, independent of organism concentration, while all of the other factors were significant including the nitrate concentration. An analysis of variance on these screening experiments - Table No.B-2 Appendix B - also showed that carbon to nitrate ratio, nitrate concentration, and pH were all significant effects at the 95% confidence level. The significant effect of nitrate contradicts the evidence of zero order reaction with respect to nitrate concentration determined from the individual plots of nitrogen concentration versus time. The reason for this was that the experimental design does not really unlock the interaction effect of carbon and nitrate, since carbon and nitrate varied as a ratio. Therefore, the average effect of carbon and nitrate concentration on the unit denitrification rate was evaluated separately in a full factorial design at the conditions given in Table No. 9.

The average effects were + 0.00055 mg/l NO_3 as N / mg/l organisms - hour for a change of nitrate concentration from 40 mg/l to 120 mg/l and + 0.0324 mg/l NO_3 as N / mg/l organisms - hour for a change in carbon concentration from

TABLE 7.

SCREENING EXPERIMENTS
UNIT DENITRIFICATION RATES.

Expt. No.	NO_3	$C:NO_3$	pH	Organisms	Unit Denitrification Rate $\frac{mg/l\ NO_3}{mg/l\ organisms\text{-}hr}$
1	-	-	-	-	0.0307
2	+	-	-	-	0.0550
3	-	+	-	-	0.0678
4	+	+	-	-	0.1270
5	-	-	+	-	0.0150
6	+	-	+	-	0.0406
7	-	+	+	-	0.0424
8	+	+	+	-	0.0613
9	-	-	-	+	0.0246
10	+	-	-	+	0.0565
11	-	+	-	+	0.0600
12	+	+	-	+	0.1430
13	-	-	+	+	0.0198
14	+	-	+	+	0.0290
15	-	+	+	+	0.0508
16	+	+	+	+	0.0710

TABLE 8.

AVERAGE EFFECT OF FACTORS ON UNIT DENITRIFICATION

16 Screening Experiments.

Factor	Change in Level	Effect on Denitrification Rate.
Carbon to Nitrate Ratio	1:1 to 5:1	+ 0.0440 $\frac{mg/l\ NO_3\ as\ N}{mg/l\ organisms\text{-}hr.}$
Nitrate Concentration	40 to 120	+ 0.0340 "
pH	6.5 to 8.5	- 0.0203 "
Organisms Concentration	50 to 100	+ 0.00107 "

TABLE 9

2^2 FACTORIAL DESIGN TO EVALUATE THE EFFECT OF CARBON AND NITRATE ON UNIT DENITRIFICATION RATE.

Expt.No.	NO_3	C	pH	Organisms	Unit Denit.Rate.
18	+	-	-	+	0.0960
10	+	+	-	+	0.0565
17	-	+	-	+	0.1290
9	-	-	-	+	0.0246

Design Level	+1	-1
Carbon Concentration	120	40
Nitrate Concentration	120	40
pH		6.5
Organism Concentration		100

40 mg/l to 120 mg/l of carbon. Nitrate concentration from these experiments appears to have little effect on the denitrification rate.

This work was then expanded to a full factorial design to investigate the effect of nitrate concentration, carbon concentration and pH at the same time. The experimental design, along with the resulting unit denitrification rates are shown in Table No. 10. Note that four of the experiments were drawn from the original 16 screening experiments.

The average effects of carbon and pH were approximately 5 times the effect of nitrate on the denitrification rate, and the analysis of variance of these eight experiments shown in Table B-3 of Appendix B demonstrated that carbon and pH and the interaction effect between carbon and nitrate were significant at the 95% confidence level.

Determination of Optimum pH Since hydrogen ion concentration was determined as a significant factor, a series of experiments was run to find an optimum pH range for denitrification. Some of the experimental data from the screening experiments was included in this investigation. Experiments were performed within a pH range of 6.0 to 9.0 the extremes of hydrogen ion concentration which might be expected at a wastewater treatment plant. The experimental temperature was 27°C with a carbon to nitrate ratio of approximately 2.5:1 to 5:1. The unit denitrification rate

TABLE 10.

2^3 FACTORIAL DESIGN TO EVALUATE THE EFFECT OF CARBON pH AND NITRATE CONCENTRATION ON THE UNIT DENITRIFICATION RATE.

Expt No.	Nitrate Conc.	Carbon Conc.	pH	Unit Denit Rate $\frac{mg/l\ NO_3}{mg/l\ Organisms\ -hr}$
9	-	-	-	0.0246
13	-	-	+	0.0198
17	-	+	-	0.1290
18	-	+	+	0.0880
10	+	-	-	0.0960
6	+	+	-	0.0565
20	+	+	+	0.0406
	+	-	+	0.0321

variation with pH is shown on Figure No. 24. An optimum pH of 7.0 with a corresponding unit denitrification rate of 0.16 to 0.18 mg/l NO_3 per mg/l of organisms per hour was established. The experimental data shown in this graph are summarized in Table No. 11. This broad optimum for Pseudomonas denitrificans is well within the normal pH of wastewater treatment.

Determination of the Effect of Carbon on the Denitrification Rate - The amount of carbon required for complete reduction of nitrate is determined by the stoichiometry of the parallel reactions of nitrate reduction and carbon degradation. The overall reaction proposed for nitrate reduction with citrate as the hydrogen or electron donor which follows, indicates a carbon requirement of 1.7 mg of carbon per mg of nitrate as N.

$$4\ C_6H_5O_7 + 12\ KNO_3 \rightarrow 12\ CO_2 + 12\ KHCO_3 + 6\ N_2 + 4\ H_2O$$

The amount of carbon utilized in the batch reactions also included the soluble carbon converted to cellular organic material during bacterial growth. In Table No. 12 the overall nitrate reduction and soluble carbon utilized during the original 16 screening experiments is tabulated. To determine the approximate carbon utilized during nitrate reduction, the change in suspended solids concentration was first related to organic carbon in the form of cells according to the approximate formula for cells of Hoover and Porges (1952) i.e. $C_5H_7NO_2$.

FIGURE NO. 24

VARIATION OF UNIT DENITRIFICATION RATE WITH HYDROGEN ION CONCENTRATION

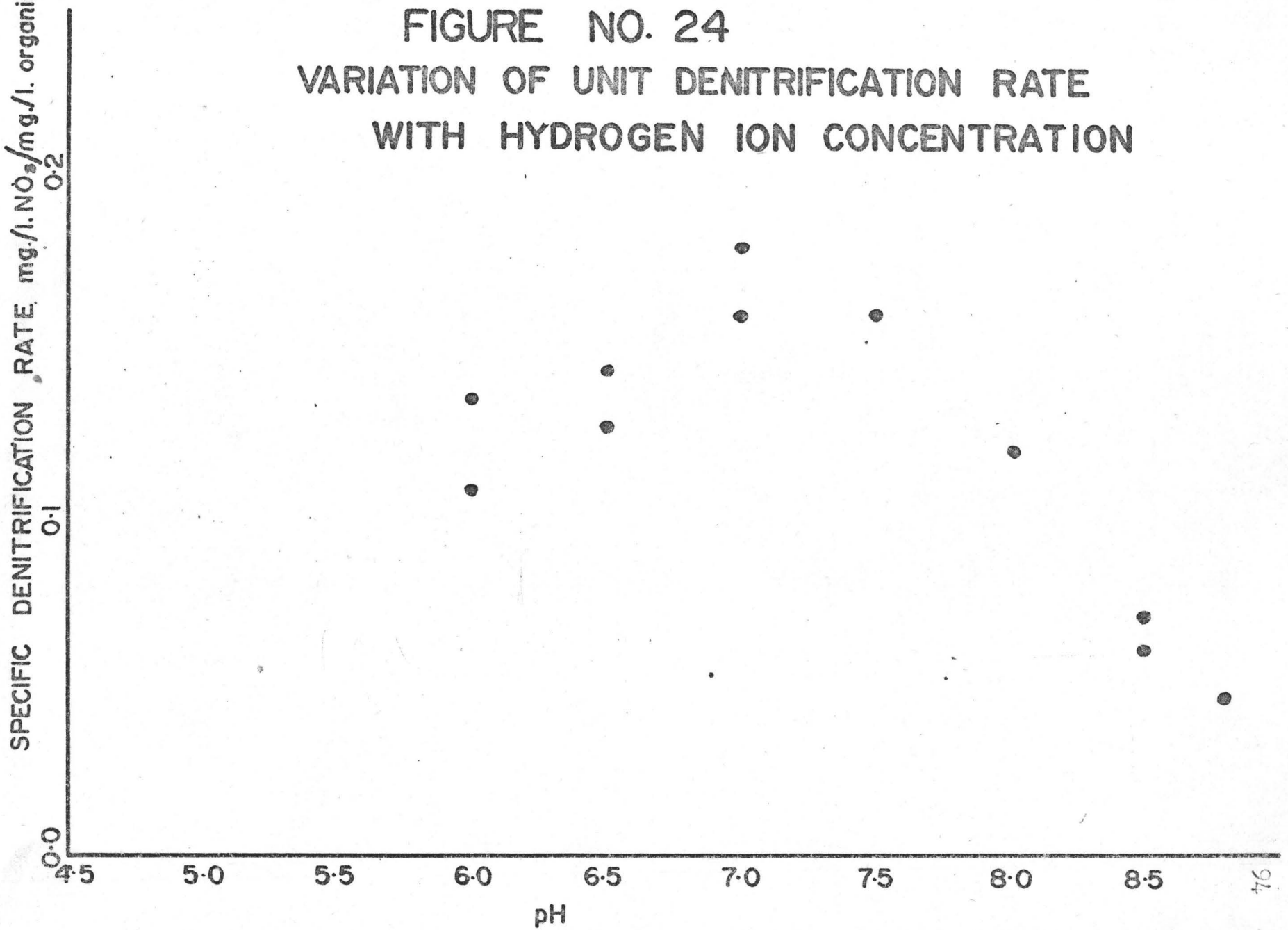


TABLE 11.

UNIT DENITRIFICATION RATE VARIATION WITH HYDROGEN ION CONCENTRATION

pH	Expt No.	Initial Nitrate Conc. mg/l	Initial Carbon Conc. mg/l	Initial Organism Conc. mg/l	Unit Denitrification Rate $\frac{mg/l\ NO_3\ as\ N}{mg/l\ organisms\text{-}hr}$
6.0	23	62.90	264	40	0.1350
6.0	24	62.00	250	30	0.1070
6.5	12	105.25	585	80	0.1430
6.5	4	113.00	625	70 *	0.1270
7.0	25	101.20	270	90	0.1600
7.0	26	101.30	272	85	0.1800
7.5	41	121.50	310	60	0.1610
8.0	21	87.60	245	25	0.1200
8.5	16	106.8	485	140	0.0710
8.5	8A	94.5	675	80	0.0613
8.8	22	80.2	210	60 *	0.143

* Initial solids not actually determined.

TABLE NO.12

CARBON UTILIZATION IN THE ORIGINAL 16 SCREENING EXPERIMENTS.

Expt. No.	Observed Carbon mg/l	Observed Nitrate mg/l	Observed Cells mg/l	Predicted Carbon in Cells	Carbon for Nitrate Reduction	Ratio Carbon to Nitrate for Reduction.
1	52	21.0	45	23.8	28.2	1.34
2	106	42.0	45	23.8	82.2	1.96
3	98	31.8	75	39.7	58.3	1.84
4	395	112.0	180	95.3	299.7	2.67
5	44	20.6	50	26.5	17.5	0.85
6	126	48.9	115	61.0	65.0	1.33
7	74	38.2	125	66.2	7.8	0.21
8	335	93.5	170	90.0	245.0	2.63
9	35	10.5	35	18.5	16.5	1.57
10	90	59.5	85	45.0	45.0	0.76
11	110	29.5	55	29.1	80.9	2.74
12	290	105.2	130	68.8	221.2	2.10
13	48	36.5	35	18.5	29.5	0.81
14	85	40.0	60	31.8	53.2	1.33
15	120	37.1	125	66.3	53.7	1.45
16	255	106.5	105	55.7	199.3	1.87

This carbon concentration was then subtracted from the observed soluble carbon consumed during each experiment, and the difference assumed to be carbon used for nitrate reduction. As shown in Table No. 12, the average ratio of soluble carbon used for reduction to nitrate as N reduced was 1.83 : 1.0. This is within 7% of the carbon to nitrate ratio predicted by the proposed equation.

The two series of screening experiments showed that the amount of carbon was the major factor affecting denitrification rate with a large interaction effect between carbon and nitrate concentration. The unit denitrification rates for all of the experiments at pH 8.5 were, therefore, plotted as a function of carbon to nitrate ratio. Data at a pH of 8.5 were used since the greatest amount of data was available at this pH level from preliminary experiments. The rates are tabulated in Table No.13. As shown on Figure No. 25 the unit denitrification rate increased with increasing carbon to nitrate ratio up to a ratio in the interval between that ratio predicted for nitrate reduction about 1.8, and the average ratio for both nitrate reduction and organism growth (2.7). Above the latter $C:NO_3$ ratio the unit denitrification rate was independent of carbon concentration. This graph agrees with the concentration independence predicted from the individual experimental plots of carbon concentration versus time.

TABLE NO.13.

UNIT DENITRIFICATION RATE VARIATION WITH CARBON TO NITRATE RATIO.

Ratio of Soluble Carbon to Nitrate as N.	Expt.No.	Original Nitrate Conc. mg/l	Original Carbon Conc. mg/l	Unit Denitrification Rate mg/l NO as N / mg/l cells-hr.
0.25	48	65.0	16	0.0038
0.70	47	67.8	14	0.0019
0.97	14	103.0	100	0.029
1.22	6	110.4	136	0.0406
1.42	5	38.0	54	0.015
1.41	13	44.0	62	0.0198
2.34	44	39.9	93	0.047
2.50	45	28.5	72	0.0365
2.95	46	29.8	88	0.049
4.74	7	38.9	184	0.0424
4.57	16	106.8	485	0.0710
5.05	8B	114.0	575	0.0700
5.40	15B	36.4	196	0.0485
5.55	15A	37.7	208	0.0508
7.15	8A	94.5	675	0.0613

FIGURE NO. 25
EFFECT OF CARBON TO NITRATE RATIO ON UNIT DENITRIFICATION RATE

In wastewater treatment, the aim is to reduce the nitrate concentration to zero. Therefore, any denitrification process must supply sufficient carbon to insure that both the nitrate reduction and organism growth requirements would be satisfied. In other words, a waste treatment process would operate under the concentration independent conditions. The exact kinetic expression at the lower carbon to nitrate ratios is, therefore, not a critical requirement of this study.

A comparison of the theoretical carbon requirements of this study with data from the literature is difficult because most other studies have been made with mixed populations, and with different carbon sources. For example, McCarty's (1969) work with methanol as the electron donor showed a carbon to nitrate ratio of 0.66 for theoretical reduction of nitrate to nitrogen gas. The difference with the present work is related to the metabolic pathway followed by the carbon, and the energy derived from each reaction. Similarly, the overall ratio of carbon used to nitrate reduced was 0.93 for McCarty's work with methanol as opposed to 2.5 for these batch studies in pure culture with citrate as the carbon source.

The Temperature Variation of Unit Denitrification Rate - As the previous experiments have established an optimum pH range and the concentration independence of denitrification,

rate with carbon and nitrate concentration an investigation of the dependence of unit rate on temperature could proceed. Duplicate batch experiments were run with initial nitrate concentration at 120 mg/l; carbon to nitrate ratio of 3:1; pH of 7.0; and initial organism concentration of approximately 100 mg/l; at temperatures of 5°C, 10°C, 15°C, 20°C, and 27°C. A single experiment was performed later at 3°C, as an extension of the work. The experimental plots of carbon and nitrate versus time were all similar in form to the previous screening experiment, i.e. a lag period followed by a linear removal with respect to time.

The results for 5°C reactions and 10°C reactions are shown in Figure 26 to Figure 29 for comparison of the lag period, and to demonstrate the linear removal characteristic. It should be noted that complete denitrification of the 5°C reactor contents from 80.0 mg/l NO_3 as N to 1.0 mg/l NO_3 was achieved within 48 hours at the low organism concentration of 100 to 200 mg/l. A simultaneous decrease in carbon concentration from 250 to 75 mg/l followed a similar pattern. These batch experiments indicate that if a significant percentage of activated sludge organisms (say 25%) were to perform at the same rate as the Pseudomonads used in these experiments at 5°C then complete denitrification of wastewater could be accomplished within 10 hours if sludge levels were maintained between 2,000 and 5,000 mg/l. The results also suggest that

FIGURE NO. 26

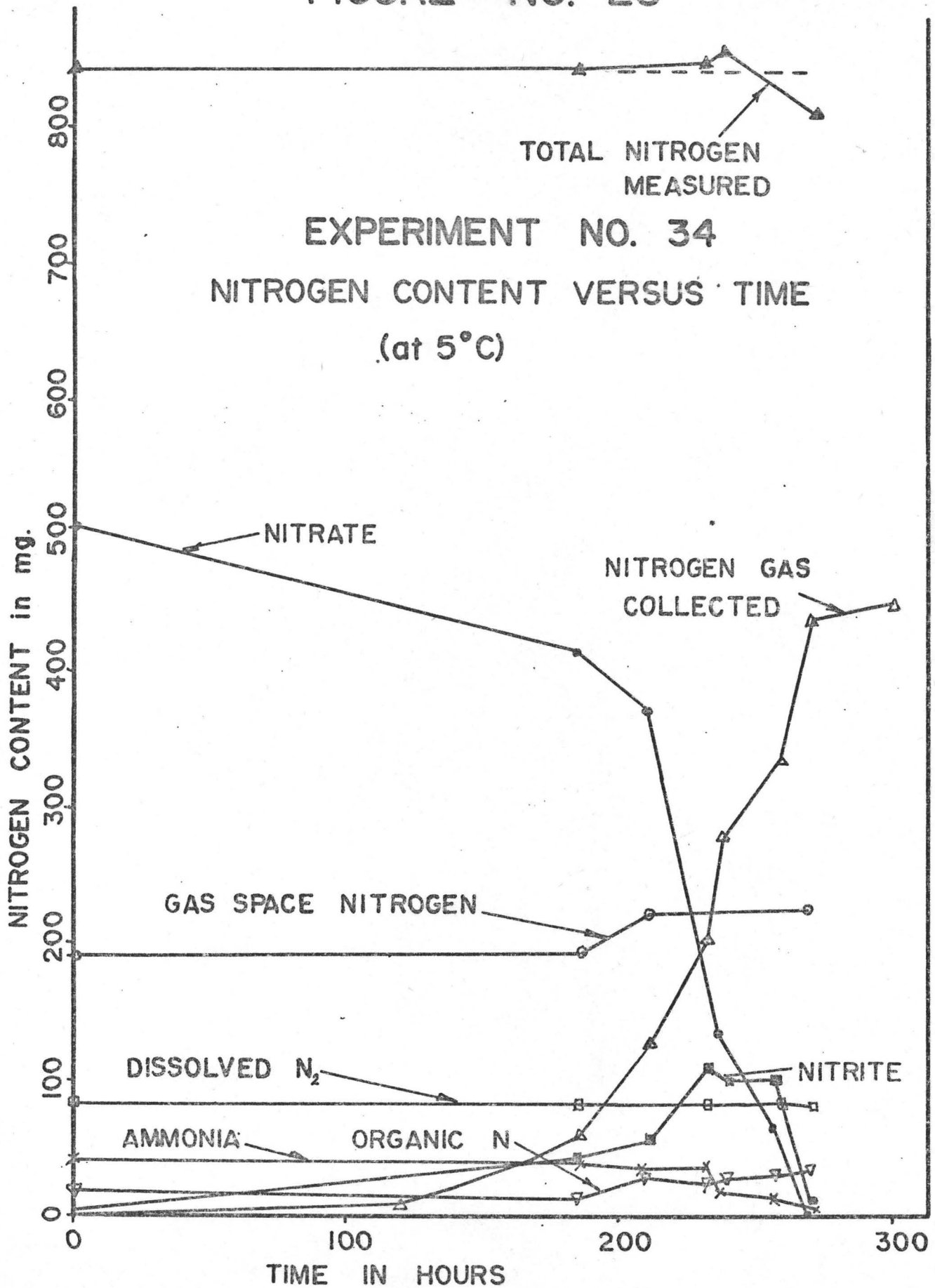
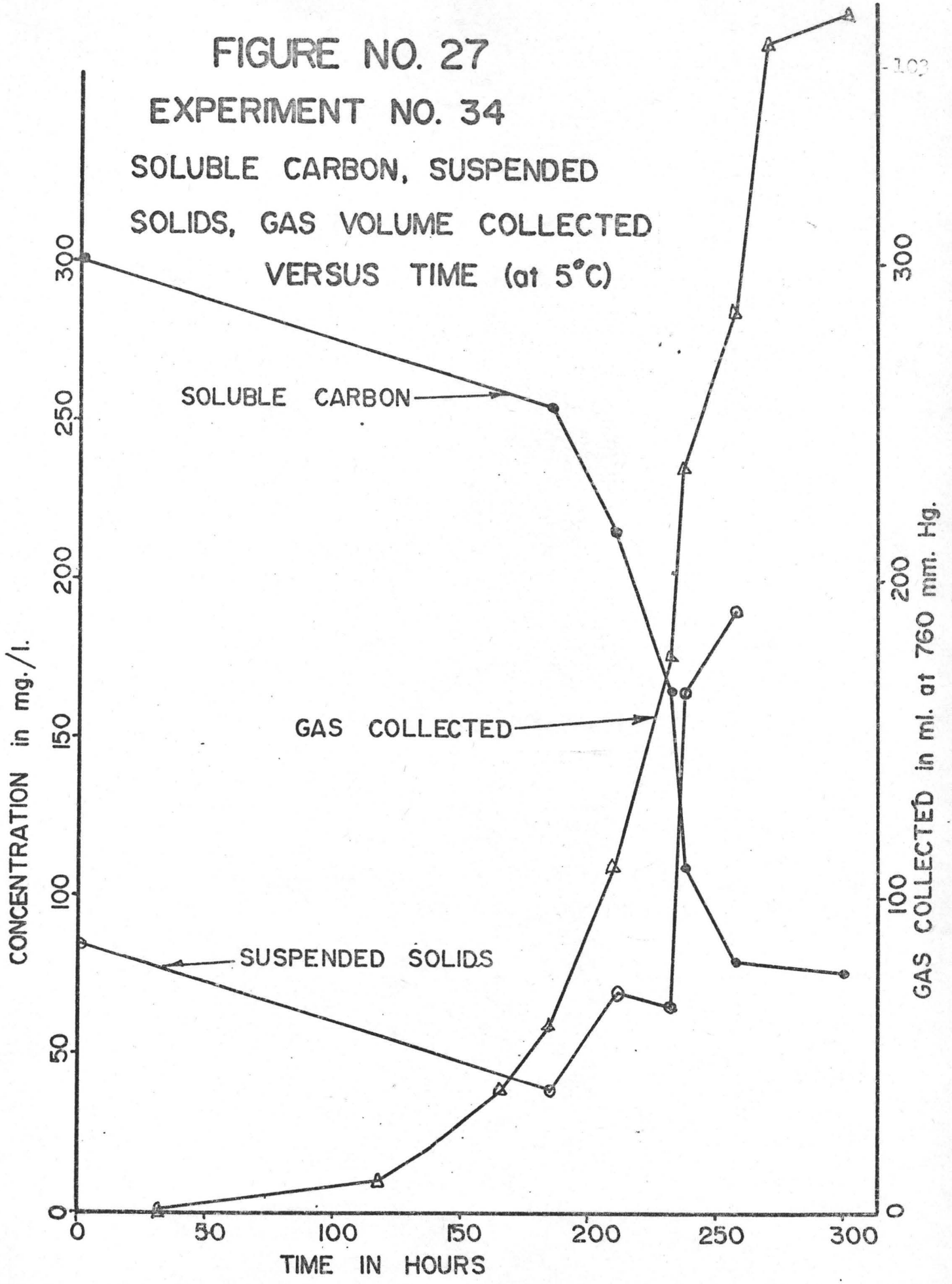

TOTAL NITROGEN MEASURED
EXPERIMENT NO. 34
NITROGEN CONTENT VERSUS TIME
(at 5°C)
NITRATE
NITROGEN GAS COLLECTED
GAS SPACE NITROGEN
DISSOLVED N_2
NITRITE
AMMONIA
ORGANIC N
NITROGEN CONTENT in mg.
TIME IN HOURS
0
100
200
300
400
500
600
700
800

FIGURE NO. 27

EXPERIMENT NO. 34

SOLUBLE CARBON, SUSPENDED SOLIDS, GAS VOLUME COLLECTED VERSUS TIME (at 5°C)



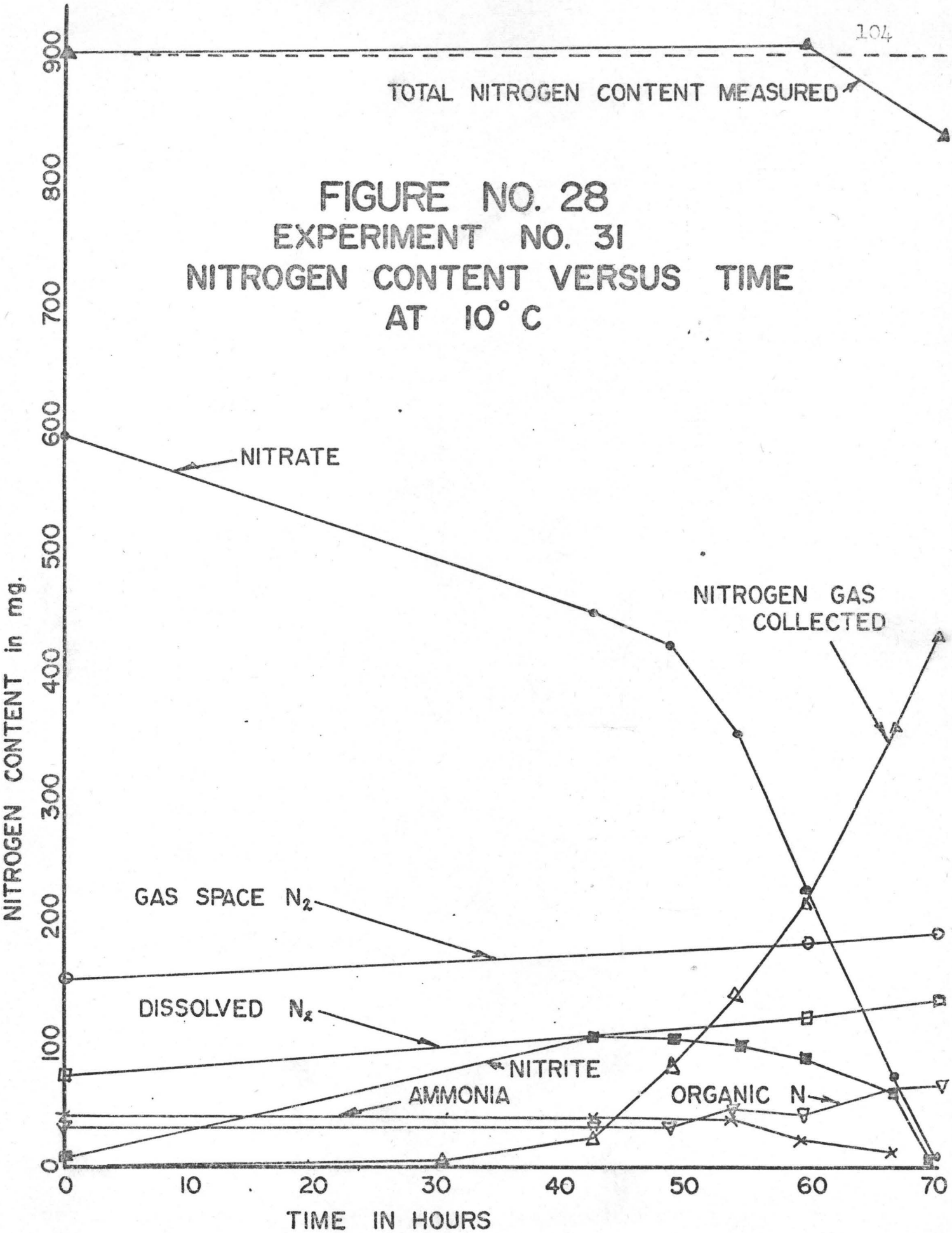
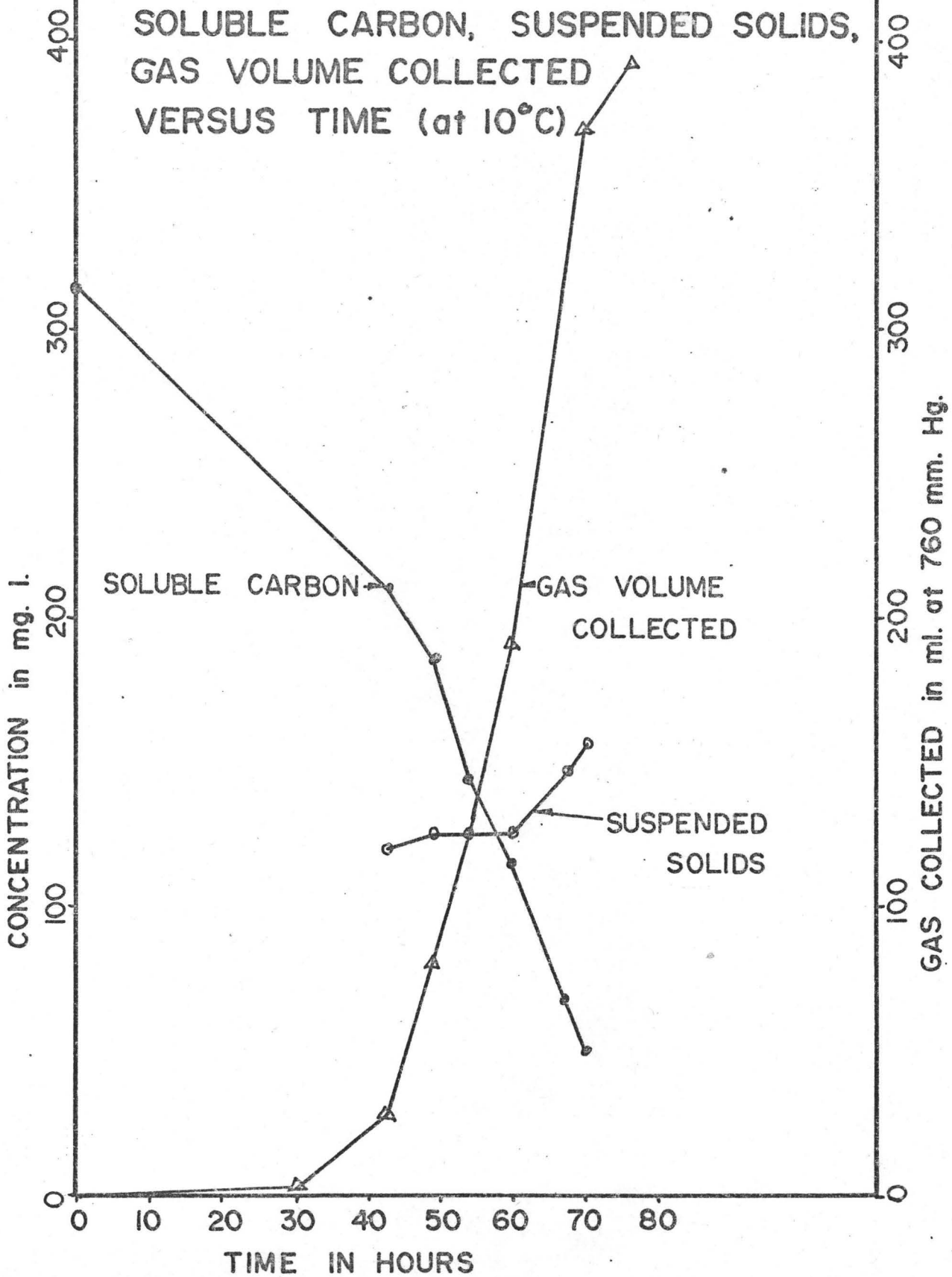


FIGURE NO. 28
EXPERIMENT NO. 31
NITROGEN CONTENT VERSUS TIME AT 10° C

FIGURE NO. 29
EXPERIMENT NO 31



denitrification would be significant in the deep water regions of lakes where temperatures drop to 4°C, the temperature of maximum water density.

The length of the lag period increased with decreasing temperature from about five hours for the 27°C to eight days for the 5°C reactors. The unit denitrification rates at the various temperatures are listed in Table No. 14, and are plotted as a function of temperature in Figure No. 30. Many enzyme reactions follow an Arrhenius temperature relationship of the form:

$$K = K_0 \, e^{-E/RT}$$

where K is the reaction rate constant in hr-1

K_0 is the frequency factor,

E is the activation energy in cal/gm mole,

R is the universal gas constant in cal/gm mole °K,

and T is the temperature in °K.

The data was then log transformed and fitted with a least squares straight line. This line of best fit is shown as the dashed line on Figure 30, along with the 95% confidence limits established from an analysis of variance. The activation energy calculated from the slope of the least squares line was 16.8 K cal/mole, which is well within the 2 to 24 cal/gm mole variation of many enzyme systems (Doelle, 1969).

Relation of Pseudomonas Denitrificans Pure Culture Work to Denitrification with a Mixed Culture. In wastewater

TABLE NO.14.

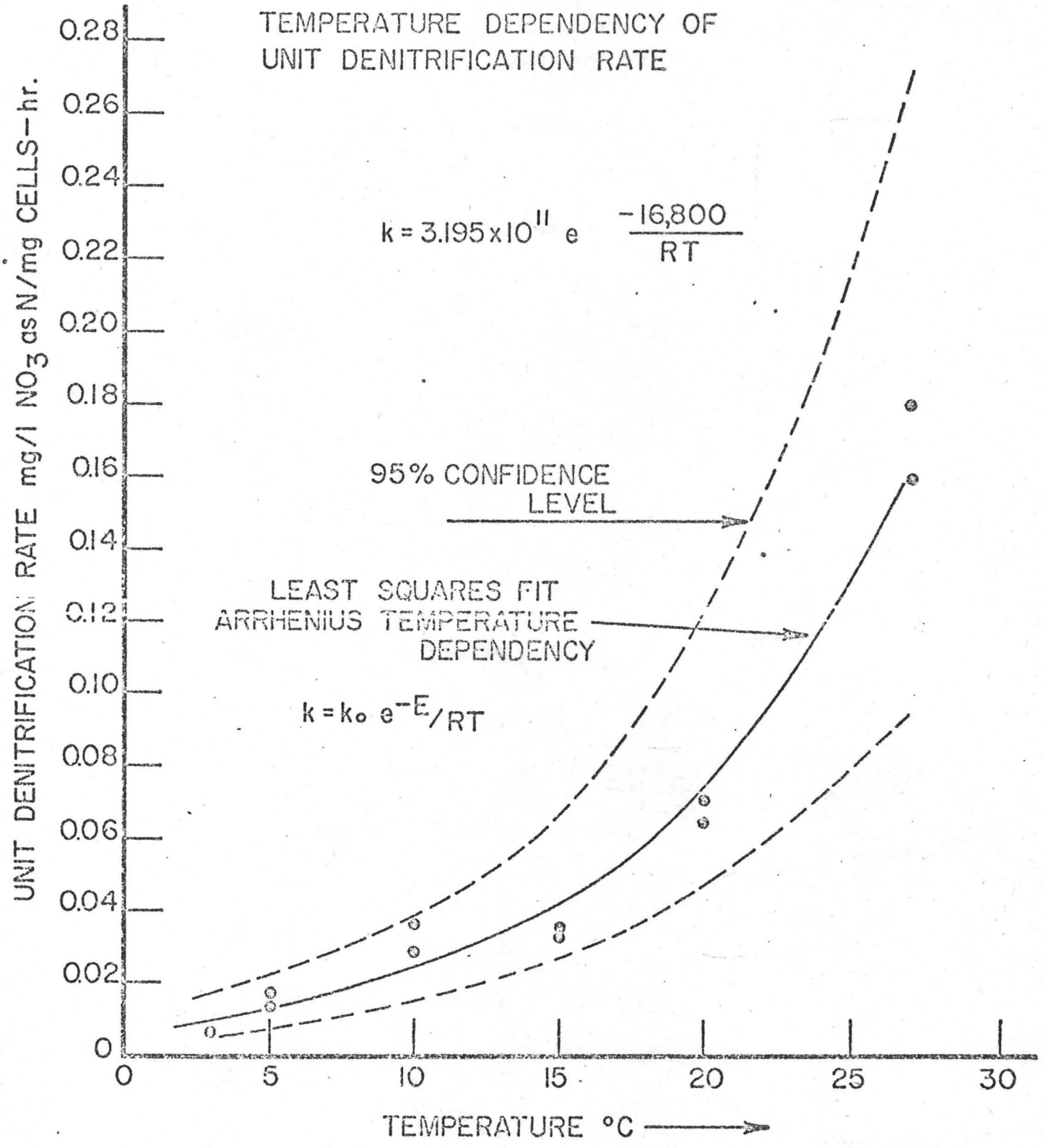
UNIT DENITRIFICATION RATE VARIATION WITH TEMPERATURE.

Temp. °C	Expt. No.	Initial Nitrate Conc. mg/l	Initial Carbon Conc. mg/l	Initial Organism Conc. mg/l	Unit Denitrification Rate mg/l NO_3 as N / mg/l organisms-hr.
27°	25	101.2	270	90	0.18
27°	26	101.3	272	85	0.16
20°	27	107.8	274	60	0.0716
20°	28	111.9	268	60	0.0656
15°	29	106.8	268	80	0.0354
15°	30	98.8	268	60	0.0348
10°	31	113.5	315	115 *	0.0285
10°	32	113.0	300	115 *	0.0284
5°	34	99.0	300	85	0.0135
5°	35	117.9	310	60 *	0.0175
3°	36	117.8	328	135 *	0.0063

* Initial solids not actually determined.

FIGURE. 30

TEMPERATURE DEPENDENCY OF
UNIT DENITRIFICATION RATE



treatment maintenance of a pure culture of denitrifying organisms would be almost impossible. It would be necessary to operate a continuous recycle system in which an enriched culture of denitrifiers would thrive. In order to relate the pure culture work of dilute dominant cultures of Pseudomonas denitrificans to waste treatment, batch experiments with activated sludge were carried out. A series of experiments with varying ratios of Pseudomonas sp to activated sludge making up a total organism population of 100 mg/l were run to determine an approximate estimate of the percentage of denitrifying organisms in activated sludge. Ratios of zero, 0.25:1.0, 0.5:1.0; 1.0:1.0 and ∞ were used in the experiments. A constant temperature of 27°C was used to provide optimum growth. The initial nitrate concentration was 120 mg/l as N. The initial carbon to nitrate ratio was varied between 3:1 and 5:1 to insure no limitation of rate due to lack of carbon and the pH was regulated at 7.5.

Figure No. 31 shows the results of nitrogen concentrations as a function of time for the 1:1 weight ratio of Pseudomonas sp to activated sludge organisms. A familiar pattern of lag period followed by the linear removal of nitrate with respect to time is demonstrated. The carbon and solids data also follows a similar pattern in Figure No. 32. As previously, the unit denitrification rates were determined by fitting the nitrate data with a least squares line, and dividing the slope of this line by the reaction mid-time solids

FIGURE NO. 31

EXPERIMENT NO. 42

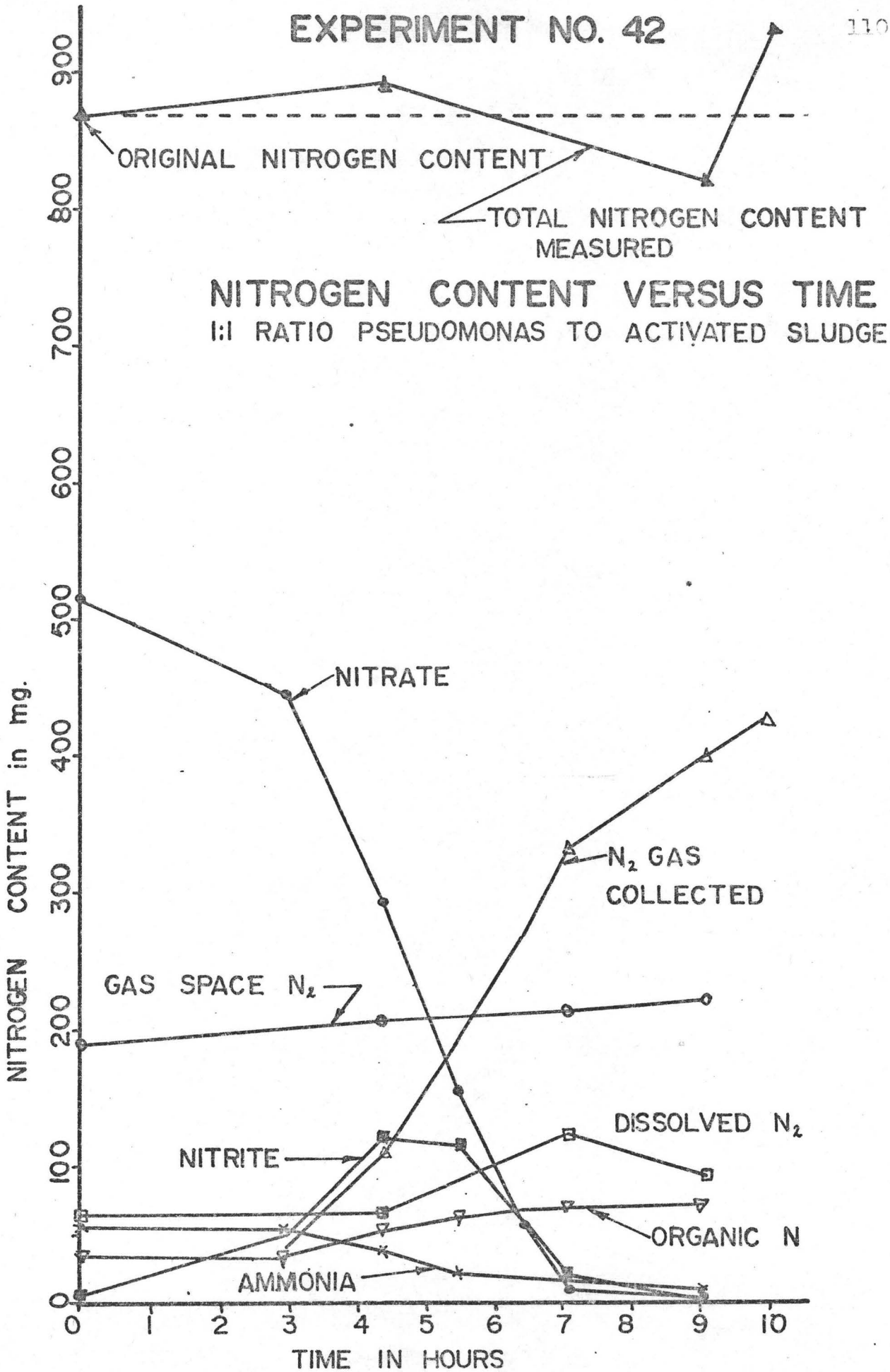
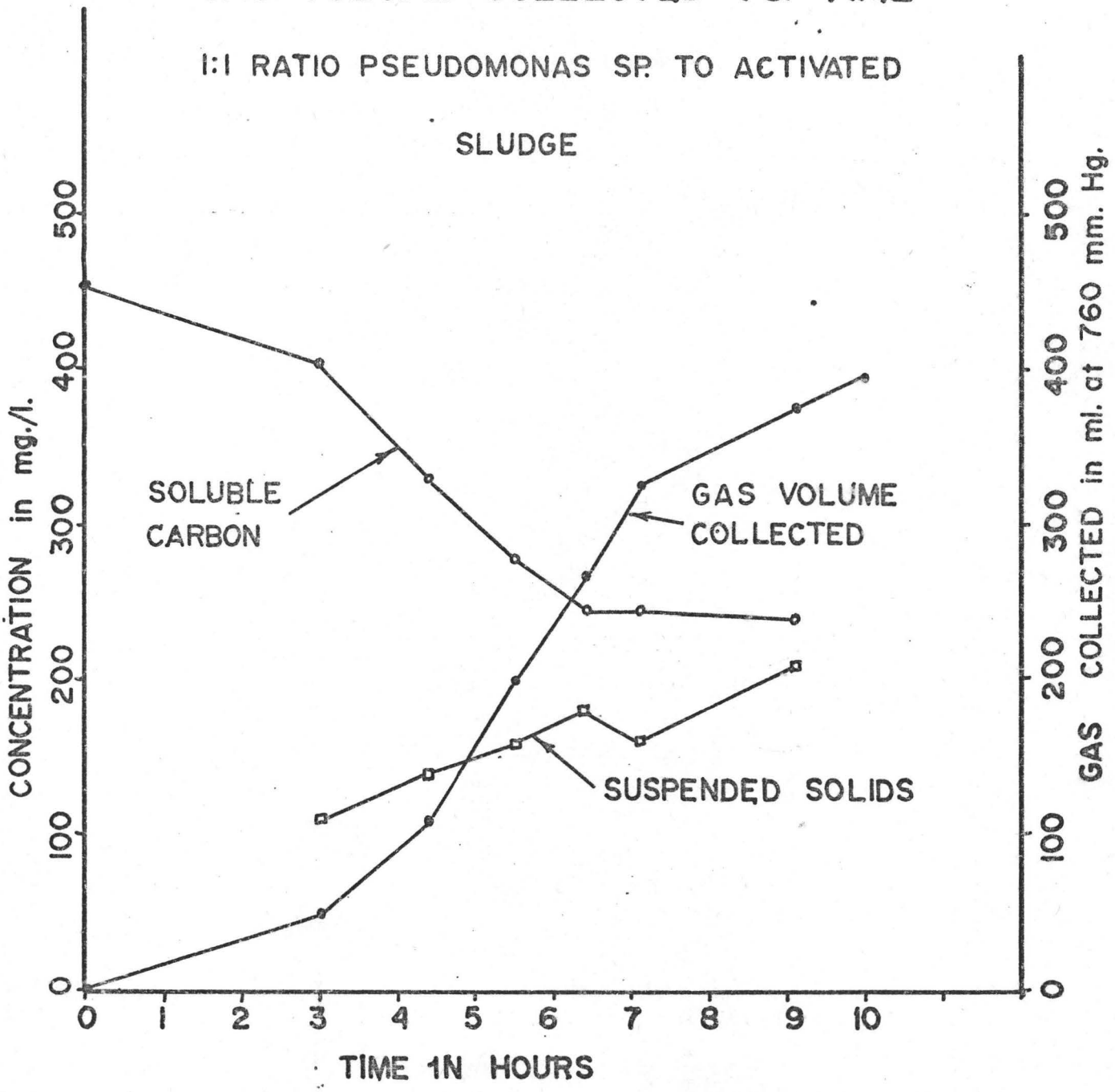


FIGURE NO. 32

EXPERIMENT NO. 42

SOLUBLE CARBON, SUSPENDED SOLIDS, GAS VOLUME COLLECTED VS. TIME

1:1 RATIO PSEUDOMONAS SP. TO ACTIVATED SLUDGE



concentrations. The experimental data for this series of runs is summarized in Table No.15, and the individual experiments are presented in tabular form in Appendix A.

In Figure No. 33, the unit denitrification rates are plotted as a function of the ratio Pseudomonas sp to activated sludge. Since the rates appeared to be increasing at a decreasing rate from the purely activated sludge condition to an asymptote at the pure culture rate, the data was fitted with a modified geometric function of the form $Y = A + BC^x$. In this case Y represents the unit denitrification rate and x the weight ratio of Pseudomonas sp to activated sludge. The constant A represents the value of unit denitrification rate to which the curve is an asymptote. The data was log transformed, values of A arbitrarily chosen, and a least squares straight line fitted to the semi-log plot Ln. (Y - A)versus X. This process was repeated until the residual sum of squares was a minimum and the corresponding value of A was chosen as the asymptote.

As shown in Figure No.33, the value of A of 0.158 which produced the minimum sum of squares was very close to the pure culture unit denitrification rate of 0.163 mg NO_3 as N/mg organisms-hr. The unit denitrification rate at pure activated sludge predicted by this curve was approximately 17% of the unit denitrification rate of the pure culture at similar conditions. The ratio of rates determined from the individual experiments was approximately 25%. In other words,

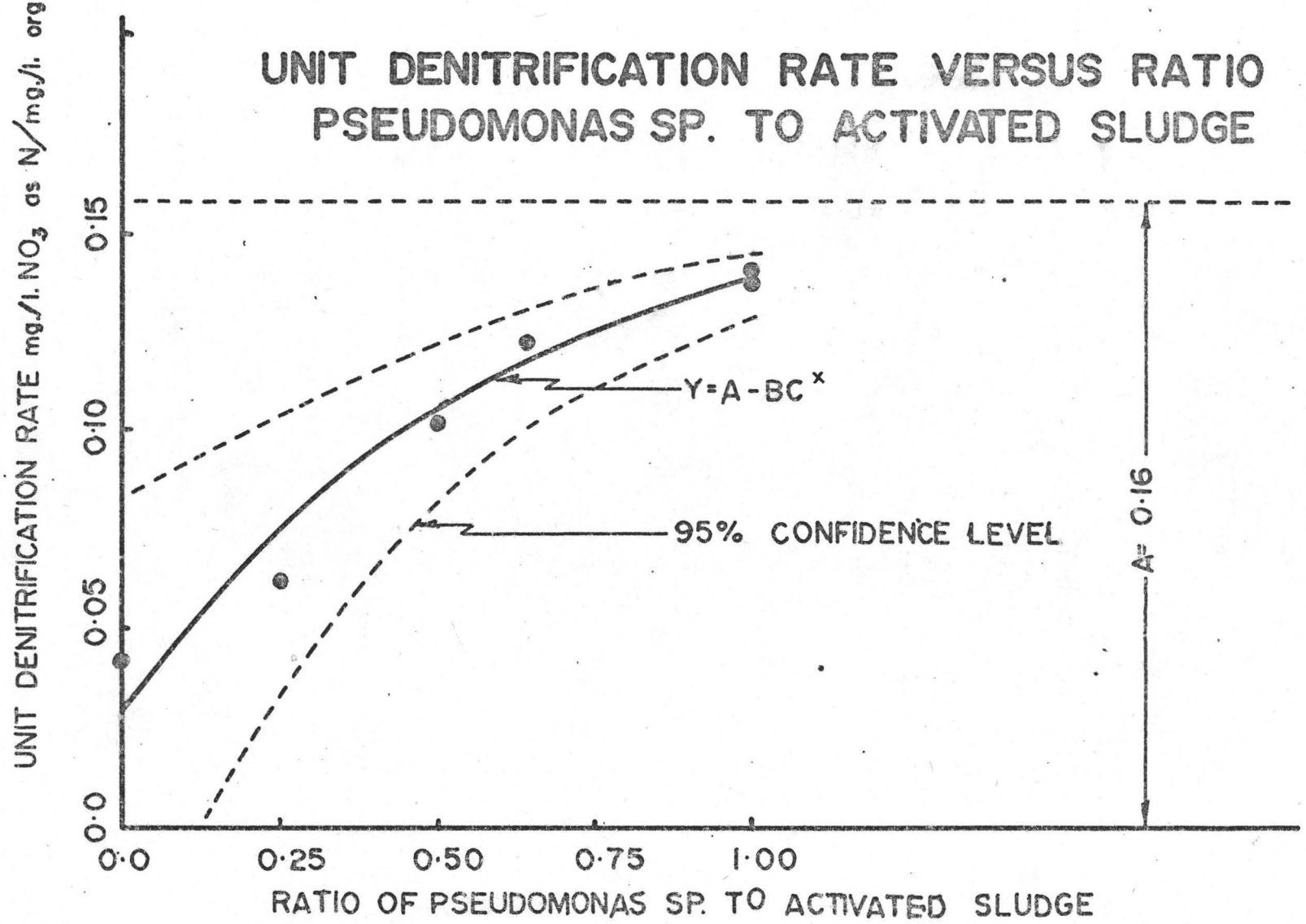
TABLE NO.15.

UNIT DENITRIFICATION RATES FOR BATCH EXPERIMENTS WITH VARYING RATIOS OF PSEUDOMONAS SP TO ACTIVATED SLUDGE.

Ratio Pseud. Act.S.	Expt. No.	Initial Nitrate Conc. mg/l	Initial Carbon Conc. mg/l	Initial Organism Conc.# mg/l	Unit Denitrification Rate $\frac{mg/l\ NO_3\ as\ N}{mg/l\ organisms\text{-}hr}$
0.0	40	134.8	420	170	0.042
0.25	38	132.0	350	135	0.062
0.50	39	129.7	335	145	0.102
0.6	43	92.5	500	95 *	0.122
1.0	37	120.0	330	130	0.136
1.0	42	96.9	455	110 *	0.140
∞	41	121.5	310	60	0.163

* Initial solids not actually determined.
\# Organism concentration measured as total suspended solids.

FIGURE NO. 33


UNIT DENITRIFICATION RATE VERSUS RATIO
PSEUDOMONAS SP. TO ACTIVATED SLUDGE
UNIT DENITRIFICATION RATE mg./l. NO_3 as N/mg./l. organisms
0·0
0·05
0·10
0·15
Y=A-BC^x
95% CONFIDENCE LEVEL
A= 0·16
0·0
0·25
0·50
0·75
1·00
RATIO OF PSEUDOMONAS SP. TO ACTIVATED SLUDGE

an activated sludge batch experiment with an organism population of 500 mg/l to 1000 mg/l would have reduced the the nitrate concentration of the medium in the same time period as a pure culture population of Pseudomonads.

Organism Growth. The ability to predict the amount of cellular growth which would occur during a biological waste treatment reaction is a necessary requirement for design and operation.. In order to produce a maximum unit denitrification rate carbon must be provided in sufficient quantity to meet the nitrate reduction requirements plus the needs for organism growth. Examination of McCarty's (1969) equations for methanol requirements for nitrate reduction and organism growth showed that approximately 0.28 gm of carbon are required for cell growth per gm of nitrate nitrogen as N reduced to nitrogen gas. In other words, approximately 0.53 gm of solids would be produced for each gm of nitrate as N reduced considering an equation for sludge solids of $C_5H_7NO_2$ (Hoover and Porges, 1952).

In Table No. 12, the average carbon requirement for organism growth can be calculated as .93 gm of carbon per gm of nitrate as N reduced. This would mean that approximately 1.74 gm of cells were produced per gm of nitrate reduced, which is roughly triple the cell growth predicted by McCarty's experiments with methanol. The volatile solids portion of the suspended solids was approximately 95% indicating that most of the solids were organisms. The

overall carbon utilization for the 16 experiments performed at 27°C was 2.74 gm soluble carbon per gm of nitrate reduced. This again is almost double the 1.4 consumptive ratio found by McCarty using methanol as a hydrogen donor. However, Johnson (1964) in his continuous denitrification experiments demonstrated a consumptive ratio of approximately 2.2 (calculated from his data), which is much closer to the current experiments. Possible explanations for this discrepancy are that organisms using citrate utilize a greater portion of the soluble carbon for organism growth. Organisms using methanol in contrast use a greater proportion of the carbon to reduce nitrate and then degrade methanol to the point where it can be incorporated into cell material. As well, the denitrifying organisms in activated sludge used in McCarty's work and other studies probably grow less efficiently due to competition with other organisms in the sludge mass.

For design of a plant scale denitrification unit the rate data should be obtained from laboratory studies using the actual carbon source to be applied in practice. As well, the experiments should be run at a range of temperatures which is to be experienced.

Nitrogen Balance. The laboratory apparatus was designed so that the system was completely closed to the atmosphere in order that the transformations of nitrogen compounds could be studied through the reaction period. Previous workers on denitrification with the exception of

Johnson (1964) have used open systems and could not account for nitrogen lost as gaseous products to the atmosphere. At several times throughout each reaction, the nitrogen content of the system in all forms was analysed, and the total weight measured related to the original nitrogen content. Table No. 16 illustrates a typical balance sheet for Experiment No. 34 carried out at 5°C. The transformations of the various nitrogen compounds with respect to time are shown in Figures, 14, 16, 18, 20, 26, 28, 31, with a nitrogen balance indicated at the top of the graphs. Most of the nitrogen balances closed within 2% to 6% with the odd reaction deviating as much as 10%. The most important sources of error in the nitrogen balances were the organic nitrogen. Total Kjeldahl analyses which were at most times 4% to 6% of the solids concentration: lower than the expected 8% to 12% predicted by Porges equation. In addition, contents of the gas space above the reaction liquid were not always at the same concentration as the gas reservoir concentration. Gas analysis of samples taken directly from the gas space and from the reservoir differed by one or two percent in nitrogen concentration. The dissolved nitrogen gas concentration made up a significant amount of the total nitrogen balance, e.g. up to 25% of the nitrogen content in the system for Experiment No. 5. As well, the change in per cent nitrogen for the gas space made up a considerable portion of the total nitrogen in the reactors.

TABLE NO.16.

TYPICAL NITROGEN BALANCE ON AN EXPERIMENTAL REACTOR. EXPERIMENT #.34.

Date	Time	NO_2 & NO_3 mg/l	T.K.Solids Nitrogen mg/l	NH_3 mg/l	Dissolved N_2 mg/l	Total Liquid N_2 mg/l	Liquid Volume ml	Gas Evolved ml	% Nitrogen	Wt. N_2 Evolved mg	Wt. of Liquid & Solid N_2 mg	Wt.of N_2 in Original Space mg	Wt.of N_2 in Samples mg	Wt.N_2 in % Change in Gas Space mg	Total Wt. of N mg	Balance Within %
Dec.11	4.15	100	4.0	8.0	16.0	128.0	5130	0	0		656	186	0.0		842.0	
19	11.05	90	2.6	7.8	16.0	116.4	5105	60.0	80.0	59.0	595	186	2.9		840.0	- 0.2
20	11.50	84	5.4	6.9			5080	110.0	92.0				5.6			
21	9.50	54	4.6	7.0	16.2	81.8	5055	175.0	94.8	204.0	413	186	7.6	34.6	845.2	+ 0.4
22	12.30	46	5.5	3.8			5030	235.0	95.6	276.0			9.4			
22	9.45	33	6.1	2.6	16.4	58.1	5005	285.0					10.9			
23	10.15	2	7.6	1.4	16.3	27.3	4980	370.0	96.5	439.0	136	186	11.6	38.6	811.2	- 3.4

Microbiological Aspects of the Work. Microbiological colony counts on plate agar after incubation at 20°C for 48 hours were carried out to check on the purity of the batch cultures. Samples were obtained upon reaction initiation after organism seeding, and upon termination of the reaction. In most cases less than 5% of the colonies counted at the end of the reaction differed from the usual circular or slightly elliptical pearly white colony typical of Pseudomonas denitrificans found at reaction initiation. This indicated that the bacterial cultures were dominated by Pseudomonas and little contamination entered the system during sampling. The colony counts were also utilized to confirm the organism growth evaluated by suspended solids measurements. In Table No. 17, the microbiological colony counts are tabulated along with the suspended solids data at the corresponding times. All of the data obtained at 27°C was used to construct Figure No. 34, a graph relating colony counts to suspended solids concentration. The very large spread of the data between the 95% confidence levels around the least squares straight line fit of the experimental points is typical of bacterial count suspended solids correlations. However, the graph does confirm the trend that increased suspended solids indicate organism growth. Considerable error exists in both the suspended solids measurements ($\pm$20%), and the bacterial colony counts. Flocculation of organisms which was visibly apparant in the reactors tended to reduce the colony counts, since many

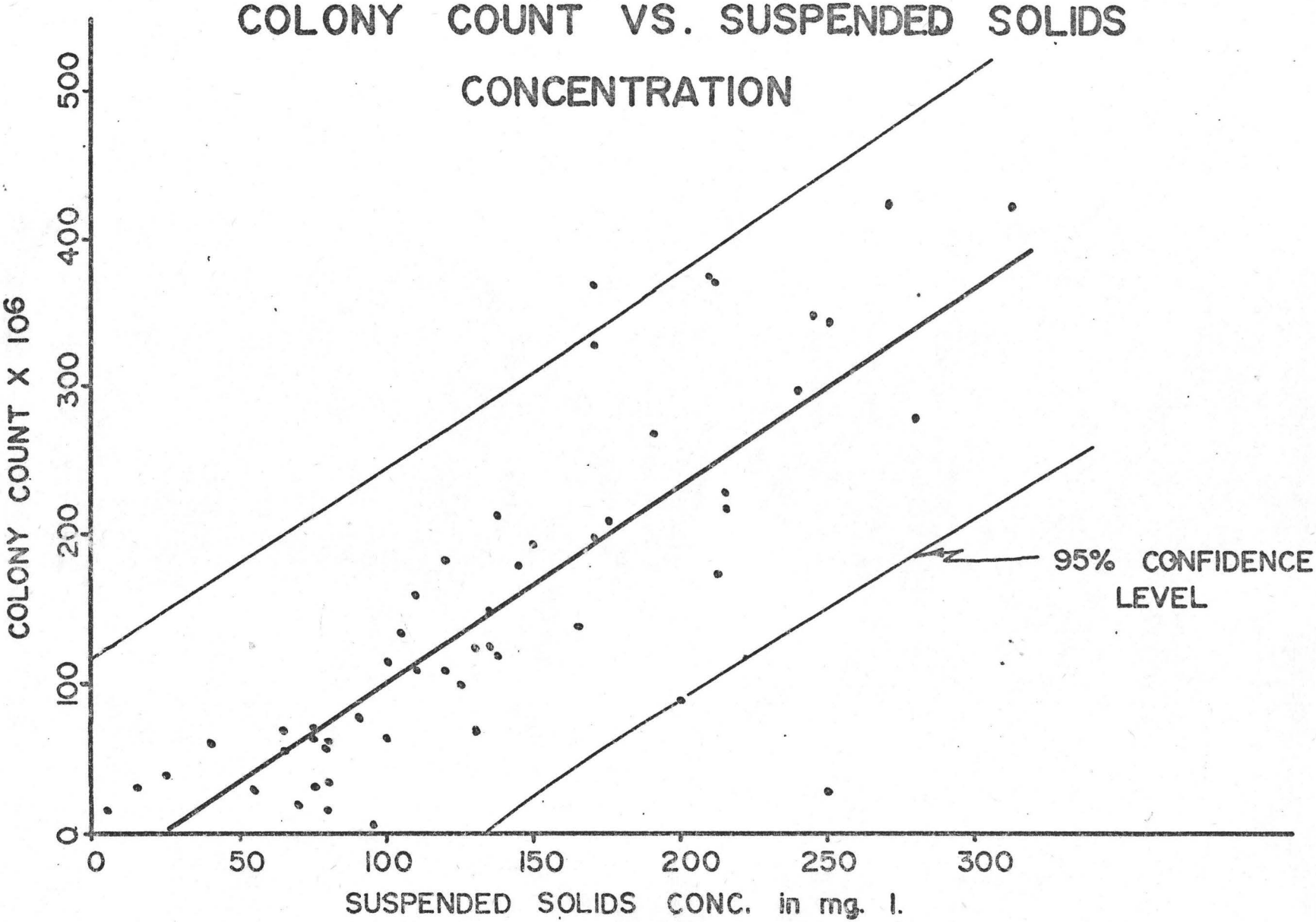
TABLE NO.17.

COLONY COUNTS AND SUSPENDED SOLIDS CONCENTRATIONS.
27°C REACTIONS

Expt. No.	Start		Middle		End.	
	Plate Count Million/ml.	S.S.mg/l	Plate Count Million/ml.	S.S.mg/l	Plate Count Million/ml.	S.S.mg/l
15	179	145			425	270
14	160	110			368	170
15B	215	140			280	280
16	120	140			350	245
8	58	80			395	250
9	118	100			150	135
6	69	65				
5	66	75				
3	57	65			68	130
1					38	80
4	20	70			29	250
7	15	80			90	200
10	125	130			229	215
11	62	80			75	135
8B	30	75			425	315
2	30	55			65	100
10B	60	80			125	135
13	80	90			100	125
May 28 (1)	122	120	202	170	375	210
(2)	16	125	197	150	212	175
(3)	33	175	373	210	300	240
(4)	12	90	62	96	110	110
May 19 (1)	40	25			188	120
(2)	17	5			60	40
(3)	33	15				
June 5 (1)	69	75			138	105
(2)	138	165			268	190
(3)	220	215			332	170

FIGURE NO. 34

COLONY COUNT VS. SUSPENDED SOLIDS CONCENTRATION



flocculated organisms initiated a colony as opposed to the assumption that each colony derived from a single organism.

The data in Table No. 17 could be utilized along with corresponding denitrification rate data to establish unit rates of nitrate reduction per cell rather than on a weight basis. It should be pointed out, however, that the rate data based on colony counts would probably vary even more widely than the rate data based on weight measurements of cells and would not be as applicable to process design.

CONCLUSIONS

(1) The experiments demonstrated that closed nitrogen balances could be performed on batch denitrification reactors to account for the bacterial conversion of nitrate to nitrogen gas.

(2) Denitrification rate is independent of nitrate concentration at the levels investigated, i.e. 120 mg/l to 1 mg/l.

(3) The rate of denitrification is also independent of organic carbon concentration provided sufficient carbon is available to meet the stoichiometric requirements of nitrate reduction, and the requirements for organism growth.

(4) The optimum pH for denitrification by pure cultures of Pseudomonas denitrificans appears to be between 7.5 and 7.0 - well within the range usually associated with wastewater treatment.

(5) The relationships between temperature and unit denitrifications rate for pure cultures of Pseudomonas denitrificans can be predicted by an Arrhenius type curve between 5°C and 27°C.

(6) The unit denitrification rate of Pseudomonas denitrificans at 5°C is approximately 20% of the rate at 20°C but is still significant at this level for

(a) wastewater treatment purposes,

(b) denitrification in deep water cold temperature regions of natural water bodies.

(7) A significant percentage of activated sludge organisms normally aerobically metabolising organic carbon can denitrify wastewater utilizing nitrate as the terminal electron acceptor if anaerobic conditions prevail. No persistent significant build-up of nitrite was observed.

ABBREVIATIONS & SYMBOLS

approx.	-	approximately
avg.	-	average
BOD.	-	biochemical oxygen demand
cal.	-	calories
conc.	-	concentration
dia.	-	diameter
DO.	-	dissolved oxygen
ft.	-	feet
ft^2.	-	square feet
gal.	-	gallons - imperial
gm	-	grams
gpd	-	gallons per day
gpm	-	gallons per minute
Hg	-	mercury
hr	-	hour
K cal	-	Kilo calories
l	-	liters
lb	-	pound
ln	-	natural logarithm.
max	-	maximum
min	-	minimum
mg	-	milligram
mg/l	-	milligrams/liter
ml	-	milli liter

MLSS	-	mixed liquor suspended solids
mm	-	millimeter
No	-	number
ppm	-	parts per million
psig	-	pounds per square inch gauge
Sp	-	species
SS	-	suspended solids
STP	-	sewage treatment plant.
TKN	-	total Kjeldahl nitrogen

SYMBOLS

@	-	at
^{o}F	-	degrees Fahrenheit
pH	-	logarithm of hydrogen ion concentration reciprocal
%	-	percent
>	-	greater than
<	-	less than
Δ	-	increment
Σ	-	sum of
μ	-	micron
$m\mu$	-	milli micron
eg	-	for example
ie	-	that is

REFERENCES

Alexander, M. "Introduction to Soil Microbiology". Wiley, New York (1964).

A.P.H.A., A.W.W.A., W.P.C.F. Standard Methods for the Examination of Water and Wastewater. 12th Edition (1965).

Balakrishnan, S., Eckenfelder, W.W., "Nitrogen Relationships in Biological Treatment Processes. I. Nitrification in the Activated Sludge Process". Water Research 3, 73-81 (1969).

Barth, E.F., Brenner, R.C., Lewis, R.F. "Combined Chemical Biological Approach to Control of Nitrogen and Phosphorous in Waste Water Effluents". Journal WPCF 40, 12, 2040 (1968).

Barth, E.F., Dean, R.B. "Nitrogen Removal from Wastewaters - Statement of the Problem". FWQA Division of Research & Development, Nitrogen Removal Workshop, July 30. 1970.

Barth, E.F., Mulbarger, M., Salatto, B.V., Ettinger, M.B. "Removal of Nitrogen by Municipal Wastewater Treatment Plants". Journal WPCF. 38, 7 1203 (1966).

Brandon, T.W., Grindley, J. "Effects of Nitrates on the Rising of Sludge in Sedimentation Tanks". Journal Proc. Inst. of Sewage Purification 175-179 (1944).

Bremmer, J.M., Shaw, K. "Denitrification in Soil". Journal of Agricultural Science, 51, 1, 21-51 (1958).

Bringmann, G., Kuhn, R., Wagner, B. "Model Experiments on the Biological Removal of Nitrogen as a Gas from Treated Sewage". Gesundheits-Ingenieur 80, 364 (1959).

Bringmann, G. "Complete Biological Elimination of Nitrogen from Clarified Sewage in Conjunction with a High Efficiency Nitrification Process". Gesundheits-Ingenieur 82, 233, (1961).

Bringmann, G. "Optimale Stickstoff-Abs-gasung durch Einsatz von nitrifizierendem Belebtschlamm und Redox-Steurung". Gesundheits-Ingenieur, 81, 5, 140 (1960).

Cantanzaro, E.W. "Continuous Automatic Chemical Analysis of Nitrate in the Presence of Ammonia and Urea". Annals New York Academy of Science. 87, 2, 808-812 (1960).

Chang, J.P., Morris, J.G. "Studies on the Utilization of Nitrate by Micrococcus denitrificans". Journal General Microbiology, 29, 301-310 (1962).

Christianson, C.W., Rex, E.H., Webster, W.M., Virgil, F.A., "Reduction of Nitrate Nitrogen by Modified Activated Sludge". U.S. Atomic Energy Commission, TID 7517, Part 1a, 264 (1956).

Davies, O.L. "Design and Analysis of Industrial Experiments". Oliver & Boyd (1967).

Delwiche, C.C. "Biological Transformations of Nitrogen Compounds". Industrial and Eng. Chemistry. 48, 1421, (1956).

Delwiche, C.C. "Nitrification". "A Symposium on Inorganic Nitrogen Metabolism". Edited by McElroy & Glass. John Hopkins Press. 218 (1956).

Delwiche, C.C. "Denitrification". "A Symposium on Inorganic Nitrogen Metabolism". Edited by McElroy & Glass. John Hopkins Press. 233 (1956).

Delwiche, C.C. "The Nitrogen Cycle". Scientific American. 223, 3, 137-146 (1970).

Doelle, H.W. "Bacterial Metabolism". Academic Press. New York & London, (1969).

Eckenfelder, W.W., O'Connor, D. "Biological Waste Treatment". Pergammon Press. (1961).

Eckenfelder, W.W., Balakrishnan, S. "Kinetics of Biochemical Nitrification and Denitrification". Report - Centre for Research in Water Resources, University of Texas, June (1968).

Eliasson, R., Tchobanoglous, G. "Chemical Processing of Wastewater for Nutrient Removal". Journal WPCF 40, 5, Part 2 R.171 (1968).

Engel, H. "Die Stickstoffentlundung". Encyclopedia of Plant Physiology. Vol.VIII 1083-1106 (Edited by Ruhland W.) Springer-Verlag, Berlin (1958).

Farrell, J.B. Ammonia Nitrogen Removal by Stripping with Air. FWQA. Division of Research and Development Nitrogen Removal Workshop July 30, 1970.

Ferrari, A. "Nitrogen Determination by a Continuous Digestion and Analysis System". Annals New York Academy of Sciences. 87, 2, 792-800 (1960).

Fewson, C.A. Nicholas, D.J.D. "Utilization of Nitrate by Micro-organisms". Nature 190, 4770 2-7 (1961(a)).

Fewson, C.A. Nicholas, D.J.D. "Nitrate Reductase from Pseudomonas aeruginosa". Biokim Bio-phys, Acta. 49 335 (1961(b)).

Finson, P.O. Sampson, D. "Denitrification of Sewage Effluents".. Water and Waste Treatment Journal. 7 298-300 (1959).

Fruh, E.G. "Biological Responses to Nutrients-Eutrophication: Problems in Freshwater". Advances in Water Quality Improvement, Vol. I. Edited by Gloyna, E.F. and Eckenfelder, W.W. University of Texas (1969).

Gauthier, D.K. "Nitrate Reducatase & Soluble Cytochrome C in Spirillum itersonii". Journal General Bacteriology 102, 3, 797-803. (1970).

Godlewski, E. Bulletin International Academy of Science Cracovie 178 (1895).

Gunderley, F.C. et al. "Dilute Solution Reactions of the Nitrate Ion as Applied to Water Reclamation". Robert A. Taft Water Research Centre Report No. TWRC-1.

Harvey, H.W. "The Chemistry & Fertility of Sea Waters". Cambridge University Press (1955).

Helmers, E.N. et al. "Nutritional Requirements in the Biological Stabilization of Industrial Wastes". Sewage & Industrial Wastes 23, 884 (1951).

Hoover, S.R. Porges, N. Assimilation of Dairy Wastes by Activated Sludge II, The Equation of Synthesis and Rate of Oxygen Utilization, "Sewage and Industrial Wastes". 23, 3, 306 (1952).

Hulme, W.J. "The Mechanism of Denitrification". Trans Chemical Society. 105, 623-632 (1914).

Johnson, W.K. Schroepner, G.L. "Nitrogen Removal by Nitrification and Denitrification". Journal WPCF 36, 8, 1015. (1964).

Johnson, W.K. "Nutrient Removal by Conventional Treatment Processes". Proc.17th Ind.Waste Conference. Purdue University 96, 151, (1958).

Johnson, W.K. "Removal of Nitrogen by Biological Treatment". Advances in Water Quality Improvement. Vol.II. Edited by Gloyna, E.F., and Eckenfelder, W.W. University of Texas, pg.178 (1969).

Kamphake, L.J. et al. "Automated Analyses for Nitrate by Hydrazine Reduction". Water Research

Lackey, J.B. "Effects of Fertilization on Receiving Waters". Sewage & Industrial Wastes 30, 11, 1411 (1958).

Lamanna, C. Mallette, M.F. "Basic Bacteriology. Its Biological & Chemical Background". Williams and Williams (1965).

Ludzack, F.J., Ettinger, M.B. "Controlling Operation to Minimize Activated Sludge Effluent Nitrogen". Journal WPCF. 34, 9, 920 (1962).

McCarty, P.L. et al. "Biological Denitrification of Wastewater by Addition of Organic Materials". Proc.24th Industrial Waste Conference Purdue University, May 6 (1964).

McCarty, P.L. et al. Treatment of High Nitrate Waters". A.W.W.A. Journal. 61, 2, 659-662 (1969).

McClean, H. "A Continuous Biochemical Reactor Study Using Mixed Microbial Cultures. Thesis, McMaster Univ.(1968).

Mechalas, B.J. et al. "A Study of Nitrification & Denitrification". U.S. Dept. of Interior. FWQA. Water Pollution Control Research Series Report 17010DR007/70 (1970).

Meyeroff, O. "Die Atmung des Nitribildners und ihre Beinflussung Durch Chemische Substanzen". Pflügers Arch ges Physiol 164, 240, (1917).

Montgomery, H.A.C., Quarmby, C. "The Extraction of Gases Dissolved in Water for Analysis by Gas Chromatography". Lab Practice 15,5, 538-43 (1966).

Moore, S.F., Schroeder, E.D. "An Investigation of the Effects of Residence Time on Anaerobic Bacterial Denitrification". Water Research 4, 685-694.(1970).

Mulbarger, M.C. et al. "Modifications of the Activated Sludge Process for Nitrification and Denitrification". 43rd Annual Conf. WPCF. Boston, Mass (1970).

Myers, J. Matsen, F.A. "Kinetic Characteristics of Warburg Manometry". Arch Biochem & Biophys.55, 373-388 (1955).

Nason, A., Takahashi, H. "Inorganic Nitrogen Metabolism". Annual Review of Microbiology. 12, 203 (1958).

Nason, A. "Enzymatic Pathways of Nitrate, Nitrite, and Hydroxylamine Metabolism".Bact.Review, 26, 16-41.(1962).

Nicholas, D.J.D. "The Metabolism of Inorganic Nitrogen and its Compounds in Micro-Organisms". Biological Review, 38, 530 (1963).

Painter, H.A. "A Review of Literature of Inorganic Nitrogen Metabolism in Micro-Organisms". Water Research, 4, 393-450 (1970).

Pichinoty, F., D'Ornano, L.D. "Sur le Mechanisme d'l'inhibition par l'oxygene de la denitrification Bacterienne". Biochem. Biophys Acta. 52, 386 (1961).

Pelczar, M.J. Reid R.D. "Microbiology". McGraw Hill, 2nd Edition, (1965).

Sacks, L.E. Barker, H.A. "The Influence of Oxygen on Nitrate and Nitrite Reductase". Journal of Bacteriology, 58, 11-22. (1949).

Sawyer, C.N. "Some New Aspects of Phosphates in Relation to Lake Eutropication". Sewage and Industrial Wastes. 24, 768 (1925).

Sawyer, C.N. "Causes, Effects & Control of Aquatic Growths". Journal WPCF. 34, 3, 279 (1967).

Sawyer, C.N. Bradney, L. "Rising of Activated Sludge in Final Settling Tanks". Sewage Works Journal 17, 6, 1191 (1945).

Sawyer, C.N. "An Evaluation of Nutrient Control Methods". Journal Eng. Inst. of Canada 37-40 (1969).

Sawyer, C.N. "Basic Concepts of Eutrophication". Journal WPCF 38, 5, 737 (1966).

Sawyer, C.N. Chemistry for Sanitary Engineers McGraw Hill (1960).

Schmidt, B., Kampf, W.D., "Uber den Einfluss des Sauerstoffs auf die Denitrifikationsleistung von Pseudomonas fluorescens Arch.Hrg.Bakt. 146, 171-182 (1962)

Skerman, V.B.D., MacRae, I.C. "The Influence of Oxygen in the Reduction of Nitrate by Adapted Cells of Pseudomonas denitrificans". Canadian Journal of Microbiology. 3, 215 (1957a).

Skerman, V.B.D., MacRae, I.C. "The Influence of Oxygen on the Degree of Nitrate Reduction by Pseudomonas denitrificans". Canadian Journal of Microbiology, 3, 505 (1957b).

Skerman, V.B.D., MacRae, I.C. "The Influence of Oxygen on the Formation of Nitratase in Pseudomonas denitrificans". Canadian Journal of Microbiology. 7, 169.

Slechta, A.F., Culp, G.L. Water Reclamation Studies at the South Tahoe Public Utilities District. Journal WPCF 39, 787 (1967).

Smith, J.M., Masse, A.N., et al "Nitrogen Removal from Municipal Wastewater by Columnar Denitrification". Report FWQA, USA. (1970a).

Smith, J.M., Masse, A.N., Miele, R.R. Renovation of Municipal Wastewater by Reverse Osmosis. Environmental Protection Agency Water Quality Office Publication 17040 (1970b).

Snell, J.R. "Anaerobic Digestion II, Nitrogen Changes and Losses During Anaerobic Digestion". Sewage Works Journal. 15, 1, 56 (1943).

Spangler, W.J., Gilmour, C.M. "Biochemistry of Nitrate Respiration in Pseudomonas stutzeri. I Aerobic & Nitrate Respiration Routes of Carbohydrate catabolism". Journal of Bacteriology. 91, 245. (1966)

Swinnerton, J.W., Linnenbom, V.J., Cheek, C.H. "Determination of Dissolved Gases in Aqueous Solution by Gas Chromatography". Analytical Chemistry 34, 4, 483-485. (1962).

Tarzwell, C.M., Gaufin, A.R. "Some Important Biological Effects of Pollution Often Disregarded in Stream Surveys". Proc.8th Ind.Waste Conf. Purdue University 295 (1953).

Watson, W., Twine, S.R. "Fundamentals in Sewage Effluent Recirculation". Journal Inst.of Sewage Purification. 107 (1949).

Wheatland, A.B., Barrett, M., Bruce, A. "Some Observations on Denitrification in Rivers and Estuaries". Journal Inst.of Sewage Purification. 149 (1959).

Wijler, J., Delwiche, C.C. "Investigations on the Denitrifying Process in Soils". Plant & Soil 5, 2, (1954).

Wilson, I.S. "Some Problems in the Treatment of Effluents from the Manufacture of Organic Chemicals". Chemistry & Industry 1278-1288 (1967).

Wuhrmann, K., Mechsner, K. "Beitrag zur Kenntnis der Mikrobiellen Denitrifikation". Path Microbiol. 26, 579-591 (1963).

Wuhrmann, K. "Microbial Aspects of Water Pollution Control". Advances in Applied Microbiology 6, 119-151. Academic Press (1964).

Wuhrmann, K. "Research Developments in Regard to Concepts of Base Values of the Activated Sludge System". Advances in Water Quality Improvement. Vol.I. Edited by Gloyna, E.F. Eckenfelder, W.W. Page 190. University of Texas Press (1969).

Wuhrmann, K. "Objectives, Technology, and Results of Nitrogen and Phosphorous Removal Processes". Advances In Water Quality Improvement. Vol.I. Edited by Gloyna, E.F., Eckenfelder, W.W. Page 121. University of Texas Press (1969).

Wuhrmann, K. "Biological Waste Treatment". Reinhold. Proc.3rd Conf.Manhattan College, New York (1960).

Wuhrmann, K. "Nitrogen Removal in Sewage Treatment Process". XVth Int.Conf. in Limnology, Madison, Wisconsin, (1962).

Wuhrmann, K. "Effects of Oxygen Tension on Biochemical Reactions in Sewage Purification Plants". Biological Waste Treatment, Reinhold. Manhattan College (1960).

Wuhrmann, K. "High Rate Activated Sludge Treatment and its Relation to Stream Sanitation". I Pilot Plant Studies. Sewage & Ind.Wastes 26, 1, 1 (1954).

APPENDIX A

TABULAR PRESENTATION OF EXPERIMENTAL RESULTS

EXPERIMENT NO. 1

Experimental Conditions.

Date: August 3/70

Initial Nitrate Concentration 40 mg/l
Initial Carbon Concentration 40 mg/l
pH 6.5

Temperature 27°C
Dissolved Oxygen 0.0. mg/l
Initial Organism Concentration 50 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	42.5	0.30	2.2	2.4	11.8	5300	0.0	79.0	62	35
6.00	38.0	0.20				5290	10.0		57	55
8.67	27.0	0.18	1.4	5.8	11.6	5265	35.0	87.0	38	55
9.70	23.5	0.10				5240	45.0		28	
12.40	21.5	0.05	1.0	7.8	11.8	5205	75.0	96.0	10	80

Gas Space: 100 ml.

EXPERIMENT NO.2.

Experimental Conditions.

Date: August 15/70

Initial Nitrate Concentration 120 mg/l
Initial Carbon Concentration 120 mg/l
pH 6.5

Temperature 27°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 50 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	94.0	0.003	5.0	5.0	11.8	5380	0.0	79.0	120	55
5.00	83.0	0.003	0.5	5.6	18.3	5355	40.0	76.5	104	75
7.33	67.0	0.003	0.4	6.0	22.0	5330	80.0	78.0	54	70
9.25	53.0	0.006	0.6	6.8	21.5	5305	145.0	78.0	28	80
11.75	52.0	0.003	0.45	7.5		5280	190.0		12	100
16.70	52.0	0.003	0.6	8.5	18.3	5255	210.0	78.5	14	100

Gas Space: 100 ml.

EXPERIMENT NO.3.

Experimental Conditions.

Date: July 21/70

Initial Nitrate Concentration 40 mg/l
Initial Carbon Concentration 200 mg/l
pH 6.5

Temperature 27°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 50 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	33.0	4.7	0.7	1.8	16.0	5350	0.0	79.0	172	55
5.43	24.6	9.0		1.2		5325	30.0	89.5	163	65
8.70	9.0	9.0	1.0	2.9		5300	80.0		112	75
9.50	4.5	7.8	1.65	3.0	17.2	5275	120.0	86.0	96	
10.50	3.6	3.4	1.10	4.8		5250	154.0		85	
11.82	1.2	0.4	1.10	3.0	16.4	5225	180.0	84.5	74	85
14.00										130

Gas Space:125 ml.

EXPERIMENT NO. 4.

Experimental Conditions.

Date: August 3/70

Initial Nitrate Concentration 120 mg/l
Initial Carbon Concentration 600 mg/l
pH 6.5

Temperature 27°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 50 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	113.0	0.20	14.4	14.4	14.5	5290	0.0	79.0	625	
5.42	102.0	0.20	14.0	14.0		5265	32.5	87.7	625	70
8.67	66.5	0.10	6.2	15.4	25.0	5240	110.0	86.7	590	110
9.92	52.5	0.10	7.9	12.1		5215	150.0	88.5	395	105
10.97	34.0	0.50	5.8		32.4	5190	215.0		350	115
12.00	16.0	0.40	3.8	14.2		5165	290.0		270	125
13.08	1.0	0.05	2.0	13.8	31.2	5140	395.0	99.0	230	250

Gas Space: 225 ml.

EXPERIMENT NO. 5.

Experimental Conditions.

Date: July 21/70

Initial Nitrate Concentration 40 mg/l
Initial Carbon Concentration 40 mg/l
pH 8.5
Temperature 27°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 50 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	38.0	5.0	2.3	0.9	13.5	5260	0.0	79.0	54	75
8.68	27.5	8.5	2.0	5.7		5235	30.0	80.0	34	85
11.00	24.8	8.8	1.0	6.0		5210	42.0	84.4	28	
13.70	19.8	9.2	0.4	7.2	14.5	5185	60.0	80.0	14	95
23.90	17.4	8.6	0.6	5.8	14.0	5160	88.0	82.0	10	125 (15.6) hrs

Gas Space 100 ml.

EXPERIMENT NO.6.

Experimental Conditions.

Date: July 21/70

Initial Nitrate Concentration 120 mg/l
Initial Carbon Concentration 120 mg/l
pH 8.5

Temperature 27°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 50 mg/l.

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	110.4	5.6	1.0	2.7	13.0	5325	0.0	79.0	136	65
8.68	102.2	11.8	0.85	2.4	14.5	5300	30.0	79.5	110	85
12.42	71.6	22.4		2.9		5275	75.0		43	95
13.92	68.6	25.4	0.90	4.0	14.2	5250	112.5	91.5	36	195
16.75	57.8	26.2	0.70	4.2		5225	150.0		34	180
22.80	61.5	18.5	0.40	4.0	14.0	5200	180.0	96.5	10	

Gas Space: 100 ml.

EXPERIMENT NO.7.

Experimental Conditions.

Date: August 3/70

Initial Nitrate Concentration 40 mg/l
Initial Carbon Concentration 200 mg/l
pH 8.5

Temperature 27°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 50 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	38.95	1.05	7.3	11.5	10.6	5270	0.0	79.0	184	75
6.00	28.00	10.80	3.4	12.2	15.5	5245	2.5	96.0	150	80
9.92	10.20	9.30	1.6	8.4		5220	30.0			105
12.00	1.70	1.75	1.0	8.4	33.0	5195	55.0	99.4	122	120
13.50	0.70	0.20	1.7	9.1		5170	77.5		110	200

Gas Space: 200 ml.

EXPERIMENT NO.8A

Experimental Conditions.

Date: August 26/70

Initial Nitrate Concentration 120 mg/l
Initial Carbon Concentration 600 mg/l
pH 8.5

Temperature 27°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 50 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	94.5	0.0	0.54	8.7	18.0	5300	0.0	79.0	675	80
1.76	88.75	0.2	0.46	11.9		5275	50.0	92.0	650	105
3.80	67.40	4.5	0.38	10.8		5250	105.0	98.6	500	
6.64	32.50	5.0	0.15	12.6		5225	225.0		465	160
7.60	16.05	4.0	0.12	12.6	29.0	5200	285.0	98.0	435	220
8.60	1.00	2.0	0.10	12.6	25.4	5175	340.0	99.0	340	250

Gas Space: 120 ml.

EXPERIMENT NO.8B

Experimental Conditions.

Date: August 15/70

Initial Nitrate Concentration 120 mg/l
Initial Carbon Concentration 600 mg/l
pH 8.5

Temperature 27°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 50 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	114.0	0.50	1.05	6.5	10.6	5300	0.0	79.0	575	75
7.00	112.0	2.00		7.0		5275	17.5			
9.25	91.4	4.60	0.35	8.5	18.9	5250	32.5	76.0	560	110
11.50	61.0	5.30	0.25	9.1		5225	72.5		490	
14.00	49.0	3.50	0.20	10.5		5200	127.5			
16.67	9.40	2.60	0.15	11.0		5175	190.0	87.6	295	265
17.67	3.90	0.10	0.15	12.5	28.9	5150	225.0		295	290
19.33	0.50	0.05	0.10	12.5	29.0	5125	250.0	87.3	295	315

Gas Space: 120 ml.
(Gas leak suspected)

EXPERIMENT NO. 9.

Experimental Conditions.

Date: August 26/70

Initial Nitrate Concentration 40 mg/l
Initial Carbon Concentration 40 mg/l
pH 6.5

Temperature 27°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 100 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	30.4	1.55	0.7	4.6	16.6	5120	0.0	79.0	65	100
1.78	23.3	1.70	0.4	6.9		5095	42.0	85.0	45	120
3.77	19.7	0.25	0.8	7.4	13.0	5070	47.0	90.3	35	125
6.57	19,9	0.06	0.75	6.7	12.6	5045	70.0	89.0	30	135

Gas Space: 180 ml.

EXPERIMENT NO 10A

Experimental Conditions.

Date: September 2/70

Initial Nitrate Concentration 120 mg/l. Temperature 27°C
Initial Carbon Concentration 120 mg/l. Dissolved Oxygen 0.00 mg/l
pH 6.5 Initial Organism Concentration 100 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	120.0	0.08	1.70	6.2	11.0	5330	0.0	79.0	102	130
3.33	100.0	0.13	2.05	6.7		5305	45.0	80.0	95	170
4.50	84.0	0.10	0.45	7.4		5280	105.0		40	130
5.08	78.5	0.10	1.30	7.7	18.0	5255	135.0	91.4	28	150
7.00	62.0	0.10	0.40	8.3	16.3	5230	240.0	83.0	14	150
9.50	60.5	0.10	1.35	7.0	13.5	5205	285.0	83.5	12	215

Gas Space: 90 ml.

EXPERIMENT NO.10B

Experimental Conditions.

Date: August 25/70

Initial Nitrate Concentration 120 mg/l
Initial Carbon Concentration 120 mg/l
pH 6.5

Temperature 27°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 100 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	112.8	0.2	3.10	4.0	10.6	5120	0.0	79.0	130	80
2.87	90.8	0.2	0.40	5.0	19.3	5095	40.0	84.0	84	125
5.00	67.8	0.2	0.25	4.4		5070	100.0	71.0	74	100
7.33	61.9	0.1	0.40	6.6		5045	160.0	79.0	68	140
9.25			1.10			5020	175.0	79.0	26	135
17.67	56.4	0.1	0.65	7.0	24.8	4995	185.0	83.0	19	

Gas Space 180 ml.

EXPERIMENT NO. 11

Experimental Conditions.

Date: September 2/70

Initial Nitrate Concentration 40 mg/l
Initial Carbon Concentration 200 mg/l
pH 6.5

Temperature 27°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 100mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	32.5	0.35	1.00	4.2	14.0	5275	0.0	79.0	183	80
3.00	26.5	0.20	0.45	3.4		5250	15.0	82.4	155	110
4.25	11.0	0.10	0.25	3.9	15.1	5225	42.0	87.2	125	
6.17	3.5	0.23	0.20	5.7		5200	107.0	83.2	90	120
8.67	3.0	0.60	0.35	6.2	13.2	5175	120.0	89.0	73	135

Gas Space: 125 ml.

EXPERIMENT NO.12

Experimental Conditions.

Date: November 26/70

Initial Nitrate Concentration 120 mg/l
Initial Carbon Concentration 600 mg/l
pH 6.5

Temperature 27°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 100 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	105.25	0.75	11.0	7.0	11.9	5130	0.0	79.0	585	80
5.08	64.20	27.80	8.0	9.8	12.0	5105	120.0	85.0	450	110
6.00	43.20	28.80	4.7	14.3	13.1	5080	175.0	95.4	385	200
6.50	18.30	25.70				5055	225.0		345	210
7.00	4.70	10.30	3.0	18.6		5030	280.0		335	215
7.50	1.25	0.75	2.0	20.0	18.1	5005	385.0	94.5	310	210
8.33	0.05	0.45	1.3	28.5	12.5	4980	445.0	90.0	295	210

Gas Space: 170 ml.

EXPERIMENT NO.13

Experimental Conditions.

Date: November 4/70

Initial Nitrate Concentration 40 mg/l
Initial Carbon Concentration 40 mg/l
pH 8.5

Temperature 27°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 100 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas. Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	44.0	2.0	3.0	5.1	11.6	5300	0.0	79.0	62.0	90
12.00	15.9	17.6	2.0	5.3		5275	17.5	86.0	38.0	120
17.20	8.1	17.9	1.5	7.0	18.3	5250	65.0	84.5	14.0	125
19.75	9.4	11.0	1.0	8.0		5225	90.0		14.0	
26.25	7.5	9.5	0.3	5.4	16.5	5200	105.0	91.0	14.0	125

Gas Space: 140 ml.

EXPERIMENT NO. 14.

Experimental Conditions.

Date: September 9/70

Initial Nitrate Concentration 120 mg/l
Initial Carbon Concentration 120 mg/l
pH 8.5

Temperature 27°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 100 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	103.0	2.0	4.5	5.5	12.6	5385	0.0	79.0	100	110
3.50	92.0	5.0				5360	10.0		68	
6.00	77.8	6.7	6.4	6.7	17.2	5335	56.0	96.2	41	170 (5,7 hr)
7.10	69.8	7.2	3.0	7.0	23.2	5310	85.0	93.0	22	165 (6.7 hr)
7.70	65.0	6.9	0.3	7.4		5285	100.0		16	
11.00	63.0	7.0	0.4	6.6	13.5	5260	150.0	94.1	15	170

Gas Space: 120 ml.

EXPERIMENT NO.15A

Experimental Conditions.

Date: September 9/70

Initial Nitrate Concentration 40 mg/l
Initial Carbon Concentration 200 mg/l
pH 8.5

Temperature 27°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 100 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	37.75	1.25	3.8	5.9	10.5	5315	0.0	79.0	208	145
2.00	37.75	0.75				5290	0.0			
3.50	24.60	0.40	4.0	6.0	18.9	5265	17.0	84.0		
4.50	12.00	0.50	4.0	7.5		5240	32.0			
5.70	2.25	0.75	5.8	7.9		5215	50.0		106	250
6.60	2.60	0.40	5.2	7.0	23.4	5190	85.0	99.0		235
8.20	3.20	0.80	4.5	5.2		5165	90.0	95.0	95	250
11.00	0.70	0.30	3.8	8.2	20.8	5140	110.0	96.2	88	270

Gas Space: 70 ml.

EXPERIMENT NO.15B

Experimental Conditions.

Date: September 15/70

Initial Nitrate Concentration 40 mg/l
Initial Carbon Concentration 200 mg/l
pH 8.5

Temperature 27°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 100 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	36.4	2.60	4.0	6.3	14.6	5495	0.0	79.0	196	140
3.20	33.0	5.00			21.6	5470	15.0			
4.50	21.2	6.80	3.5	8.9		5445	25.0	87.5	150	144
5.20	17.0	4.30				5420	45.0			
5.67	7,5	3.50	2.7	6.9		5395	57.5	91.5	126	
6.93	3.5	0.50	2.0	7.0		5370	85.0		120	205
8.05	3.0	0.50	1.6	6.9		5345	100.0	89.5	112	260
8.67	2.1	0.45	1.4	6.3	18.1	5320	105.0	87.5		
10.90									112	280

Gas Space: 140 ml
(Gas leak detected).

EXPERIMENT NO.16

Experimental Conditions.

Date: September 15/70

Initial Nitrate Concentration 120 mg/l
Initial Carbon Concentration 600 mg/l
pH 8.5

Temperature 27°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 100 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	106.8	1.20			10.6	5140	0.0	79.0	485	140
4.50	86.2	7.00	2.5	8.8	15.0	5115	42.5	83.5	395	200
6.17	70.5	6.65	1.0	8.2		5090	100.0		365	155
6.90	58.4	6.65		10.5	15.1	5065	137.5	83.5	330	
8.03	48.9	7.30	0.75	10.15		5040	195.0		310	235
9.08	31.8	7.15	0.15	10.85	15.2	5015	245.0	88.0	270	195
10.10	7.5	5.00	0.10	10.7	16.5	4990	302.0	95.0	260	200
10.70	0.25	0.25	0.10	10.8	21.5	4965	327.0	99.5	230	245

Gas Space: 125 ml.

EXPERIMENT NO.17

Experimental Conditions.

Date: Janurary 5/71

Initial Nitrate Concentration 120 mg/l
Initial Carbon Concentration 40 mg/l
pH 6.5

Temperature 27°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 100 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	116.9	3.1	3.2	3.2	11.7	5385	0.0	79.0	50	65
1.00	112.3	5.7	2.5	4.0		5360	7.5	76.5	46	
4.00	87.5	20.5	2.0	4.4	16.3	5335	30.0	80.0	12	80
5.00	85.0	21.0	1.5	4.5		5310	35.0			
8.00	83.5	24.5	2.2	4.2	12.5	5285	45.0	87.0	9	88
13.25	81.8	22.2	3.4	3.0	11.5	5260	60.0	96.0	7	55

Gas Space: 75 ml.

EXPERIMENT NO.18

Experimental Conditions.

Date: Janurary 5/71

Initial Nitrate Concentration 40 mg/l
Initial Carbon Concentration 120 mg/l
pH 6.5

Temperature 27°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 100 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	54.0	2.0	3.4	2.8	12.3	5310		79.0	126	75
2.00	43.0	8.0				5285				
5.00	13.3	25.7				5260	Gas	89.0	74	65
5.70	6.0	28.0				5235				
9.25	4.4	1.6			18.2	5210	Leak	96.0	53	100 (8hr)
10.25	2.9	1.1	7.6	1.6		5185				
13.25	2.9	1.1.	5.4	2.4	17.7	5160		95.0	30	125

Gas Space: 100 ml.

EXPERIMENT NO.19

Experimental Conditions.

Date: January 5th, 1971

Initial Nitrate Concentration 120 mg/l
Initial Carbon Concentration 40 mg/l
pH 8.5

Temperature 27°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 100 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	117.6	2.4	3.9	5.4	12.2	5220	0.0	79.0	42	122
3.00	105.0	16.0	2.5	5.2		5195	2.5			118
5.50	102.0	12.0	2.0	5.8		5170	12.5	86.3	35	
8.00	84.8	21.2	1.8	6.0	18.0	5145	25.0	93.0	30	136
13.25	84.2	19.8	2.2	5.6	17.5	5120	32.5	87.5	9	145

Cas Space: 120 ml.

EXPERIMENT NO.20

Experimental Conditions.

Date: Janurary 5/71

Initial Nitrate Concentration 40 mg/l
Initial Carbon Concentration 120 mg/l
pH 8.5

Temperature 27°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 100 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	47.7	2.3	5.3	2.1	11.5	5290	0.0	79.0	126	85
2.00	42.5	3.5				5265	7.5		120	
4.40	23.0	18.0	3.0	5.0	12.3	5240	17.5	97.6		115
5.00	16.8	18.2	2.9	4.7		5215	37.5		68	
6.30	5.5	15.5				5190	107.5		50	
8.00	4.5	0.5	2.0	6.4	12.5	5165	145.0	98.6	30	95
13.25	1.7	0.3	1.8	5.6		5140	190.0	97.5	24	120

Gas Space: 60 ml.

EXPERIMENT NO.21

Experimental Conditions.

Date: May 19 1970

Initial Nitrate Concentration 120 mg/l
Initial Carbon Concentration 200 mg/l
pH 8.0

Temperature 27°C
Dissolved Oxygen 0.0. mg/l
Initial Organism Concentration 50 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	87.6	0.4	2.4	3.6	15.2	5340	0.0	79.0	245	25
6.30	82.3	2.7	1.2			5315	25.0		220	
9.20	68.7	2.3	0.9	4.0	22.2	5290	75.0	96.0	175	
10.80	61.0	4.0	0.4	4.4		5265	110.0		125	
13.10	34.6	9.4	0.0	6.2	25.7	5240	185.0	98.5	65	70
15.10	10.7	12.3	0.0			5215	250.0	98.5	25	120
16.40	0.4	9.6	0.0	7.1	25.5	5190	295.0	95.3	18	85

Gas Space: 180 ml.

EXPERIMENT NO 22

Experimental Conditions.

Date: June 5/70

Initial Nitrate Concentration 100 mg/l
Initial Carbon Concentration 200 mg/l
pH 8.8

Temperature 27°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 100 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	80.2	5.34	4.5	5.4	13.5	5410	0.0	79.0	210	
0.85	77.1	6.44	4.0	5.9		5385	10.0	86.5	150	60
2.18	72.2	5.28	3.6	6.0		5360	20.0		155	50
3.18	69.3	4.23	3.3	7.8	22.7	5335	35.0	95.0	147	65
4.85	64.7	2.84	3.5	7.6		5310	55.0		144	90
8.33	62.8	0.15	3.0	9.0	17.5	5285	65.0	99.0	138	105

Gas Space: 125 ml

EXPERIMENT NO 23

Experimental Conditions.

Date: June 19/70

Initial Nitrate Concentration 120 mg/l
Initial Carbon Concentration 300 mg/l
pH 6.0

Temperature 27°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 50 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	62.9	0.1	0.2	3.7	13.6	5360	00.0	79.0	264	40
6.60	51.8	5.2	0.1	6.0		5335	30.0	81.5	164	50
9.00	45.8	4.7	0.1	3.7	14.1	5310	50.0	85.0	140	90
10.00	31.5	4.5	0.1			5285	120.0	89.5	96.	
12.20	2.2	0.8	0.1			5260	155.0	94.0	84	100
12.60	0.6	0.4	0.1	7.0		5235	170.0			70
26.60	0.0	0.2	0.2	7.3	15.2	5210	220.0	99.5	62	

Gas Space: 145 ml.

EXPERIMENT NO 24

Experimental Conditions.

Date: June 19/70

Initial Nitrate Concentration 100 mg/l
Initial Carbon Concentration 300 mg/l
pH 6.0

Temperature 27°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 50 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	62.0	0.5	0.1	3.0	12.3	5320	0.0	79.0	250	30
6.60	53.0	4.5	0.2	3.3		5295	20.0		180	50
9.00	45.5	5.0	0.1	5.3	12.9	5270	45.0	82.5	145	60
12.20	25.8	4.7	0.1	7.2		5245	122.5	90.0	100	70
12.60	10.5	3.5	0.1			5220	127.5		95	60
26.60	1.8	0.2	2.1	5.3	14.0	5195	195.0	99.0	55	40

Gas Space: 170 ml.

EXPERIMENT NO.25

Experimental Conditions.

Date: November 4/70

Initial Nitrate Concentration 120 mg/l
Initial Carbon Concentration 300 mg/l
pH 7.0

Temperature 27°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 100 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	101.2	5.3	4.4	6.6	11.5	5125	0.0	79.0	270	90
3.00	88.4	19.6	3.0	5.0	11.0	5100	65.0	86.5	170	165
4.92	72.5	20.0	4.7	7.9		5075	195.0		110	195
5.59	21.1	14.9	3.0	10.0	19.0	5050	250.0	89.0	63	180
6.09	7.8	5.7	3.2	5.8		5025	315.0		48	
7.01	0.9	0.6	1.7	6.35	22.4	5000	395.0	94.5	42	

Gas Space: 225 ml.

EXPERIMENT NO.26

Experimental Conditions.

Date: November 4/70

Initial Nitrate Concentration 120 mg/l
Initial Carbon Concentration 300 mg/l
pH 7.0

Temperature 27°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 100 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	101.3	5.1	3.3	7.7	11.5	5245	0.0	79.0	272	85
3.00	77.3	19.6	1.8	6.2	11.6	5220	55.0	93.0	157	110
4.92	36.2	19.4	3.8	10.6		5195	190.0		85	175
5.59	4.5	3.5	3.2	9.8	20.0	5170	255.0	96.5	42	200
6.09	0.95	0.55	2.6	6.1		5145	310.0		42	190
7.01	0.45	0.3	1.6	13.2	27.2	5120	375.0	97.5	42	

Gas Space: 125 ml.

EXPERIMENT NO.27

Experimental Conditions.

Date: October 20/70

Initial Nitrate Concentration 120 mg/l
Initial Carbon Concentration 300 mg/l
pH 7.0
Temperature 20°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 50 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	107.80	0.20	5.8	3.0	12.0	5280	0.0	79.0	274	60
12.92	82.25	0.25	4.1	3.3		5255	50.0		172	75
18.08	50.65	0.35	1.3	4.5	20.2	5230	155.0	94.0	94	105
20.84	29.70	0.30	1.0	4.2		5205	255.0		66	105
22.58	12.80	0.20	0.6	6.2	31.0	5180	305.0	98.0	38	110
24.75	1.35	0.15	0.4	8.0		5155	365.0		32	120
26.33	0.40	0.10	0.8	7.0	28.1	5130	385.0	99.0	30	

Gas Space: 120 ml.

EXPERIMENT NO.28

Experimental Conditions.

Date: October 20/70

Initial Nitrate Concentration 120 mg/l
Initial Carbon Concentration 300 mg/l
pH 7.0

Temperature 20°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 50 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	111.9	0.10	4.8	3.0	11.8	5245	0.0	79.0	268	60
9.42	94.7	0.30	3.0	3.2		5220	30.0		204	50
12.92	81.25	0.25	2.0	3.4	16.5	5195	40.0	91.0	178	65
18.08	50.60	0.40	1.2	3.6		5170	175.0		98	95
20.84	27.05	0.45	1.0	3.2	31.3	5145	265.0	97.0	62	115
22.70	15.00	0.50	0.5	4.7		5120	315.0		42	
24.75	7.80	0.22	0.6	6.2		5095	370.0		30	120
26.33	0.0	0.25	0.6	6.0	27.8	5070	395.0	99.0	24	

Gas Space: 125 ml.

EXPERIMENT NO 29

Experimental Conditions.

Date: October 22/70

Initial Nitrate Concentration 120 mg/l
Initial Carbon Concentration 300 mg/l
pH 7.0

Temperature 15°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 100 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	106.75	0.75	7.3	7.6	12.8	5235	0.0	79.0	268	80
21.33	84.25	8.75	7.4	6.4		5210	45.0		200	150
26.00	78.75	9.75	7.3	5.4	16.7	5185	75.0	84.0	172	130
31.83	71.35	10.65	5.2	5.2		5160	140.0		130	135
36.08	59.25	10.75	3.9	7.1		5135	322.0		86	125
45.50	2.40	0.10	1.1	9.2	25.4	5110	315.0	98.5	18	155

Gas Space: 225 ml

EXPERIMENT NO.30

Experimental Conditions.

Date: October 22/70

Initial Nitrate Concentration 120 mg/l
Initial Carbon Concentration 300 mg/l
pH 7.0

Temperature 15°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 100 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	98.75	0.25	6.6	4.4	14.2	5205	0.0	79.0	268	60
21.33		8.50	6.6	6.6		5180	35.0		200	140
26.00	71.40	9.60	6.7	6.0		5155	110.0		167	120
31.83	61.60	10.40	4.8	7.0		5130	195.0		132	125
36.08	50.35	10.65	3.8	5.8	14.3	5105	245.0	82.0	88	195
45.50	0.85	1.65	1.7	9.0	14.5	5080	395.0	97.4	24	145

Gas Space: 135 ml.

EXPERIMENT NO. 31

Experimental Conditions.

Date: October 27/70

Initial Nitrate Concentration 120 mg/l
Initial Carbon Concentration 300 mg/l
pH 7.0

Temperature 10°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 100 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	113.5	1.5	8.0	6.2	14.3	5260	0.0	79.0	315	
43.15	86.4	20.6	7.8	6.3		5235	27.5		210	115
49.15	82.0	20.0	7.5	6.5		5210	80.0		185	125
54.52	68.6	19.4	7.8	9.6		5185	125.0		145	125
60.24	43.8	17.2	4.3	8.3	23.5	5160	190.0	94.0	115	125
67.16	14.2	11.8	2.5	12.5		5135	310.0		70	145
70.41	1.2	0.8	1.0	13.0	26.0	5110	370.0	97.0	50	155

Gas Space: 160 ml.

EXPERIMENT NO.32

Experimental Conditions.

Date: October 27/70

Initial Nitrate Concentration 120 mg/l
Initial Carbon Concentration 300 mg/l
pH 7.0

Temperature 10°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 100 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	113.0	2.0	10.7	5.6	14.4	5150	0.0	79.0	300	
43.15	83.8	21.2	7.3	6.0		5125	40.0		170	115
49.15	76.7	20.8	7.0	7.4	15.2	5100	115.0	93.0	155	125
54.52	55.0	19.0				5075	170.0		115	125
60.24	30.5	17.0	5.1	9.6	22.5	5050	245.0	92.0	85	120
67.16	1.8	1.2	5.0	10.0		5025	365.0		50	150
70.41	0.7	0.3	2.3	10.3	25.0	5000	385.0	94.5	45	145

Gas Space: 250 ml.

EXPERIMENT NO33

Experimental Conditions.

Date: November 24/70

Initial Nitrate Concentration 120 mg/l
Initial Carbon Concentration 300 mg/l
pH 7.0

Temperature 5°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 100 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
48.00	91.1	13.9	7.2	4.4	16.4	5250	20.0	95.6	236	80
55.00	68.1	27.9	7.7	6.6		5225	65.0		200	135
62.10	55.6	27.4	6.1	4.3	18.2	5200	100.0	93.0	180	120
72.10			5.2	9.8		5175	160.0		174	
78.80	39.2	26.8	4.9	12.7	19.1	5150	205.0	93.0	124	160
84.70	23.2	26.8	3.6	11.4		5125	245.0		106	155
98.10	7.0	3.0	4.9	11.9	28.3	5100	330.0	96.0	54	

Gas Space: 110 ml.

EXPERIMENT NO.34

Experimental Conditions.

Date: December 11/70

Initial Nitrate Concentration 120 mg/l
Initial Carbon Concentration 300 mg/l
pH 7.0

Temperature 5°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 100 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	99.0	1.0	8.0	4.0	16.0	5130	0.0	79.0	300	85
186.80	81.6	8.4	7.8	2.6	16.0	5105	60.0	80.0	255	40
210.60	73.2	10.8	6.9	5.4		5080	110.0	92.2	215	70
233.60	32.4	21.6	7.0	4.6	16.2	5055	175.0	94.8	165	
238.30	26.0	20.0	3.8	5.5		5030	235.0	95.6	110	65
257.50	13.0	20.0	2.6	6.1	16.4	5005	285.0		80	165
270.00	0.0	2.0	1.4	7.6	16.3	4980	370.0	96.5		190

Gas Space: 195 ml.

EXPERIMENT NO.35

Experimental Conditions.

Date: December 11/70

Initial Nitrate Concentration 120 mg/l
Initial Carbon Concentration 300 mg/l
pH 7.0

Temperature 5°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 100 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	117.9	2.6	8.9	3.5	16.2	5155	0.0	79.0	310	
138.10	102.0	4.0	7.8	2.6		5130	45.0	95.5	270	60
186.80	75.4	21.6	6.9	5.4	17.0	5105	100.0	97.3	205	100
210.60	58.2	24.8	7.0	4.6		5080	180.0	95.0	185	70
221.50	37.6	25.4	3.8	5.5	20.0	5055	230.0	99.0	105	85
233.70	6.2	23.8				5030	285.0	99.0	50	140
240.60	1.2	5.8	2.6	6.1		5005	345.0	95.5	30	170
257.50	0.4	1.6	1.4	7.6	28.0	49.80	390.0	99.0	25	225

Gas Space: 250 ml.

EXPERIMENT NO. 36

Experimental Conditions.

Date: March 5/71

Initial Nitrate Concentration 120 mg/l
Initial Carbon Concentration 300 mg/l
pH 7.0

Temperature 3°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 100 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	117.0	0.2	3.0	7.8	14.5	5360	0.0	79.0	328	
220.00	110.8	0.1				5335	60.0		308	135
340.00	194.5	0.2				5310	95.0		288	150
440.00	93.0	8.0				5285	120.0		288	155
510.00	92.2	9.5				5260	130.0	85.5	280	145
630.00	57.5	27.5				5235	175.0		218	180
680.00	44.0	35.0				5210	220.0	93.0	164	175
700.00	38.5	33.5				5185	270.0		114	225
725.00	0.5	3.5	0.0	12.2	21.0	5160	420.0	99.5	46	270

Gas Space: 150 ml.

EXPERIMENT NO.37

Pseudomonas sp : Activated Sludge 1:1

Experimental Conditions.

Date: January 26/71

Initial Nitrate Concentration 120 mg/l
Initial Carbon Concentration 300 mg/l
pH 7.5

Temperature 27°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 10 0 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	120.0	1.0	8.0	11.9	13.8	5130	0.0	79.0	330	130
3.50	112.0	5.0	7.8			5105	125.0		255	
5.75	60.0	11.5		12.9		5080	225.0		220	
7.50	7.0	4.5				5055	340.0		150	215
8.50	8.5	2.0	5.3	16.9	31.2	5030	370.0	92.5	53	
10.00	6.5	1.5	4.5	19.5		5005	420.0		15	325
10.80	0.5	2.5	3.0	19.5	23.1	4980	440.0	99.0		305

Gas Space: 170 ml.

EXPERIMENT NO.38 Pseudomonas sp : Activated Sludge
0.25 : 1.0

Experimental Conditions.

Date: January 26/71

Initial Nitrate Concentration 120 mg/l
Initial Carbon Concentration 300 mg/l
pH 7.5
Temperature 27°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 100 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	132.0	3.0	5.3	10.8	14.0	5130	0.0	79.0	350	135
3.70	117.0	7.5				5105	35.0			
7.50	67.5	13.5	4.2	15.7	19.5	5080	135.0	85.0	175	145
8.50	59.0	15.0				5055	190.0		120	185
9.90	34.0	19.0	3.4	17.8	25.2	5030	265.0	93.0	55	255
13.70	6.0	4.0				5005	380.0		45	
21.00	3.0	1.5	2.5	27.2	25.3	4980	420.0	98.5	10	

Gas Space: 170 ml.

EXPERIMENT NO39 — Pseudomonas sp : Activated Sludge.

Experimental Conditions. — 0.5 : 1.0

Date: January 26/71

Initial Nitrate Concentration 120 mg/l
Initial Carbon Concentration 300 mg/l
pH 7.5

Temperature 27°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 100 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	129.7	0.3	4.5	9.7	13.9	5215	0.0	79.0	335	145
5.00	117.0	8.0				5190	35.0			
7.50	58.8	22.2	3.2	14.5	25.1	5165	110.0	96.0	120	150
8.50	40.5	23.5				5140	165.0		85	215
10.00	7.8	22.2				5115	250.0		55	205
10.75	3.5	11.5	2.7	19.8	29.3	5090	325.0	97.5	40	220
11.75	3.5	2.0				5065	375.0		25	330
16.50	5.0	2.1	1.8	25.1	22.2	5040	440.0	98.0		
18.80							470.0			

Gas Space: 165 ml.

EXPERIMENT NO.40 Pseudomonas sp : Activated Sludge

Experimental Conditions. 0.0 : 1.0

Date: January 26/71

Initial Nitrate Concentration 120 mg/l
Initial Carbon Concentration 300 mg/l
pH 7.5

Temperature 27°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 100 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	134.8	2.2	4.2	11.5	13.9	5200	0.0	79.0	420	170
7.50	125.1	6.9				5175	25.0		390	160
21.00	100.4	21.6	4.1	15.6		5150	75.0	87.3	320	
26.50	87.0	25.0				5125	135.0		260	225
32.50	38.2	22.8				5100	320.0		135	
33.09	13.7	17.8	3.5	24.2	22.3	5075	390.0	96.2	85	
34.50	14.1	3.4				5050	405.0		35	400
40.50	3.2	8.8				5025	475.0		15	430
43.75	1.5	1.0	1.5	25.2	21.5	5000	500.0	99.0		

Gas Space: 200 ml.

EXPERIMENT NO.41 Pseudomonas sp : Activated Sludge

Experimental Conditions. 1.0 : 0.0

Date: January 26/71

Initial Nitrate Concentration 120 mg/l
Initial Carbon Concentration 300 mg/l
pH 7.5
Temperature 27°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 100 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	121.5	0.5	3.5	4.8	13.8	5275	0.0	79.0	310	60
7.50	98.5	15.5		4.9		5250	25.0		260	
9.50	91.0	22.0				5225	50.0		245	85
12.00	87.5	25.0	2.3	5.1	15.2	5200	75.0	87.3		
13.20	68.0	22.0				5175	150.0		155	
14.50	22.5	17.0	0.9	13.5	28.3	5150	275.0	93.0	65	
16.50	1.5	6.0				5125	350.0		35	
22.00	1.0	4.0	0.5	14.6	27.2	5100	400.0	98.0	25	180

Gas Space: 125 ml.

EXPERIMENT NO. 42 Pseudomonas sp : Activated Sludge

Experimental Conditions. 1.0 : 1.0

Date: September 30/70

Initial Nitrate Concentration 120 mg/l
Initial Carbon Concentration 600 mg/l
pH 7.5

Temperature 27°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 100 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	96.9	1.1	10.6	6.8	12.4	5320	0.0	79.0	455	
3.00	84.4	9.6	10.3	6.3		5295	50.0		405	110
4.40	55.8	23.2	7.0	10.4	12.5	5270	110.0	86.5	330	140
5.50	29.8	22.2	4.5	12.1		5245	200.0		280	160
6.40	12.2	11.8	4.0	13.2		5220	265.0		245	180
7.10	2.6	4.4	3.5	13.7	24.2	5195	325.0	90.5	245	160
9.10	1.2	0.8	2.6	14.2	18.3	5170	375.0	94.5	240	210
10.00							395.0			

Gas Space: 210 ml.

EXPERIMENT NO. 43 Pseudomonas sp : Activated Sludge

Experimental Conditions. 0.6 : 1.0

Date: September 30/70

Initial Nitrate Concentration 120 mg/l
Initial Carbon Concentration 600 mg/l
pH 7.5

Temperature 27°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 100 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	92.5	5.5	10.5	7.7	12.6	5220	0.0	79.0	500	
3.00	89.4	13.6	10.6	5.4		5195	50.0		470	95
4.40	68.8	23.2	8.6	10.8		5170	120.0		380	95
5.50	56.2	24.8	6.5	12.3		5145	160.0	88.5	310	130
6.40	43.6	24.4	3.0	11.5	13.5	5120	217.5	86.0	295	165
7.10	28.2	23.8	5.3	13.9		5095	275.0		265	155
8.10	3.2	5.8	4.2	15.2	18.6	5070	340.0	89.0	250	180

Gas Space: 90 ml.

EXPERIMENT NO. 44

Experimental Conditions.

Date: June 5/70

Initial Nitrate Concentration 40 mg/l
Initial Carbon Concentration 100 mg/l
pH 8.5

Temperature 27°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 100 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	39.9	6.1	2.6	8.8	41.5	5445	0.0	79.0	93	
0.50	37.8	6.8	0.5			5420	75.0		91	150
1.00	30.4	6.7	0.7			5395	85.0		77	150
2.30	21.2	4.9	0.1			5370	165.0		37	175
3.30	11.7	6.4	0.2	11.2	32.4	5345	210.0	91.5	27	160
5.00	0.9	7.1	0.2			5320	260.0		24	190
8.50	0.0	6.6	0.2	9.5	14.5	5295	320.0	95.0	16	215

Gas Space: 85 ml.

EXPERIMENT NO.45

Experimental Conditions.

Date: May 28/70

Initial Nitrate Concentration 40 mg/l
Initial Carbon Concentration 100 mg/l
pH 8.5

Temperature 27°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 100 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	28.5	4.1	0.4	10.0	25.2	5250	0.0	79.0	72	120
1.00	25.3	5.7	0.3	10.1		5225	95.0		56	
2.40	16.4	6.1	0.2	10.4	23.2	5200	Oil	99.8	42	164
4.50	12.1	5.4	0.2	11.4		5175	Leak		18	192
6.50	7.7	4.8	0.2	11.4	14.5	5150		98.0	18	230

Gas Space: 250 ml.

EXPERIMENT NO.46

Experimental Conditions.

Date: May 28/70

Initial Nitrate Concentration 40 mg/l
Initial Carbon Concentration 100 mg/l
pH 8.5

Temperature 27°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 100 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	29.8	6.4	0.3	7.7	24.9	5410	0.0	79.0	88	130
0.50	23.6	6.4	0.3	8.5		5385	50.0		78	130
1.50	18.9	6.1	0.2	8.8	22.8	5360	100.0	87.0	72	130
2.40	13.8	6.2	0.2	9.0		5335	130.0		54	155
5.00	7.5	6.3	0.2	7.8		5310	160.0	98.0	42	175
7.00	0.0	3.8	0.1	9.4	13.8	5285	190.0	98.0	26	165

Gas Space: 110 ml.

EXPERIMENT NO.47

Experimental Conditions.

Date: January 10/71

Initial Nitrate Concentration 20 mg/l
Initial Carbon Concentration 15 mg/l
pH 8.5

Temperature 27°C
Dissolved Oxygen 0.0. mg/l
Initial Organism Concentration 100mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	20.0	2.0	2.0	8.8	13.8	5400	0.0	79.0	16	125
5.20	18.2	1.0				5375	11.5		10	
10.40	17.4	0.8	0.9			5365	20.0		9	130
20.00	14.1	0.2	0.4	8.7	14.0	5340	37.5	85.0	4	130

Gas Space: 150 ml.

EXPERIMENT NO48

Experimental Conditions.

Date: January 10/71

Initial Nitrate Concentration 60 mg/l
Initial Carbon Concentration 20 mg/l
pH 8.5

Temperature 27°C
Dissolved Oxygen 0.0 mg/l
Initial Organism Concentration 100 mg/l

Time hrs.	NO_3 mg/l	NO_2 mg/l	NH_3 mg/l	Solids Total Kjeldahl mg/l	Dissolved Nitrogen mg/l	Liquid Volume ml.	Gas Collected ml	% Nitrogen Gas	Soluble Carbon mg/l	Suspended Solids mg/l
0.00	65.0	0.5	3.0	8.3	16.2	5350	0.0	79.0	16	165
3.30	63.0	1.2				5325	21.0		10	170
6.70	60.5	0.8	0.7	8.2		5315	35.0		8	
10.00	59.00	0.1	0.1	8.5	13.8	5290	55.0	84.2	4	175

Gas Space: 90 ml.

APPENDIX B

DATA ANALYSIS.

APPENDIX B.

DATA ANALYSIS

Least Squares Analysis.

All of the experimental batch data produced curves which could be interpreted as an initial lag or acclimation period followed by a relatively linear removal rate with respect to time. The linear portion of the curve was, therefore, fitted with a least squares straight line using the program listed as Program 1. Note that least squares fits of polynomials greater than first order were also a feature of this program, and a comparison of the degree of applicability of successively higher orders of polynomials was determined by an F-test.

In the case of the temperature dependency of the unit denitrification rate, the data was first transformed to 1/T versus log of the unit denitrification rate, and the semi-logarithmic plot was then fitted with a straight line using the same program. Log transformation of the data does weight the fit at one end of the data range, but a more accurate search technique to determine the curve constants was not considered necessary for the purposes of this work.

Experimental Design.

A complete factorial design as performed for the four factors of pH, nitrate concentration, carbon to nitrate ratio, and organism concentration, at two levels was used to

```
      PROGRAM TST (INPUT,OUTPUT,TAPE5=INPUT,TAPE6=OUTPUT)
C R.N.DAWSON
C PROGRAM FOR LINEAR LEAST SQUARES FITTING OF Y VS X BY
C POLYNOMIALS IN X
C PROGRAM TAKES THE N DATA POINTS (X,Y) AND FITS POLYNOMIALS OF
C INCREASING ORDER FOR INCREASING NUMBERS OF PTS FROM 3 TO N BY 2
C AN F TEST IS PERFORMED FOR EACH INCREASE IN ORDER
      DIMENSION T(200),Z(200),A(200),B(200),X(200),Y(200)
      DIMENSION YY(200),W(200)
      DIMENSION R(200)
      READ(5,1) NDATA
      DO 102 KKK=1,NDATA
      READ(5,1) NPTS
      DO 100 I=1,NPTS
      READ(5,2) T(I),Z(I)
  100 CONTINUE
      J=NPTS
      N=J
      DO 106 I=1,J
      X(I) = T(I)
      Y(I) = Z(I)
  106 CONTINUE
      DO 101 I=1,200
      A(I)=0.0
      B(I)=0.0
  101 CONTINUE
      WRITE(6,3) KKK,NPTS
      JJJ=J-2
      IF(JJJ.GT.7) JJJ=7
      WRITE(6,4)
      DO 102 M=1,JJJ
      CALL LESQ(A,B,X,Y,M,N)
      MM=M+1
      YYY=0.0
      DO 104 I=1,N
      YYY=YYY+Y(I)
      YY(I)=B(1)
      DO 104 L=2,MM
      LL=L-1
      W(I)=B(L)*(X(I))**LL
      YY(I)=YY(I)+W(I)
  104 CONTINUE
      SUM=0.0
      SIG=0.0
      YMEAN=YYY/FLOAT(N)
      DO 105 I=1,N
      RS=(Y(I)-YMEAN)**2
      SS=(Y(I)-YY(I))**2
      SUM=SUM+RS
      SIG=SIG+SS
  105 CONTINUE
      RMS=SIG/FLOAT(N-MM)
      R(1)=0.0
      R(MM)=RMS
      NN=N-MM
      F=(R(M)*FLOAT(NN+1)-R(MM)*FLOAT(NN))/R(MM)
      FF=((SUM-SIG)/FLOAT(MM-1))/RMS
      WRITE(6,5) N,M,RMS,F,NN,FF,(B(L),L=1,MM)
  102 CONTINUE
      STOP
    1 FORMAT(I5)
    2 FORMAT(F9.4,F11.4)
    3 FORMAT(H0,8H RUN NO=,I3,9H NO DATA=,I4)
    4 FORMAT(1H0,5X,4H PTS,3X,6H ORDER,5X,12H MEAN SQUARE,4X,7H F TEST,1
     1X,3H DF,20X,6H COEFF)
    5 FORMAT(1H ,3X,I3,3X,I3,3X,E14.6,F10.5,I3,F9.4,/,8F14.6)
    6 FORMAT(E14.6,2I5,2E14.6)
      END
```

study the response (unit denitrification rate) at all possible combinations of the variables and levels of the variables. Factorial designs are the most efficient experimental programs for evaluating the effect of the factors on the response as well as providing the interaction effect of variables on the response.

The general method for constructing a factorial design at two levels of each variable is itemized as follows:

1. For factor 1, start with -1, +1, -1, +1 ---, and continue for all the 2^k tests.
2. For factor 2 start with the first two tests as -1, the next two as +1, and the next two as -1 etc.
3. For factor 3, start with the first four tests as -1, the next four as +1, and the next four as -1, etc. i.e. -1, -1, -1, -1, +1, +1, +1, +1.
4. For factor 4, start with the first eight tests as -1, the next eight as +1, etc.
5. Proceed in a similar way for all variables, i.e. x_5, x_6 --- etc. until finally reaching the kth variables, x_k.
6. For the kth variable, start with the first 2^{k-1} tests as -1, and the next 2^{k-1} tests as +1.

This method will yield all 2^k distinct combinations of the variables without repetition. Table No. 7 in the

main body represents a typical design for four factors at two levels of each factor, i.e. 16 tests constituting a 2^4 factorial design.

Calculation of Effects. For a factorial design the main effects, i.e. the effect on the response (in our case unit denitrification rate) in moving from a low level of a factor to a high level of a factor, e.g. nitrate concentration can be evaluated by the following formula.

$$E = \frac{1}{n/2} \sum_{i=1}^{n} yi$$

E = effect
n = number of tests
yi = response
response = value of dependent variable multiplied by +1 or -1, whichever applies for the particular experiment.

For example, for the first 16 screening experiments run, the average effects follow:-

$$\text{Nitrate Concentration} = \frac{1}{8} \times \begin{bmatrix} -.0307 & +.0550 \\ -.0678 & +.1270 \\ -.0150 & +.0406 \\ -.0424 & +.0613 \\ -.0246 & +.0565 \\ -.0600 & +.1430 \\ -.0198 & +.0290 \\ -.0508 & +.0710 \end{bmatrix}$$

$$-.3111 \quad +.5834$$

$$= \frac{.2723}{8} \text{ or } 0.0340$$

i.e. In increasing the nitrate concentration from 40 to 120 mg/l the unit denitrification rate increased 0.0340 mg/l NO_3 asN/mg/l organisms-hr.

Carbon to Nitrate Ratio

$$\frac{1}{8} \times \begin{bmatrix} -.0307 + .0678 \\ -.0550 + .1270 \\ -.0150 + .0424 \\ -.0406 + .0613 \\ -.0246 + .0600 \\ -.0565 + .1430 \\ -.0198 + .0508 \\ -.0290 + .0710 \end{bmatrix}$$

$$-.2712 + .6233$$

$$= \frac{.3521}{8} \text{ or } 0.0440$$

i.e. In increasing the carbon to nitrate ratio from 1:1 to 5:1, the unit denitrification rate increased 0.0440 mg/l NO_3 as N/mg/l organisms-hr.

pH

$$\frac{1}{8} \times \begin{bmatrix} -.0307 + .0150 \\ -.0550 + .0406 \\ -.0678 + .0424 \\ -.1270 + .0613 \\ -.0246 + .0198 \\ -.0565 + .0290 \\ -.0600 + .0508 \\ -.1430 + .0710 \end{bmatrix}$$

$$-.5646 + .3299$$

$$= -0.0293$$

i.e. In increasing the pH from 6.5 to 3.5, the unit denitrification rate decreased 0.0293 mg/l NO_3 as N/ mg/l organisms-hr.

$$\text{Organism Concentration} = \frac{1}{8} \times \left[\begin{array}{l} -.0307 + .0246 \\ -.0550 + .0565 \\ -.0678 + .0600 \\ -.1270 + .1430 \\ -.0150 + .0198 \\ -.0406 + .0290 \\ -.0424 + .0508 \\ -.0613 + .0710 \end{array}\right]$$

$$-.4398 + .4547$$

$$= \frac{.0149}{8} \text{ or } .0019$$

i.e. In increasing the organism concentration from 50 to 100 mg/l the unit denitrification rate increased .0019 mg/l NO_3 as N/mg/l organisms-hr.

Average Effects of 2^2 Factorial Design from Table 9.

$$\text{Nitrate Concentration} = \frac{(.0960 + .0565 - .1290 - 0.0246)}{2}$$

$$= \frac{.0011}{2} = -.0006 \text{ mg/l } NO_3/\text{mg/l organisms-hr}$$

$$\text{Carbon Concentration} = \frac{(.0565 + .1290 - .0960 - .0246)}{2}$$

$$= \frac{.0649}{2} = +.0324 \frac{\text{mg/l } NO_3}{\text{mg/l organisms-hr}}$$

Average Effects of 2^3 Factorial Design from Table 10.

Nitrate Concentration =

$$\frac{1}{4}(-.0246 - 0.0198 - 0.1290 - 0.088 + 0.0960 + 0.0565 + 0.0406 + 0.0321)$$

$$= .0090 \frac{\text{mg/l } NO_3}{\text{mg/l organisms-hr.}}$$

Carbon Concentration =

$$\frac{1}{4}(-0.0246 - 0.0198 - 0.0960 - 0.0321 + 0.1290 + 0.0880 + 0.0565 + 0.0406) = .0354$$

$$pH = \frac{1}{4}(-0.0246 - .1290 - .0960 - .0565 + .0198 + .0880 + .0406 + .0321)$$

$$= .0314 \text{ mg/l } NO_3 / \text{ mg/l organisms-hr.}$$

The interaction effects in a full factorial design can be determined by multiplying the coded levels of interacting factors by the response and then evaluating the interaction in a similar manner to the main effects. Usually interactions between more than two factors are considered to be negligible. A table for determining the multipliers for the response for interaction effects for the 16 screening experiments follows.

TABLE NO. B-1.

SCREENING EXPERIMENTS - INTERACTION EFFECTS CALCULATION.

Expt. No.	A NO_3	B C:NO_3	C pH	D Organisms	AB	AC	AD	BC	BD	CD
1	-1	-1	-1	-1	+1	+1	+1	+1	+1	+1
2	+1	-1	-1	-1	-1	-1	-1	+1	+1	+1
3	-1	+1	-1	-1	-1	+1	+1	-1	-1	+1
4	+1	+1	-1	-1	+1	-1	-1	-1	-1	+1
5	-1	-1	+1	-1	+1	-1	+1	-1	+1	-1
6	+1	-1	+1	-1	-1	+1	-1	-1	+1	-1
7	-1	+1	+1	-1	-1	-1	+1	+1	-1	-1
8	+1	+1	+1	-1	+1	+1	-1	+1	-1	-1
9	-1	-1	-1	+1	+1	+1	-1	+1	-1	-1
10	+1	-1	-1	+1	-1	-1	+1	+1	-1	-1
11	-1	+1	-1	+1	-1	+1	-1	-1	+1	-1
12	+1	+1	-1	+1	+1	-1	+1	-1	+1	-1
13	-1	-1	+1	+1	+1	-1	-1	-1	-1	+1
14	+1	-1	+1	+1	-1	+1	+1	-1	-1	+1
15	-1	+1	+1	+1	-1	-1	-1	+1	+1	+1
16	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1

Calculated of interaction effect of nitrate concentration and carbon to nitrate ratio -

$$\text{Interaction effect} = \frac{1}{8} \times \begin{bmatrix} -.0550 + 0.0307 \\ -.0678 + 0.1270 \\ -.0406 + 0.0150 \\ -.0424 + 0.0613 \\ -.0565 + 0.0246 \\ -.0600 + 0.1430 \\ -.0290 + 0.0198 \\ -.0508 + 0.0710 \\ \hline -.4021 + 0.4924 \end{bmatrix} \quad \text{or } \frac{.0903}{8} = .0113$$

Analysis of Variance.

The analysis of variance of the effects and interactions in a factorial design assesses by means of significance tests whether or not the observed effects can be accounted for by experimental error. The methods used in an analysis of variance lead to mean squares for the factors and interactions between factors. If the F-test shows a mean square to be significantly greater than the error mean square at a predetermined confidence level, it can be inferred that changing the level of that factor significantly affects the response.

The Yates Method (Davies 1967) of analysis of variance is simply a tabular method which is particularly convenient for estimating the mean squares when four or more factors affect the response.

An analysis of variance for the three factors, nitrate, carbon, and pH is shown in Table B-3. An estimate of the error variance was obtained by determining the variance of five repeat experiments performed under the same conditions within the experimental design.

Experiment No. 3	0.0678
11	0.0600
45	0.0365
46	0.0490
44	0.0240

Confidence Levels

The 95% confidence levels for the regression lines,

TABLE NO. B-2.

ANALYSIS OF VARIANCE
SCREENING EXPERIMENTS

Factors Listed According to Yates Method (Davies 1967)

See Table No.7 for rate data in mg. NO_3 as N/mg of organisms-hr

		Organisms Concentration			
		I (50) pH		d(100) pH	
		I(6.5)	c(8.5)	I(6.5)	c(8.5)
I(40) c/NO_3	I(1:1)	.0307	.015	.0246	.0198
	b(5:1)	.0678	.0424	.0600	.0508
a(120) c/NO_3	I(1:1)	.0550	.0406	.0565	.0290
	b(5:1)	.1270	.0613	.1430	.0710

Calculation of Effects

	Denit. Rate.						Mean Square	
I	.0307	.0857	.2805	.4398	.8945	.1118		
a	.0550	.1948	.1593	.4547	.2723	.0340	.004627	*
b	.0678	.0556	.2841	.1280	.3521	.0440	.007748	*
ab	.1270	.1037	.1706	.1443	.0903	.0113	.000509	
c	.0150	.0811	.0835	.1572	-.2347	-.0293	.003443	*
ac	.0406	.2030	.0445	.1949	-.1245	-.0155	.0009687	
bc	.0424	.0488	.1149	.0282	-.1099	-.0137	.0007549	
abc	.0613	.1218	.0294	.0621	-.0817	-.0102	.0004171	
d	.0246	.0243	.1091	-.1212	.0149	.0018	.0000138	
ad	.0565	.0592	.0481	-.1135	.0163	.0020	.0000166	
bd	.0600	.0256	.1219	-.0390	.0377	.0047	.00008883	
abd	.1430	.0189	.0730	-.0855	.0339	.0042	.00007182	
cd	.0198	.0319	.0349	-.0610	.0077	.0009	.00000371	
acd	.0290	.0830	-.0067	-.0489	-.0465	-.0058	.00013514	
bcd	.0508	.0092	.0511	-.0416	.0121	.0015	.00000915	
abcd	.0710	.0202	.0110	-.0401	.0015	.0002	.00000014	

- Outside estimate of error variance .0001872
- F test, 2-tailed at 95% confidence level for 5,1 degrees of freedom 6.6 x 0001872 = .00123.
- All mean squares greater than .00123 are significant at the 95% confidence level and are marked with an *.

TABLE NO.B-3.

ANALYSIS OF VARIANCE
CARBON, NO_3 AND pH
EFFECTS ON DENITRIFICATION RATE.

Factors Listed According to Yates Method (Davies 1967).

See Table No.10 for rate data in mg/l NO_3 as N/mg. of cells-hr.

		pH			
		I (6.5) Nitrite Concentration		C (8.5) Nitrate Concentration	
		I(40)	a(120)	I(40)	a(120)
Carbon Concentration	I(40)	.0246	.096	.0198	.0321
	b(120)	.1290	.0565	.0880	.0406

Calculation of Effects.

	Denit.Rate				Mean Square	
I	.0246	.1206	.3061	.4866		
a	.0960	.1855	.1805	-.0342	.0011	
b	.1290	.0519	-.0011	.1416	.0200	*
ab	.0565	.1286	-.0351	-.2036	.0415	*
c	.0198	.0714	.0649	-.1256	.0157	*
ac	.0321	-.0725	.0769	-.0340	.0011	
bc	.0880	.0123	-.1439	.0118	.0001	
abc	.0406	-.0474	-.0597	.0842	.0071	*

- Outside estimate of error variance .0001872
- F test, 2-tailed at 95% confidence level for 4,1 degrees of freedom 7.71 x.0001872 = .00145
- All mean squares greater than .00145 are significant at the 95% confidence level and are marked with an *.

solids-organism count data, log transformed data of unit denitrification rate versus temperature reciprocal, log transformed data of unit denitrification data versus weight ratio of Pseudomonas/Activated Sludge, were calculated from an analysis of variance of the data. A sample calculation for the 95% confidence limits on the regression line through the solids, colony count data follows:-

Regression Line Equation.

$$Yi = -32.44 + 1.338\ Xi$$

calculated from data tabulated in Table 17.

where Xi = suspended solids concentration in mg/l

Yi = colony count $x10^6$

The residual sum of squares is calculated from the analysis of variance below.

Source of Variance	Sum of Squares	Degrees of Freedom	Mean Square
Regression	$b_1\left(\Sigma XiYi - \frac{(\Sigma Xi)(\Sigma Yi)}{n}\right)$	1	MS_n
About Regression (residual)	By Subtraction	n-2	$S^2=\frac{(SS)}{(n-2)}$
About mean (total corrected for mean)	$\Sigma Yi^2 - \frac{(\Sigma Yi)^2}{n}$	n-1	

$$b_1 = \frac{\Sigma(Xi - \bar{X})(Yi-\bar{Y})}{\Sigma(Xi - \bar{X})^2}$$

for the suspended solids versus colony count line

Source of Variance	Sum of Squares	Degrees of Freedom	Mean Square
Regression	487,832	1	487,832
About Regression (residual)	257,912	51	5,056.233
About Mean	745,744	52	

The variance for any future observation was calculated according to the formula

$$V(Y_k) = S^2 \cdot \left\{ 1 + \frac{1}{n} + \frac{(X_k - \bar{X})^2}{\sum (X_i - \bar{X})^2} \right\}$$

The confidence limits were then established from the following -

$$\text{Limit} = \hat{Y}_k \pm (\text{value of 2 tailed } t\text{-test}) \sqrt{V(Y_k)}$$

At the 95% confidence level the value of the two tailed t - test is tabulated as 2.011

$$95\% \text{ confidence limit} = \hat{Y}_k \pm 2.011 \sqrt{V(Y_k)}$$

APPENDIX C.

ANALYTICAL METHODS & CALIBRATION.

APPENDIX C.

Analytical Methods and Calibration.

1. Carbon - The soluble carbon concentration was determined using the Beckman carbon analyzer as outlined in the main body of the report. A typical carbon analysis calibration curve of sodium oxalate standards is shown in Figure C-1.

2. Nitrite - The nitrite samples in the range 0-1.3 mg/l nitrite as N were performed according to Technicon Auto-Analyzer Industrial Method 35-69 W. Samples with a higher nitrite concentration were diluted to bring them within range. A schematic of the auto-analyzer flow sheet is shown in Figure C-2. The reagents and standards utilized in the tests are itemized below -

Colour Reagent -

Sulfanilamide $C_6H_8N_2O_2S$ 20 gm.

Phosphoric Acid H_3PO_4 (conc.) 200 ml.

N-1 Napthylethylenediamine dihydrochloride $C_{12}H_{14}N_2 \cdot 2HCl$ 1 gm.

Distilled Water q.s. 2 litres.

To approximately 1500 ml of distilled water add 200 ml of concentrated phosphoric acid and 20 gm of sulphanilamide. Dissolve completely. (Heat if necessary). Add 1 gm of N-1 naphthylethylenediamine dihydrochloride and

FIGURE NO. C-1

TYPICAL SOLUBLE CARBON CALIBRATION CURVE

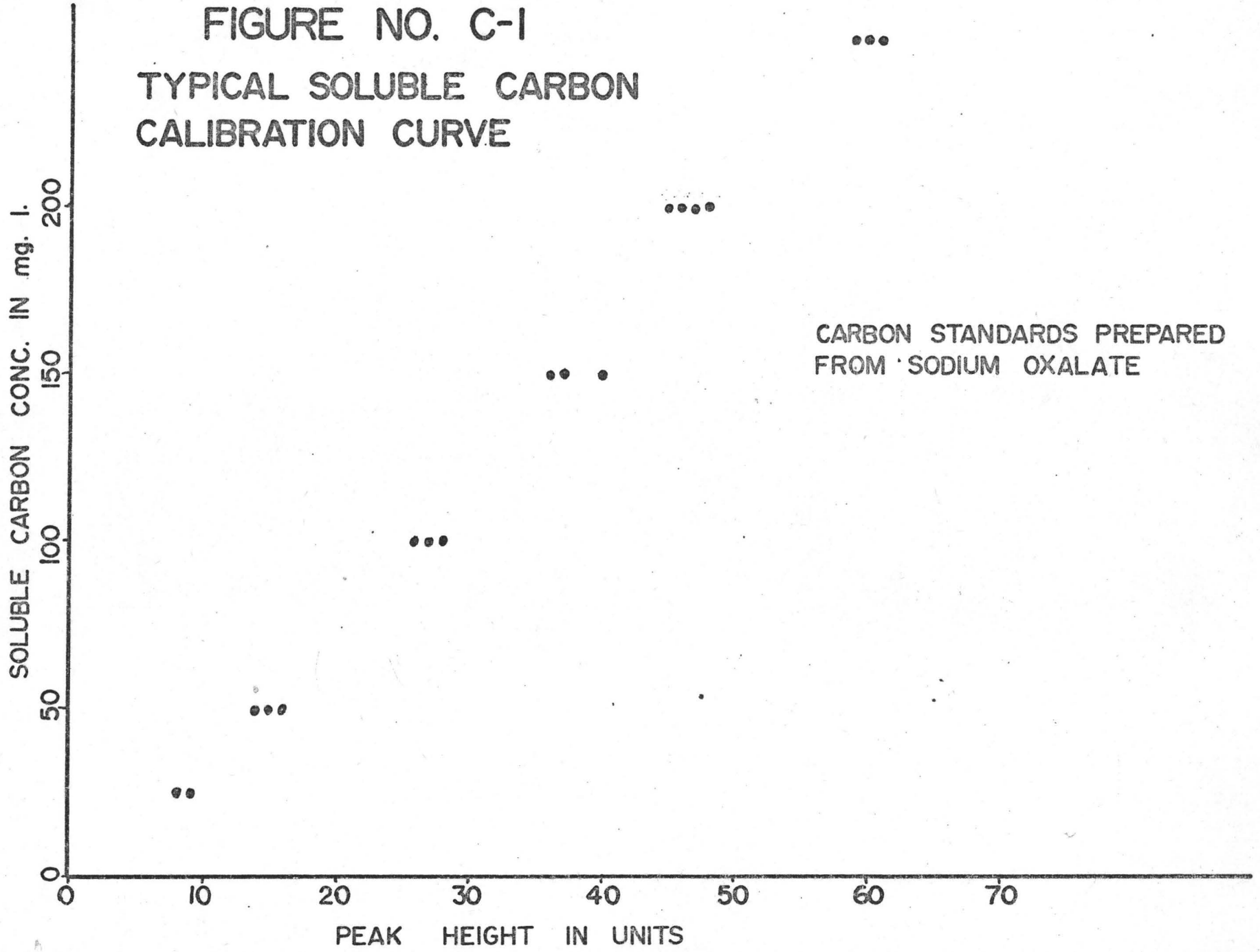
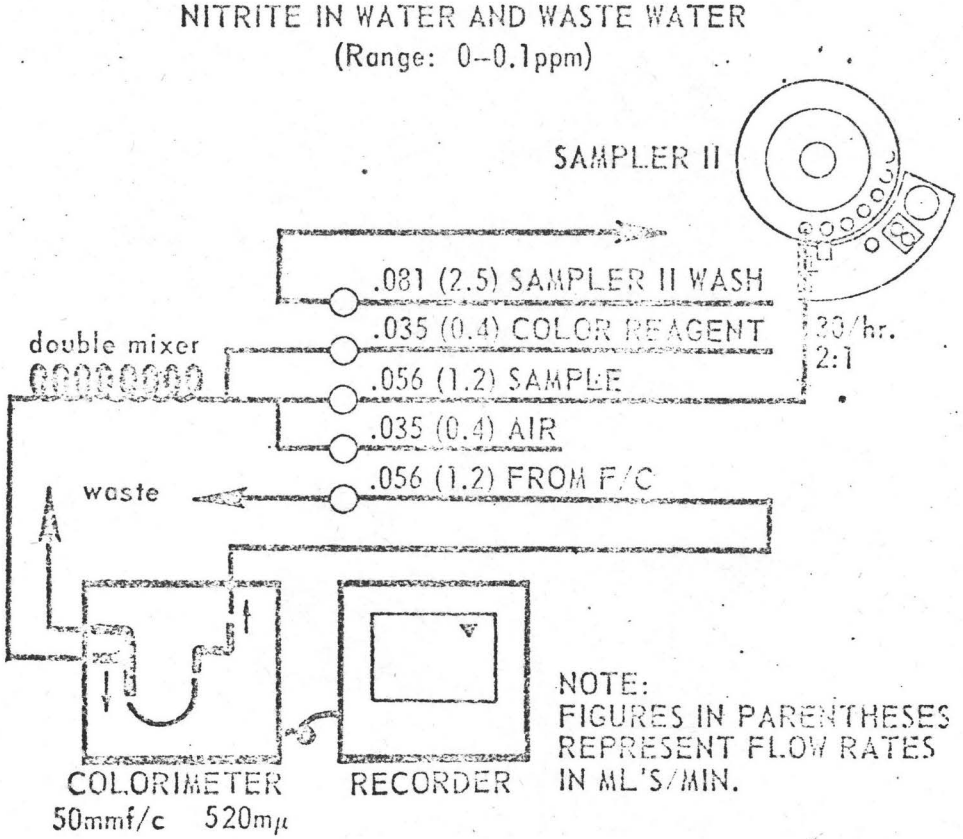


FIGURE NO. C-2


NITRITE IN WATER AND WASTE WATER
(Range: 0--0.1ppm)
SAMPLER II
.081 (2.5) SAMPLER II WASH
.035 (0.4) COLOR REAGENT
.056 (1.2) SAMPLE
.035 (0.4) AIR
.056 (1.2) FROM F/C
30/hr.
2:1
double mixer
waste
COLORIMETER
50mmf/c 520mμ
RECORDER
NOTE:
FIGURES IN PARENTHESES
REPRESENT FLOW RATES
IN ML'S/MIN.

dissolve. Dilute to 2 litres. Add 1.0 ml of Brij-35. Store in a cold dark place. Stability one month.

Standards 100 mg/l

Sodium nitrite $NaNO_2$	0.4926 gm.
Distilled water q.s.	1 litre.

Dissolve 0.4926 gm of sodium nitrite in distilled water and dilute to one litre. Add 2 ml of purified chloroform per litre as a preservative. Prepare working standards in the range 0.01 mg/l to 1.3 mg/l in serial dilution for calibration. A typical calibration curve for the analyses performed on November 4th 1970 is shown in Figure C-3. No difficulty was experienced in obtaining a stable baseline and smooth peaks.

3. Total Nitrogen Kjeldahl - Nitrogen analyses in the range 0-40 mg/l were performed according to Technicon Auto-analyzer. Industrial Method 30-69A. All reactor samples fell within this range. A schematic of the auto-analyzer flow sheet is shown in Figure C-4. The reagents and standards utilized in the tests are itemized as follows -

Digestion Mixture.

Selenium Dioxide	3.0 gm.
Sulphuric Acid (conc)	900 ml.
Perchloric Acid 68-70%	20 ml.

Dissolve 3.0 gm selenium dioxide in approximately 50 ml of distilled water and add 20 ml of perchloric acid.

FIGURE NO. C-3

TYPICAL NITRITE ANALYSIS
CALIBRATION CURVE NOV. 7/70

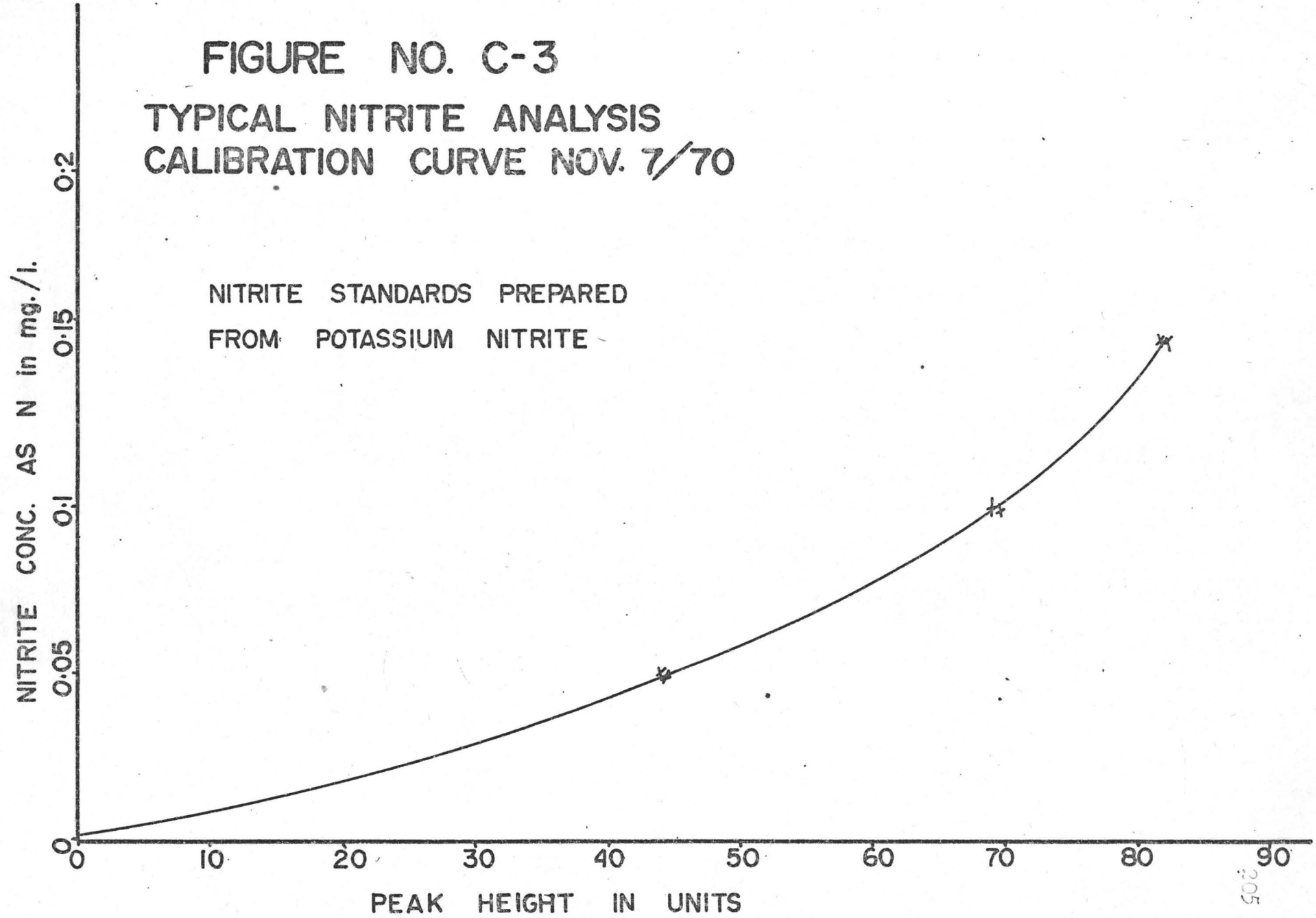
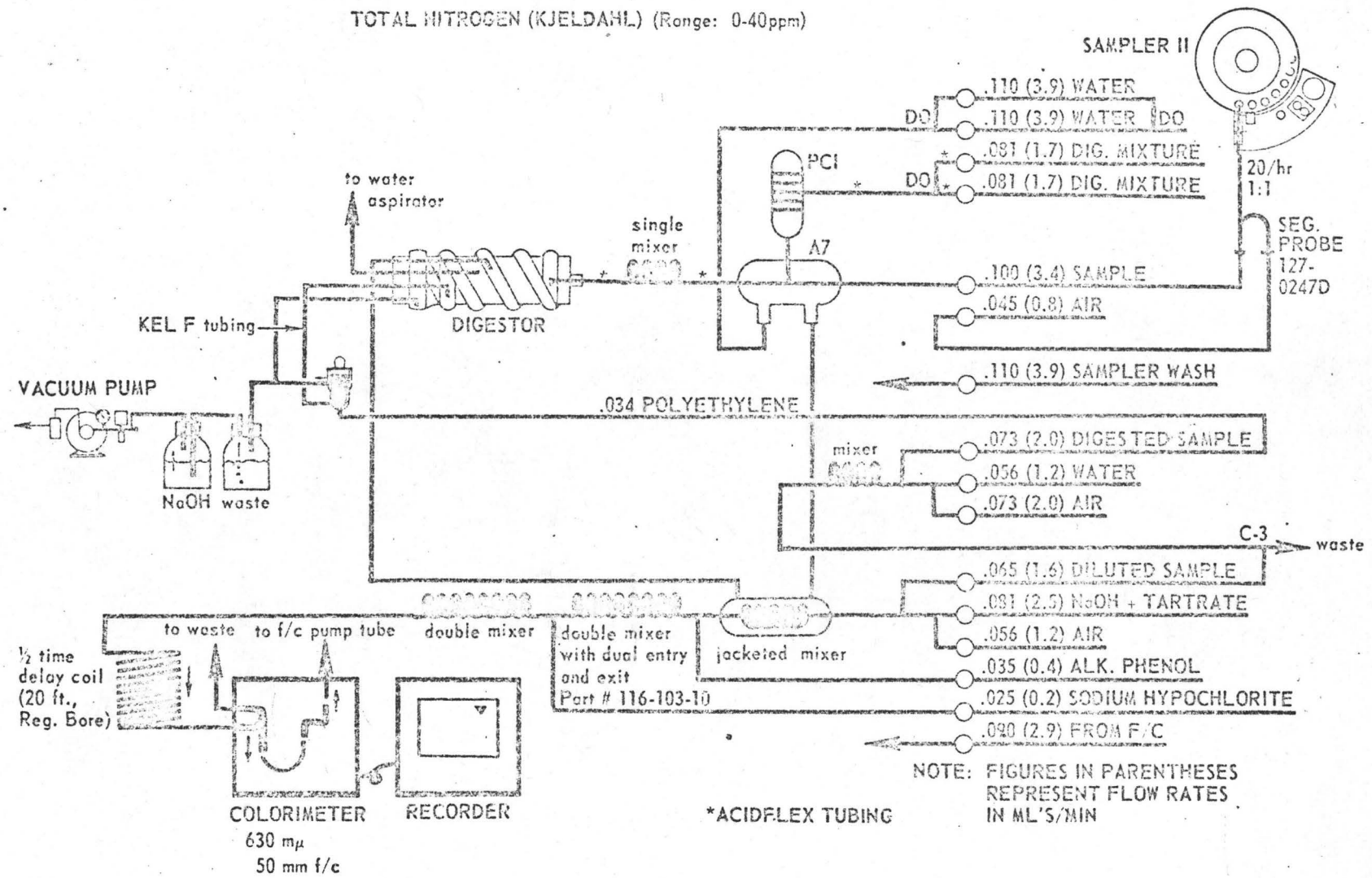


FIGURE NO. C-4


TOTAL NITROGEN (KJELDAHL) (Range: 0-40ppm)
SAMPLER II
.110 (3.9) WATER
DO
.110 (3.9) WATER
DO
.081 (1.7) DIG. MIXTURE
DO
.081 (1.7) DIG. MIXTURE
20/hr
1:1
PCI
to water aspirator
single mixer
A7
SEG. PROBE 127-0247D
.100 (3.4) SAMPLE
.045 (0.8) AIR
KEL F tubing
DIGESTOR
.110 (3.9) SAMPLER WASH
VACUUM PUMP
.034 POLYETHYLENE
mixer
.073 (2.0) DIGESTED SAMPLE
.056 (1.2) WATER
.073 (2.0) AIR
NaOH waste
C-3
waste
.065 (1.6) DILUTED SAMPLE
.081 (2.5) NaOH + TARTRATE
.056 (1.2) AIR
.035 (0.4) ALK. PHENOL
.025 (0.2) SODIUM HYPOCHLORITE
.090 (2.9) FROM F/C
to waste
to f/c pump tube
double mixer
double mixer with dual entry and exit Part # 116-103-10
jacketed mixer
½ time delay coil (20 ft., Reg. Bore)
COLORIMETER
630 mμ
50 mm f/c
RECORDER
*ACIDFLEX TUBING
NOTE: FIGURES IN PARENTHESES REPRESENT FLOW RATES IN ML'S/MIN

Add 900 ml of concentrated sulphuric acid and dilute to one litre. Mix and allow to cool. Adjust the volume and store in an amber glass bottle.

Sodium Hydroxide Reagent.

Sodium Hydroxide (NaOH)	350 gm.
Potassium Sodium Tartrate $KNaC_4H_4O_6 \cdot 24H_2O$	50 gm.

Dissolve 350 gm sodium hydroxide and 50 gm potassium sodium tartrate in about 700 ml distilled water in a one litre volumetric flask. Allow to cool and dilute to volume. Store in a polythylene bottle. The potassium sodium tartrate is added to prevent precipitation of heavy metal contaminants in the alkaline medium.

Alkaline Phenol.

Sodium Hydroxide, 5N (400 gm/litre)	500 ml.
Phenol, liquified, about 88%	276 ml.

Slowly add 276 ml of liquified phenol from a separatory funnel to 500 ml 5N sodium hydroxide contained in a vessel surrounded by circulating cold water, stirring the mixture continuously. Dilute to one litre with distilled water. Store in a polyethylene bottle.

Sodium Hypochloride.

Any good commercially available household bleach having 0.5% available chlorine is suitable.

Standards - In order to achieve the greatest accuracy from the system it is essential that a carefully assayed

nitrogen containing material having the same matrix as the samples is used for calibration. A 100 mg/l solution of ammonium chloride was used as a stock solution to prepare serial dilutions for system calibration.

Ammonium Chloride	NH_4Cl	3.79 gm.
Distilled Water	qs	1 litre.

A typical calibration curve for the analyses performed on October 25, 1970 is shown in Figure C-5.

4. Nitrate:

The nitrate plus nitrite in the samples was determined according to Technicon Auto-Analyzer Industrial Method 33-69W for samples in the range of 0 to 2.0 mg/l or samples diluted with distilled water to fall within this range. The nitrite concentration was determined separately and subtracted to give the nitrate concentration. A schematic of the auto-analyzer flow sheet is shown in Figure C-6. The reagents and standards utilized in the tests are itemized below.

Colour Reagent.

Sulfanilamide	$C_6H_8NO_2S$	20 gm.
Phosphoric Acid	H_3PO_4 (conc).	200 ml.
N-1 Napthylethylenediamine Dihydrochloride	$C_{12}H_{14}N_2 \cdot 2HCl$	1 gm.
Distilled Water	qs	1 litre.

To approximately 1500 ml of distilled water add 200 ml of concentrated phosphoric acid and 20 gm of

FIGURE NO. C-5

TYPICAL TOTAL KJELDAHL ANALYSIS CALIBRATION CURVE OCT. 25/70

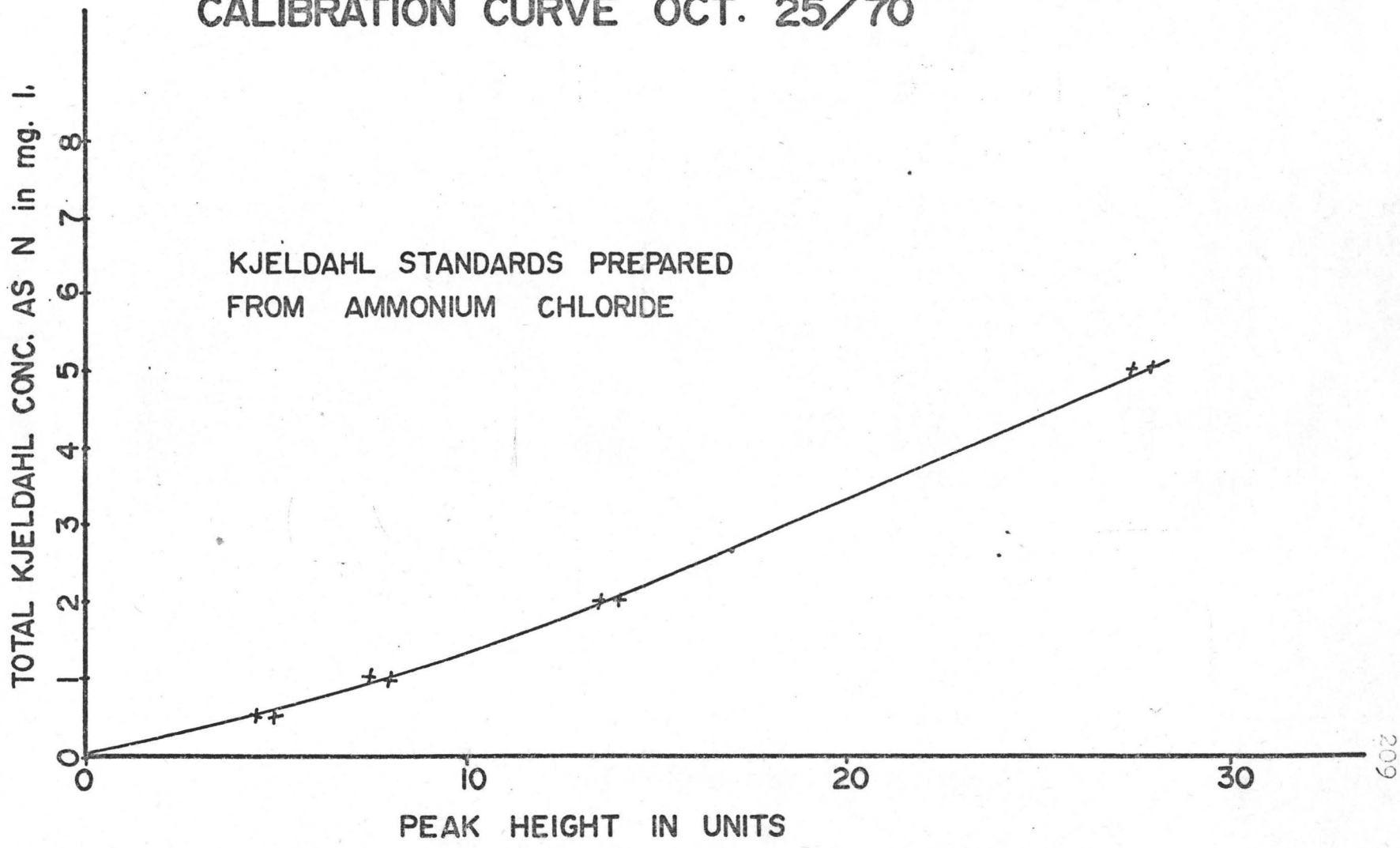
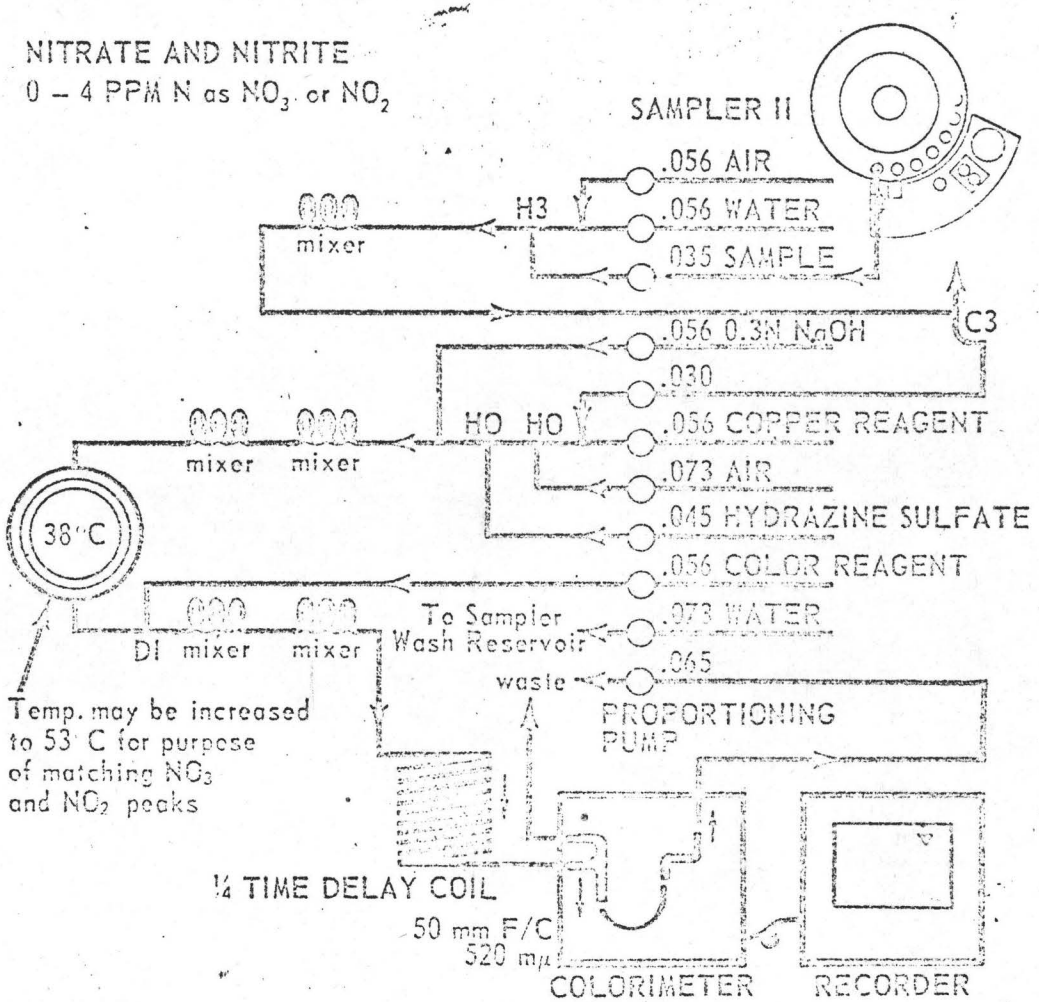


FIGURE NO. C-6


NITRATE AND NITRITE
0 – 4 PPM N as NO_3 or NO_2
SAMPLER II
.056 AIR
.056 WATER
.035 SAMPLE
H3
mixer
C3
.056 0.3N NaOH
.030
HO HO
.056 COPPER REAGENT
mixer mixer
.073 AIR
.045 HYDRAZINE SULFATE
38°C
.056 COLOR REAGENT
To Sampler
Wash Reservoir
.073 WATER
DI mixer mixer
waste
.065
Temp. may be increased
to 53° C for purpose
of matching NO_3
and NO_2 peaks
PROPORTIONING
PUMP
¼ TIME DELAY COIL
50 mm F/C
520 mμ
COLORIMETER
RECORDER

sulfanilamde. Dissolve completely (heat if necessary). Add 1 gm of N-1 Napthylethylenediamine Dihydrochloride and dissolve. Dilute to 2 litres. Add 1.0 ml Brij-35. Store in a dark cold place.

Stock Copper Solution.

Anhydrous Cupric Sulphate	$CuSO_4$	2.5 gm.
Distilled water	qs	1 litre.

Dissolve 2.5 gm of cupric sulphate in distilled water and dilute to one litre. Working copper solution was then prepared by diluting 6.25 ml of the stock solution to 2 liters with distilled water.

Stock Sodium Hydroxide	3N	
Sodium Hydroxide	NaOH	120 gm.
Distilled Water	qs	1 litre.

Dissolve 120 gm of sodium hydroxide in 750 ml of distilled water. Allow to cool and dilute to one litre. Working NaOH solution was then prepared by diluting 100 ml of the stock solution to 1 litre.

Stock Hydrazine Sulphate

Hydrazine Sulphate	$N_2H_4H_2SO_4$	54.00 gm
Distilled Water	qs	2 litre.

Dissolve 54.00 gm of hydrazine sulphate in 1800 ml of distilled water. Dilute to 2 litres. The solution is stable for six months. Working solution was then prepared by diluting 25 ml of stock solution to one litre. The solution, when stored in an amber bottle, had a useful

life of one month.

Standards.

Potassium Nitrate	KNO_3	0.7218 gm.
Distilled Water	qs	1 litre.

Dissolve 0.7218 gm of KNO_3 in distilled water and dilute to 1 litre. Add 2 ml of purified chloroform per litre and prepare serial dilutions for standards. A typical calibration curve for the analyses performed on September 15 1970 is shown in Figure C-7.

5. Ammonia.

Samples for free ammonia analysis in the range 0-10 mg/l were performed according to Technicon Auto Analyzer Industrial Method 18-69W. No dilution of samples was required for our reactor studies. A schematic of the auto-analyzer flow sheet is shown in Figure C-8. The reagents and standards utilized are itemized below.

Alkaline Phenol.

Similar to total Kjeldahl analysis.

Sodium Hypochlorite

As per total Kjeldahl analysis.

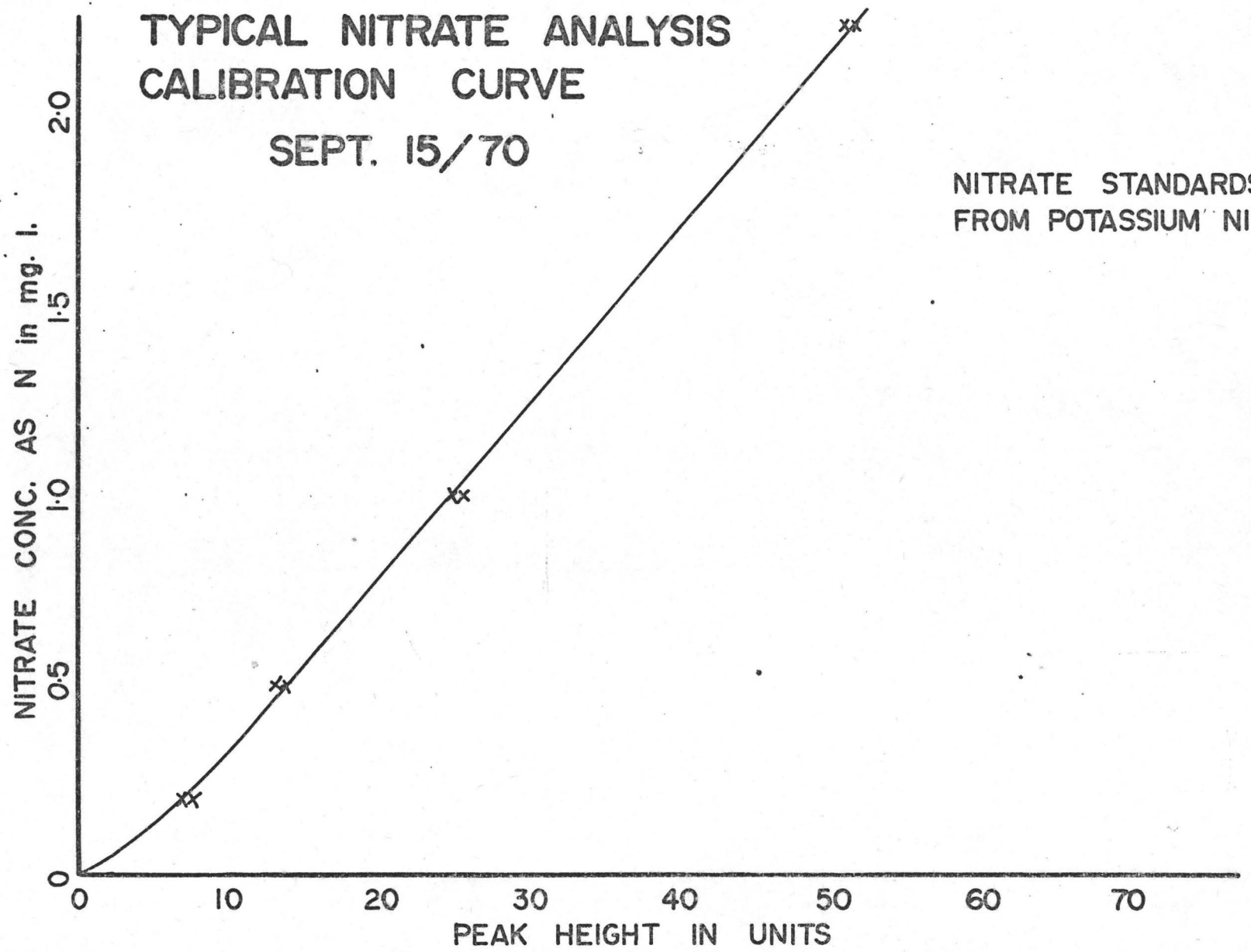
Potassium Sodium Tartrate.

Potassium Sodium Tartrate	$KNaC_4H_4O_6 \cdot 2H_2O$	200 gm.
Distilled Water	q.s.	1 litre.

Dissolve 200 gm of potassium sodium tartrate in 850 ml of distilled water and dilute to 1 litre.

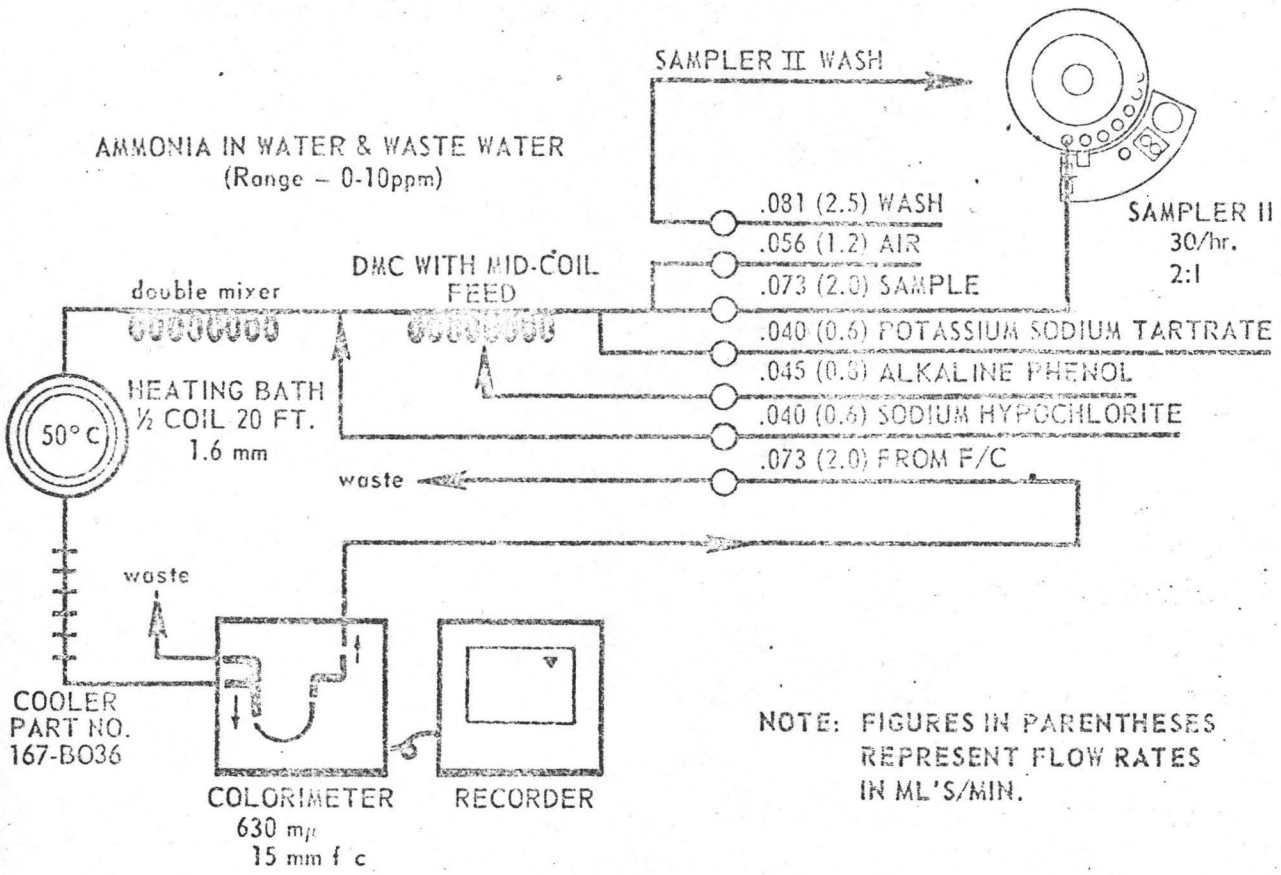
FIGURE NO. C-7

TYPICAL NITRATE ANALYSIS CALIBRATION CURVE SEPT. 15/70



NITRATE STANDARDS PREPARED FROM POTASSIUM NITRATE

FIGURE NO. C-8


SAMPLER II WASH
AMMONIA IN WATER & WASTE WATER
(Range – 0-10ppm)
.081 (2.5) WASH
.056 (1.2) AIR
.073 (2.0) SAMPLE
.040 (0.6) POTASSIUM SODIUM TARTRATE
.045 (0.8) ALKALINE PHENOL
.040 (0.6) SODIUM HYPOCHLORITE
.073 (2.0) FROM F/C
SAMPLER II
30/hr.
2:1
DMC WITH MID-COIL FEED
double mixer
HEATING BATH
½ COIL 20 FT.
1.6 mm
50° C
waste
waste
COOLER
PART NO.
167-B036
COLORIMETER
630 mμ
15 mm f c
RECORDER
NOTE: FIGURES IN PARENTHESES REPRESENT FLOW RATES IN ML'S/MIN.

Standards.

NH_4Cl as per total Kjeldahl analysis. A typical calibration curve for the analyses performed on June 19 1970 is shown in Figure C-9. The ammonia concentration usually checked closely with the filtered total Kjeldahl analysis.

6. Gas Analysis.

The chromatographic system used during the experiments (Fisher Gas Partitioner)is specifically designed for analysis of mixtures of specific groups of gases, e.g. hydrogen, oxygen, nitrogen, methane, carbon monoxide, and carbon dioxide. However, the system could easily be extended to mixtures of other gases by replacement of the standard columns by others which could separate the required gas mixtures.

The operation of the instrument is shown schematically in Figure C-10.

In effect, the apparatus contains two chromatographic systems which are inter-dependent, since the presence of one column influences the performance of the other. Each column has the unique properties that are necessary for the separation of several of the components of the sample mixture. In each case the properties of the column are carefully selected to give the optimum results with the gases it separates.

FIGURE NO. C-9

TYPICAL AMMONIA ANALYSIS
CALIBRATION CURVE JUNE 19/70

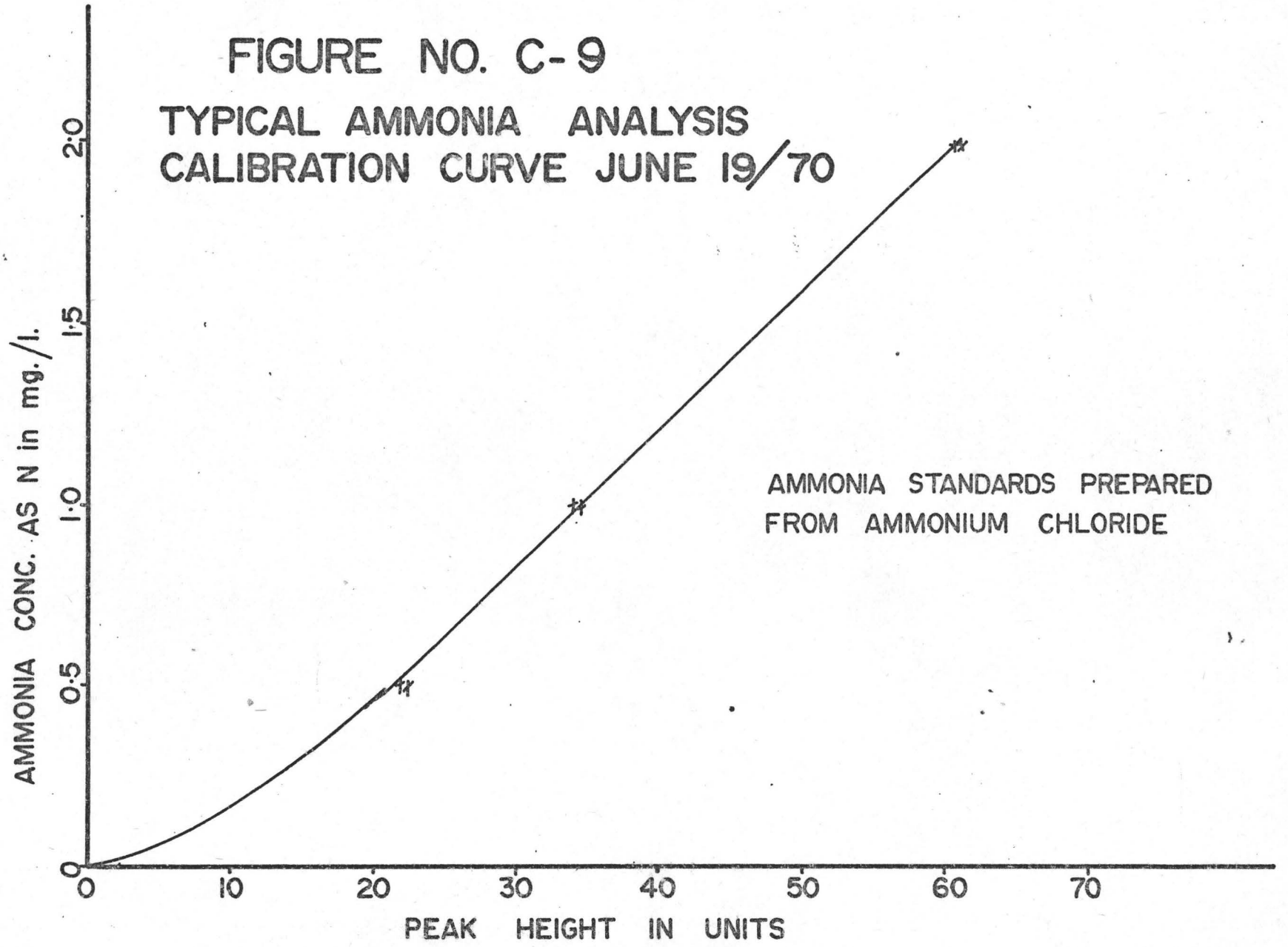
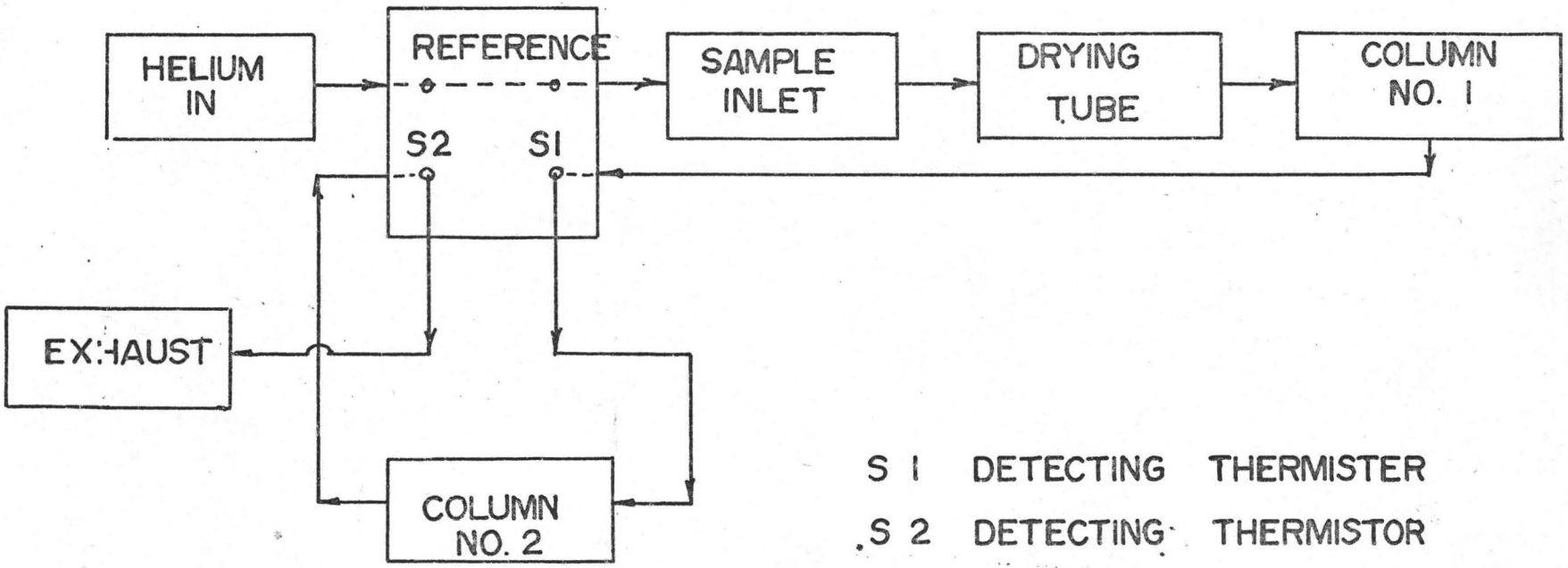


FIGURE NO. C-10

SCHEMATIC DIAGRAM OF FLOW THROUGH GAS PARTITIONER SHOWING TWO COLUMN DETECTOR SYSTEM

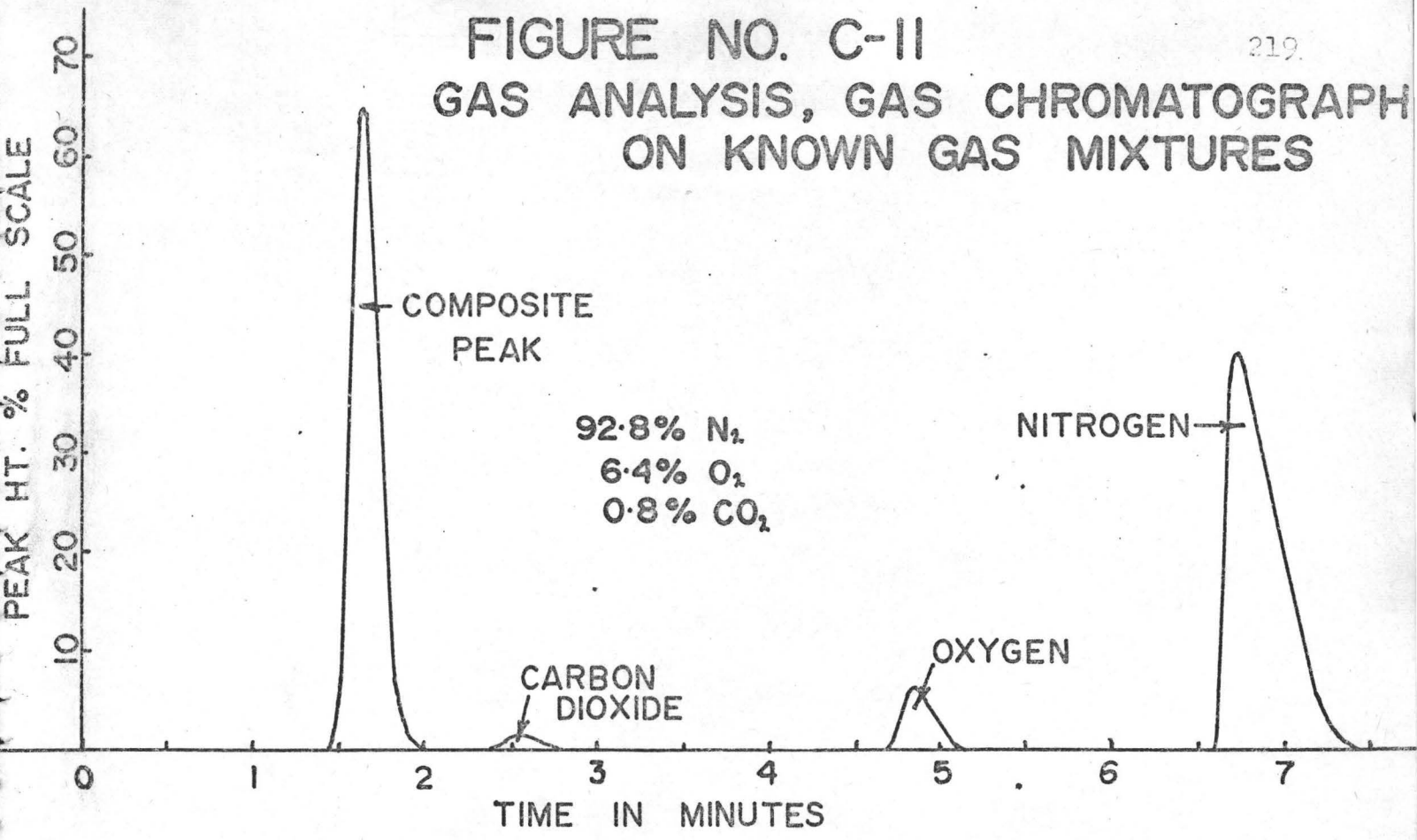


The primary requirement for the system to work is that at any given time only one component should be passing through one of the two detectors. This is accomplished by regulation of the carrier gas flow rate and the column length. A second requirement is that the component separated by Column 1 and recorded by Detector 1 should be absorbed by the second column. Thirdly, the compounds separated by Column 2 should not be separated or affected by Column 1. Consequently there will be only one peak for each component.

A column system consisting of 21" of silica gel in the column 1 position, followed by 13 feet of molecular sieve 13X, the silica gel column separates carbon dioxide from air mixtures while the moleculor sieve column separates nitrogen oxygen and nitrous oxide while absorbing carbon dioxide and water vapour. Using a helium carrier gas flow rate of 50 ml/min with a chromatograph sensitivity of 100% adequate separation of these gases could be achieved with a recorder speed of 1 inch per minute and a sensitivity of 100 millivolts full scale deflection as shown in Figure C-11. The columns were calibrated periodically by the injection of known percentage mixtures of gases. A linear relationship was obtained between area under the peaks versus percent sample concentration for the 0.5 ml samples as shown in Figure C-12 for nitrogen gas. Due to

FIGURE NO. C-11

GAS ANALYSIS, GAS CHROMATOGRAPH ON KNOWN GAS MIXTURES



0·5 ml. SAMPLE
HELIUM FLOW RATE 40 ml. min.
100 % SENSITIVITY

1·0 INCH MIN. RECORDER SPEED
100 MILLIVOLT SENSITIVITY

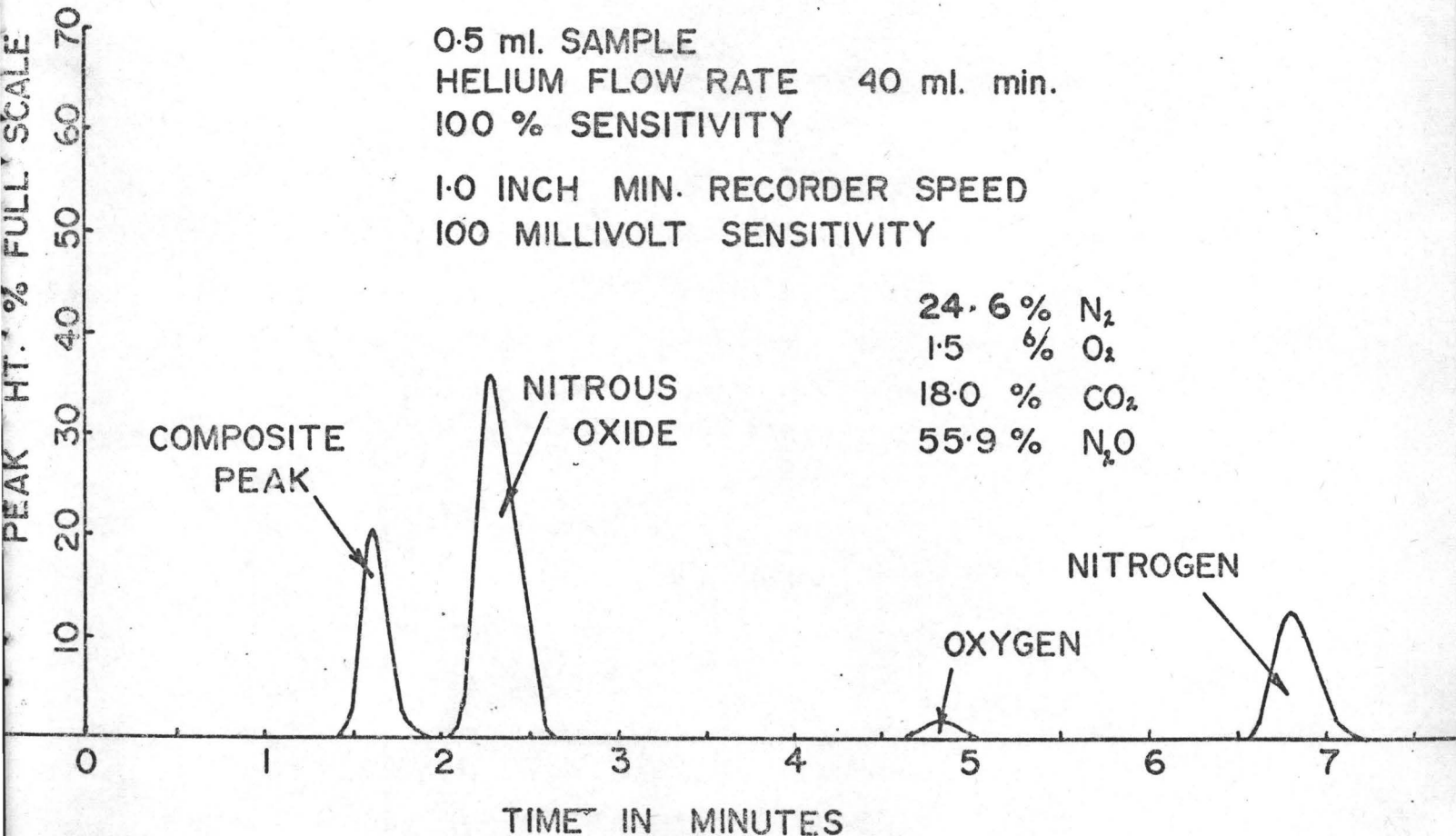
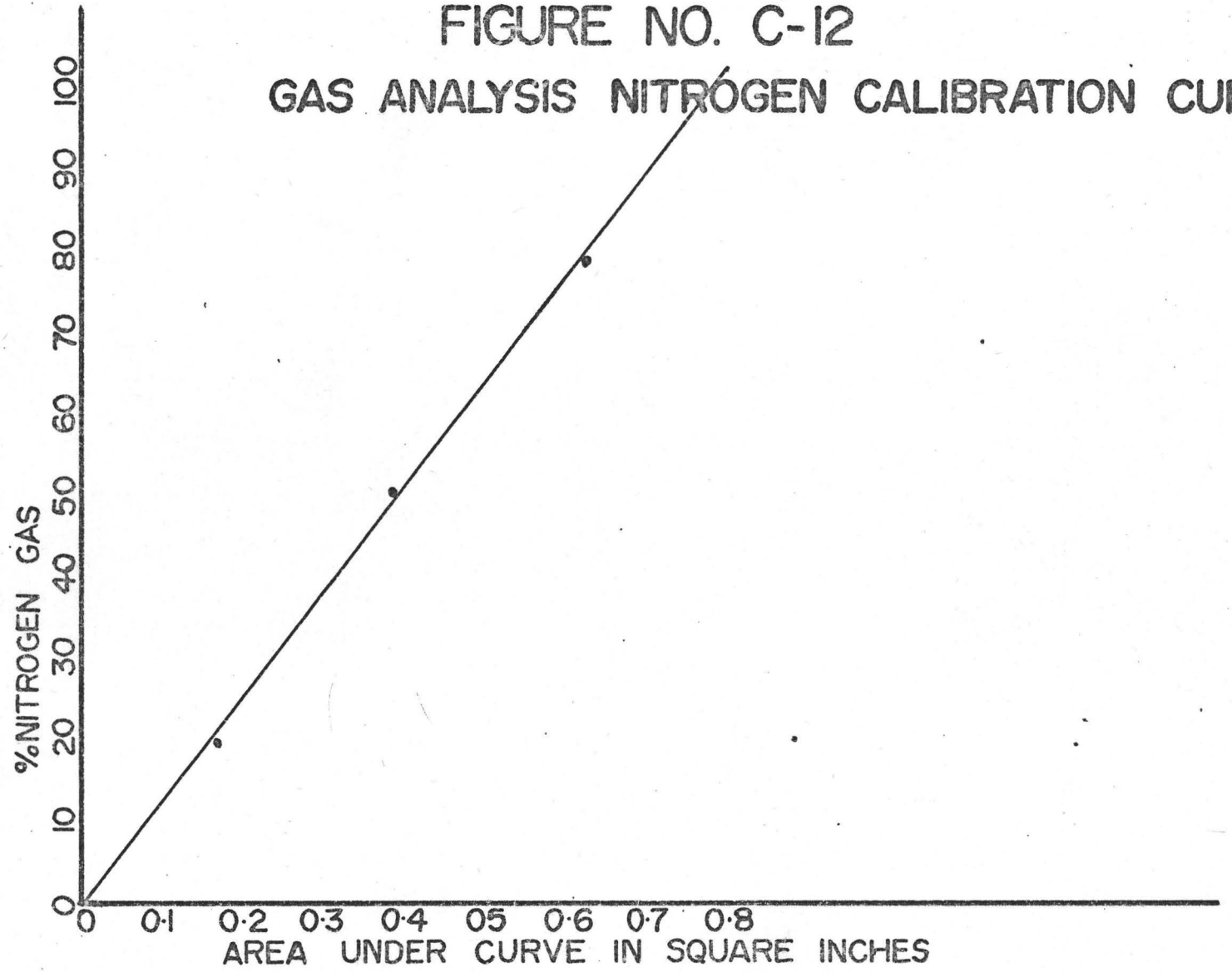


FIGURE NO. C-12

GAS ANALYSIS NITROGEN CALIBRATION CURVE



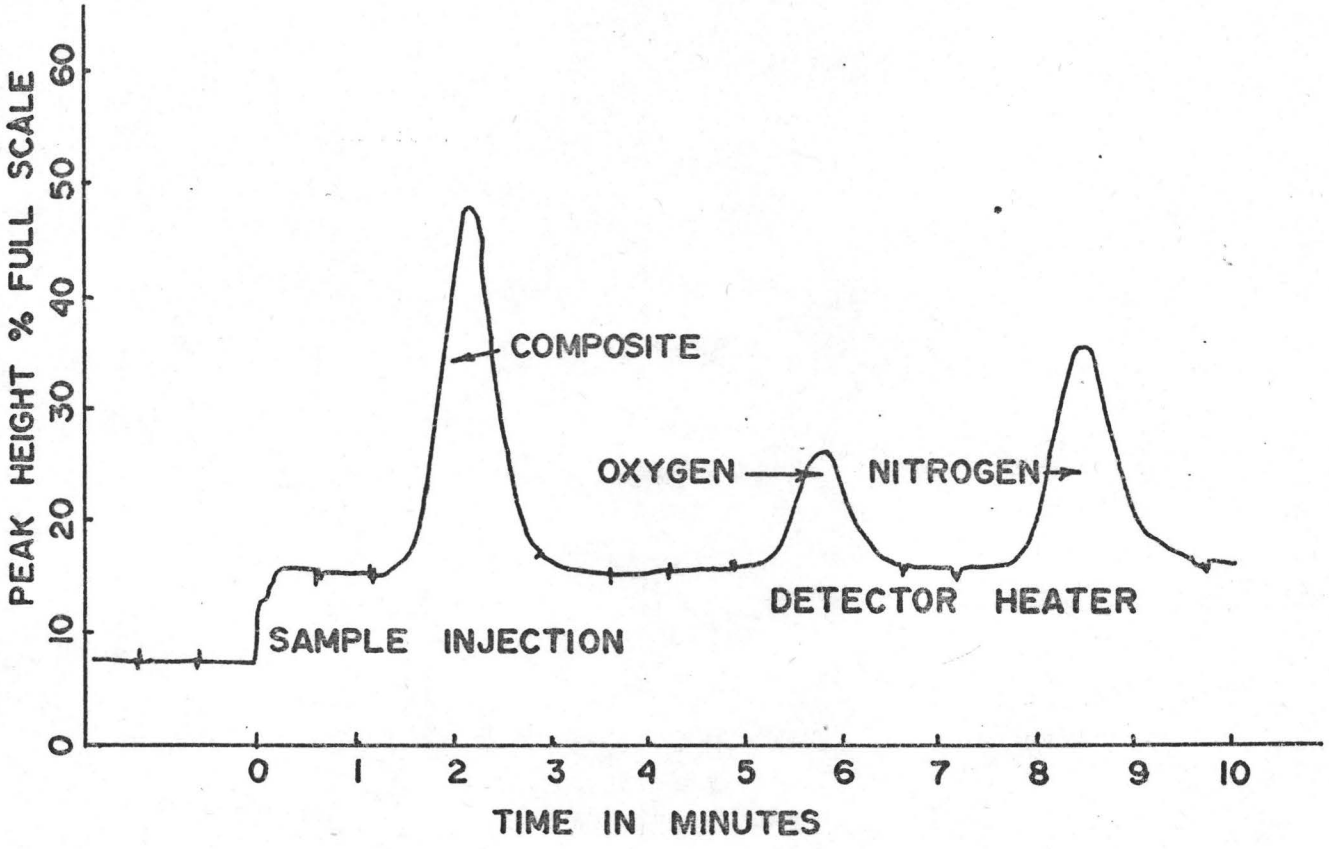
unavoidable absorbance of quantities of water vapour on the molecular sieve columns separation of the peaks decreased. A check was maintained on the effectiveness of the calibration curves by running an air sample through with each unknown gas concentration. As necessary, the molecular sieve columns were regenerated by heating the columns to 400°C in a muffle furnace for 24 hours while carrier gas was passed through them.

7. Dissolved Nitrogen Analysis - after Swinnerton et al.

The dissolved nitrogen concentration was determined by injecting a 0.5 ml liquid sample into a 40 mm long by 10 mm diameter glass chamber through which helium carrier gas was bubbled at the rate of 50 ml/min. The gases scrubbed from the liquid sample by the helium were then carried through a 3/8" diameter polyethylene tube filled with 14 to 20 mesh $CaSO_4$ which absorbed any water vapour carried along with the gases. The gases were then passed through the same chromatographic columns in the Fisher partitioner as were used for gas analysis. With the chromatograph sensitivity set at 100% and the recorder at 1 inch per minute and the 1 MV sensitivity separate peaks were produced for composite, oxygen, nitrogen, and carbon dioxide. As shown in Figure C-13, the peaks were sufficiently smooth to allow measurement of area under the curve. Comparison with a calibration curve developed from

FIGURE NO. C-13
DISSOLVED GAS ANALYSIS, WATER AT 27° C

SAMPLE SIZE 0·5 ml.
CHROMATOGRAPH SENSITIVITY 100 %.
RECORDER SENSITIVITY 1·0 mv.
CHART SPEED 2 min / inch



water saturated with air at various temperatures was used for determination of sample nitrogen concentration. A linear relationship existed between dissolved nitrogen concentration and area under the peaks. The calibration curve was checked frequently with water samples saturated with air at a known temperature.

A diagram of the flow pattern used for dissolved nitrogen analysis is shown in Figure C-14.

FIGURE NO. C-14

GAS FLOW DIAGRAM, DISSOLVED GAS ANALYSIS