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Nitrification Enhancement by Powdered Activated Carbon Addition In Activated Sludge

A dissertation submitted in partial satisfaction of the requirements for the Doctor of Philosophy in Engineering

by

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This is dedicated to my parents, family and B.

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List of Abbreviations

AI	Adsorbable, Inhibitory
ANI	Adsorbable, Non-inhibitory
Ben	Bentonite Reactor
DO	Dissolved Oxygen
GC	Glucose Activated Carbon Reactor
GNC	Glucose Non-Activated Carbon Reactor
HRT	Hydraulic Retention Time, days
ISA	Ionic Strength Adjuster
KN	Kjeldahl Nitrogen
MCRT	Mean Cell Retention Time, days
MLSS	Mixed Liquor Suspended Solids, mg/l
MLVSS	Mixed Liquor Volatile Suspended Solids, mg/l
NAI	Non-adsorbable, inhibitory
NANI	Non-adsorbable, non-inhibitory
RW	Refinery Wastewater
RC	Refinery Wastewater Fed PAC Reactor
RNC	Refinery Wastewater Fed Non-PAC Reactor
SOC	Soluble Organic Carbon
SCFH	Standard Cubic Feet per Hour

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ABSTRACT OF THE DISSERTATION

Nitrification Enhancement in Powdered
Activated Carbon-Activated Sludge

by

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Previous investigators provided evidence that the addition of powdered activated carbon (PAC) to nitrifying activated sludge (AS) can improve nitrification rates. Plausible but unsubstantiated mechanisms proposed to explain these observations include adsorption of compounds toxic to nitrifiers; enhanced growth of nitrifiers and/or concentration of trace nutrients on the carbon surface.

The major objective of this research is to further define the mechanism of nitrification enhancement in PAC-AS. Using refinery and synthetic wastewater feed, a series of acute and chronic experiments, as well as experiments with variable carbon dosages, was conducted to evaluate the relative importance of adsorption, suspended solids, and microbial acclimation on AS nitrification rates. The general procedure was to compare the effect of a "spiked" adsorbable/non-adsorbable, inhibitory compound on nitrification rates in AS and in AS supplemented

either with PAC or inert suspended solids (bentonite clay).

With "spiked" adsorbable inhibitors, the acute experiments demonstrated nitrification enhancement due to PAC addition in unacclimated sludge cultures. Statistically significant enhancement due to either PAC or bentonite addition was not evident in any experiment where a nonadsorbable inhibitor was added.

A chronic experiment gave evidence that the addition of PAC to AS can indirectly inhibit nitrification by virtue of desorption of a previously adsorbed inhibitor. In this same experiment, it was shown that an adequate dose of virgin PAC can dramatically arrest the effect of an adsorbable inhibitor and restore full nitrification capability.

Results from the variable PAC dose experiments suggested that there may be an optimal dose of PAC required to negate the effects of an adsorbable inhibitor. For adsorbable inhibitors, PAC addition resulted in virtual complete nitrification and enchancements of 75 to 100% over that of the controls (i.e., no PAC addition). No significant loss of nitrification capability was observed at PAC concentrations greater than 2-4000 mg/l PAC for all adsorbable inhibitors tested. For relatively non-adsorbable inhibitiors, enhancements of only 3 to 30% were observed at the highest PAC dosage evaluated.

The overall results provide strong evidence that the major mechanism of nitrification enhancement in PAC-AS is related to the carbon's ability to adsorb inhibitors.

INTRODUCTION

The modern day activated sludge process was originally conceived in 1914 by Arden and Lockett, who were the first to realize that the naturally occurring microbial process could be captured and controlled in a slurry type reactor. Heretofore biological wastewater treatment had been restricted to processes using microorganisms growing in fixed-films. At the time of their development understanding of process kinetics and stoichiometry was non-existent, and the mechanisms of treatment such as "adsorption on the concrete walls" were frequently proposed.

Presently the activated sludge process is usually the process of choice for treating domestic and industrial wastewaters, especially for larger applications. We now understand the process kinetics and stoichiometry much better than in the time of Arden and Lockett, but a understanding of its behavior still eludes us.

Over the past decade, several important developments have been made to enhance activated sludge process performance and energy conservation. One of the important enhancements for improved performance is the addition of powdered activated carbon. The motive for adding activated carbon was the notion that the carbon would remove biologically resistant compounds that were not degradable by the microorganisms, providing levels of treatment which were previously only obtainable with physicochemical treatment techniques.

Early work in the powdered activated carbon-activated sludge process is described in various patents of Derleth (1927) and Statham

(1938), while the modern patent was held by DuPont (Hutton and Robertaccio, 1975) until recently, and sold to Zimpro Corporation. Since the development of the activated sludge-powdered activated carbon process, a number of evaluations have been made of the process' efficacy on various types of wastewaters.

The success of PAC research is demonstrated by the current operation of a number of full scale PAC-activated sludge (PAC-AS) treatment plants in both the municipal and industrial sectors. Operational data from bench, pilot and full scale plants have provided evidence that PAC has the following benefits over conventional activated sludge systems:

- Improved organics removals
- Improved removal of EPA priority pollutants
- Improved sludge thickening/dewatering
- Reduced sludge bulking
- Increased color removal
- Suppressed aerator foaming
- Improved nitrification

The primary objective of early PAC-AS research was to determine the carbon's ability to remove biologically resistent organic compounds. Although some or all of the advantages of PAC addition have been routinely reported in a number of studies, there is a lack of fundamental understanding of the process mechanisms responsible for these benefits. This is especially true in the relatively few cases where improved nitrification was noted. To date, almost all PAC-AS studies have focused

upon the elucidation of the mechanism(s) involved in increased organic removals. An equally important benefit of PAC addition is nitrification enhancement and although a number of mechanisms have been proposed to explain this phenomena, no research has been conducted to substantiate any one mechanism. Mechanisms proposed to explain nitrification enhancement are adsorption of compounds toxic or inhibitory to nitrifiers; enhanced growth of nitrifiers on the surface of the carbon; and concentration of trace nutrients at the carbon surface, among others.

The major objective of this research is to further define the mechanism of PAC-AS nitrification enhancement. To differentiate among the proposed mechanisms an experimental program was devised with emphasis placed upon evaluation of the adsorption and surface growth theories. This was done because previous researchers have provided tentative support for these theories.

The experimental program, carried out in four phases, was devised to evaluate the relative influences of 1) adsorption, 2) the presence of suspended particles and 3) acclimation on activated sludge nitrification rates, subjected to a variety of compounds with known characteristics. The effect of sludge acclimation to an industrial wastewater feed is included in this study because the literature review suggested that acclimation ability may be an important variable in the ultimate degree of nitrification inhibition.

The bench scale study was conducted in the UCLA Water Quality Control Laboratory. In an effort to gain practical results, an arrangement was made to obtain wastewaters with a west coast refiner operating a

large, integrated oil refinery. The wastewater was periodically obtained (approximately every four weeks), and three 15 liter bench scale treatment plants (reactors) were assembled and operated using this wastewater. In addition, two bench scale plants were operated treating a synthetic glucose-based wastewater, in order to provide for a source of unacclimated sludge cultures for experimentation. Reactors were operated with and without carbon, using both types of wastewaters. Additionally a reactor treating refinery wastewater was operated with bentonite clay. Bentonite clay is a suspended solid which is known to have suitable surface chemistry for microbial attachment, but limited ability for adsorption of organics. A clay was chosen which had approximately the same mesh size as the powdered activated carbon.

An intensive survey of all know nitrification-inhibiting compounds and their carbon adsorption properties was made. From this survey a group of compounds were selected for evaluation. Selection was based upon a compound's adsorptive and nitrification inhibitory properties and the likelihood of finding it in refinery wastewaters.

The specific objective of the study was to evaluate the mechanism of nitrification enhancement. The procedure was to evaluate a series of compounds which were could be grouped as adsorbable-inhibitory (AI), nonadsorbable-inhibitory (NAI), nonadsorbable-noninhibitory (NANI), and adsorbable-noninhibitory (ANI). Compounds selected were aniline (AI), phenol (AI), cyanide (NAI), acrylonitrile (NANI) toluene (ANI) and ethanol (NAI). Most of these compounds are traditionally associated with refinery or petrochemical processes, and their presence in the west

coast refinery wastewater was confirmed with gas chromatography/mass spectrometry.

A series of experiments using both refinery and synthetic wastewaters was conducted to distinguish enchancement due to the adsorption of inhibitory compounds from preferential microbial attachment. The effect of a "spiked" adsorbable/non-adsorbable, inhibitory/non-inhibitory compound on nitrification rates was compared for control activated sludge units and those containing either PAC or bentonite. If higher nitrification rates are observed in PAC units compared to bentonite units when a spiked compound with both adsorbable and inhibitory characteristics is tested, the adsorption theory is supported. Conversely, if equal or higher nitrification rates are observed in the bentonite units when a non-adsorbable, inhibitory compound is used, the surface attachment theory is supported. The use of non-inhibitory compounds for testing served the major purpose of verifying experimental techniques since the ratio of nitrification rates should be close to unity in these cases.

The effects of selected compounds on nitrification rates in acclimatized and non-acclimatized sludges were also examined in parallel to the experiments discussed above. The overall experimental program was divided into four phases:

Phase I : Preliminary experiments

—— Three GC/MS analysis for acid/neutral/base extractable organics were performed on separate batches of refinery wastewaters to screen for potential nitrification inhibitors.

—— A heavy metal analysis was conducted on a single batch of refinery wastewater.

—— Isotherm studies were conducted on PAC and bentonite to determine the efficacy for its use in subsequent experiments.

Phase II: Batch experiments

—— A series of experiments was run to determine the short term effects of specific compounds on nitrification rates in PAC, bentonite, acclimated and unacclimated activated sludges. Nitrification rates were compared to that of the corresponding control (i.e., no spiked compound) in order to form a basis of comparison among different reactor types.

Phase III: Chronic experiments

—— It is generally agreed that short term acute tests do not give a good indication of how a given biological system will react under actual field conditions. As such, experiments were conducted to determine the long-term effects of a specific adsorbable inhibitor on nitrification rates with PAC and bentonite added to nitrifying activated sludge.

Phase IV: Carbon Dose Experiments

—— A series of experiments using nitification inhibitors of known adsorptive characteristics was performed in the presence of variable powdered activated carbon dosages.

LITERATURE REVIEW

NITRIFICATION

Biochemistry

The biological process of nitrification is carried out sequentially under aerobic conditions principally by two chemoautotrophic genera Nitrosomonas and Nitrobacter. These are responsible for the oxidation of ammonia to nitrite and nitrite to nitrate, respectively. Both organisms depend on membrane-bounded oxidative electron transport chains in which chemical energy is derived from their respective inorganic sources. These energy-yielding oxidations are coupled to cellular biosynthetic reactions which involve the fixation of carbon dioxide through mechanisms very similar to those of the Calvin cycle in photosynthetic organisms. The general stoichiometric reaction sequence occurring during nitrification can be written as follows (USEPA, 1975):

$$NH_4^+ + \frac{3}{2}\,O_2 \rightarrow NO_2^- + 2H^+ + H_2O + 58\text{-}84\ \text{Kcal} \qquad (1)$$

$$NO_2^- + \frac{1}{2}\,O_2 \rightarrow NO_3^- + 15.5 - 20.9\ \text{Kcal} \qquad (2)$$

Although it is well established that certain heterotrophic organisms are also capable of nitrification, it is generally considered that heterotrophs contribute insignificant quantities of nitrate in natural systems (Wallace and Nichols, 1969; Verstraete and Alexander, 1973). The basic metabolic mechanisms and intermediate biochemical pathways of nitrifiers has been reviewed previously by Wallace and Nichols (1969), Aleem (1970) and Painter (1970). The sections that follow will focus

upon the recent developments in the biochemistry of nitrification.

Ammonia Oxidation by Nitrosomonas

The oxidation of ammonia by Nitrosomonas involves a complex pathway which is not yet completely understood. Hofman and Lees (1953) were among the first investigators to obtain experimental evidence that the oxidation of ammonia to nitrite proceeds via hydroxylamine (NH_2OH) since it was observed that hydroxylamine accumulated during ammonia oxidation in the presence of hydrazine, an inhibitor of hydroxylamine oxidation. These findings were supported in later studies conducted by Engel and Alexander (1958) and Anderson (1965).

Lees (1954) speculated that the oxidation of ammonia to nitrite should theoretically occur in three steps since the conversion of ammonia to nitrate involves a six electron transfer causing the valence change of nitrogen from -3 to +3. Assuming a two electron transfer for each oxidation-reduction reaction, at least two intermediates with the oxidation states of -1 and +1 are expected in the reaction sequence. Aside from the initial reaction to form hydroxylamine, many intermediate products have been proposed, such as nitroxyl (NOH), nitric oxides (NO or N_2O) and hyponitrite (N_2O_2). Unfortunately, this other intermediate(s) has not yet been conclusively identified. The reason is probably due to its extreme chemical instability.

The fact that Nitrosomonas cells are incapable of oxidizing ammonia under anaerobic conditions in the presence or absence of an electron acceptor led Rees and Nason (1966) to suspect that molecular

oxygen is essential at least in the formation of hydroxylamine. Indirect evidence for this suspicion was provided through ^{18}O tracer studies conducted by Verstraete and Alexander (1972) which demonstrated that the oxidation of ammonia to hydroxylamine through heterotrophic nitrification involved the incorporation of molecular oxygen. More recently, Hollocher, et al. (1981) presented definitive evidence through isotopic methods that ammonia to hydroxylamine oxidation proceeds by way of a direct insertion (mono-oxygenase) reaction involving O_2. They observed that of the two atoms in O_2, one is inserted in NH_4^+ to form NH_2OH while the other is presumably reduced to water. Furthermore, they theorized that since the only source of reducing equivalents for the reduction of that oxygen atom is NH_2OH, the oxidation of NH_4^+ and NH_2OH must be functionally linked. This theory is not novel as it has been proposed by earlier investigators (Hooper, et al., 1972; Hooper and Terry, 1977) and was recently further advanced by Suzuki, et al.(1981).

The intermediate(s) involved in hydroxylamine oxidation to nitrite remains highly speculative at this time. Work by Nicholas and Jones (1960) with Nitrosomonas cell-free extracts which produced nitrite from hydroxylamine aerobically in the presence of mammalian cytochrome c, provided direct evidence of the involvement of an electron transport chain. A subsequent investigation by Anderson (1964) revealed that under anaerobic conditions in the presence of Nitrosomonas cell extracts and a hydrogen acceptor (methylene blue), nitrous and nitric oxides were produced in the amounts equivalent to the hydroxylamine added. However, under aerobic conditions in the presence of methylene blue or mammalian cytochrome c, conversion of hydroxylamine to nitrite was observed.

Moreover, in the presence of cytochrome c, hydroxylamine and cyanide under aerobic conditions, nitrite production was inhibited. This inhibition was removed when methylene blue replaced cytochrome c in the reaction media. These results led Anderson to suggest that the metabolism of NH_2OH to nitrite is a two step process involving in formation of nitroxyl and its subsequent nonenzymatic decomposition to nitrous oxide. Nitrous oxide was then converted to nitrite by an enzyme system requiring oxygen.

Almost concurrent to Anderson's findings, Aleem and Lees (1963) demonstrated that NH_2OH oxidation by Nitrosomonas was mediated by cytochrome b, c, and a-type components. They suggested that NH_2OH oxidation is activated by the enzyme hydroxylamine-cytochrome c reductase (now referred to as hydroxylamine oxidoreductase) which they had partially purified and studied. This enzyme was found to be sensitive to various metal-binding agents which led the investigators to conclude that the oxidation step involves the participation of electron transport carriers and is mediated by a cytochrome enzyme system requiring a metal. It was shown by Nicholas (1962) using paramagnetic resonance on Nitrosomonas particles, that the essential metal involved in the oxidase system is copper.

Since then, the enzyme complex hydroxylamine oxidoreductase has been extensively purified and studied further (Ritchie and Nicholas, 1974; Hooper, et al., 1978; Terry and Hooper, 1981) The enzyme has been shown to be able to use either cytochrome c or phenazine methosulfate as an electron acceptor and carries out the removal of at least two elec-

trons from hydroxylamine:

$$NH_2OH \rightarrow (NOH) + 2e^- + 2H^+ \quad (3)$$

Although intact Nitrosomonas cells can oxidize NH_2OH to NO_2^- stoichiometrically, the nitrite yielded by the enzyme system is less than 50% even aerobically, the remainder being nitrate (NO_3^-), nitric oxide(NO) and nitrous oxide(N_2O) (Hooper, et al., 1979). These results are in contrast to the nitrite yields of 80 to 90% obtained by Aleem and Lees (1963) with a 85-fold purified enzyme.

A number of cytochromes have been purified and characterized from Nitrosomonas europeae recently. These include: cytochrome c(CO)-550, c(CO)-552 (Miller and Wood, 1982, 1983); c-552, c-554 (Yamanaka and Shinra, 1974), a1 (Erickson and Hooper, 1972b);p-460 (Erickson and Hooper, 1972a) and hydroxylamine oxidoreductase. Current research (Suzuki, et al., 1981) hypothesizes that cytochrome c-552 is reduced by NH_2OH in the presence of hydroxylamine oxidoreductase and cytochrome c-554 and the reduced c-552 is oxidized by cytochrome a1 in air. Thus, the electron flow from NH_2OH is suggested to proceed as follows:

$$NH_2OH + \text{Hydroxylamine reductase} \rightarrow C\text{-}554 \rightarrow C\text{-}552 \rightarrow a1 \rightarrow O_2$$

The more recent purification of CO-binding cytochromes (Miller and Wood , 1983) which are frequently associated with the function of a terminal oxidase, may shed further light into the mechanism of hydroxylamine oxidation.

Suzuki, et al. (1981) advanced a hypothetical scheme of ammonia oxidation based on the immediate formation of peroxonitrite (ONO_2^-). According to Suzuki, peroxonitrite is an attractive intermediate in that its presence could explain the formation of nitrate from hydroxylamine nonenzymatically. It was shown by Hughes and Nichlin (1970) that peroxonitrite, when protonated, is unstable and isomerizes to nitrate:

$$ONOO^- + H^+ \rightarrow ONOOH \rightarrow HNO_3^-$$

Moreover, studies of NH_2OH oxidation in alkaline solutions demonstrated the production of nitroxyl ions (NO^-) which forms peroxonitrite in combination with O_2. Copper ions can catalyze the reaction of peroxonitrite with NH_2OH to nitrite. If ammonia can be hydroxylated by peroxonitrite, then Suzuki suggests the following reactions for ammonia oxidation:

$$NH_3 + ONOOH \rightarrow NH_2OH + HNO_2 \qquad (4)$$

$$NH_2OH \rightarrow NOH + 2e^- + 2H^+ \qquad (5)$$

$$NOH + O_2 \rightarrow ONOOH \qquad (6)$$

Totaling:

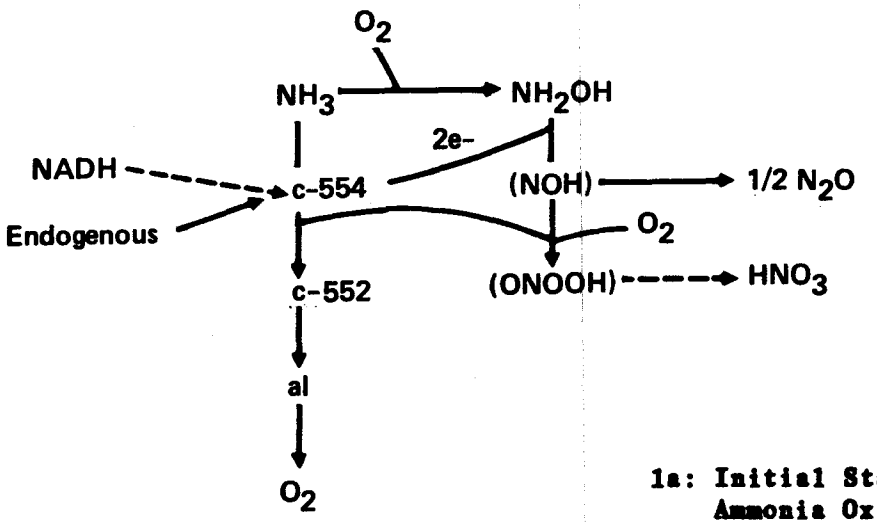
$$NH_3 + O_2 \rightarrow HNO_2 + 2H^+ + 2e^- \qquad (7)$$

where reaction (4) and (6) are tightly coupled. This is in accordance

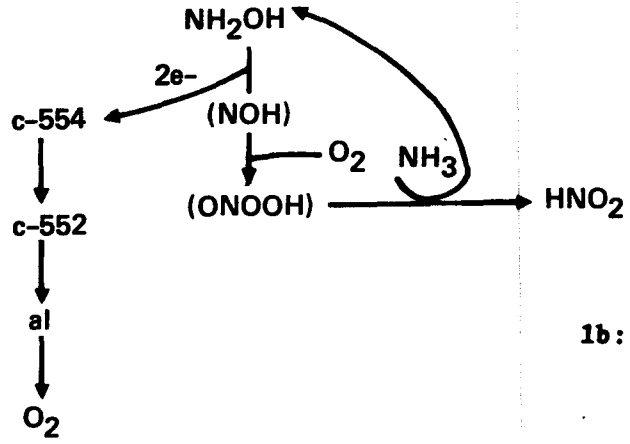
to earlier theories proposing that ammonia and hydroxylamine oxidations are functionally linked. The hypothetical scheme for ammonia oxidation is illustrated in Figure 1. In Figure 1a., ammonia is initially hydroxylated by O_2 plus electrons derived from endogenous metabolism or added NADH. Once the hydroxylation is initiated, electrons from NH_2OH oxidation become available for further hydroxylation of ammonia. At steady state (Figure 1b.), the oxidation of nitroxyl (NOH) is coupled to ammonia hydroxylation with peroxonitrite as the possible intermediate. The only electrons going through the electron transport chain system to molecular oxygen for possible energy generation are the two electrons derived from hydroxylamine.

Oxidation of Nitrite to Nitrate by Nitrobacter

The oxidation pathway of nitrite to nitrate by the Nitrobacter species is relatively simple compared to that of Nitrosomonas and is better understood. The oxidative mechanism proceeds via a series of enzymatic steps involving the sequential transfer of electrons from nitrite to molecular oxygen whose role is strictly that of a terminal electron acceptor (Lees and Simpson, 1957). It was also demonstrated by these same investigators that the oxidation involves the cytochrome-electron transport enzyme nitrite oxidase and that the transfer of electrons is mediated by c and a1-type cytochromes. Later, Aleem, et al. (1965), working with ^{18}O and $H_2^{18}O$ isotopes, discovered that the oxygen atom in nitrate was generated from water and not from molecular oxygen. These important findings led to the current understanding of nitrite oxidation. The oxidation of nitrite to nitrate by Nitrobacter can be



1a: Initial State of Ammonia Oxidation



1b: Steady State

Figure 1: Proposed Oxidation Pathway for Ammonia to Nitrate (After Suzuki, 1980)

represented by the following scheme (Aleem, 1970) and redox equations (Sundermeyer and Bock, 1981):

$$NO_2^- \rightarrow \text{cyt. c} \rightarrow \text{cyt. A1} \rightarrow O_2$$

Nitrite Oxidase — Cytochrome Oxidase

$$NO_2^- + H_2O + 2 \text{ cyt. a1(+3)} \rightarrow NO_3^- + 2H^+ + 2 \text{ cyt. a1(+2)} \quad (8)$$

$$2H^+ + 2 \text{ cyt. a1(+2)} + 0.5\ O_2 \rightarrow H_2O + 2 \text{ cyt. a1(+3)} \quad (9)$$

Totaling:

$$NO_2^- + 0.5\ O_2 \rightarrow NO_3^-$$

In equation 8, oxygen is not involved, nitrite is oxidized to nitrate by water, protons and electrons released, and the electrons are transferred to cytochrome a1, an essential component of the nitrite-oxidizing system, and then subsequently to oxygen. Equation 8 is the energy-yielding step, which is coupled to the electrotransport phosphorylation. No intermediates of nitrite oxidation have ever been found (Painter, 1977).

Energetics and Energy Assimilation

The standard free energy changes for various nitrogen reactions have been calculated by Aleem (1970) and relevant reactions are presented here in Table 1. The initial oxidation of ammonia to hydroxylamine is slightly endergonic (see Table 1) and it has been suggested by Anderson (1965) that some "energy-linked" activation of ammonia prior to its oxidation is necessary. This contention has yet to be substantiated by experimental evidence. It is well established however, that the subsequent oxidation of hydroxylamine to nitrate (ΔG^{o} = -68.89 Kcal/mole-N), which is coupled to the electron transport chain, is the major energy yielding source for Nitrosomonas.

As with most autotrophic bacteria, Nitrosomonas must reduce pyridine nucleotides (i.e., NADH) because the latter participates in the reduction of carbon dioxide in biosynthetic reactions. Inspection of the free energy changes in Table 1 shows that the direct reduction of NAD+ by either ammonia or hydroxylamine is highly unfavorable from a thermodynamic point of view. From this, it would appear that the reduction of NAD+ is energy dependent and would more likely involve hydroxylamine than ammonia (ΔG^{o} =+35.6 vs. ΔG^{o} =+56.3 Kcal/mole for ammonia). The experimental evidence for the energy-dependent reduction of NAD+ by hydroxylamine was provided by Aleem (1966), who showed that the required energy could be supplied through the oxidation of hydroxylamine or by exogenously supplied ATP. According to current theories regarding autotrophic respiration, it appears that chemoautotrophs use a process called reversed electron transport (Pelczar, et al., 1977). Electrons

Table 1: Standard Free Energies of Reactions

Reaction	ΔG^{o} (Kcal/mole-N)	E(volts)
$NH_4^+ + 1/2\ O_2 \longrightarrow NH_2OH + H^+$	+3.85	+0.899
$NH_2OH + 1/2\ O_2 \longrightarrow NO_2^- + H_2O + H^+$	-68.89	+0.066
$NH_4^+ + 3/2\ O_2 \longrightarrow NO_2^- + H_2O + 2\ H^+$	-65.04	+0.344
$NO_2^- + 1/2\ O_2 \longrightarrow NO_3^-$	-18.18	+0.420
$NH_4^+ + NAD^+ + H_2O \longrightarrow NH_2OH + NADH + 2H^+$	+56.3	---
$NH_2OH + 2\ NAD^+ + H_2O \longrightarrow NO_2 + 2\ NADH + 3\ H^+$	+35.6	---

normally flow from NADH to oxygen through the electron transport chain, thereby generating ATP. In reversed electron transport the opposite occurs in that part of the ATP generated by the cell must be utilized to form NADH for use in the assimilation of carbon dioxide via the Calvin cycle. It is still unclear whether reverse electron transport involves the same, a separate or a branched electron transport pathway in Nitrosomonas.

Aleem (1966) deduced and concluded from experimental observations that reverse electron reaction was approximately 40% efficient. Although this is a relatively high energy efficiency for biological systems, this requirement coupled with the fact that the Calvin cycle requires 9 moles of ATP and 6 moles of $NADH_2$ (to be provided through the oxidation of ammonia or nitrite) to produce 1 mole of glyceraldehyde-3-phosphate (Stanier, et al., 1976) clearly demonstrates that the reductive process requires a great deal of energy. The high energy requirements for nitrifiers undoubtly accounts for their relatively low cellular yields as compared to those of heterotrophic organisms. Table 1 also shows that the oxidation of nitrite to nitrate nets approximately 18.2 Kcal/mole-N. Thus, Nitrosomonas obtains more energy per mole of nitrogen oxidized than does Nitrobacter. This fact is reflected in their relative cell yields (i.e., 0.06 for Nitrosomonas and 0.02 for Nitrobacter; Painter, 1977). Cell yield is defined as weight of cells formed per mole of N oxidized.

The mechanism of energy assimilation in Nitrobacter is better understood than that in Nitrosomonas. Kiesow (1972) suggested that the

interrelationship between the assimilation of energy from nitrite and the generation of reducing power in Nitrobacter is as represented in Figure 2. Figure 2 illustrates the mechanism of reversed electron flow in Nitrobacter. The reduction of NAD+ by nitrite is not possible directly because the NO_3^-/NO_2^- redox system has an Eo of +0.42 (pH=7) which is higher than that of the NAD+/NADH+H+ system (Eo=-0.32, pH=7). To circumvent this thermodynamically "uphill" difficulty, electrons flow from NAD with the consumption of ATP. According to Kiesow, energy released from nitrite oxidation can be used for two purposes depending on need. If the generation of ATP is required, it is accomplished through the lower electron transport system in Figure 2 (cytochrome 605, cytochrome oxidase and oxygen) with the production of H_2O. If carbon reduction is required, the upper part of the mechanism of Figure 2 is employed, resulting in the reduction of NAD to NADH. In both cases, nitrite is oxidized to nitrate.

Microbiology of Nitrification

General Characteristics of Nitrifiers

Watson (1974) has listed all known genera of autotrophic bacteria associated with naturally occurring nitrification. Of the four genera of ammonia oxidizers given, only Nitrosomonas (i.e., N. europeae) has been linked to nitrification in sewage. Similarly, only Nitrobacter, of the three genera reported to be nitrite oxidizers, (i.e., N. agilis) have been isolated from wastewaters (Fliermans, et al., 1974). These highly specialized physiological groups of bacteria are gram negative

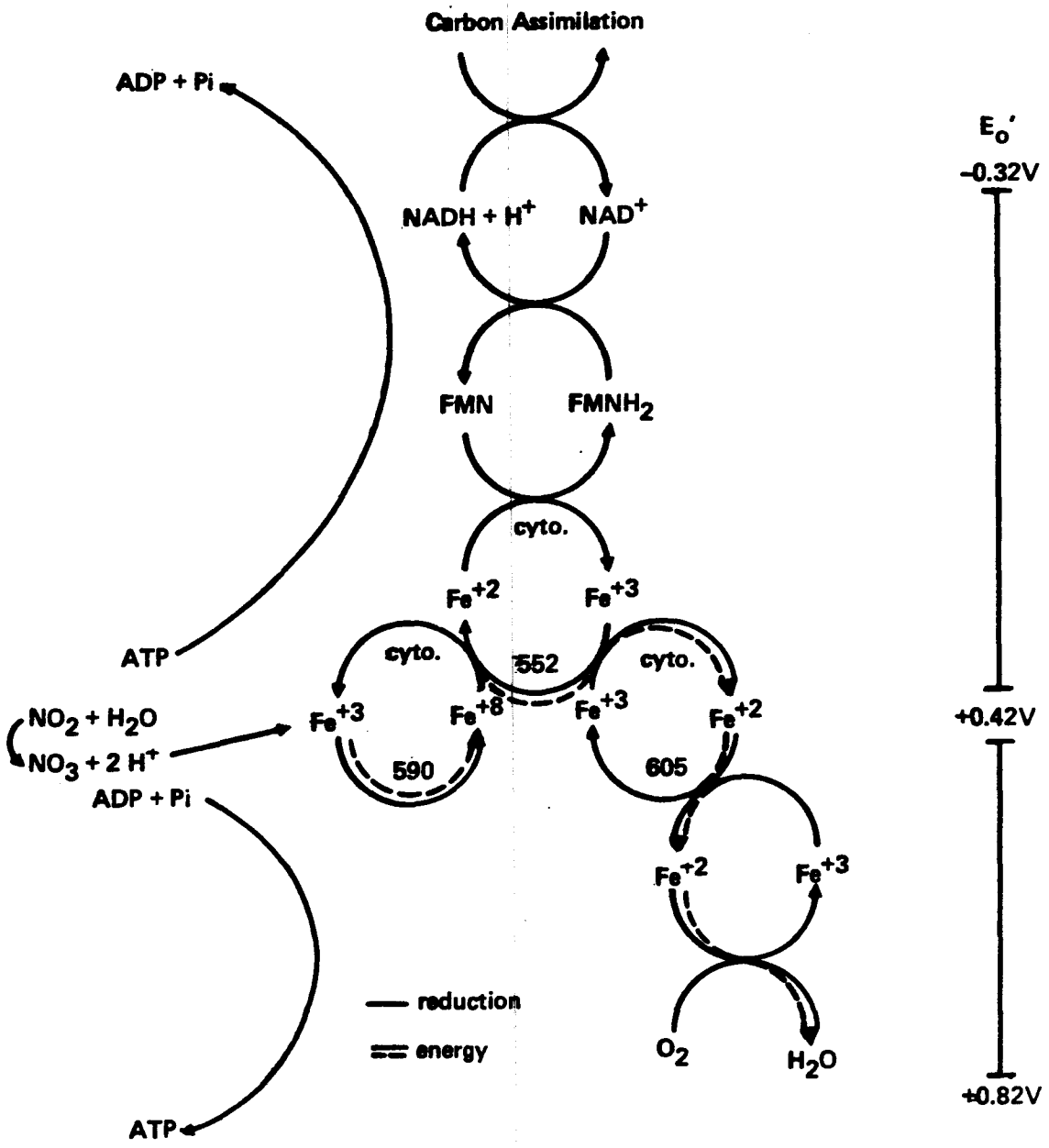


Figure 2: Proposed Mechanism of Energy Assimilation and Generation of Reducing Power in Nitrobacter (After Kiesow, 1972)

and strict aerobes with respect to their ability to nitrify. Other morphological and physiological characteristics of nitrifiers are presented in Table 2 (Stanier, et al., 1976).

Certain strains of Nitrobacter have been shown to assimilate organic compounds such as acetate (Delwiche and Finstein, 1965) and formate (Van Gool and Laudelout, 1966) as their sole energy and carbon source, respectively; however, these strains grew much more slowly than with nitrite and CO_2. The basis of obligate autotrophy in Nitrosomonas and most species of Nitrobacter has been attributed to their lack of certain key enzymes (i.e., α-ketoglutaric dehydrogenase) of the tricarboxylic acid cycle (Hooper, 1969); although it was also proposed (Pan and Umbrett, 1972) that growth of nitrifiers on organic compounds is prevented by the formation of toxic organic products (i.e. pyruvic acid).

Nutritional Growth Requirements

Aside from carbon dioxide, ammonia or nitrite, a minimal amount of dissolved oxygen and micronutrients are obligate requirements for nitrifier growth. The requirement of dissolved oxygen will be discussed in detail in a later section. Up until 1955, it was generally considered that particulate materials such as calcium carbonate, which was utilized as a buffer in early culture experiments, were necessary for growth. It was postulated that the solid surface in the growth media provided obligate surface sites to which nitrifying organisms adsorbed and multiplied. Although this "obligate surface" theory was later disproved by

Table 2: The Genera of Nitrifying Bacteria
(After Stanier, et. al., 1976)

Genus	Cell Form	Flagella	Membrane Intrustions	DNA Composition (Moles % G+C)	Obligate Autotroph
Nitrosomonas	Rod	Subpolar	Lamellar	50-51	+
Nitrospira	Tight Spiral	Peritrichous	None	54	+
Nitrosococcus	Sphere	Peritrichous	Lamellar	5--51	+
Nitrosolobus	Irregular, Lobed	Peritrichous	Vesicular	54-55	+
Nitrobacter	Rod, often pear shape	*	Lamellar	60-62	+ or -
Nitrospina	Long, slender rod	*	None	58	+
Nitrococcus	Sphere	Polar	Tubular	61	+

workers who successfully grew Nitrobacter (Goldberg and Gainey, 1955) and Nitrosomonas (Engel and Alexander, 1958) in clear media, the role of suspended solids in the nitrification process remains unclear. A stimulatory effect of suspended particles on nitrification rates has been reported by a number of investigators in studies with surface waters (Seppanen, 1970; 1972; Kholdebarin and Oertli, 1977) and soils (Lees and Quastel, 1946a). Kholdebarin and Oertli (1977) maintain that although nitrifiers can function very well in the absence of suspended material, their activities can be enhanced as the amount of particulates increases in solution. Their results indicated that particle sizes of 1 to 3 micrometers were most beneficial in enhancing nitrification rates. They suggested that the enchancement mechanism involves the particle's ability to provide a physical support medium for the proliferation of nitrifiers. This is in contrast to Lees and Quastel's theory of substrate (ammonium ion) concentration at the surface of particles. Conflicting evidence was presented by Aleem and Alexander (1960) who reported no detectable effects on nitrification rates due to suspended particles. Goldberg and Gainey (1955) asserted that while suspended solids were not essential for nitrifier growth, attachment to particles will occur if suspended solids are present.

The ability to cultivate nitrifiers in clear media accelerated research efforts by other investigators since prior metabolic and mineral nutritional studies on nitrifiers were greatly hindered by the presence of large quantities of insoluble ingredients in the culture medium. Consequently, a large amount of information on the nutritional requirements of nitrifiers was acquired in the subsequent years after

the clear growth medium had been defined. Sharma and Ahlert (1977), in a comprehensive review on nitrification, have summarized much of of this work. This summary along with other results are presented in Table 3. The results in Table 3 indicate that phosphate, magnesium, iron and copper (0.03 mg/1 for Nitrosomonas; Painter, 1977) are absolute growth requirements for both Nitrosomonas and Nitrobacter. In addition, sodium is probably required for Nitrosomonas (Loveless and Painter, 1968) while calcium has been shown to be required by Nitrobacter (Lees, 1954). Painter (1977) contented that the optimal phosphate concentration (310 mg/1) reported by Van Droogenbroek and Laudelout was excessively high and perhaps could be explained by carryover of the element in the inocula and/or the effect of phosphate on the pH of the medium.

Kinetics of Nitrification

Monod Kinetics

The most popular model used to describe substrate limiting nitrifier growth is that of the empirical expression proposed by Monod(1949):

$$\mu = \frac{\mu_{max} S}{K_S + S} \qquad (10)$$

where μ = specific growth rate, time^{-1}

μ_{max} = maximum specific growth rate, time^{-1}

S = limiting substrate concentration, mass/volume

K_S = saturation constant, numerically equal to the

Table 3: Substances Required or Stimulatory for Nitrification
(After Sharma and Alhert, 1977)

Substance	Concentration	Effect*	Reference
Phosphate	-	Reqd for Ns G & Nb G	Lees (1955)
	310 as P	Reqd for Ns G & Nb G	Van Droogenbroeck & Laudelout(1967)
	5 as P	Reqd for Nb G	Aleem (1959, cited in Painter,1970)
Magnesium	-	Reqd for Ns G & Nb G	Lees (1955)
	5	Reqd for Nb G	Aleem (1959, cited in Painter, 1970)
	10.5-50.5 (as $MgSO_4$-$7H_2O$)	No effect on Ns A	Skinner & Walker (1961)
	12.5-50	Ns A+	Loveless & Painter (1968)
	50-100	Slight Ns A-	
Molybdenum	-	Nb A+	Aleem (1959, cited in Painter, 1970)
	10^{-9}M(0.0001)	11-fold increase in Nb A and G	Finstein & Delwiche (1965)
	10^{-2}M(1000)	Slight Nb A,G-	
Iron	-	Reqd for Ns G & Nb G	Lees (1955)
	0.5-0.6	Ns G+	Skinner & Walker (1961)
	7	Reqd for Nb G	Aleem (1959, cited in Painter,1970)
Calcium	-	Reqd for Nb G	Lees (1955)
	0.5-10	No effect by itself on Ns A; + in presence of 5 mg/l EDTA	Loveless & Painter (1968)
	10.5-50.5 (as $CaCl_2$-$2H_2O$)	No effect on Ns A	Skinner & Walker (1961)
Copper	-	Reqd for NbG	Lees (1955)
	0.0.06	Ns A+; Added Ns A+ along with 5 mg/l EDTA	Loveless & Painter (1968)
	0.1	Slight Ns A+; With higher concentration Ns A-	Tomilinson et al (1966)
	0.1-0.5	Increasing Ns A	Skinner & Walker (1961)

(continue on next page)

Table 3 Cont: Substances Required or Stimulatory for Nitrification
(After Sharma and Alhert, 1977)

Substance	Concentration	Effect+	Reference
Sodium	0.6-1.5	Ns A+; Ns G-	Loveless & Painter
	1.5-7.0	Ns A-; Ns G+	(1968)
Marine Salts	-	Reqd by some estuarine or littoral cultures of ammonia oxidizers	Finstein & Bitzky (1972)
Vitamins			
A-Palmitate	50,000 USP ml^{-1}	Ns A+; Nb G+	Pan (1971)
Pantothenic Acid	0.05 mg/ml	Nb G+	
	0.0025 µg/ml	Nb A+	Gundersen (1955)
Nicotinic Acid	0.05 mg/ml	Nb G+	Pan (1971)
Ascorbic Acid	0.05 mg/ml	Nb G+	
Biotin	0-150 mµg	2-4 fold Nb A+; 100-1000-fold Nb G+	Krulwich & Funk (1965)
	2	Slight Ns A,G+	Clark & Schmidt(1967)
Adenine Sulfate	0.05 mg/ml	Nb G+	Pan (1971)
Sodium Glutamate	1720 mg/ml	Ns G+; Nb G+	
Yeast Extract	2 mg/ml	Nb G+	
L-Serine	4 µg/ml	Ns A+; Ns G+	Clark & Schmidt (1967)
	1050 mg/ml	Nb G+	Pan (1971)
L-Glutamine	4 µ/ml	Ns G+; Ns A+	Clark & Schmidt (1967)
	1450 mg/ml	Nb G+	Pan (1971)
L-Glutamic Acid	4 µ/ml	Ns G+; Ns A+	Clark & Schmidt (1967)
L-Aspartic Acid	4 µ/ml	Ns G+; Ns A+	
Ash of corn steep liquor		Ns G+	Gundersen (1958)
Glucose, p-amino benzoic acid	2-5	Ns A+; Nb A+; impure, mixed culture	Cooper & Catchpole (1973, cited in Painter, 1977, and in Stafford, 1974)
Zinc	1.0	Nb G+	Aleem (1959, cited in Painter, 1970)

* All results are for pure cultures unless indicated otherwise. In mg/l unless specified otherwise. Ns = Nitrosomonas; Nb = Nitrobacter; G+ = Growth; A = Activity; + = Simulation; - = Inhibition, e.g., Ns A+= stimulation of Nitrosomonas activity.

lowest concentration of substrate at which

$\mu = 1/2\mu_{max}$, mass/volume

The Monod expression provides for the continuous transition between first and zero order kinetics based on substrate concentration. Inspection of equation 10 reveals that if K_S is much greater with respect to S, the expression may be approximated as a first order equation:

$$\mu = \frac{\mu_{max} S}{K_S} \tag{11}$$

Alternately, if K_S is much less that S, the expression may be reduced to a zero-order equation:

$$\mu = \mu_{max} \tag{12}$$

The rate of substrate removal is related to the specific growth rate by the following equation:

$$\frac{-dS}{dt} = \mu \frac{X}{Y} = \frac{\mu_{max} S}{K_S + S} \cdot \left(\frac{X}{Y}\right) = qX = q_m \frac{XS}{K_S + S} \tag{13}$$

where Y = yield coefficient, mass organisms grown/mass mass substrate used

q = specific rate of substrate utilization, mass substrate/ mass organisms/time = μ/Y

q_m = maximum specific rate of substrate utilization, mass substrate/mass organisms/time= μ_{max}/Y

X = microbial mass concentration, mass/volume

Just as in the case of the Monod equation, eqn. 13 can be approximated by a first order equation when K_S is much greater than S;

$$q = q_m \frac{S}{K_S} \tag{14}$$

and a zero-order equation when S is much greater than K_S:

$$q = q_m \tag{15}$$

Lineweaver-Burke Linearization (1934) is one common method of determining kinetics constants μ_{max} and K_S for the Monod expression from kinetic data. This involves taking the reciprocal of both sides of eqn. 10; rearranging and recognizing that the parameter MCRT or mean cell retention time (to be discussed later) is related to the growth rate as follows;

$$\mu = \frac{1}{\theta_c} = \frac{\mu_{max} S}{K_S + S} \tag{16}$$

where θ_c = MCRT,

= mass organisms in reactor/mass organisms wasted/day

Linearizing,

$$\frac{1}{\mu} = \theta_c = \frac{1}{\mu_{max}} + \left(\frac{K_S}{\mu_{max}} \right) \left(\frac{1}{S}\right) \tag{17}$$

A linear plot of θ_c vs. 1/S yields $1/\mu_{max}$ as the intercept and K_S/μ_{max} as the slope. The yield coefficient Y can then be estimated for a

similar plot of equation 13 (1/q vs. 1/S). Several other methods are available for the determination of Monod kinetic parameters, including those of Hanes (1932) and Hofstee (1955). Each method has its own inherent advantages and disadvantages (Dixon and Webb, 1979) and adoption of any particular method will depend largely on the nature of the data to be analyzed. These methods are summarized in Table 4.

There is substantial literature supporting the use of the Monod expression (eqn. 10 and 13) to describe nitrifier growth and/or substrate uptake in a variety of environments. The equations have been successfully applied to nitrification in soils (McLaren, 1971); pure cultures (Skinner and Walker, 1961, Boon and Laudelout, 1962, Loveless and Painter, 1968); estuaries and streams (Knowles, et al., 1965, Stratton and McCarty, 1967); and activated sludge systems (Dowing, et al., 1964, Poduska and Andrews, 1975, Williamson and McCarty, 1975). In addition, dynamic models of nitrifying activated sludge systems, based on Monod kinetics, are available to simulate effects of changing influent conditions on effluent quality (Poduska and Andrews, 1975; Murphy, et al., 1977; Batchelor, 1983)

The growth limiting nutrient in equations 10 and 13 has been generally considered to be the energy source for Nitrosomonas or Nitrobacter, and less frequently, the electron acceptor (dissolved oxygen). Typical values for Monod kinetic constants obtained from the literature are shown in Table 5. Table 6 summarizes Table 5 in terms of ranges of kinetic constants reported for differing environmental conditions. For comparison, Table 6 includes typical kinetic constants (Sharma and

Table 4: Popular Techniques used to Estimate Kinetic Parameters For the Monod Function

Technique	Linearized Form	Disadvantages
Lineweaver-Burke Analysis	$\frac{1}{\mu} = \frac{K_S}{\mu_{max}} \frac{1}{S} + \frac{1}{\mu_{max}}$	Most accurate known values will be centered near the origin, while those which are least accurately known will be far from the origin and thus will have most influence on the slope, $\frac{K_S}{\mu}$.
Hanes Analysis	$\frac{S}{\mu} = \frac{K_S}{\mu_{max}} + \frac{S}{\mu_{max}}$	Points near μ are spread out so the slope, $1/\mu_{max}$ may be accurately determined. However, the intercept appears near the origin which makes accurate determination of K_S difficult. (Good for least squares analysis)
Hofstee Analysis (multiply eqn. 16 by $(K_S + S)$ and divide by S)	$\mu = \mu_{max} - K_S \frac{\mu}{S}$	μ is in both coordinates, therefore least squares analysis is impossible.

Table 5: Typical Kinetic Constants for Nitrifying Bacteria
(Nitrosomonas)

μ_M (day^{-1})	Y ($\frac{\text{vicell}}{\text{wt N oxidized}}$)	K_s Energy Source (mg/l)	K_s O_2 (mg/l)	Culture Condition	pH	Reference
2.2 (30 °C)	0.06	10 (30 °C) 9.5(25 °C) 1.2(25 °C)	0.5 (30 °C)	pure	7.0-7.4	Skinner & Walker (1961)
0.7 (20 °C)				pure	7.5	As cited by Painter (1977)
1.5 (30 °C)				pure	8.0	Engel & Alexander (1968)
1.38 (25 °C)	0.03-0.1	1.0 (20 °C)	0.3 (20 °C)	pure	8.0	Loveless & Painter (1968)
0.69 (25 °C)				pure	8.0	Loveless & Painter (1968)
0.88 (25 °C)				pure	7.6	Loveless & Painter (1968)
0.25 (10 °C)				pure		Buswell (1954)
0.50 (15 °C)				pure		
0.94 (20 °C)				pure		
1.74 (30 °C)				pure		
1.50 (30 °C)				pure		
0.46 (30 °C)				pure	8.0	Lees (1952)
			0.5	pure		Poeters (1969)
0.33 (20 °C)	0.05	1.0		As	7.5-8.0	Downing, et. al. (1964)
0.59 (20 °C)						Hall (1974)
		1.0		As		Briggs, et. al. (1968) (as cited by Painter (1977)
0.3 (20 °C)				As		Jenkins (1969)
0.4 (20 °C)	0.2+	2.0		As	7.2	Stall & Sherrard (1974)
1.08 (20 °C)		0.063		As	7.2	Poduska & Andrews (1975)
0.32* (20 °C)				As	7.3	Hall & Murphy (1980)
0.45 (23 °C)	0.15 (23 °C)			As	7.5-8.0	Boscari, et. al (1980)
0.54 (20 °C)			0.3	As	7.0	Williamson & McCarty (1975)
0.1 (20 °C) to 0.61 (25 °C)**				As	6.5 to 8.5	Painter & Loveless (1983)
(10 to 35°C)		0.61** to 2.5		As	7.5	Charley, et. al (1980)
	0.29	2.59 to 4.59		Syn. River Water		Stratton & McCarty (1967)
0.65	0.05	0.6		Thames Esturary Water		Knowles, et. al. (1965)

* — Activated sludge process
+ — mg VSS g^{-1} substrate
◊ — mg TSS/mg influent TKN
** — Multiple Determinations over the range of temperature or pH

Table 5 Continued: Typical Kinetic Constants for Nitrifying Bacteria

(Nitrobacter)

μ_N (day^{-1})	Y ($\frac{wt\ cell}{wt\ N\ oxidized}$)	K_S Energy Source (mg/l)	K_S O_2 (mg/l)	Culture Condition	pH	Reference
1.39 (32 °C)	0.02	8.0 (32 °C) 5.0 (25 °C)	0.34 (20°C)++ 0.48 (29°C)++ 0.72 (32°C)++	pure	7.7	Boon & Laudelot (1962)
	0.04-0.07			pure		Gould & Lees (1960)
	0.02			pure		Lees & Simpson (1957)
		1.4 (14 °C) 2.1 (18 °C) 9.4 (32 °C)		pure		Laudelot & Van Tricholen (1960)
			2.0	pure		Peeters, et. al. (1969)
0.14 (20 °C)				As	7.0	Downing, et. al. (1969)
1.44 (20 °C)				As	7.2	Poduska & Andrews (1975)
0.68 (20°C)•				As	7.3	Hall & Murphy (1980)
0.27 (23 °C)	0.07			As	7.5-8.5	Boccarri, et. al. (1980)
			0.42	As (pure O_2)		Stankovich (1972)
			2.0	As		Nagel & Haworth (1969)
0.19 (20 °C)				As	8.0	Painter &
0.32 (20 °C)				As	7.5	Loveless (1983)
0.1 (20 °C)				As	7.0	
0.22 (20 °C)				As	6.5	
0.27 (25 °C)				As	8.0	
0.23 (25 °C)				As	6.5	
	0.084 (20 °C)	0.34-1.77		Syn. River Water		Stratton & McCarty (1967)
0.84 (20 °C)	0.02	1.9		Thames River Water		Knowles, et. al. (1965)

• —— Activated sludge process

++ —— As calculated by Stenstrom & Poduska, (1980)

● —— specific activity (mg N/mg TSS-hr)

Table 6: Summary of Ranges of Kinetic & Stoichiometric Coefficients

	μ_M (day^{-1})	Y (wt. cell per wt N oxidized)	K_s Energy Source (mg/l)	K_s O_2 (mg/l)	Culture Condition	Temperature (^0C)	pH
Nitrosomonas							
	0.7-2.2	.03-0.1	1.0-10.0	0.5	pure	20-30	7-8
	0.3-1.08	0.05-0.15	0.063-2.5	0.3	As	16-35	7.2-8.5
Nitrobacter							
	1.39	0.02-0.07	1.4-9.4	0.34-2.0	pure	14-32	7.7
	0.1-1.44	0.07	-	0.42-2.0	As	20-35	6.5-8.5
Heterotrophs							
	7.2-17.0	0.37-0.79	< 1-181	0.0007-0.1	As	20	7.0

Ahlert, 1977) for heterotrophic bacteria grown on glucose substrate.

Several important points can be made with respect to the tables. For example, Table 6 shows the the values of μ_{max} for nitrifiers are at least an order of magnitude smaller than that of heterotrophs, indicating that nitrification in activated sludge processes will occur only where the specific growth rate is low. Furthermore, the values of K_S (energy source) are seen to be relatively low for both nitrifying genera as compared to the substrate concentrations normally encountered in a typical wastewater treatment plant (i.e., 20-40 mg/1 NH_4^+-N). This would mean that nitrifier growth rates are essentially independent of substrate concentration (zero-ordered reaction) over a broad range. Such zero-order substrate kinetics have been reported by numerous investigators under various conditions (Engel and Alexander, 1959; Nicholas and Jones, 1960; Wild, et al., 1971; Kiff, 1972; Huang and Hopson, 1974; Hall and Murphy, 1980; Sutton, et al., 1981) over the composite range of 1.6 to 673 mg/1 NH_4^+-N). Tomlinson, et al. (1966) has suggested that the rates of nitrification will not be limited by energy sources as long as the concentration of ammonia and nitrite exceeds 3 and 10 mg/1-N, respectively.

However, there appears to be some disagreement in the literature with respect to the true reaction kinetics of nitrification. A recent investigation by Charley, et al. (1980) gave evidence that nitrification rates followed strict Monod type kinetics at all operational concentrations at a treatment plant. They reported that the ammonia concentration at which μ is 90% of μ_{max} (S_{90}) can be as high as 22.5 mg/1 (35°

C). At the S90 value reaction kinetics can be regarded as changing from first to zero order. They also reported first order reaction kinetics for Nitrobacter up to 20 mg/l nitrite.

Until the recent work of Painter and Loveless (1983), there was a paucity of data for μ_{max} values of Nitrobacter in activated sludge. In over half the μ_{max} values reported in Table 3, μ_{max} for Nitrosomonas is greater than that of Nitrobacter in activated sludge. According to Painter and Loveless, in these cases it is Nitrobacter rather than Nitrosomonas that could be the deciding factor as to whether or not full nitrification will occur under any given set of conditions. Moreover, they implied that in some circumstances nitrite accumulation should occur even under optimal nitrifying conditions. However, situations such as those proposed by the authors are rarely observed. One possible explanation for this may lie in the energetics of nitrification. Nitrosomonas obtains approximately four times the energy per mole of nitrogen oxidized than does Nitrobacter (see eqns. 1 and 2). As such, this would suggest that Nitrobacter would have to oxidize nitrite approximately 4 times as fast to generate the same mass (assuming equal cell mass/unit energy produced). Although μ_{max} for Nitrosomonas may be greater than Nitrobacter for a given set of conditions, their specific growth rates, μ may be equal in magnitude due to a lower K_S value for Nitrobacter. It should be pointed out that no K_S value has yet been reported for Nitrobacter in activated sludge systems. The common observation that nitrite rarely accumulates in natural biological systems points to the fact that the Nitrobacter species have developed a capacity for rapid nitrite oxidation. And by doing so, the current general consensus is that the

overall nitrification process is governed by the activity of Nitrosomonas.

Several investigators (Sharma and Ahlert, 1977; Beccari, et al., 1980; Painter, 1977; Hall and Murphy, 1980) have noted the broad ranges of kinetic constants reported in the literature for nitrifiers. In particular, the wide range of values for μ_{max} is disturbing since the design of wastewater nitrification systems depends on the accurate estimation of this parameter. It is generally accepted that the estimation of nitrifier kinetic parameters in mixed cultures of autotrophs and heterotrophs is difficult at best. Most of this difficulty apparently stems from inadequate methods of estimating the fraction of microorganisms which are autotrophs. It is necessary to determine nitrifier organism mass or to precisely know yield coefficients to determine the values of μ in equation 13 for differing culture conditions.

Sharma and Ahlert (1977) reviewed several methods used previously to estimate nitrifier concentrations in both pure and mixed cultures. Included in their discussion are the drawbacks of the various methods. They concluded that most methods were based on invalid assumptions while others are insufficiently tested to warrant general use. More recently, Hall and Murphy (1980) reviewed several techniques that have emerged within the last 5 years or so for nitrifier concentration measurement. Among these methods are techniques based on most probable number (MPN); fluorescent antibodies (FA); and ^{14}C-bicarbonate incorporation. They dismissed the MPN and FA techniques on the basis of their inherent practical limitations and the ^{14}C-bicarbonate assimilation technique

because of its questionable assumption that the relationship between carbon uptake and nitrification is constant.

Instead they proposed a relatively simple technique based on pure culture equivalent activities to estimate the nitrifier kinetic constants. Their technique, which is essentially a modification of an earlier method proposed by Srinath, et al. (1976), involves the direct addition of known quantities of pure nitrifiers in a mixed liquor sample, along with a selective inhibitor (N-serve) of Nitrosomonas activity. Substrate concentration or products formed are measured as a function of time and compared to concurrent assays consisting of 1) mixed liquor, and 2) mixed liquor with known pure culture additions. Increments of activity resulting from the known addition of nitrifiers are used to calculate μ based on observed zero order kinetics. In the presence of N-serve, any changes in ammonia concentration could be attributed to heterotrophic effects since N-serve, at the concentrations used, is known to inhibit Nitrosomonas activity completely while Nitrobacter or heterotrophic activity remain unaffected. The proposed estimation method appears to hold promise, particularly in wastewater analysis, since in situ determinations can be performed in a short period with relative ease.

From the foregoing discussion, it is clear that there is a critical need for improved and perhaps standardized techniques for the measurement of nitrifier kinetic constants. It is well established that the accurate determination of specific growth rate or specific substrate removal rate is essential for the proper design of biological nitrifica-

tion systems.

It is likely that the variability of reported kinetic constants can be attributed not only to inadequate measurement techniques but also to differing culture conditions. The exact kinetics occurring during nitrification are governed by any number of complex interacting factors relating to prevailing culture conditions. These factors include pH, dissolved oxygen concentration, presence or absence of micronutrients or inhibitors, temperature and interactions with heterotrophic organisms in mixed culture. No relationship is available to allow the prediction of μ at specified levels of these parameters.

Nitrification in Activated Sludge Processes

To date, biological nitrification in activated sludge systems is the form of ammonia removal generally considered the most attractive in terms of process applicability and cost effectiveness (USEPA, 1975; Focht and Chang, 1975). It is also recognized however, that nitrifying organisms are extremely sensitive to prevailing environmental conditions and process variables. Sufficient information based on literature reports and years of operational experience is now available to provide some basis for the design of nitrifying systems (USEPA, 1975). In addition, two excellent reviews have appeared to address the subject of nitrification and/or denitrification as applied to wastewater treatment systems (Focht and Chang, 1975; Sharma and Ahlert, 1977).

Nitrification in activated sludge systems can be obtained if the conditions suitable for the retention and accumulation of nitrifying organisms are maintained. The necessary conditions may be expressed in terms of inorganic substrate concentration, MCRT, pH, temperature, dissolved oxygen concentration, presence of required nutrients and absence of inhibitory or toxic substances. The interplay among these various factors plus the presence of a diverse heterotrophic population in any given system makes for a unique and extremely complex ecosystem from which only gross generalizations can be made. The purpose of this section is to review the various factors affecting the nitrification process, with emphasis on activated sludge systems. It is important to note that the interpretation of the results presented herein should be approached with caution since they were obtained under different sets of conditions. Generalizations are made when deemed appropriate.

MCRT, OLR and HRT

Downing, et al. (1964) were among the first investigators to attempt a quantitative description of nitrification in wastewater treatment plants. The most important finding in this work was that the growth rate of nitrifiers can be related to a manipulative process variable referred to as mean cell retention time (MCRT) or MCRT. The growth rate of microorganisms can be related to the design of activated sludge systems by the following expression:

$$\theta_c = \frac{1}{\mu} \qquad (18)$$

where θc = mean cell retention time

MCRT can be calculated from operating data by dividing the quantity of biological mass in the reactor by the quantity of biological mass wasted daily. As discussed earlier, the growth rate of nitrifiers is much lower than that of heterotrophs, consequently in combined carbon-nitrogen oxidation systems, a solids wasting program must be established in order that the slower nitrification population can be retained and utilized efficiently. The MCRT is the critical parameter in this regard.

The minimum MCRT required to achieve nitrification consistently for any given system is largely dependent upon the growth rate of nitrifiers and the net biological solids production rate of the process. As noted previously, the growth rate of nitrifiers is influenced by and sensitive to many environmental factors and process operating parameters. The net solids production rate, however, is largely dependent on heterotrophic growth rate and yield which are primarily determined by process operating parameters. The parameter MCRT is inversely proportional to the organic loading rate (OLR) which, along with hydraulic retention time (HRT) determines the concentration of organic substrate in the waste stream under dynamic conditions. Higher OLR's will produce increased sludge quantities that must be wasted from the system. An increased wasting rate will then result in a decreased fraction of the slower growing nitrifying population in the reactor. Continued operation at higher OLR's may ultimately lead to the cessation of nitrification due to the "washout" of the nitrifying population.

The maximum F/M ratio, defined as OLR divided by the mass of reactor biological solids, required to maintain consistent nitrification appears to be in the range of 0.3 to 0.4 lb BOD/lb MLVSS/day (Balakrishnan and Eckenfelder, 1969), and for increasing values above that range, the degree of nitrification has been found to decrease correspondingly (Prakasam and Loehr, 1972). Johnson and Schroepfer (1964) and later Prakasam and Loehr (1972) reported that a minimum MCRT of 3 days is required for nitrification in laboratory scale activated sludge units. Poduska (1973) and Poduska and Andrews (1974) summarized previous results on the effects of MCRT in relation to the degree of nitrification obtainable (see Figure 3) and also noted that at temperatures less than 20° C the MCRT required to maintain high nitrification efficiences increases markedly.

Figure 3 points out an interesting fact in that under steady state conditions, activated sludge systems will tend to give an "all or none" response to nitrification for a given MCRT. The high efficiencies of nitrification are associated with the low K_S (energy source) values of nitrifiers. Poduska and Andrews (1974) pointed out that there are occasions when this phenomenon will not occur. One is when the reactor is subjected to dynamic loads, in which case a slightly higher MCRT will be required. Other occasions can be attributed to the presence of inhibitory substances.

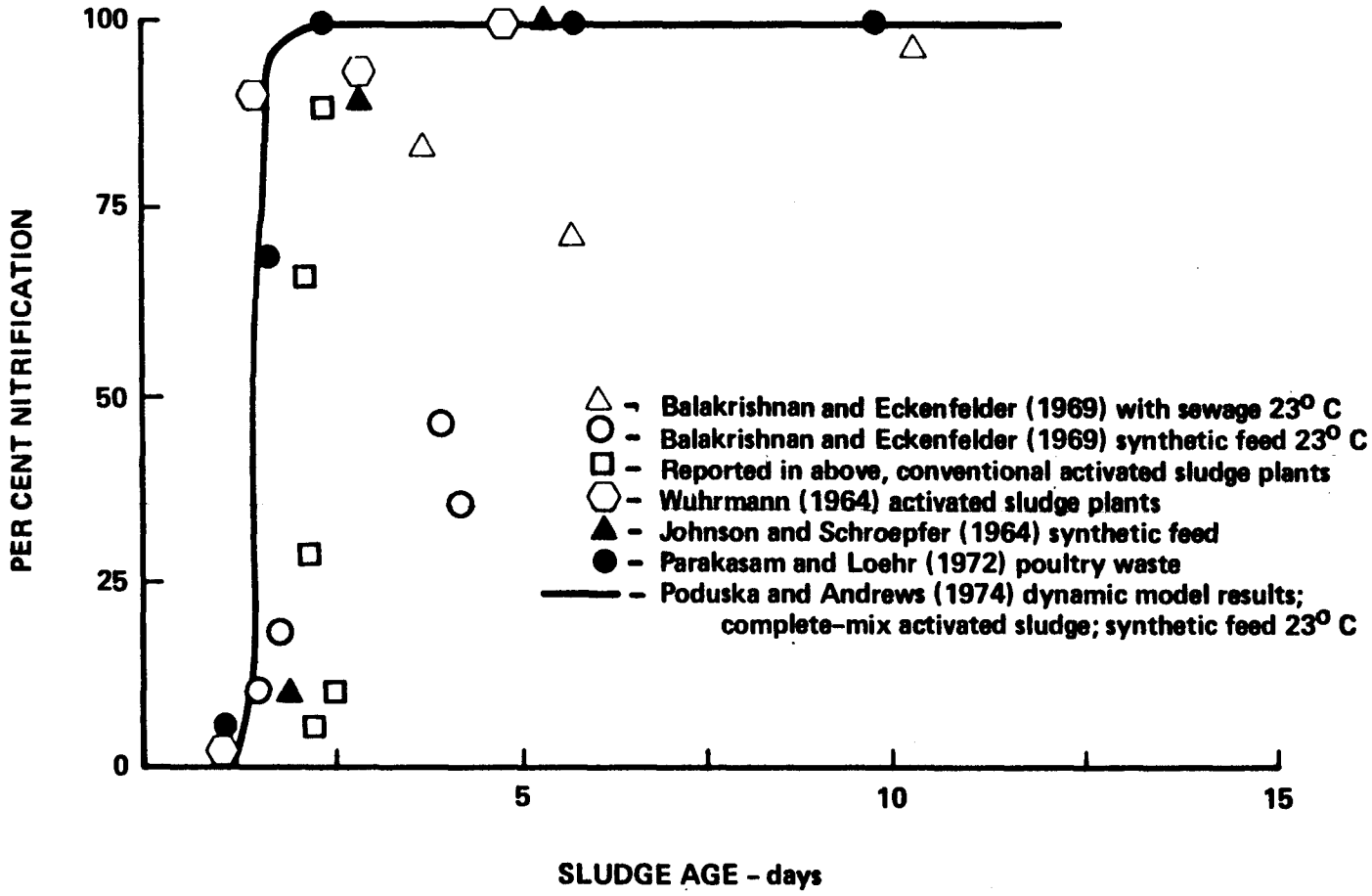


Figure 3: Comparison of Nitrification Data taken from the Literature and Results Predicted by the Steady State Model of Poduska and Andrews (After Poduska and Andrews, 1974)

Effect of Temperature on Nitrification

Temperature has a profound effect on nitrification rates. In a full scale municipal treatment plant, Beckman, et al. (1972) observed 90 to 95% nitrification at 18° C with a MCRT of 6.5 days and only 75% nitrification at 10° C. Sutton, et al. (1981), in studies conducted in a full scale Canadian industrial treatment plant, reported that the degree of nitrification was seasonal with the loss of nitrification each winter as the aerobic basin temperature approached 10° C. In addition, they observed that complete nitrification could not be sustained during winter months even under conditions of good process control with MCRT greater than 70 days. As a result of these findings, it was decided to proceed with a project to raise the aerobic basin temperature during winter months.

Early studies with mixed and pure cultures of nitrifying organisms revealed that the growth rate of nitrifiers, as well as that of heterotrophs, is a strong function of temperature. Knowles, et al. (1965) fit kinetic data collected from suspended growth cultures and determined the following mathematical relationships between nitrifier growth rate and temperature(8 to 28° C):

$$\text{Log}_{10}\ \mu_{max} = 0.413(T) - 0.944 \qquad (19)$$

for Nitrosomonas (where T is expressed in degrees Celsius) ; and

$$\text{Log}_{10}\ \mu_{max} = 0.0255(T) - 0.492 \qquad (20)$$

for Nitrobacter.

They also reported that nitrifier K_S (energy source) values were dependent upon temperature and established empirical expressions for these relationships as well:

$$\text{Log}_{10}\ K_S(NH_4^+\text{-}N) = 0.051(T) - 1.158 \tag{21}$$

for Nitrosomonas; and

$$\text{Log}_{10}\ K_S(NO_2^-\text{-}N) = 0.063(T) - 1.149 \tag{22}$$

for Nitrobacter.

Charley, et al. (1980) reported that the relationship between K_S and temperature as proposed by Knowles does not always hold. From kinetic data generated from laboratory scale activated sludge units, they observed a general increase of K_S (NH_4^+-N) with temperature over the range of 10 to 35°C, but found that the K_S for Nitrosomonas was lowest (0.61 mg/1) at 15°C. Moreover, they reported that the S(90) value (5.49 mg/1) at 15°C was the lowest among all the temperatures tested. These findings suggests that the nitrification reaction kinetics at this temperature are more efficient than at any other temperature studied. However, these findings do not mean that treatment plants nitrify the fastest at this particular temperature since the overriding factor governing nitrification in activated sludge is the fraction of nitrifiers present. This, in turn is largely dependent on μ_{max} or MCRT and temperature. Their findings do indicate however, that at 15°C, a given population size of nitrifiers would oxidize ammonia more efficiently than the same size population at any other temperature studied. A further implication is that nitrifiers apparently have different optimum

temperatures for carbon assimilation (growth) and energy production (nitrification).

Because both μ and K_S are a function of temperature, it is important that both parameters be considered when attempting to determine the effect of temperature dependent nitrification. The empirical equations presented above can be used to approximate temperature effects but the actual effects can only be determined through experimentation.

In wastewater treatment systems, temperature is the most difficult environmental parameter to control. This difficulty is reflected in many cases where nitrifying ability is partially or completely lost in treatment plants situated in colder climates. Studies involving nitrification in activated sludge have indicated that the nitrification rate approximately doubles for a rise in temperature from 10 to 20°C (USEPA, 1975). The optimum temperature for nitrification in activated sludge is reported to be in the range of 30 to 35°C with a pronounced decrease at temperatures below 18°C and above 35°C (USEPA, 1975). Therefore, in many treatment plant environments nitrification proceeds at suboptimal temperatures. In order to compensate for low or suboptimal temperatures, operation at higher retention times and/or higher MCRT are often required to maintain reasonable nitrification efficiencies. For example, Sutton, et al. (1981) reported, based on bench scale studies, that the minimum MCRT required to nitrify Du Pont wastewater was 25 to 30 days at 20°C; compared to 55 to 60 days at 10°C.

pH and Alkalinity Effects on Nitrification

In general, functions relating pH to nitrification rates in pure, mixed or activated sludge cultures are fairly flat over a limited pH range and steadily decrease on either side of the plateau. For pure cultures of Nitrosomonas, plateau ranges of 8.0 to 8.5 (Buswell, et al. 1954); 7.2 to 9.2 (Engel and Alexander, 1958) and 7.0 to 8.2 (Loveless and Painter, 1968) have been reported. For pure cultures of Nitrobacter, Lees (1954) and Boon and Laudelout (1962), reported the optimal pH range to be 8.3-8.6 and 7.3 to 8.4, respectively. Similarly, optimal pH ranges for nitrification in activated sludge have been reported to be 8.4 to 8.6 (Rimer and Woodward, 1972); 7.5 to 8.5 (Downing, et al., 1964) and 7.5 to 8.0 (Painter and Loveless, 1983). Although there are apparent differences among reported optimal pH ranges, in all cases the optimal pH is at least 7.0. The differences in reported ranges are likely attributable to differences in culture conditions (i.e., trace-element status in the medium; Loveless and Painter, 1968) and/or nitrifier strains. It is generally accepted that nitrification will occur optimally at a neutral to slightly alkaline pH (Focht and Chang, 1975; Sharma and Ahlert, 1977) of 7.0 to 8.0.

Most of the controversy regarding pH effects on nitrification rates appear to be reported outside of the optimal range. Nitrification rates for Nitrosomonas have been reported to occur with 50% optimal activity at pH values as low as 6.2 to as high as 9.6 (Engel and Alexander, 1958), although much narrower ranges have been observed (Hofman and Lees, 1952; Loveless and Painter, 1968). For activated sludge Hall

(1974), Downing, et al. (1964) and Painter and Loveless (1983) reported that the lower limit at which nitrification can occur is 6.2, 6.5 and 6.0 respectively. In contrast, nitrification at pH values as low as 4.0 (Prakasam, et. al., 1974) and 4.9 (Prakasam and Loehr, 1972) have been reported to occur in poultry wastewaters. Disalvo and Sherrard (1980) and Stankewich (1972) reported nitrification occurring in activated sludge systems at the pH of 5.1 and 5.8 respectively. It has been shown however, that nitrifiers have the ability to acclimate to pH values outside their optimal ranges while retaining similar nitrification abilities (Haug and McCarty, 1972).

Anthonisen, et al. (1976) has suggested that the mechanism responsible for lowered nitrification rates at pH values above or below the optimal range involves pH dependent NH_3/NH_4^+ and NO_2^-/HNO_2 equilibria in aqueous environments. He proposed that free ammonia (NH_3) and free nitrous acid (HNO_2) which increase in concentration at higher and lower pH's, respectively, inhibit nitrifying organisms. This theory has been disputed by Poduska (1973) who performed impulse nitrite spiking tests at various pH levels and demonstrated that the overall effect of a pH decrease was significantly greater on nitrite oxidation rate than that attributed to the increase in concentration of HNO_2 alone. From this, it would appear that the mechanism responsible for the effect of pH on nitrification rates is more complex than that proposed by Anthonisen, and merits further study. The subject of substrate and end product inhibition will be reviewed in greater detail in a later section.

The effect of CO_2 produced from the degradation of organic matter in the treatment of domestic wastewaters is generally not a problem since the CO_2 is the equilibrium with the atmosphere. Although the optimal pH for nitrification may be greater than 8.0, Wang, et. al (1978) recommended operating treatment plants at 7.6 to 7.8 in order to allow CO_2 to escape to the atmosphere.

If highly nitrogenous wastes are present in the waste stream, depressed nitrification rates may be observed in cases where inadequate buffering capacity exist. The effect of nitrification on pH depression is readily apparent by inspection of the following synthesis and oxidation equations for nitrifiers (USEPA, 1975):

$$55\ NH_4^+ + 76\ O_2 + 109\ HCO_3^- \rightarrow \tag{23}$$

$$C_5H_7NO_2 + 54\ NO_2^- + 57\ H_2O + 104\ H_2CO_3$$

(Nitrosomonas)

$$400\ NO_2^- + NH_4^+ + 4\ H_2CO_3 + HCO_3^- + 195\ O_2 \rightarrow \tag{24}$$

$$C_5H_7NO_2 + 3\ H_2O + 400\ NO_3^-$$

(Nitrobacter)

Development of equations 23 and 24 was based on a reasonable cell yields and the commonly used empirical formulation for microbial cells. These equations indicate that a large amount of alkalinity (HCO_3^-) will be utilized during nitrification. Although a small part of this will be

utilized in autotrophic biosynthesis, the majority will be used to neutralize the production of free acid (H^+) in context of the carbonic acid system. A ratio of alkalinity destroyed (as mg/l $CaCO_3$) per mg of ammonia oxidized of approximately 7.1 has been calculated (USEPA, 1975). Wang, et al. (1978), neglecting biosynthesis, showed that one mole of calcium bicarbonate is needed to neutralize every two moles of nitric acid produced from the nitrification process:

$$2\ NH_4^+ + 2\ HCO_3^- + 4\ O_2 + Ca(HCO_3)_2 \rightarrow \qquad (25)$$

$$Ca(NO_3)_2 + 4\ CO_2 + H_2O$$

In wastewaters where insufficient buffering capacity exists, eqn. 25 can be used to readily calculate the amount of added alkalinity (lime) required.

It has been shown that pH strongly influences the toxic effects of heavy metals in activated sludge. This will be discussed more fully in a later section.

Effect of Dissolved Oxygen of Nitrification

Equations 23 and 24 indicate that oxygen is required for the oxidation-reduction reactions occurring in nitrification. These equations correspond to an oxygen consumption ratio of approximately 3.15 mg O_2/mg NH_4^+-N oxidized and 1.11 mg O_2/mg NO_2^--N oxidized (USEPA, 1975). It remains a point of controversy as to whether or not stoichiometric amounts of oxygen are used during nitrification (Sharma and Ahlert,

1977) and it has been suggested (Kiesow, 1972 as cited in Sharma and Ahlert, 1977) that the variable oxygen consumption rates for nitrification reported in the literature may be explained by variable stoichiometry exhibited by Nitrobacter during inorganic assimilation (see Figure 2).

The concentration of dissolved oxygen (DO) in wastewater treatment plants has a significant effect of nitrification rates. Murphy (1974, as cited in USEPA, 1975) observed that nitrification was more efficient at 7 to 8 mg/1 DO than at 1 mg/1. Nagel and Haworth (1969) determined that the relationship between DO and nitrification rates could be fitted with the Monod expression over the concentration range studied. These experiments, carried out in full-scale treatment plants, indicated that the K_S (O_2) value was a surprisingly high value of 2.0 mg/1.

A number of other experimental and theoretical studies involving pure and mixed and activated sludge cultures have been reported on the effects of DO on nitrification rates. Notable among these investigations is that by Stenstrom and Poduska (1980) who reviewed and summarized much of the previous work in this respect. This summary is presented in Table 7. Several important generalizations can be made or inferred from inspection of Table 7:

1. Oxygen substrate limitation exists in nitrification processes and maintenance of a minimal dissolved oxygen concentration is essential to achieve a sustained degree of nitrification.

2. The $K_S(O_2)$ values for nitrifiers are reportedly higher than that

Table 7: Summary of the Effects of DO on Nitrification
(Taken from Stenstrom & Poduska, 1980)

Organism	K_s(DO) mg/l	DO mg/l	Conditions and remarks	References
Nitrosomonas	0.3		20 C Pure culture	Loveless & Painter (1968)
	0.25		Pure culture	Peeters et al. (1969)
		1.0	Zero-order[1], pure culture	Schoberl & Engel (1964)
		2.0	Zero-order	Knowles et al. (1965)
Nitrobacter	1.84,2.46		Pure culture, by respirometric	Peters et al. (1969) and
	0.83		amperometric and micro calorimetric technique	Laudelout et al. (1976)
	0.34,0.48		at 20,29,35 C respectively	
	0.72		Calculated from Boon & Laudelout (1962)	
		2.0	Zero-order	Schoberl & Engel (1964)
		4.0	Zero-order	Knowles et al. (1965)
Nitrosocytis		7.5	Zero-order	Gundersen (1966)
Oceanous		0.5-0.7	Inhibitory	Forster (1974)
Nitrifiers, generally		1.0-1.5	Proper operation of ASP[2]	Jenkins (1969), Balakrishnan & Echenfelder (1969), Wild et al. (1971), Wuhrman (1968)
	0.8		In soil	Calculated by Shah & Coolman (1978)
		0.5	Inhibitory[3] in ASP	Bragstad & Bradney (1937)
		0.3	Inhibition[4] in ASP	Downing & Seragg (1958)
		0.3	Inhibition[4] in ASP	Downing & Boon (1963)
		0.2	Inhibitory in ASP	Downing et al. (1964)
		0.3-1.0	Proper operation in ASP	Downing et al. (1964)
		4.0	Maximum rate of nitrification	Wuhrman (1963)
	0.42		In pure oxygen ASP	Stankowich (1972)
	2.0		In ASP	Nagel & Haworth (1969)

1 Zero-Order MIN DO concentration for zero-order kinetics
2 ASP= activated sludge process
3 Inhibitory, no reaction
4 Inhibition, reaction depends upon concentration

of heterotrophic organisms. This implies that the presence of organic matter can directly inhibit nitrifiers by virtue of the competition for available DO at low concentrations in activated sludge.

3. A wide range of critical dissolved oxygen concentrations and half-saturation constants have been reported in the literature. Consequently, the factors affecting the effect of DO on nitrification have not yet been well established.

Stenstrom and Poduska (1980) addressed this later point by exploring several mechanisms that might explain the variability of results reported in Table 7. The mechanisms considered were the effects of oxygen diffusion in flocs, variation between measured results due to steady-state and dynamic measuring techniques and double-substrate limited kinetics. They reasoned that although experimental evidence was lacking, the first two mechanisms are both theoretically plausible explanations and warranted further investigation. The authors then demonstrated, through computer simulations, that the mechanism of double-substrate limited kinetics could account for much of the variable results seen in Table 7.

The concept of simultaneous multiple substrate kinetics was proposed before by Bader (1978) and applied in the following form by the authors to describe double substrate limited nitrification:

$$\mu' = \mu_{max}\left(\frac{S_1}{K_{S_1} + S_1}\right)\left(\frac{S_2}{K_{S_2} + S_2}\right) - K_D \qquad (26)$$

where S_1 = substrate 1 concentration (energy source)

S_2 = substrate 2 concentration (O_2)

K_{S1} = half-saturation coefficient for S_1

K_{S2} = half-saturation coefficient for S_2

K_D = decay or maintenance coefficient, $time^{-1}$

μ' = net specific growth rate, $time^{-1}$

Figure 4 (taken from Stenstrom and Poduska, 1981) illustrates the effects of double substrate limiting kinetics on the growth rate (MCRT) of nitrifiers assuming typical kinetic constants. The investigators concluded, based on their analysis, that the lowest DO concentration at which nitrification could occur is approximately 0.3 mg/l. They further propose that at a low MCRT, it is possible to nitrify as efficiently as at a higher MCRT, although in such cases the required DO concentration is much greater.

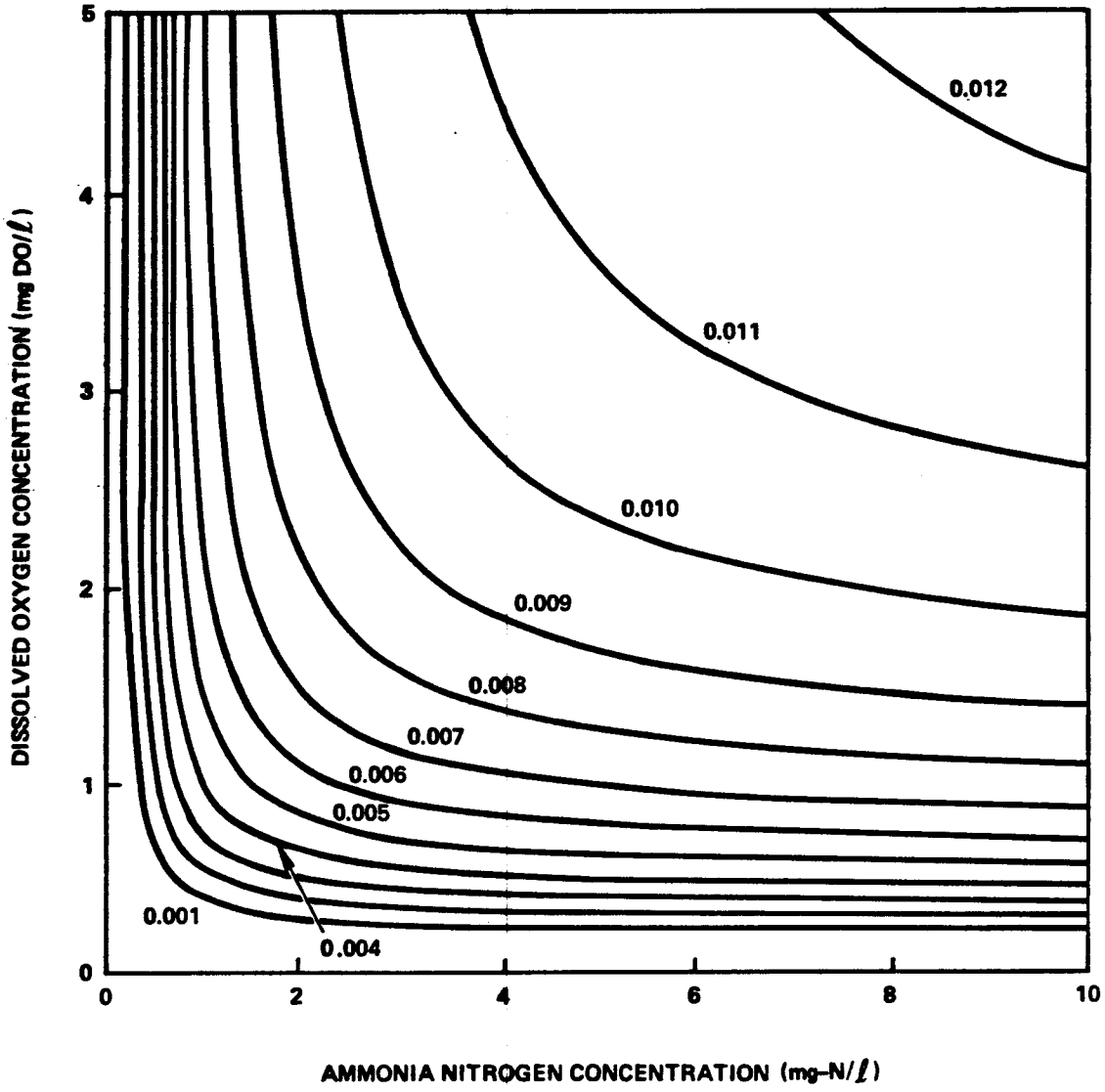


Figure 4: Net Growth Rate Contours Versus Ammonia Nitrogen and Dissolved Oxygen Concentration. (Numbers on curves indicate growth rate, hrs^{-1}. (After Stenstrom and Poduska, 1980).

$$\mu' = \mu_{max}\left(\frac{S_1}{K_{S_1} + S_1}\right)\left(\frac{S_2}{K_{S_2} + S_2}\right) - K_D$$

$\mu_{max} = 0.02\ hrs^{-1}$ $K_{SDO} = 0.5$ mg DO/1

$K_S = 1.0$ mg NH_4^+-N/1 $K_D = 0.005\ hrs^{-1}$

Inhibition of Nitrification

For academic and practical reasons, it is important to have a concept of how nitrifiers are inhibited or killed. Many physical processes and chemical compounds may alter or destroy nitrifier activity. Typical physical processes include low or high temperature and osmotic pressure or surface tension effects, while a great number of chemical agents in appropriate concentrations are capable of inhibiting or ceasing nitrification completely. The purpose of this section is to review much of the literature relating to the inhibition of nitrifying organisms, with special emphasis on specific inhibitory compounds and the classification of their effects through kinetic data. Knowledge of the mode of action for particular inhibitors and its threshold concentration required to exert appreciable effects may make it possible to predict and perhaps modify conditions under which nitrification can continue.

The inhibitory effects of chemical compounds on microbiological processes in general may take on one or more of many possible modes of action. These include:

1. Damage to the cell wall
2. Inhibition of cell wall synthesis
3. Alteration of cell permeability
4. Alteration of protein and nucleic acid molecules
5. Blockage of biosynthetic reaction (antimetabolites)

6. Inhibition of enzyme action

The relative importance among these mechanisms of action have been shown for specific classes of chemical substances in studies with certain heterotrophic microorganisms (Hugo, 1971). Much less is known about the general action of inhibitors on nitrifying organisms although it is likely that certain inhibitors will act in a similar manner to that of heterotrophs.

For the sake of generality, Carpenter (1971) has arbitrarily grouped major inhibitory chemical agents along with their possible mechanisms of action on microorganisms. This is summarized in Table 8. Most common among the modes of inhibitory or toxic action is that of disruption of cell membranes and enzyme function. It is probable that many of the inhibition effects (short of complete and irreversible toxicity) due to chemical substances can be explained by enzyme inhibition. As will be discussed later, numerous attempts have been made to quantify nitrification inhibition through the use of existing kinetic theories and models describing enzyme inhibition.

Enzyme Inhibition

There is vast amount of literature (i.e., Webb and Leyden, 1966; Laidler and Bunting, 1973; Dixon and Webb, 1979) available which describes quite elaborately the various theories and kinetic mechanisms of enzyme inhibition in pure systems. In this report the discussion will be limited to the "classical mechanisms" relating to steady-state single substrate enzyme inhibition. This is done in recognition of the

Table 8: Major Toxic or Inhibitory Chemical Agents and Possible Modes of Microbial Action

Group	Examples	Possible Mode of Action
Soaps		Disrupt cell membranes and increase permeability
Detergents	Sodium Lauryl Suflate (anions) Quaternary Ammonium Halides	Disrupt cell membranes, probably by combining with lipids and proteins; N and P compounds leak from cell
Acids	H_2SO_4, organic acids	Destroy cell walls & membranes
Alalies	Lye (NaOH), Quicklime	Destroy cell walls and membranes
Alcohols	Methyl, Ethyl, Prophyl, Butyl, etc.	Denatures and coagulate proteins
Phenols	Phenols, cresols, Lysols, etc.	Denatures and precipitates proteins
Halogenic Compounds	Cl, I, Br, Hypochlorite, etc.	Oxidizes -SH, $-NH_2$ of enzymes or coenzymes and membranes
Heavy Metals	Hg, Ag, Cu, Cr, etc.	Reacts with -SH groups of enzymes or coenzymes; precipitates proteins
Basic Dyes	Crystal violet, Brillant Green	Probably forms salts with nucleic acids

fact that nitrifier enzyme systems are not yet well defined and that it is currently impossible to express the more sophisticated mechanistic models in terms of the complex enzymatic processes involved in nitrification. It is acknowledged that the basic mechanisms to be reviewed can strongly influence the development of future empirical models characterizing nitrification inhibition.

Michaelis-Menten Kinetics

The theoretical basis of almost all current enzyme inhibition models is Michaelis-Menten kinetics. As such, it is worthwhile to briefly review the elementary concepts involved in this theory. The basic assumption of Michaelis-Menten kinetics is that enzymatic catalysis occurs through a series of elementary reactions involving the formation of an enzyme-substrate complex:

$$S + E \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} ES \xrightarrow{K_2} P$$

where S, E, ES, P represent the substrate, free enzyme, enzyme-substrate complex, and product, respectively. Assuming equilibrium conditions (i.e., when the rate of formation of ES is equal to its rate of breakdown);

$$K_1(E)(S) - K_{-1}(ES) - K_2(ES) = 0 \qquad (27)$$

The total enzyme concentration E_0 can be expressed as,

$$(E_0) = (E) + (ES)$$

(28)

and the rate of reaction as,

$$V = K_2(ES) \tag{29}$$

Using equations 27, 28, 29, V can be expressed as,

$$V = \frac{K_2 E_0 S}{K_m + S} \tag{30}$$

where K_m= Michaelis-Menten constant, $(K_{-1} + K_2)/K_1$ and $K_2(E_0)$ is usually written as the maximum reaction velocity V_{max}, thus the final form of the Michaelis-Menten equation can be written as:

$$V = V_{max} \frac{S}{K_m + S} \tag{31}$$

Figure 5 shows a typical plot of this equation. Although this equation is similar in form to that of the Monod equation discussed earlier, the former has theoretical basis whereas the Monod equation is strictly empirical. Nevertheless, the parameters V_{max} and K_m can be determined with experimental data using the previously described techniques for the Monod equation.

Classical Enzyme Inhibition Mechanisms

An enzyme inhibitor can have the effect of binding with the substrate and/or the enzyme-substrate complex, thus influencing the rate of the reaction. When inhibition occurs in enzyme systems, the following three different types of behavior are to be distinguished depending upon

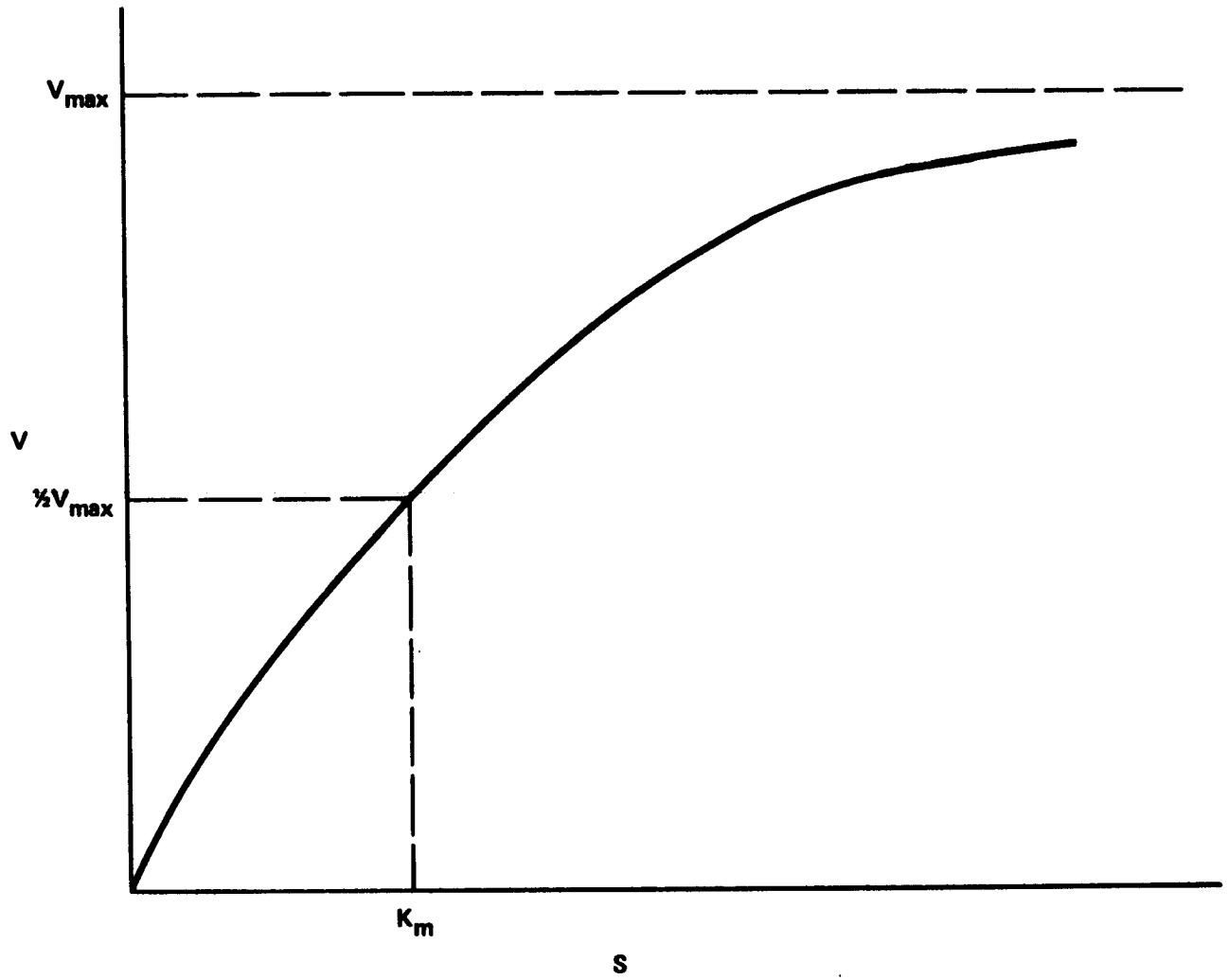


Figure 5: Plot of Michealis-Menten Equation

the manner in which the degree of inhibition is affected by the substrate concentration (i.e. ammonia or nitrite):

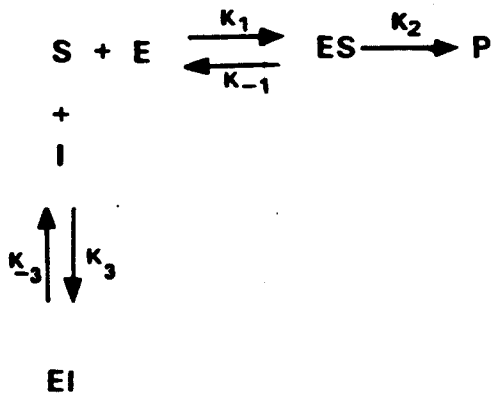
1. In pure competitive inhibition, the degree of inhibition is decreased as substrate concentration increases.

2. In pure non-competitive inhibition, the degree of inhibition is unaffected by the concentration of substrate.

3. In pure uncompetitive inhibition, the degree of inhibition is increased as substrate concentration is increased.

The rate expressions relating the velocity of reaction to substrate and inhibitor concentration for each particular type of inhibition can be derived according to its corresponding mechanism of inhibition.

In the case of pure competitive inhibition, the proposed mechanism is as follows;



where I = concentration of inhibitor

K_{-3}/K_3 = dissociation constant for the enzyme-inhibitor complex = K_I

In this scheme, the degree of inhibition observed will be dependent on the relative amount of substrate and inhibitor present. The presence of the inhibitor will not have an influence on V_{max} since at high substrate concentrations it will not compete effectively for sites on the enzyme. However, because the equilibrium between enzyme and inhibitor has to be displaced, more substrate will be necessary to reach V_{max} and hence K_m will be increased. This effect can be expressed mathematically in terms of the equilibrium equations for the mechanism;

$$K_1 S (E_0 - ES - EI) = (K_{-1} + K_2) ES$$

$$K_3 I (E_0 - ES - EI) = K_{-3}(EI)$$

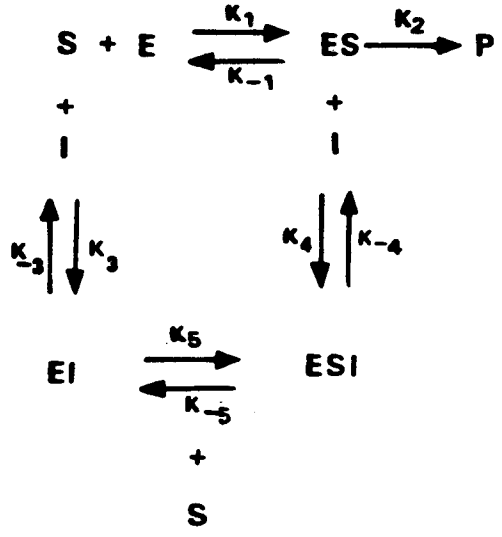
$$V = K_2 ES$$

Solving for V using these relationships:

$$V = V_{max} \frac{S}{K_m(1 + \frac{I}{K_I}) + S} \qquad (31)$$

Thus the effect of competitive inhibition is to increase K_m by a factor of $(1+I/K_I)$ without affecting the value of V_{max}.

The mechanistic pathway representing pure non-competitive inhibition is as follows:

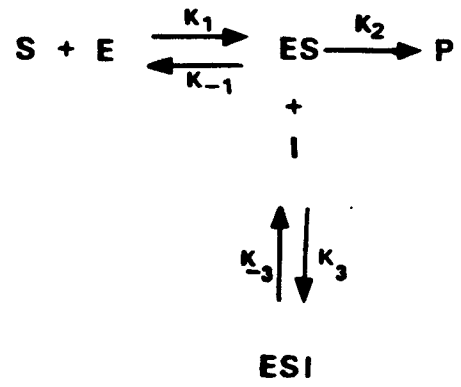


The lengthy derivation of the equilibrium rate expression has been presented by Dixon and Webb (1979) and will not be reproduced here. The resulting equation is,

$$V = V_{max}\frac{S}{(S + K_m)(1 + \frac{I}{K_I})} \qquad (32)$$

Inspection of equation (32) reveals that the effect of non-competitive inhibition is to divide V_{max} by the factor $(1+I/K_I)$, leaving K_m unaffected. If I becomes infinite, V_{max} may become zero and it would be impossible to alter the inhibitory effect by simply increasing the substrate concentration.

The mechanism for uncompetitive inhibition is shown below,



and it corresponding rate expression can be derived as,

$$V = \frac{V_{max}\, S}{K_m + S(1 + \frac{I}{K_I})} \qquad (33)$$

In this case, the effect of the inhibitor reduces both V_{max} and K_m. Here, a high substrate concentration will not overcome the inhibition effect since the inhibitor binds with ES rather than E.

Characterization of Inhibition

The effect of inhibitors can be distinguished by the use of reciprocal linear plots similar to those described in Table 4 for the estimation of Monod kinetic parameters. Experiments are run in which the effect of substrate concentration on reaction velocity is measured in the presence and absence of the inhibitor thereby yielding two linear plots. The relative positions of the two lines will be characteristic of the type of inhibition. Table 9 and Figure 6 shows the linearized equations for Lineweaver-Burke analysis and the typical resulting plots. Following the characterization of inhibition type, the value of K_I can be estimated by using the values of K_m' and V_{max}' obtained from the graph

Table 9: Classical Enzyme Inhibition Mechanisms and the Determination of Kinetic and Inhibition Constants by Lineweaver-Burke Plots.

Inhibition type	Equation (V=)	Effect on V_{max}	Effect on K_m	Inverted form $\frac{1}{V}$ =	Intercept on vertical axis	Intercept on base line	Slope
none	$\frac{V_{max} S}{K_m + S}$	—	—	$\frac{K_m}{V_{max}} \frac{1}{S} + \frac{1}{V_{max}}$	$\frac{1}{V_{max}}$	$\frac{-1}{K_m}$	$\frac{K_m}{V_{max}}$
competitive	$\frac{V_{max} S}{K_m \left[1 + (\frac{I}{K_i}) \right] + S}$	none	increase	$\frac{K_m}{V_{max}} \frac{1}{S} \left[1 + (\frac{I}{K_i}) \right] + \frac{1}{V_{max}}$	$\frac{1}{V_{max}}$	$\frac{-1}{K_m \left[1 + (\frac{I}{K_i}) \right]}$	$\frac{K_m \left[1 + (\frac{I}{K_i}) \right]}{V_{max}}$
noncompetitive	$\frac{V_{max} S}{(K_m + S) \left[1 + (\frac{I}{K_i}) \right]}$	decrease	none	$\frac{K_m}{V_{max}} \left[1 + (\frac{I}{K_i}) \right] \frac{1}{S} + \frac{\left[1 + (\frac{I}{K_i}) \right]}{V_{max}}$	$\frac{1 + (\frac{I}{K_i})}{V_{max}}$	$\frac{-1}{K_m}$	$\frac{K_m \left[1 + (\frac{I}{K_i}) \right]}{V_{max}}$
uncompetitive	$\frac{V_{max} S}{K_m + S \left[1 + (\frac{I}{K_i}) \right]}$	decrease	decrease	$\frac{K_m}{V_{max}} \frac{1}{S} + \frac{\left[1 + (\frac{I}{K_i}) \right]}{V_{max}}$	$\frac{1 + (\frac{I}{K_i})}{V_{max}}$	$- \left[\frac{1 + (\frac{I}{K_i})}{K_m} \right]$	$\frac{K_m}{V_{max}}$

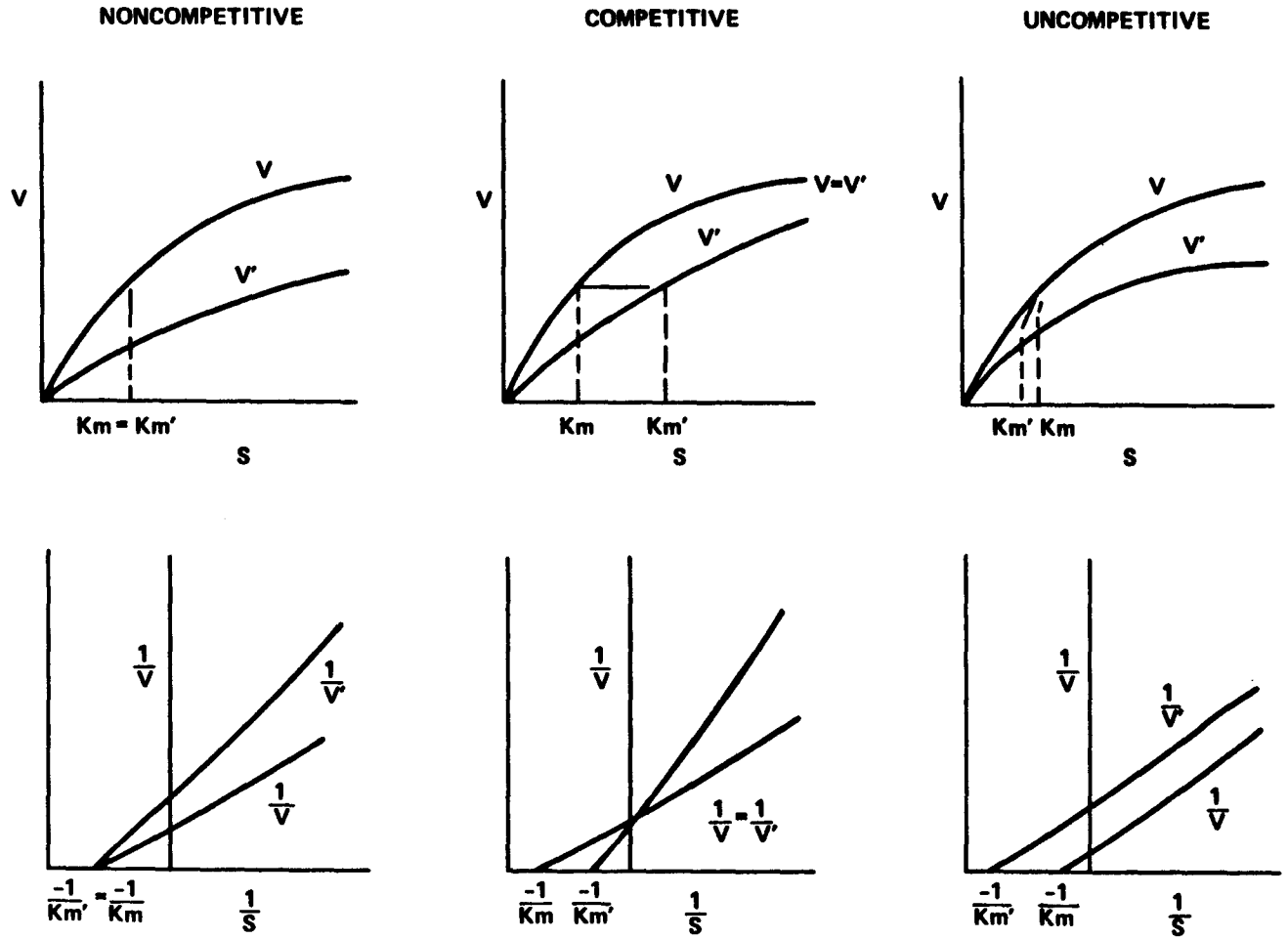


Figure 6: Effect of Inhibitors of Various Types on Lineweaver-Burke Plots of Substrate Concentration on Enzyme Reacttion Velocity V. (V' and K_m' = V and K_m in the presence of the inhibitor)

in conjunction to the values K_m and V_{max} obtained from the uninhibited data. The values of K_m' and V_{max}' are defined as $K_m(1+I/K_I)$ and $V_{max}(1+I/K_I)$, respectively.

The distinction among the types of inhibition is often not easily made based on graphical analysis due to the difficulties associated with the given techniques (see Table 4). For example, exact experimental data is often necessary to conclude that the lines are truly parallel (as in uncompetitive inhibition) rather than weakly intersecting (as in non-competitive inhibition).

The Hunter-Downs plot is another method frequently used to characterize inhibition effects (Dixon and Webb, 1964). This technique depends on the ratio of substrate concentration to inhibitor concentration and thus is less subject to the disadvantages of the reciprocal linearized methods described previously. A Hunter-Downs plot may be constructed by plotting the term $I(\alpha/(1-\alpha))$ against substrate concentration, where α = V(in the presence of the inhibitor)/V(in the corresponding control). A large value of $I(\alpha/(1-\alpha))$ represents a weak inhibitor and a small value indicates strong inhibition. If the inhibition is competitive, the Hunter-Downs plot will slope upward to the right whereas in non-competitive inhibition, the data will lie on a horizontal line. For uncompetitive inhibition, the line should slope downward. Although this technique cannot be used to quantify K_m or K_I, it has its advantages over other methods in identifying the type of inhibition exhibited.

A more accurate determination of K_I can be obtained graphically from reciprocal linearized plots made at different inhibitor concentrations (Dixon and Webb, 1979). If the slopes or intercepts of these lines are plotted against If the slopes or intercepts of these lines are plotted against inhibitor concentration, K_I can frequently be determined directly. The results of such plots for the three classical inhibition mechanisms is shown below:

Type of Inhibition	Plot	intercept on base line
competitive	slope vs. I	K_I
non-competitive	intercept on vertical axis	K_I
un-competitive	intercept on vertical axis	K_I

Alternately, Dixon and Kornberg (1962, as cited in Dixon and Webb, 1979) presented a simple graphical procedure for the determination of K_I. In this method, the velocity of the reaction V is determined with a series of inhibitor concentrations at constant substrate concentration S_1 to yield a straight line when 1/V vs. I is plotted. Then a second series of points are determined at another substrate concentration S_2, yielding a second line which cuts the first line at K_I. This method was found to be applicable for competitive and non-competitive types of inhibition. In the case of un-competitive inhibition, a plot of 1/V vs. I will yield a series of parallel lines at different substrate concentrations making it impossible to determine K_I. Proof of the applicability of this technique is presented by Dixon and Webb (1979).

Specific Inhibitors of Nitrification

The results of many studies performed with pure, mixed or activated sludge cultures have clearly established that nitrifiers are sensitive to a variety of compounds commonly found in wastewaters, especially those of industrial origin. In addition, both nitrifying genera have been reported to be subject to substrate and end product inhibition.

The effect of inhibition on nitrification processes usually implies that of lowered nitrifier specific growth rates and maximum substrate utilization rates. The ramifications of these effects are obvious in relation to wastewater treatment systems, however what might not be as obvious is that the degree of inhibition is contingent upon many factors aside from the mere presence of a particular inhibitor. Painter (1970) provided an overall perspective on nitrification inhibition in this respect. He notes that the degree of effectiveness of an inhibitor depends on:

1. presence of microorganisms other than nitrifiers
2. concentration of the inhibitor
3. duration of exposure to the inhibitor
4. circumstances of exposure (i.e., whether nitrifiers are in a batch or continuous system)
5. presence of other inhibitors which may exert antagonistic or

synergistic effects

In addition, pH and the total concentration of biological solids in the system has been shown to affect the ultimate degree of inhibition expressed in nitrification processes.

Substrate and End Product Inhibition in Nitrification

Both nitrifying organisms are reportedly subject of substrate and product inhibition although the actual inhibitory species have not yet been conclusively identified. The most generally accepted theory of substrate-product inhibition is based upon ammonia/ammonium and nitrite/nitrous acid equilibria in aqueous solutions. Given that such equilibria are dependent upon pH and temperature, it has been difficult to distinguish these effects from that of a concentration change in nitrogen species. According to the theory, aqueous ammonia undergoes the following reaction in water:

$$NH_3(aq) + H_2O \rightleftharpoons NH_4^+ + OH^-$$

with an equilibrium expression of,

$$K_1 = \frac{[NH_4^+]\ [OH^-]}{[NH_3]\ (aq)} \tag{34}$$

for water:

$$K_w = [H^+]\ [OH^-] \tag{35}$$

For practical purposes, the total ammonia is the sum of the ionized and unionized form (free ammonia):

$$[NH_3](total) = [NH_3](unionized) + [NH_4^+] \tag{36}$$

Using equations 33, 35, and 36, the following expression may be derived for the ratio of unionized ammonia to total aqueous ammonia:

$$\frac{[NH_3]\ (unionized)}{[NH_3](total)} = \frac{1}{1 + \frac{(K_1)\ [H^+]}{K_w}} \tag{37}$$

Using the same analysis for nitrite/nitrous acid equilibria, the following expression can be obtained,

$$\frac{[HNO_2]}{[NO_2]\ (total)} = \frac{1}{1 + \frac{(K_w)(K_2)}{[H^+]}} \tag{38}$$

where K_2 = dissociation constant for the

HNO_2/NO_2^- equilibrium

Thus at progressively lower pH's, more and more nitrite is in the form of undissociated nitrous acid (HNO_2). Conversely, at progressively higher pH's increasing amounts of ammonia is in the form of free ammonia (NH_3). For example, at pH 7 only about 0.5% of the total ammonia is in the free form compared to 5% at pH 8. Although it has been reported that nitrifiers are capable of withstanding a wide range of substrate concentrations (i.e., up to 1960 mg/l of NH_3 and 5000 mg/l NO_2^- ; Sharma and Ahlert, 1977) with only slight inhibitory effects, results from

other studies have suggested that the formation of free ammonia or nitrous acid in minute quantities can have a very powerful inhibitory effect.

Boon and Laudelout (1962) studied the influence of nitrite and nitrate concentration on the activity of Nitrobacter and reported that optimal activity occurs at 280 mg/l nitrite, while nitrate inhibited the rate of oxidation non-competitively. This inhibitory effect was shown to follow the substrate inhibition model of Haldane (1930). They also theorized that the depressed rates of oxidation at higher nitrite concentrations was due to the formation on HNO_2 at lower pH's. This theory was subsequently supported by Prakasam and Loehr (1972) and Anthonisen (1976) who reported nitrite oxidation inhibition above HNO_2 concentrations of 0.3 and 0.22 mg/l respectively. Both studies also indicated that free NH_3 was inhibitory to Nitrobacter activity, although Prakasam and Loehr suggested that this effect was less important than that due to HNO_2. Additional observations by Prakasam and Loehr on the removal of inhibitory effects in the presence of denitrification led them to propose that the incomplete ammonia oxidation was due to the end products NO_2^- and NO_3^-.

Anthonisen (1976) used data collected from laboratory batch and continuous nitrifying systems to devise an operational graph for the evaluation of substrate and product inhibition. He proposed that both ammonia and nitrite oxidation were inhibited by free ammonia and nitrous acid by virtue of the substrate's propensity to permeate nitrifier cell walls. He concluded that free NH_3 in the concentration ranges of 0.1 to

1.0 and 10 to 150.0 mg/l was inhibitory to Nitrobacter and Nitrosomonas, respectively and that the tolerances within these ranges depended upon the initial concentration of exposure. Moreover, he reported complete inhibition beyond HNO_2 concentrations of 2.6 mg-N/1.

Neufeld, et. al. (1980), in studies conducted with pure ammonia oxidizers, reported that the inhibitory effects of free NH_3 began at a concentration of 10 mg/1 and that these effects could also be described by Haldane's substrate inhibition model based on classical Michaelis-Menten kinetics:

$$V = \frac{V_{max}}{(1 + \frac{K_m}{S} + \frac{S}{K_I})} \qquad (39)$$

where V = nitrification rate, mg NH_3/mg VSS-day

V_{max} = maximum nitrification rate, mg NH_3/mg VSS-day

K_m = half saturation constant, mg NH_3/1

S = substrate concentration, mg NH_3/1

K_I = inhibition constant, numerically equal to the highest substrate concentration at which $V = 1/2\ V_{max}$.

The values of K_I, based on empirically developed equations were reported to be 500 mg/1 (pH 7); 500 mg/1 (pH 8) and 200 mg/1 (pH 9).

Keenan, et al. (1979) conducted studies in a leachate treatment plant and reported conflicting results with those of the preceding studies. These investigators observed no relationship between the specific

oxidation rate and the concentration of free ammonia, but instead obtained a reasonable fit to the Haldane's inhibition model using ionized ammonium (NH_4^+) as the inhibitory substrate.

To further complicate the subject of substrate inhibition, Kholdebarin and Oertli (1977) reported data from batch nitrification studies with surface waters, suggesting that ionized ammonium exerts a stimulatory effect on nitrification rates. More significantly, their experiments on nitrite oxidation at different pH's in the initial absence of free ammonia demonstrated that elevated pH's (i.e., pH = 9.5) exerted the inhibitory effect rather than the presence of free NH_3. These later results are consistent with those cited previously (Poduska, 1974) and support the contention that the overriding effect seen at higher substrate concentration might be due to a yet unknown mechanism relating to pH in general rather than to the elevated concentrations of free ammonia or undissociated nitrous acids.

Specific Inhibitors of Nitrification in Pure Culture Studies

Many pure culture studies have demonstrated that nitrifiers, especially Nitrosomonas, are sensitive to a large number of inorganic and organic compounds as well as many heavy metals. It has been postulated (Tomilinson, et. al., 1966) that the greater susceptibility of Nitrosomonas may be due to its relatively complicated enzyme system compared to that of Nitrobacter. Much of the reported work on specific inhibitors of nitrification was performed with pure cultures of Nitrosomonas.

Lees (1952) showed that oxygen uptake rates of Nitrosomonas are inhibited in the presence of chelating agents (i.e., histidine, allythiourea, thiourea, 8-hydroquonline) in support of similar results previously obtained with soil organisms (Quastel and Scholefield, 1949). Jensen and Sorensen (1952) studied the effect of a variety of known enzyme inhibitors and herbicides on pure cultures of Nitrosomonas isolated from manure. Their results indicated that the most powerful inhibitors were organic sulfur compounds containing SH-groups (i.e., cysteine; diethyldithiocarbamate) or those giving rise to them through tautomerism (i.e., thiourea). They suggested that the mechanism of toxicity might be due to the inactivation of those enzymes requiring heavy metals such as copper or nickel. Anderson (1965) reported that micromolar concentrations of cyanide inhibited ammonia oxidation whereas thiourea had no effect on hydroxylamine oxidation. This indicated that certain inhibitors can be specific to the pathway of ammonia to nitrite oxidation.

Hooper and Terry (1973) undertook an extensive investigation on ammonia oxidation inhibition in which they categorized a wide range of inhibitors according to its possible mode of action. They presented the grouping of inhibitors as follows:

1. Metal binding compounds- Those compounds which may disrupt enzymatic function due to a strong affinity for essential metals (i.e., copper). A partial listing of these compounds include allythiourea, KCN, Diethyldithiocarbamate as specific inhibitors of ammonia oxidation and NaN_2, Na_3S as inhibitors

to both ammonia and hydroxylamine oxidation.

2. Enzyme and heme-binding compounds- Those compounds which have a high affinity for enzymes of the electron transport chain (i.e., cytochromes or catalysis). These compounds include CO, thiosemicarbazide, diphenylthiocarbazone, N-serve, and ethyl xanthate.

3. Uncouplers of oxidative phosphorylation and inhibitors of electron transport. In bacteria, this group of compounds presumably inhibits one or more of the following: oxidative phosphorylation, ATP-dependent NADP reduction; proton pumping, or ion transport. This group of compounds may consist of 2-4 dinitrophenol, N-N' dicyclohexylcarbodiimide, phenazine methosulfate and 2-6 dichlorophenolindephenol.

4. Short chain alcohols and amines- These compounds are inhibitors of ammonia but not of hydroxylamine oxidation. The possible mode of inhibitory action may be due to the disruption of membrane function and/or reaction with catalysis. Among the more powerful inhibitors studied in the group were methanol, ethanol, n- and i- propanol, methylamine and ethyl acetate.

5. Miscellaneous factors such as N_2O, light and low temperature are also inhibitory to nitrification, although the mode of action is considered distinct from the other categories listed.

In another major study of nitrification inhibition, Hockenbury and Grady (1977), investigated the effect of selected organic compounds on nitrifying cultures with the major objective of identifying and quantifying the effects of industrially significant inhibitors. Their results indicated that of the 52 compounds tested, 20 were inhibitory at a concentration of 100 mg/l and of these 20, 12 compounds contained one or more benzene rings in the structure. The most powerful inhibitors were found to be dodecylamine, aniline and methylaniline, all of which inhibited nitrification 50% or greater at 1.0 mg/l. Among the 13 most industrially significant compounds tested, aniline, ethylenediamine and hexamethylene were reported to be the most potent inhibitors. Only 3 compounds (at 100 mg/l), p-nitrobenzaldehyde; p-nitroaniline and n-methylaniline, all of which were inhibitory to Nitrosomonas, were found to be inhibitory to Nitrobacter. These investigators also characterized the inhibition of aniline, dodecylamine and ethyldiamine, all inhibitors of ammonia oxidation. The results, obtained from Hunter-Downs plots, revealed that all three inhibitors followed a uncompetitive type of inhibition. They suggested that these effects may be related to substrate inhibition.

Neufeld, et al. (1980) evaluated the effect of phenol on a culture of strict nitrifiers using respirometric techniques. The concentrations of phenol tested (2 to 75 mg/l) were found to cause progressive inhibition of ammonia oxidation. In an attempt to characterize inhibition type, a series of experiments was run at various substrate concentrations. Analyzing the resulting data with Lineweaver-Burke plots, they showed that the influence of phenol followed a modified non-competitive

type of inhibition in which K_m' varied with the square root of phenol concentration. In separate experiments run at various inhibitor concentrations, slope and intercept analysis revealed a K_I value of $(2.1 mg/1)^{1/2}$. The overall results were then used to obtain a kinetic expression for relating the MCRT to effluent ammonia concentration for a CSTR activated sludge reactor.

Several investigators have reported the effects of a variety of heavy metals in pure nitrifying cultures. Skinner and Walker (1961) found that chromium and nickel at concentrations of less than 0.25 mg/1 had an inhibitory effect on the growth of Nitrosomonas. Painter and Loveless (1968) demonstrated the inhibition of Nitrosomonas growth by copper, zinc, and cobalt at concentration ranges of 0.05-0.56 mg/1; 0.08-0.5 mg/1 and 0.08-0.5 mg/1, respectively. They noted that although copper is an essential element for Nitrosomonas growth as evidenced by its stimulatory effects at low concentrations (0.005-0.03 mg/1), its presence at higher concentrations can result in complete but reversible (with the addition of EDTA) inhibition. They also provided evidence showing that the toxic effects of heavy metals can be influenced by pH.

Beg, et al. (1982) recently studied on the toxic effects of three inorganic compounds; sodium fluoride, sodium arsenite and potassium dichromate, on nitrifying organisms in a packed bed, fixed-film biological reactor. Their data indicated that the inhibited ammonia oxidation rates, due to all the compounds tested, followed zero-order kinetics and exhibited a non-competitive type of inhibition. Assuming that K_m is much less than S, the investigators reduced the non-competitive equation

to the following form;

$$V = \frac{V_{max} K_I}{K_I + I} = \gamma_i$$

They then used the observations of zero-ordered kinetics and the following equation relating the velocities of uninhibited reaction (γ_o) to the inhibited reaction (γ_i);

$$i = 1 - \frac{\gamma_i}{\gamma_o}$$

to derive an equation for non-competitive zero-ordered nitrification inhibition kinetics;

$$1/i = (K_I/I) + 1.0 \qquad (40)$$

The authors correlated the data using equation 40 and found that the concentrations required to inhibit nitrification 75% were 3654 mg/1 (K_I =1218 mg/1), 876 mg/1 (K_I = 292 mg/1) and 150 mg/1 (K_I= 50 mg/1) for sodium fluoride (as F), sodium arsenite (as As) and potassium dichromate, respectively.

Specific Inhibitors of Nitrification in Activated Sludge

The autotrophic nature of nitrifying organisms led to an earlier belief that organic matter, in general, was inhibitory to nitrification. (see reviews by Painter, 1970 ; Focht and Chang, 1970). It is now generally accepted that this is not the case. Although peptone (1-10 mg/1)

was reportedly inhibitory to Nitrosomonas (Buswell, et. al., 1954), more recent studies (Painter and Jones, 1963; Beccari, et. al., 1980) have indicated that the peptone concentrations studied (up to 60 mg/l) exert no appreciable effect of nitrification rates. Beccari, et al. (1980) extended the study of the effect of carbonaceous substrates on nitrification to include various compounds commonly found in sewage (i.e., glucose, glutamic acid, starch, etc.). They reported no significant effects on nitrification due to the substrates tested.

The earlier observations commonly made of depressed nitrification rates in municipal and industrial wastewater treatment plants can be attributed to unsuitable operating conditions (i.e., low DO or MCRT) rather than to the presence of organic matter (Downing, et al. 1964; Kiff, 1972; Hockenbury and Grady, 1977). Moreover, it has been shown that the presence of organic matter in sewage can have a stimulatory effect on nitrification processes (Hockenbury, et al., 1977). It is becoming increasingly more apparent that the detrimental effects of organic matter on nitrification are specific rather than general.

There have been a number of studies performed to determine the effect of specific inhibitors on nitrification in activated sludge cultures. The most comprehensive study to date is that conducted by Tomilinson, et. al. (1966). A summary of the results of this exhaustive study is presented in Table 10 along with other relevant studies. Also included in this table are the results from pure culture studies cited previously. Several important generalizations can be made from the work of Tomilinson:

Table 10: Effects of Various Compounds on Nitrification

Compound (1)	Concentration (2)	Effect (3)	Reference (4)
2,2',-bipyridine	100_{3}	91%-/AS	Hockenbury and Grady(1977)
N,N,-dicyclohexylcarbodiimide	10^{-4}M	60%-/EC	Hooper et al (1975)
a a,dipyridyl (2,2 - bipyridine)	10^{-4}M	--/EC	Hooper et al (1975)
a a -bis(hexafluoroacetonyl)cyclohexanone	$2x10^{-5}$M	74%-/EC	Hooper et al (1975)
β-diethylaminoethyldiphenylpropylacetate	$5x10^{-5}$M	65%-/EC	Hooper et al (1975)
(4-chloro-o-tolory) acetic acid methozone (as above)	0.01M	98%-7/80	Jensen et al (1952)
1-5-diphenylcarbonhydrazide	100	0%-/AS	Hockenbury and Grady(1977)
1-amino-2-naphtol-4-sulfonic A	100	0%-/AS	Hockenbury and Grady(1977)
1-chloro-4-nitrobenzene	100	0%-/AS	Hockenbury and Grady(1977)
1-isonicotinyl-2-isopropyl-hydrazine (iproniazid)	0.01M	88%-/EC	Hooper et al (1975)
1-naphtylamine	100	81%-/AS	Hockenbury and Grady(1977)
2,4 dinitrophenol	460_{4}	75%-/AS	Tomlinson et al (1966)
2,4-dichloro(6-phenylphenoxy)ethylamine HBr	10^{-4}M	96%-/EC	Hooper et al (1975)
2,4-dichlorophenoxyacetic acid (2-4 D)	0.002M	0/80	Jensen et al (1952)
2,4-dichlorophenoxyacetic acid (2-4 D)	0.005M	61%-7/80	Jensen et al (1952)
2,4-dichlorophenoxyacetic acid (2-4 D)	0.01M	78%-7/80	Jensen et al (1952)
2,4-dichlorophenoxyacetic acid (2-4 D)	0.02M	99%-7/80	Jensen et al (1952)
2,4-dinitrophenol (aldifen)	$2x10^{-4}$M	75%-/EC	Hooper et al (1975)
2,4-dinitrophenol (aldifen)	0.0005M	50%-7/80	Jensen et al (1952)
2,4-dinitrophenol (aldifen)	0.001M	70%-7/80	Jensen et al (1952)
2,4-dinitrophenol (aldifen)	0.001M	87%-7/80	Jensen et al (1952)
2,4-dinitrophenol (aldifen)	0.002M	97%-7/80	Jensen et al (1952)
2,4dichloro-6-phenylphenoxyethyldiethylamine	10^{-4}M	96%-/EC	Hooper et al (1975)
2,6-dichlorophenolindophenol (Tillman's reag)	10^{-3}	--/EC	Hooper et al (1975)
2-amino-3,5-diiodobenzoic acid	100	0%-/AS	Hockenbury and Grady(1977)
2-amino-4-chloro-6-methyl-pyrimidine	10mg/kg	0Acolin/80	Brenner & Bundy (1974)
2-amino-4-chloro-6-methyl-pyrimidine	10mg/kg	34%-5/80	Brenner & Bundy (1974)
2-chloro-6-trichloromethyl-pyridine (N-serve)	0.05	1%-28/80	Goring (1962)
2-chloro-6-trichloromethyl-pyridine (N-serve)	1	95%-28/80	Goring (1962)
2-chloro-6-trichloromethyl-pyridine (N-serve)	10	--	Voets et al (1975)
2-chloro-6-trichloromethyl-pyridine (N-serve)	10	95%-28/80	Goring (1962)
2-chloro-6-trichloromethyl-pyridine (N-serve)	100	75%-/AS	Tomlinson et al (1966)
2-chloro-6-trichloromethyl-pyridine (N-serve)	10mg/kg	--	Voets et al (1975)
2-chloro-6-trichloromethyl-pyridine (N-serve)	0.2	75%-28/80	Goring (1962)
2-methyl pyridine (a picoline)	100	40% Ns A-, Nb A-/AS	Stafford (1974)
2-methyl-4-chlorophenoxyacetic acid	0.005M	87%-7/80	Jensen et al (1952)
2-methyl-4-chlorophenoxyacetic acid	0.01M	98%-7/80	Jensen et al (1952)
2-methyl-4-chlorophenoxyacetic acid	0.002M	40%-7/80	Jensen et al (1952)
3-aminotriazole (amitrole)	10^{-3}M	--/EC	Hooper et al (1975)
4-methyl pyridine (γ-picoline)	100	90% Ns A-,>99%Nb A-/AS	Stafford (1974)
8-hydroxyquinoline (8-quinolinol,oxyquinoline,	72.5_{3}	75%-/AS	Tomlinson et al (1966)
8-quinolinol (8-quinolinol)	10^{-3}M	--/EC	Hooper et al (1975)
EDTA (EDETIC acid)	$>350_{3}$	75%-/AS	Tomlinson et al (1966)
N-serve	$5x10^{-5}$M	86%-/EC	Hooper et al (1975)

N-serve	10	—	Veets et al (1975)
acetamide	100	0/AS	Hockenbury and Grady(1977)
acetone (2-propanone,dimehtyl keytone)	2000.	75%-/AS	Tomlinson et al (1966)
acetone (2-propanone,dimethyl keytone)	0.14M	--/EC	Hooper et al (1975)
acetonitrile	100	0%-/AS	Hockenbury and Grady(1977)
acrylonitrile	100	0%-/AS	Hockenbury and Grady(1977)
alanine	0.01M	0,Acclim	Quastel & Scholenfield (1949)
allyl alcohol (2-propen-1-ol,vinyl carbinol)	19.5	75%-/AS	Tomlinson et al (1966)
allyl chloride (chloroallylene)	100	75%-/AS	Tomlinson et al (1966)
allyl isothiocyanate	1.9	75%-/AS	Tomlinson et al (1966)
allylthiourea (thiosinamine,allylthiocarbamide)	10^{-6}M	82%-/EC	Hooper et al (1975)
aminoethanol (ethanolamine)	0.2M	43%-/EC	Hooper et al (1975)
aminoguanidine (hydrazinecarboximidamide)	10^{-3}M	74%-/EC	Hooper et al (1975)
aniline	100	86%-/AS	Hockenbury and Grady(1977)
aniline (benzenamine,phenylamine)	7.7	75%-/AS	Tomlinson et al (1966)
anthranilic acid	100	0%-/AS	Hockenbury and Grady(1977)
arsenite,Na	$1.3x10^{-2}$M	75%-/AS	Tomlinson et al (1966)
aspartate	0.01M	0,Acclim	Quastel & Scholenfield (1949)
azide, Na(S325)	10^{-3}M	90%-/EC	Hooper et al (1975)
azide, Na(S325)	0.00001M	50%-12/20	Jensen et al (1952)
azide, Na(S325)	0.00001M	50%-7/20	Jensen et al (1952)
azide, Na(S325)	0.00002M	70%-7/20	Jensen et al (1952)
azide, Na(S325)	0.00002M	74%-12/20	Jensen et al (1952)
azide, Na(S325)	0.00005M	70%-12/20	Jensen et al (1952)
azide, Na(S325)	0.00005M	85%-7/20	Jensen et al (1952)
azide, Na(S325)	0.0001M	99%-12/20	Jensen et al (1952)
azide, Na(S325)	0.0001M	99%-7/20	Jensen et al (1952)
azobenzene	100	0%-/AS	Hockenbury and Grady(1977)
benzothiazole disulphide	98	75%-/AS	Tomlinson et al (1966)
benzidine dihydrochloride	100	84%-/AS	Hockenbury and Grady(1977)
benzocaine	100	30%-/AS	Hockenbury and Grady(1977)
benzyl thiuronium chloride	49.	75%-/AS	Tomlinson et al (1966)
benzylamine	100	26%-/AS	Hockenbury and Grady(1977)
brucine	100	0%-/AS	Hockenbury and Grady(1977)
butanol,n (n-butyl alcohol,1-butanol)	0.11M	--/EC	Hooper et al (1975)
butanol,t (ter-butyl alcohol)	0.11M	35%-/EC	Hooper et al (1975)
calcium	0.5-20.	0	Loveless & Painter (1968)
carbon disulfide (carbon bisulfide)	10mg/kg	95%-14/20	Bromner & Bundy (1974)
carbon disulfide (carbon bisulfide)	10mg/kg	97%-5/20	Bromner & Bundy (1974)
carbon disulfide (dithiocarbonic anhydride)	35.	75%-/AS	Tomlinson et al (1966)
chlorate,K (peterate)	0.00001-0.001M	Nb G-,No A-	Loos & Quastel (1945)
chlorate,K (peterate)	0.00001-0.001M	Nb G-,No A-	Veets et al (1975)
chlorate,K (peterate)	0.002M	75%-/AS	Tomlinson et al (1966)
chlorination	0.6-2.0mg/l	+ than EColi	Strom et al (1976)
chlorobenzene	100	0%-/AS	Hockenbury and Grady(1977)
chloroform	18	75%-/AS	Tomlinson et al (1966)
chrome,trivalent	118	75%-AS	Skinner & Walker (1961)
chrome,trivalent	>0.25	No G-	Skinner & Walker (1961)
citric acid	100	0%-/AS	Hockenbury and Grady(1977)
cobalt	0.08-0.5	-	Loveless & Painter (1968)
copper	0.05-0.56	-/EC	Loveless & Painter (1968)
copper	0.05-0.56	No A-	Loveless & Painter (1968)

copper	150.	75%-AS	Tomlinson et al (1966)
copper	4.	0AS	Tomlinson et al (1966)
copper	4.	75%Na A-	Tomlinson et al (1966)
copper with EDTA	0.005-0.48	0	Loveless & Painter (1968)
cresol, meta (3-methylphenol)	11.4	75%-/AS	Tomlinson et al (1966)
cresol, ortho (2-methylphenol)	12.8	75%-/AS	Tomlinson et al (1966)
cresol,para (4-methylphenol)	16.5	75%-/AS	Tomlinson et al (1966)
cyanate, Na	$2.5x10^{-3}$M	75%-/AS	Tomlinson et al (1966)
cyanide, K	$5.x10^{-6}$M	78%-/EC	Hooper et al (1975)
cyanide, Na	$3.5x10^{-3}$M	75%-/AS	Tomlinson et al (1966)
cyanide, Na	0.00001M	17%-7/80	Jensen et al (1952)
cyanide, Na	0.00002M	45%-12/80	Jensen et al (1952)
cyanide, Na	0.00002M	63%-7/80	Jensen et al (1952)
cyanide, Na	0.00005M	55%-12/80	Jensen et al (1952)
cyanide, Na	0.00005M	98%-7/80	Jensen et al (1952)
cyanide, Na	0.0001M	33%-12/80	Jensen et al (1952)
cyanide, Na	0.0001M	69%-12/80	Jensen et al (1952)
cyanide, Na	0.65	75%-/AS	Tomlinson et al (1966)
cyanides	20mg/l		Metcalf & Eddy (1979)
cysteine	0.01M	0,Acclim	Quastel & Scholenfield (1949)
cysteine-HCl (β-mercaptoalanine)	0.0001M	16%-7/80	Jensen et al (1952)
cysteine-HCl (β-mercaptoalanine)	0.0002M	97%-7/80	Jensen et al (1952)
cysteine-HCl (β-mercaptoalanine)	0.0005M	100%-12/80	Jensen et al (1952)
cysteine-HCl (β-mercaptoalanine)	0.01M	100%-7/80	Jensen et al (1952)
cytosine	0.05mg/ml	Mb 0-	Pan (1981)
di-allyl ether	100	75%-/AS	Tomlinson et al (1966)
dicyandiamide (cyanoguanidine)	250	75%-/AS	Tomlinson et al (1966)
diethanolamine	100	0%-/AS	Heckenbury and Grady(1977)
diethylamine	100	0%-/AS	Heckenbury and Grady(1977)
diethyldithiocarbamate (diethylcarbamodithioic acid)	10^{-3}M	--/EC	Hooper et al (1975)
diguanide (biguanide)	50	75%-/AS	Tomlinson et al (1966)
dimethyl-p-nitrosoaniline (neocelorine)	19	75%-/AS	Tomlinson et al (1966)
dimethylamine HCl	100	0%-/AS	Heckenbury and Grady(1977)
dimethylaminoazobenzene	100	0%-/AS	Heckenbury and Grady(1977)
dimethylglexime	100	30%-/AS	Heckenbury and Grady(1977)
dinitrocthocresol (3,5-dinitro-2-hydroxytoluene)	0.0002M	0/80	Jensen et al (1952)
dinitrocthocresol (3,5-dinitro-2-hydroxytoluene)	0.0005M	61%-7/80	Jensen et al (1952)
dinitrocthocresol (3,5-dinitro-2-hydroxytoluene)	0.001M	97%-7/80	Jensen et al (1952)
diphenyl guanidine	50.	75%-/AS	Tomlinson et al (1966)
diphenylamine	100	0%-/AS	Heckenbury and Grady(1977)
diphenylcarbazone	100	0%-/AS	Heckenbury and Grady(1977)
diphenylthiocarbazone (phenyldiazenecarbothioic A)	$9x10^{-3}$M	50%-/EC	Hooper et al (1975)
dithio oxamide (ethane dithioamide)	1.1	75%-/AS	Tomlinson et al (1966)
dithiocarbamate,Na cyclopentamethylene	23	75%-/AS	Tomlinson et al (1966)
dithiocarbamate,Na diethyl	0.000002M	33%-7/80	Jensen et al (1952)
dithiocarbamate,Na diethyl	0.000004M	66%-7/80	Jensen et al (1952)
dithiocarbamate,Na diethyl	0.00002M	100%-7/80	Jensen et al (1952)
dithiocarbamate,Na diethyl	0.00002M	99%-12/80	Jensen et al (1952)
dithiocarbamate,Na dimethyl	13.6	75%-/AS	Tomlinson et al (1966)
dithiocarbamate,Na methyl	0.9	75%-/AS	Tomlinson et al (1966)
dithiocarbamate,dimethyl NH4 dimethyl	19.3	75%-/AS	Tomlinson et al (1966)
dithiocarbamate,piperidinium cyclopentamethylene	57	75%-/AS	Tomlinson et al (1966)

dithiol	10^{-3}M	--/EC	Hooper et al (1975)
dl-methionine	0.0002M	50%-7/90	Jensen et al (1952)
dl-methionine	0.0005M	72%-7/90	Jensen et al (1952)
dl-methionine	0.001M	93%-12/90	Jensen et al (1952)
dl-methionine	0.001M	86%-7/90	Jensen et al (1952)
dl-methionine	0.002M	90%-7/90	Jensen et al (1952)
dl-methionine	0.004M	98%-7/90	Jensen et al (1952)
dodecylamine	100	96%-/AS	Hockenbury and Grady(1977)
ethanol	0.09M	--/EC	Hooper et al (1975)
ethanol	2400.	75%-/AS	Tomlinson et al (1966)
ethyl acetate (acetic acid ethyl ester)	0.23M	--/EC	Hooper et al (1975)
ethyl urethane (urethan)	1750.	75%-/AS	Tomlinson et al (1966)
ethyl xanthate (xanthogenate)	10^{-4}M	--/EC	Hooper et al (1975)
ethylenediamine	100	75%-/AS	Hockenbury and Grady(1977)
ethylurethane	0.1%	--18/meso	Quastel & Scholenfield (1949)
glutamate	0.01M	0,Acclim	Quastel & Scholenfield (1949)
glycine	0.01M	0,Acclim	Quastel & Scholenfield (1949)
guanidine carbonate (carbamamidine)	16.5	75%-/AS	Tomlinson et al (1966)
guanine (4416)	0.05mg/ml	Nb G-	Pan (1981)
hexamethylene diamine	100	52%-/AS	Hockenbury and Grady(1977)
hexamethylenetetramine	100	0%-/AS	Hockenbury and Grady(1977)
hydrazine	58.	75%-/AS	Tomlinson et al (1966)
l-arginine	4mg/l	Ns A-,Ns G-	Clark & Schmidt (1967a)
l-histidine	100mg/l	Ns A-	Gunderson (1955)
l-histidine	4mg/l	Ns A-,Ns G-	Clark & Schmidt (1967a)
l-lysine (2,6-diaminohexanoic A)	1460mg/ml	Ns G-	Pan (1981)
l-lysine (2,6-diaminohexanoic A)	4mg/l	Ns A-,Nb G-	Clark & Schmidt (1967a)
l-methionine	0.0005-0.01M	--16/meso	Quastel & Scholenfield (1949)
l-methionine	4mg/l	Ns A-,Ns G-	Clark & Schmidt (1967a)
l-threonine	1200 mg/ml	Ns G-	Pan (1981)
l-threonine	4mg/l	Ns A-,Ns G-	Clark & Schmidt (1967a)
l-valine (2-aminoisovaleric A)	4mg/l	Ns A-,Ns G-	Clark & Schmidt (1967a)
m-chlorocarbonylcyanidephenylhydrazone	10^{-5}M	83%-/EC	Hooper et al (1975)
magnesium	50-100	-	Loveless & Painter (1968)
melamine	100	0%-/AS	Hockenbury and Grady(1977)
mercaptobenzothiazole (MBT,2-Benzothiazolethiol)	3	75%-/AS	Tomlinson et al (1966)
methanol	$5x10^{-2}$M	--/EC	Hooper et al (1975)
methionine	0.01M	0,Acclim	Quastel & Scholenfield (1949)
methyl isothiocyanate (iso thiocyanatomethane)	0.8	75%-/AS	Tomlinson et al (1966)
methyl thiuronium sulphate	6.5	75%-/AS	Tomlinson et al (1966)
methylamine (aminomethane)	0.01M	82%-/EC	Hooper et al (1975)
methylamine HCl	1550	75%-/AS	Tomlinson et al (1966)
methylene blue	10^{-4}M	--/EC	Hooper et al (1975)
methylene blue	100.	75%-/AS	Tomlinson et al (1966)
monoethanolamine	100	16%-/AS	Hockenbury and Grady(1977)
n-methylaniline	100	90%-/AS	Hockenbury and Grady(1977)
naphthylethylenediamine diHCl	100	93%-/AS	Hockenbury and Grady(1977)
nickle	0.00004M	75%-/AS	Tomlinson et al (1966)
nickle	0.0004M	75%-/AS	Tomlinson et al (1966)
ninhydrin	100	30%-/AS	Hockenbury and Grady(1977)
p-aminobenzoic acid	100	0%-/AS	Hockenbury and Grady(1977)
p-aminopropiophenone	100	80%-/AS	Hockenbury and Grady(1977)

p-diphenylamine-H^2SO_4	100	0%-/AS	Hockenbury and Grady(1977)
p-nitroaniline	100	64%-/AS	Hockenbury and Grady(1977)
p-nitrobenzaldehyde	100	76%-/AS	Hockenbury and Grady(1977)
p-phenylazoaniline	100	54%-/AS	Hockenbury and Grady(1977)
pantothenic acid (vit. B)	0.05mg/ml	Nb G-	Pan (1981)
phenazine methosulfate	$5x10^{-3}$M	--/EC	Hooper et al (1975)
phenol	100	0,Acclim,AS	Stafford (1974)
phenol	5.6	75%-/AS	Tomlinson et al (1966)
phenol,o-,m-,p-cresols	100	0,Acclim,AS	Stafford (1974)
phenolpyridine)			
phloridzin	0.004M	12%-12/20	Jensen et al (1952)
phloridzin	0.004M	66%-7/20	Jensen et al (1952)
phloridzin	0.005M	85%-12/20	Jensen et al (1952)
phloridzin	0.005M	99%-7/20	Jensen et al (1952)
polymyxin B	10^{-4}U/ml	--/EC	Hooper et al (1975)
potassium thiocyanate (potassium sulfocyanate)	>300	75%-/AS	Tomlinson et al (1966)
propanol,i	0.13M	81%-/EC	Hooper et al (1975)
propanol,n	0.33M	--/EC	Hooper et al (1975)
propylamine	100	0%-/AS	Hockenbury and Grady(1977)
purines	0.05mg/ml	Nb G-	Pan (1981)
pyridine	100	50% Ns A-,>95% Nb A -/AS	Stafford (1974)
pyrimidines (metadiazine)	0.05mg/ml	Nb G-	Pan (1981)
pyruvate (2-oxopropanoic A)	0.04mg/ml	Nb G-	Pan (1981)
pyruvate (2-oxopropanoic A)	>0.00005	Nb G-	Pan (1981)
rhodamine B	100	0%-/AS	Hockenbury and Grady(1977)
sec-butylamine	100	0%-/AS	Hockenbury and Grady(1977)
skatole (3-methyl-1H-indole)	7.0	75%-/AS	Tomlinson et al (1966)
sodium azide(E325)	23.	75%-/AS	Tomlinson et al (1966)
sodium citrate	100	0%-/AS	Hockenbury and Grady(1977)
sodium flouride	0.02M	0%-7/20	Jensen et al (1952)
sodium flouride	0.03M	75%-7/20	Jensen et al (1952)
sodium flouride	0.05M	99%-7/20	Jensen et al (1952)
sodium flouride	0.05M	99%-7/20	Jensen et al (1952)
sodium iodoacetate	0.000002M	22%-7/20	Jensen et al (1952)
sodium iodoacetate	0.000005M	92%-7/20	Jensen et al (1952)
sodium iodoacetate	0.00001M	96%-12/20	Jensen et al (1952)
sodium iodoacetate	0.00001M	99%-7/20	Jensen et al (1952)
sodium sulfide	10^{-4}M	--/EC	Hooper et al (1975)
stearic acid	100	0%-/AS	Hockenbury and Grady(1977)
streptomycin	400.	75%-/AS	Tomlinson et al (1966)
strychnine HCl	175.	75%-/AS	Tomlinson et al (1966)
sulfamic Acid	100	0%-/AS	Hockenbury and Grady(1977)
sulfanilamide	100	0%-/AS	Hockenbury and Grady(1977)
sulfathiazole	10mg/kg	0Acclim	Brenner & Bundy (1974)
sulfathiazole	10mg/kg	33%-5/20	Brenner & Bundy (1974)
tannic acid	100	20%-/AS	Hockenbury and Grady(1977)
tannin & derivatives, phenolic acids , flavonoids	10^{-6}--10^{-8}	--	Rice & Pancholy (1974)
taurine (2-aminoethanesulfonic A)	0.05M	99%-7/20	Jensen et al (1952)
taurine (2-aminoethanesulfonic A)	0.075M	66%-12/20	Jensen et al (1952)
taurine (2-aminoethanesulfonic A)	0.075M	66%-7/20	Jensen et al (1952)
tetra chlorosalicylanilide	10^{-5}M	5%-/EC	Hooper et al (1975)
tetramethyl thiuramdisulphide (thiram)	30	75%-/AS	Tomlinson et al (1966)

tetramethyl thiurammonosulphide	16.	75%-/AS	Tomlinson et al (1966)
thiamine	5mg/ml	No A-	Gunderson (1955)
thioacetamide (ethanethioamide)	0.5$	75%-/AS	Tomlinson et al (1966)
thiosemicarbazide (hydrazinecarbothioamide)	10^{-3}M	95%-/EC	Hooper et al (1975)
thiosemicarbazide (hydrazinecarbothioamide)	0.1$	75%-/AS	Tomlinson et al (1966)
thiourea (thiocarbamide)	0.000002M	98%-7/SO	Jensen et al (1952)
thiourea (thiocarbamide)	0.000005M	98%-12/SO	Jensen et al (1952)
thiourea (thiocarbamide)	0.0003M	--16/mo	Quastel & Scholenfield (1949)
thiourea (thiocarbamide)	0.01M	75%-/meso	Quastel & Scholenfield (1949)
thiourea (thiocarbamide)	0.076	75%-/AS	Tomlinson et al (1966)
tributylamine	100	0%-/AS	Hockenbury and Grady(1977)
triethanolamine	100	0%-/AS	Hockenbury and Grady(1977)
triethylamine	100	35%-/AS	Hockenbury and Grady(1977)
trimethylamine (N-N-dimethylmethanamine)	11$	75%-/AS	Tomlinson et al (1966)
urea	100	0%-/AS	Hockenbury and Grady(1977)
vitamins,b6,b12,etc.	0.05mg/ml	Nb G-	Pan (1981)
zinc	0.08-0.5	-	Loveless & Painter (1968)

all concentrations in mg/l unless otherwise noted. Alternate names are in paranthesis and separated by commas.

A in the compound field is an abbreviation for acid

Key for symbols in "EFFECT" column.

- inhibitory -- completely inhibitory, no nitrification -n or --n where n is a number corresponds to inhibitory or completely inhibitory after n days exposure percent preceeding the "-" sign indicates level of inhibition No G or Nb G preceeding the "-" indicates inhibition of growth of Nitrosomonas or Nitrobacter, respectively.
No A or Nb A preceeding the "-" indicates inhibition of activity of Nitrosomonas or Nitrobacter, respectively.
/AS measurements made in activated sludge
/EC measurements made in an enrichment culture
/PC measurements made in pure culture
/SO measurements made in soil

1. The most inhibitory class of compounds were found to be nitrogenous organosulfur compounds such as thiourea, thioacetamide and dithioxide. These compounds generally inhibited the oxidation of ammonia as opposed to hydroxylamine or nitrite oxidation.

2. Although the short term effects of inhibitory compounds on ammonia oxidation were found to be similar to that reported for pure culture work, there were considerable differences in tolerances to cyanide and heavy metals. In this respect activated sludge cultures exhibited a much greater tolerance.

3. Except for cyanide, the compounds found to be powerful inhibitors of Nitrosomonas had relatively little effect on Nitrobacter. However, chlorate, hydrazine, and sodium azide were more toxic to Nitrobacter than Nitrosomonas.

4. The compounds studied generally appeared to follow the non-competitive mode of inhibition.

5. The effect of mixtures of inhibitors was generally greater than the effects of individual compounds.

6. Activated sludges have the ability to acclimate to certain inhibitory substances (i.e., thiourea, cyanide) and this ability may be related to the initial concentration to which the sludge is exposed.

7. Short term inhibitory tests do not generally give a good

indication of actual conditions in a treatment plant. Acclimation to a "powerful" inhibitors can result in much lower effects under treatment plant conditions. Conversely, heavy metals may exert a more severe effect, possibly due to the exhaustion of the adsorptive capacity of organic matter.

8. The inhibitory effects of the compounds examined varied from day to day according to the nitrification capability of the sludge.

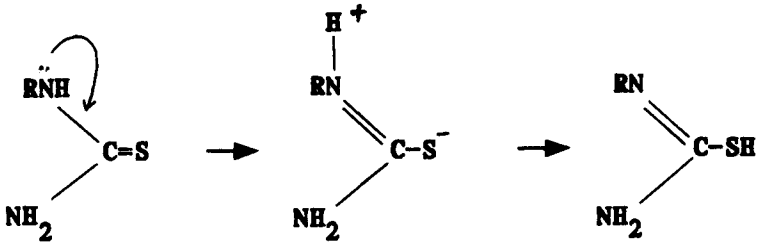
Since the work of Tomilinson, et. al. (1966), a number of studies on specific inhibitors in activated sludge have been reported, each of which have supported one or more of the points cited above. Stafford (1974) investigated the effect of phenolic compounds on continuous nitrifying activated sludge units using ammonium thiocyanate (500 mg/1) as feed. His results showed that activated sludges were capable of nitrifying ammonium thiocyanate and that the addition of 10 mg/1 phenol completely inhibited ammonia oxidation while nitrite oxidation was unaffected at phenol concentrations up to 100 mg/1. Although the reported concentration of phenol required to produce 75% inhibition was the same as that reported by Tomilinson, the required concentrations of o, m and p-cresols were approximately 3 times lower. They also provided evidence for acclimation of activated sludge to nitrifier inhibitors and showed that acclimated sludges handled shock loads of phenol (up to 100 mg/1) much more readily than unacclimated sludges.

Joel and Grady (1977) examined the nitrifying ability of activated sludge in a series of experiments using continuous flow and fill and draw reactors receiving aniline as feed. In the continuous flow experiments, where aniline eventually served as the sole carbon/nitrogen source for organism growth, it was shown that a MCRT of 7 days was sufficient to remove any inhibitory effect aniline had on the nitrifiers. They reported that COD removal and nitrification could occur simultaneously provided that the heterotrophs had degraded aniline to a non-inhibitory concentration. The authors noted however, that these results were obtained under carefully controlled steady state conditions. Complete inhibition of nitrification occurred if the reactor was subjected to a shock load of aniline, raising the concentration in the reactor a few mg/l. In the fill and draw experiments, it was shown that, within the range of 4 to 13 days, MCRT had no effect on aniline degradation, although longer MCRT resulted in greater nitrification efficiencies. In these experiments aniline degradation and nitrification were found to be sequential with nitrification commencing as soon as aniline was reduced to non-inhibitory concentrations. Furthermore, it was observed that the metabolic by-products of aniline degradation enhanced the rates of nitrification in separate stock cultures of nitrifiers. The overall results in the study demonstrated that a severely inhibitory compound such as aniline could be degraded sufficiently to allow nitrification to occur if the proper conditions were met.

Wood, et al. (1981) examined the toxic effects of various compounds on nitrifying activated sludge in batch test procedures similar to that used by Tomilinson. They noted, as Tomilinson did, that the

inhibitory effects for a given compound varied from batch to batch. Thus, their data, which are also presented in Table 10, represent mean effects from several tests. They also observed that in many cases the total inorganic nitrogen at the end of the test period varied directly with the extent of nitrification. There was a loss of inorganic nitrogen during the aeration period of the test and this loss was found to be proportional to the extent of nitrification. The authors discussed several mechanisms by which this loss of inorganic nitrogen could be accounted for and concluded that the most likely mechanism involves the reduction of nitrite by the copper containing enzyme hydroxylamine nitrite reductase , previously isolated by Hooper (1968). The investigators also presented evidence in support of the theory of enzymatic copper chelation as being the major mechanism of inhibition by thiourea and its analogues.

According to this theory, the inhibitory mechanism operates via a reaction of the thiol tautomer with copper of an essential enzyme resulting in the formation of a stable Cu-S bond:



Wood reasoned that if this theory holds, the extent of inhibition should be proportional to the electron density of the sulphur atom. This, in turn should be inversely proportional to the -I (inductive) effect of

substituents (i.e., those with a tendency to withdraw electrons) on the nitrogen atom. Using both Tomlinson's experimental data as well as their own, the investigators found these postulates to be valid.

Braam and Klapwijk (1981) recently presented results on the effect of added copper on nitrification rates in activated sludge with different mixed liquor concentrations and different pH's. These results showed that the inhibitory effect of copper is dependent upon the free copper concentration in the system rather than the total copper concentration. The free copper concentration in activated sludges was demonstrated to be strongly correlated to pH and sludge concentration, with the free copper concentration increasing as pH and/or sludge concentration decreased. They suggested that these findings could not only explain the effect of pH on nitrification by means of heavy metals (as cited originally by Painter and Loveless, 1968) but also the wide range of reported tolerances to heavy metal inhibition in the literature.

PAC-ENHANCED NITRIFICATION

Among the first reports of enhanced nitrification in the powdered activated carbon-activated sludge process was that made by Stenstrom and Grieves (1977), who reported improved nitrification in 42 liter reactors operated over a 15-month period at the Amoco Oil Texas City Refinery. The objective of their investigations was to determine the quality of an experimental powdered carbon produced from petroleum coke. They reported that PAC reactors nitrified much more reliably than non-PAC reactors, producing a mean effluent NH_3-N concentration of less than 1.0 mg/l. The

non-PAC reactors produced a mean effluent concentration of 5.1 mg/1, while the influent NH_3-N was 19.8 mg/1. The relative improvement in ammonia oxidation was much greater than the improvement in removal in other contaminants.

Subsequent tests (Grieves, et. al. 1979) showed that the PAC reactors did not always nitrify better than non-PAC reactors. Over a summer period at the Texas City Refinery non-PAC and PAC reactors produced virtually identical nitrification rates; however, over the next cool period the PAC reactors nitrified more efficiently. During the latter phases of this research a reactor was operated with catalytic-cracker catalyst "fines" added in lieu of activated carbon. The nitrification efficiency of this reactor, and the nitrification efficiency of another reactor using a very poorly performing carbon, were no better than the efficiency of the non-carbon control reactor. They concluded that a probable mechanism of nitrification enhancement was adsorption of inhibitory compounds, since reactors operating with non-adsorbing suspended solids nitrified no better than the control reactor. They also noted that the enhancement was most observable under microbiologically stressed conditions, such as lower temperature, or higher influent chemical oxygen demand (COD).

Further experiments at the Amoco Wood River Refinery in larger scale reactors (pilot scale, approximately 10,000 liter reactors) demonstrated less nitrification enhancement; however, the general level of nitrification in the pilot reactors at this refinery was always better than the results found previously at the Texas City Refinery. The non-

carbon reactors produced mean NH_3-N effluent concentrations less than 1.0 mg/1. Therefore it would be difficult to enhance nitrification.

Other investigators have reported variable findings with respect to improved nitrification with PAC addition in activated sludge. Thibault, et. al. (1977) investigated the claims of increased treatment efficiencies due to PAC addition with 4 liter bench-scale refinery wastewater fed activated sludge units. Their objective was to evaluate different carbon types and treatment performance using various carbon doses. They reported marginally improved organic removals at high carbon levels regardless of carbon dose. Both the carbon and non-carbon reactors did not nitrify throughout the study period. Their results on shock organic loads experienced throughout the test period indicated that certain carbons (i.e., wood-based units) are able to withstand and recover from upsets better than other carbons (i.e., lignin or coke based units). In addition, their results suggested that PAC addition could have detrimental effects on treatment efficiency due to desorption of accumulated toxic compounds during shock loads.

Bettens (1979) conducted industrial tests with the addition of PAC to an activated sludge plant treating textile-finishing plant wastewaters. His results, based on observations "before and after PAC addition" over a three year study period, showed that almost complete and stable nitrification efficiencies were achieved only after many months of uninterrupted PAC dosing (50-100 mg/1 influent). Moreover, denitrification ability was found to improve in the single stage reactor. Bettens attributed the increased stability of the nitrification/ denitrifi-

cation system to the "high concentration of nitrifying organisms attached to the carbon particles".

In treatability studies of chemical production wastewaters, Leipzig (1980) reported that stable nitrification efficiencies were possible in PAC pilot reactors operating at high MCRT; whereas nitrification was found to be variable and less efficient in parallel non-PAC reactors. The major portion of this study was divided into two test periods devoted to testing the effect of hydraulic retention time (HRT) and carbon dosage on nitrification efficiency. A general increase in nitrification efficiency with HRT and carbon dosage was noted in the first test period, however, this relationship deteriorated during the second test period. Nitrification was found to be most efficient in a reactor operating at an intermediate HRT (2 days) and carbon dose (167 mg/l influent) in the latter period. In all cases, the PAC reactors outperformed the parallel control reactors in terms of nitrification efficiency. Leipzig suggested that this was due to the adsorption of inhibitory compounds and/or nitrifier attachment to the carbon surface.

In the same report, Leipzig performed additional experiments on PAC-AS reactors to examine the effects of cold temperature and equalized and unequalized wastewater feed over an extended period of time. A temperature shock from 22° C to 7° C over 24 hours resulted in the complete cessation of nitrification within 16 days; whereas nitrification in a similarly operating reactor gradually subjected to the same temperature decrease over 90 days was unaffected. In two separate experiments, conducted in series over an eleven month period, a PAC-AS reactor achieved

an average nitrification efficiency of 97% with equalized wastewaters as feed compared to 82% with unequalized wastewater. Unfortunately, the latter series of experiments are less convincing evidence for PAC enchanced nitrification since no control reactor was operated for the purpose of comparison.

Specchia and Gianetto (1984) recently demonstrated increased nitrification/denitrification capacity due to PAC addition in a full scale industrial plant treating dye-works wastewater. Their comparisons were based on "before and after PAC addition" to the aeration basin (800 mg/l reactor concentration) over a four month period. The investigators suggested that observed results were due to either low dissolved oxygen concentrations at the bottom of the aeration basin created by PAC addition or to the high concentration of nitrifying-denitrifying bacteria on the carbon surface.

In summary, previous reports have provided strong evidence to indicate that the addition of PAC can result in improved nitrification, especially under "stressful conditions" such as low temperature and/or the presence of inhibitory compounds. Although mechanisms such as adsorption of toxic compounds and preferential surface growth have been suggested for explaining PAC enchanced nitrification, no studies have attempted to define the mechanism by which the enhancement phenomenon occurs.

Proposed Mechanisms for PAC-Enhanced Nitrification

Various mechanisms for explaining PAC-enhanced nitrification have been proposed. All are possible and in fact it is quite difficult to disprove any mechanism, or to show that one mechanism is dominant. The following sections describe proposed mechanisms.

Adsorption of Inhibitory Compounds

As shown previously many compounds inhibit nitrification. Some of these compounds are biologically resistant while others are resistant as well as highly adsorbable by activated carbon. Therefore, it is likely that the presence of activated carbon protects the nitrifiers from the adsorbable compounds. Evidence to support this theory was demonstrated to a limited degree in the previous work of Stenstrom and Grieves (1977) in that non-adsorbing suspended solids added to the reactors did not enhance nitrification. However, one could argue that the same carbon characteristics which promote adsorption of organic compounds also promote attachment of nitrifying bacteria.

Preferential Microbial Attachment

In the early days of anaerobic digestion it was thought that suspended solids, in the form of an inorganic inert material such as soda ash or calcium carbonate, were required as a medium to grow the anaerobic bacteria (Buswell and Hatfield, 1939). This theory may have also been applied to nitrifiers because the earliest isolations of nitrifiers in pure culture were always made on precipitates such as calcium

carbonate (see Nutritional Requirements). This theory applied to anaerobic digesters still has its followers today, and inert materials ("Digestaid") are frequently added to "sour" or "stuck" digesters.

The existence of surface area in the mixed liquor may promote stronger floc—organism associations which retain the nitrifying organisms longer in the activated sludge reactor.

Enhanced Bioactivity

It has been proposed that activated carbon may actually concentrate trace elements or nutrients at its surface which may stimulate microorganism growth. There is very little experimental evidence to support this theory, but it is plausible. For example, it is known that some inhibitors, such as thiourea, inhibit nitrification by complexing free copper, effectively "starving" the organisms of copper. The presence of carbon may provide a mechanism of overcoming this type of inhibition.

Bioregeneration

"Bioregeneration" is a term which is being used by some researchers to describe the synergism which is often observed in PAC reactors. Various researchers have noted that for certain cases a PAC reactor removes an organic compound more efficiently than would be expected from either adsorption or biodegradation alone. Explanatory theories have been proposed which require that the compound first be adsorbed to the carbon surface, making it available to the microorganisms at higher

concentrations than would be expected in the mixed-liquor, thereby stimulating biological growth.

This theory is questionable, since it is not known whether the adsorbed compounds are actually available to the microorganisms. Furthermore the theory's applicability to enhanced nitrification is even more questionable, since adsorption of ammonia at the pH and concentration found in wastewaters is negligible; however, it has been shown that certain amino acids and growth factors required by nitrifying bacteria need not be synthesized by the cell if they are present in solution because they can be transported through the cell walls (Clark and Schmidt, 1967; Delwiche and Finstein, 1965). This is in direct contradiction to the known strict chemoautotrophy metabolism of the nitrifying bacteria and the phenomenon is not well-documented or accepted (Ida and Alexander, 1965) however, if true, carbon could be stimulatory to the nitrifiers by concentrating these growth factors and making them more available. Bioregeneration may also be important in the acclimation of heterotrophic microorganisms to certain slowly biodegradable inhibitors of nitrification. Once adsorbed, these compounds are in contact with the biomass for a length of time equal to the system's MCRT. This would allow more time for heterotrophic acclimation and ultimately for the removal of nitrifier inhibition.

The various mechanisms discussed are all realistic ways of explaining carbon-enhanced nitrification. The most plausible is the adsorption of toxic organics theory based upon the previous work, but other theories may still be important.

CHARACTERISTICS OF ACTIVATED CARBONS

There are various ways of describing activated carbons. These methods have arisen over the years from operational tests performed by various industries using carbon in manufacturing processes. Many of the tests are strictly empirical, while other are based upon isotherm results. The need for tests to describe carbon arises because manufacturing techniques and carbon sources vary considerably, producing carbons with very different properties and activities. The proliferation of the various tests resulted because industries have rather special needs which can only be quantified by specific tests. Carbons tend to be classified by the test results.

Two broad classifications are granular and powdered. Granular carbons generally are retained by 40 mesh or smaller screens, while powdered carbons generally pass 100 mesh screens. The mesh size is an indicator of the distribution of surface area available to adsorption. Granular carbon has the vast majority of its surface area in the interstices of the particle, while the majority of powdered carbon's surface area is on or near the particle's true outside surface. This distribution of areas affects kinetics, and powdered carbon is used in applications which require the most rapid rates of adsorption. An example is in potable water treatment where powdered carbon is added to flocculators and mixers and subsequently removed by filters. The contact time for removal in this application is quite short, thereby requiring rapid reaction rates for efficient operation.

Isotherms

Isotherms are the most common way of describing the ability of carbon to adsorb a chemical substance. There are several types, but the most commonly used are the Langmuir, Freundlich, and the Branauer, Emmett, and Teller (BET) isotherms.

Langmuir

The Langmuir isotherm is based upon physical adsorption theory and is described as follows:

$$q_e = \frac{Q\ b\ C_e}{(1 + bC_e)} \tag{41}$$

where:

q_e = adsorbed material (mass material/ mass carbon)

Q = maximum value of q as C_e approaches ∞

C_e= equilibrium concentration of the material (mass/volume)

b = experimentally determined constant, equaling the rate constant for adsorption divided by the rate constant for desorption.

To estimate the values of Q and b, the isotherm is frequently plotted in "linearized" form, as follows:

$$\frac{1}{q_e} = \frac{1}{Q\ b\ C_e} + \frac{1}{Q} \tag{42}$$

This equation can be plotted as a straight line if $\frac{1}{q_e}$ is plotted against $\frac{1}{C_e}$. The slope of the best-fit straight line through the data points is $\frac{1}{Q\ b}$ and the intercept is $\frac{1}{Q}$. This is the commonly accepted method of

parameter estimation for this isotherm and others (with modification of the linearized equation). However, it has been shown that the use of the reciprocal form biases the data and parameter estimates, which is the same problem encountered with the estimation of growth kinetics parameters. Fortunately, adsorption data are usually easier to collect than biological growth data, and a variety of computer-aided parameter estimation techniques are available (Sweeny, et. al. 1982).

Freundlich Isotherm

The Freundlich Isotherm is an empirical isotherm which makes it less desirable from a theoretical viewpoint, but it often describes the experimental isotherm more accurately. For this reason it finds more frequent application in environmental engineering. The isotherm is described as follows:

$$q_e = K\, C_e^{\frac{1}{n}} \tag{43}$$

where

K and $\frac{1}{n}$ are experimentally described parameters.

The linearized form of the isotherm is as follows:

$$\ln(q_e) = \ln(K) + \frac{1}{n} \ln(C_e) \tag{44}$$

This equation is very easily plotted as a straight line on log-log graph paper, with the slope of the best fit line approximating $\frac{1}{n}$. To define K one must also define the units of C_e. For environmental

engineering work, C_e is most frequently defined in units of mg/l. Again, the comments made earlier about bias and the method of Sweeny et. al. (1982) apply to parameter estimation for this isotherm.

BET

The BET isotherm can be thought of as a multilayer Langmuir isotherm and takes the following form:

$$q_e = \frac{BC_eQ^o}{(C_s - C_e)\left[1 + (B - 1)\frac{(C_e)}{(C_s)}\right]} \tag{45}$$

where:

C_s = saturation concentration of solute

Q^o = moles of solute adsorbed at equilibrium concentration when a complete monolayer of adsorbent is formed.

B = experimentally determined parameter relating to the energy of interaction of the surface and solute.

The linearized form is as follows:

$$\frac{C_e}{(C_s - C_e)\,q_e} = \frac{1}{B\,Q^o} + \left[\frac{B - 1}{B\,Q^o}\right]\left[\frac{C_e}{C_s}\right] \tag{46}$$

The BET isotherm is of major importance in defining activated carbon properties. The surface areas of various carbons are determined by the BET isotherm of nitrogen adsorption.

Specific Indicators of Carbon Properties

As mentioned previously there are many indicators of carbon efficacy. The commonly used ones are tabulated as follows:

1. BET specific surface area: The BET surface area, usually reported as m^2/g carbon, is calculated from the adsorption of N_2. The surface area can be used to directly compare carbons; however, the adsorption of compounds on different carbons is usually not the same, resulting in different quantities adsorbed per unit of surface area.

2. Pore volume: The pore volume is usually reported in cm^3/gram and is usually reported for two pore sizes: less that 15 $\mathring{A}$ and greater than 15 $\mathring{A}$. The volume of larger pores is important when trying to remove contaminates with greater molecular weights.

3. Phenol Number: The phenol number is primarily used in water treatment to evaluate a carbon's potential for removing phenol from drinking waters (phenols form chlorophenols during disinfection by chlorine; therefore it is highly desirable to remove phenols prior to chlorination in order to reduce the health effects and taste and odor problems associated with chlorophenols). The phenol number is amount of carbon in mg/l required to reduce a phenol solution from 100 micromoles/l to 10 micromoles/l in 30 minutes.

4. Iodine number: The iodine number is the amount of iodine, in milligrams, adsorbed per gram of carbon when the equilibrium concen-

tration (C_e) of iodine is 0.02N. Therefore the iodine number is a point on the experimental isotherm.

5. Molasses number: The molasses number relates to the ability of a carbon to remove high molecular weight, color producing materials from molasses. The test requires the use of a standard carbon with a known or specified molasses number. Molasses samples are then decolorized with specified weights of each carbon, and the optical density of the decolorized samples is measured. The molasses number is calculated as follows:

$$Mn_{\text{test carbon}} = Mn_{\text{known carbon}} \times \frac{\text{Optical Density known carbon}}{\text{Optical Density test carbon}} \qquad (47)$$

6. Methylene Blue number: The methylene blue number is similar to the iodine number is that it is a point on the experimental isotherm, numerically equal to the milligrams adsorbed per gram of carbon when the equilibrium concentration is 1.0 mg/l.

Some typical carbons and an experimental carbon were described by Grieves, et. al. (1980). Table 11 shows two carbons. Carbon B is very similar to the carbon used in this study.

Table 11: Activated Carbon Properties

Property	Carbon A	Carbon B
Surface Area BET m^2/g	3183	1518
Pore Volume, cm^3/g		
> 15 Å	0.15	0.56
< 15 Å	2.08	1.33
Iodine Number	2933	1663
Methylene Blue Number	533	-
Phenol Number	2.02	-
Bulk Density, g/cm^3	0.315	0.401
Molasses Number	12	350
Screen Analysis (wt %)		
Passes 100 mesh	99.97	>97
Passes 200 mesh	95.5	85.95
Passes 325 mesh	72.7	65.85

After Grieves, et. al. (1980)

Adsorption Characteristics of Various Organic Compounds

In order to determine the candidate nitrification inhibitors, a search was made of adsorption characteristics of various organic compounds. These were later matched with the nitrification characteristics. Table 12 shows the results of the search. The values reported are generally Freundlich isotherm coefficients, although some are q_e values at specified equilibrium concentrations. Care should be taken when using these results. Many of the results are contradictory or quite variable. Some of the variability is probably due to the method of estimating $\frac{1}{n}$ and K. Also many of the references cited are for review papers of handbooks; the original source should be consulted prior to using the results from this table.

Table 12: Summary of Carbon Adsorption Capacities for Various Compounds

Compound (1)	Adsorption Parameter* (2)	Reference (3)
3,3',-dichlorobenzidine	240/0.952	Fochtman and Dobbs (1981)
4,4',-methylene-bis(2-chloroaniline)	190/0.64	Dobbs and Cohen (1980)
4,4 -methylene-bis (2 chloroaniline)	240/0.596	Fochtman and Dobbs (1981)
α-BHC (hexachlorocyclohexane/isolindane)	303/0.43	Dobbs and Cohen (1980)
α-endosulfan	194/0.50	Dobbs and Cohen (1980)
α-naphthol	180/0.32	Dobbs and Cohen (1980)
α-naphthylamine	160/0.34	Dobbs and Cohen (1980)
β-BHC (hexachlorocyclohexane/iso lindane)	220/0.49	Dobbs and Cohen (1980)
β-endosulfan	615/0.83	Dobbs and Cohen (1980)
β-naphthol	200/0.26	Dobbs and Cohen (1980)
β-naphthylamine	150/0.30	Dobbs and Cohen (1980)
β-naphthylamine	166/0.23	Fochtman and Dobbs (1981)
γ-BHC (isolindane)	256/0.49	Dobbs and Cohen (1980)
1,1,1-trichloroethane	2.48/0.348	Dobbs and Cohen (1980)
1,1,2,2-tetrachloroethane	10.37/0.37	Dobbs and Cohen (1980)
1,1,2-trichloroethane	5.81/0.60	Dobbs and Cohen (1980)
1,1-dichloroethane	1.8/0.53	Dobbs and Cohen (1980)
1,1-dichloroethylene	4.9/0.54	Dobbs and Cohen (1980)
1,1-diphenylhydrazine	135/0.16	Dobbs and Cohen (1980)
1,1-diphenylhydrazine	149/0.232	Fochtman and Dobbs (1981)
1,2,3,4-teterahydornaphthalene	74/0.81	Dobbs and Cohen (1980)
1,2,4-trichlorophenol	157/0.31	Dobbs and Cohen (1980)
1,2-dibromo-3-chloro-propane (DBCP/3-chloro-1,2-dibromopropane)	53/0.47	Dobbs and Cohen (1980)
1,2-dichlorobenzene	129/0.43	Dobbs and Cohen (1980)
1,2-dichlorobenzene	300/0.43	Weber et. al. (1980)
1,2-dichloroethane	3.6/0.83	Dobbs and Cohen (1980)
1,2-dichloropropane	5.9/0.60	Dobbs and Cohen (1980)
1,2-dichloropropene	8.2/0.46	Dobbs and Cohen (1980)
1,2-trans-dichloroethene	3.1/0.51	Dobbs and Cohen (1980)
1,3-dichlorobenzene	118/0.45	Dobbs and Cohen (1980)
1,4-dichlorobenzene	121/0.47	Dobbs and Cohen (1980)
1,4-dichlorobenzene	226/0.37	Weber et. al. (1977)
1-chloro-2-nitrobenzene	130/0.46	Dobbs and Cohen (1980)
2,3-dimethylphenol	552/0.248	Singer and Yen (1981)
2,4,6-trichlorophenol	155/0.40	Dobbs and Cohen (1980)
2,4-dichlorophenol	157/0.35	Dobbs and Cohen (1980)
2,4-dimethylphenol	78/0.44	Dobbs and Cohen (1980)
2,4-dimethyphenol	184/0.09	Mathews (1981)
2,4-dinitrophenol	33/0.61	Dobbs and Cohen (1980)
2,4-dinitrotoluene	146/0.31	Dobbs and Cohen (1980)
2,6-dimethylphenol	552/0.248	Singer and Yen (1981)
2,6-dinitrotoluene	145/0.32	Dobbs and Cohen (1980)
2-acetylaminofluorene	318/0.12	Dobbs and Cohen (1980)
2-chloroethyl vinyl ether	3.9/0.80	Dobbs and Cohen (1980)

2-chloronaphthalene	280/0.46	Dobbs and Cohen (1980)
2-chlorophenol	51/0.41	Dobbs and Cohen (1980)
2-ethyl butanol	170	Perrich (1981)
2-ethyl hexanol	138	Perrich (1981)
2-ethylphenol	532/0.222	Singer and Yen (1981)
2-isopropylphenol	671/0.245	Singer and Yen (1981)
2-methylphenol	383/0.172	Singer and Yen (1981)
2-nitrophenol	99/0.26	Dobbs and Cohen (1980)
3,3-dichlorobenzidine	300/0.20	Dobbs and Cohen (1980)
3,4-benzofluoranthene	57/0.37	Dobbs and Cohen (1980)
3,4-dimethylphenol	532/0.248	Singer and Yen (1981)
3-ethylphenol	532/0.222	Singer and Yen (1981)
3-methylphenol	383/0.172	Singer and Yen (1981)
4,6-dinitro-o-cresol	169/0.32	Dobbs and Cohen (1980)
4-aminobiphenyl	200/0.26	Dobbs and Cohen (1980)
4-bromophenyl phenyl ether	144/0.68	Dobbs and Cohen (1980)
4-chlorophenyl phenyl ether	111/0.26	Dobbs and Cohen (1980)
4-dimethylaminoazobenzene	249/0.24	Dobbs and Cohen (1980)
4-ethylphenol	532/0.222	Singer and Yen (1981)
4-methylphenol	383/0.172	Singer and Yen (1981)
4-nitrobiphenyl	370/0..27	Dobbs and Cohen (1980)
4-nitrophenol	76/0.17	Dobbs and Cohen (1980)
5-bromouracil	44/0.47	Dobbs and Cohen (1980)
5-chlorouracil	25/0.58	Dobbs and Cohen (1980)
5-fluorouracil	5.5/1.0	Dobbs and Cohen (1980)
DDE	232/0.37	Dobbs and Cohen (1980)
DDT	322/0.50	Dobbs and Cohen (1980)
EDTA	0.86/1.5	Dobbs and Cohen (1980)
N-butylphthalate	220/0.45	Dobbs and Cohen (1980)
N-dimethylnitrosamine	6.8×10^{-3}/6.6	Dobbs and Cohen (1980)
N-nitrosodi-n-propylamine bis(2-chloroisopropyl) ether	24/0.26	Dobbs and Cohen (1980)
N-nitrosodiphenylamine	220/0.37	Dobbs and Cohen (1980)
PCB-1016	338/0.66	Weber et. al. (1977)
PCB-1221	242/0.70	Dobbs and Cohen (1980)
PCB-1232	630/0.73	Dobbs and Cohen (1980)
PCB-1254	1,920/1.14	Weber et. al. (1977)
acenaphthene	140/0.43	Mathews (1981)
acenaphthene	190/0.36	Dobbs and Cohen (1980)
acenaphthylene	115/0.37	Dobbs and Cohen (1980)
acetaldehyde	22	Perrich (1981)
acetic acide	48	Perrich (1981)
acetone	43	Perrich (1981)
acetone cyanohydrin	0.	Dobbs and Cohen (1980)
acetophenone	194	Perrich (1981)
acetophenone	74/0.44	Dobbs and Cohen (1980)
acridine orange	190/0.29	Dobbs and Cohen (1980)
acridine yellow	230/0.12	Dobbs and Cohen (1980)
acrolein	1.2/0.65	Dobbs and Cohen (1980)
acrotein	61	Perrich (1981)
acrylic acid	129	Perrich (1981)
acrylonitrile	1.4/0.51	Dobbs and Cohen (1980)
adenine	71/0.38	Dobbs and Cohen (1980)

adipic acid	0.	Dobbs and Cohen (1980)
adipic acid (ph=3)	20/0.47	Perrich (1981)
aldrin	651/0.92	Dobbs and Cohen (1980)
allylalcohol	24	Perrich (1981)
allylamine	63	Perrich (1981)
anethole	300/0.42	Dobbs and Cohen (1980)
anthracene	376/0.70	Dobbs and Cohen (1980)
benzaldehyde	188	Perrich (1981)
benzene	0.70/2.9	Perrich (1981)
benzene	1.0/1.6	Dobbs and Cohen (1980)
benzene	16.6/0.39	Weber,et. al. (1980)
benzene	17.8/0.40	Weber,et. al. (1980)
benzene	18.5/1.158	Fochtman and Dobbs (1981)
benzene	36/0.48	El Dib and Broadway (1980)
benzidine	173/0.288	Fochtman and Dobbs (1981)
benzidine diHCl	220/0.37	Dobbs and Cohen (1980)
benzo(a)pyrene	33.6/0.44	Dobbs and Cohen (1980)
benzo(ghi)perylene	10.7/0.37	Dobbs and Cohen (1980)
benzo(k)fluoranthene	181/0.57	Dobbs and Cohen (1980)
benzoic acid	0.76/1.8	Dobbs and Cohen (1980)
benzoic acid	183	Perrich (1981)
benzothiazole	120	Dobbs and Cohen (1980)
benzothiazole	120/0.27	Perrich (1981)
bis(2-chloroethoxy) methane	11/0.65	Dobbs and Cohen (1980)
bis(2-ethylhexyl) phthalate	11,300/1.5	Dobbs and Cohen (1980)
bis-2-chloroethylether	11/0.94	Perrich (1981)
bromodichloromethane	19/0.76	Perrich (1981)
bromoform	19.6/0.52	Dobbs and Cohen (1980)
bromoform	200/0.83	Perrich (1981)
bromoform	73.1/0.62	Fochtman and Dobbs (1981)
butanol	107	Perrich (1981)
butyl acrylate	193	Perrich (1981)
butyl ether	99	Perrich (1981)
butylamine	0.	Dobbs and Cohen (1980)
butylamine	103	Perrich (1981)
butylbenzyl phthalate	1,920/1.26	Dobbs and Cohen (1980)
butyraldehyde	106	Perrich (1981)
butyric acid	119	Perrich (1981)
caproic acid	194	Perrich (1981)
carbon tetrachloride	11.1/0.83	Dobbs and Cohen (1980)
carbon tetrachloride	25.3/0.58	Weber et. al. (1977)
carbon tetrachloride	29.5/0.70	Weber et. al. (1977)
carbon tetrachloride	40/0.84	Perrich (1981)
chlordane	245/0.38	Dobbs and Cohen (1980)
chlorobenzene	91/0.99	Dobbs and Cohen (1980)
chlorobenzene	93/0.98	Perrich (1981)
chloroethane	0.59/0.95	Dobbs and Cohen (1980)
chloroform	11/0.84	Perrich (1981)
chloroform	2.6/0.73	Dobbs and Cohen (1980)
chloroform	6.3/0.73	Weber et. al. (1977)
choline chloride	0.	Dobbs and Cohen (1980)
chrysene	6/0.50	Mathews (1981)

crotonaldehyde	92	Perrich (1981)
cyclohexanone	134	Perrich (1981)
cyclohexanone	6.2/0.75	Dobbs and Cohen (1980)
cyclohexylamine	0.	Dobbs and Cohen (1980)
cytosine	1.1/1.6	Dobbs and Cohen (1980)
di-N-butylamine	174	Perrich (1981)
di-N-propylamine	174	Perrich (1981)
dibenzo(a,h)anthracene	69.3/0.75	Dobbs and Cohen (1980)
dibromochloromethane	63/0.93	Perrich (1981)
dibromochloromethane	4.8/0.34	Dobbs and Cohen (1980)
dichloroethane (ph=10)	4/0.67	Perrich (1981)
dichloroethane (ph=5)	5/0.55	Perrich (1981)
dichloroethane (ph=6)	4/0.60	Perrich (1981)
dichlorobromomethane	7.9/0.61	Dobbs and Cohen (1980)
dichloroisopropyl ether	200	Perrich (1981)
dieldrin	131/0.56	Weber et. al. (1977)
dieldrin	606/0.51	Dobbs and Cohen (1980)
diethyl phthalate	110/0.27	Dobbs and Cohen (1980)
diethylene glycol	0.	Dobbs and Cohen (1980)
diethylene glycol	53	Perrich (1981)
diethylene glycol (monobutylether)	166	Perrich (1981)
diethylene glycol (monoethylether)	87	Perrich (1981)
diisobutyl keytone	60	Perrich (1981)
dimethyl phthalate	97/0.41	Dobbs and Cohen (1980)
dimethylnitrosamine	9×10^{-6}/7.91	Fochtman and Dobbs (1981)
dimethylphenylcarbinol	210/0.33	Dobbs and Cohen (1980)
dimethylphthalate	97/0.41	Perrich (1981)
diphenylamine (3325)	120/0.31	Dobbs and Cohen (1980)
dipropylene glycol	93	Perrich (1981)
endosulfan sulfate	686/0.81	Dobbs and Cohen (1980)
endrin	666/0.80	Dobbs and Cohen (1980)
ethanol	0.	Dobbs and Cohen (1980)
ethanol	20	Perrich (1981)
ethoxytriglycol	139	Perrich (1981)
ethylacrylate	157	Perrich (1981)
ethylbenzene	100/0.40	El Dib and Broadway (1980)
ethylbenzene	53/0.79	Dobbs and Cohen (1980)
ethylchloride	36/1.5	Perrich (1981)
ethylene dichloride	163	Perrich (1981)
ethylene glycol	19.6	Perrich (1981)
ethylene glycol (monobutylether)	112	Perrich (1981)
ethylene glycol (monoethylether)	63	Perrich (1981)
ethylene glycol (monohexylether)	170	Perrich (1981)
ethylene glycol (monomethylether)	28	Perrich (1981)
ethylene glycol monoethyl acetate	132	Perrich (1981)
ethylenediamine	21	Perrich (1981)
fluoranthene	664/0.61	Dobbs and Cohen (1980)
fluoranthene	88/0.38	Mathews (1981)
fluorene	196/0.37	Mathews (1981)
fluorene	330/0.28	Dobbs and Cohen (1980)
formaldehyde	18	Perrich (1981)
formic acid	47	Perrich (1981)

guanine (4416)	120/0.40	Dobbs and Cohen (1980)
heptachlor	1,220/0.95	Dobbs and Cohen (1980)
heptachlor epoxide	1,038/0.70	Dobbs and Cohen (1980)
hexachlorobenzene	450/0.60	Dobbs and Cohen (1980)
hexachlorobutadiene	360/0.63	Perrich (1981)
hexachlorobutradiene	258/0.45	Dobbs and Cohen (1980)
hexachloroethane	96.5/0.38	Dobbs and Cohen (1980)
hexamethylenediamine	0.	Dobbs and Cohen (1980)
hexylene glycol	122	Perrich (1981)
hydroquinone (4705)	0.	Dobbs and Cohen (1980)
hydroquinone (4705)	90/0.25	Perrich (1981)
isobutanol	84	Perrich (1981)
isobutyl acetate	164	Perrich (1981)
isophorone	92/0.39	Dobbs and Cohen (1980)
isopherone	193	Perrich (1981)
isopropanol	25	Perrich (1981)
isopropyl ether	162	Perrich (1981)
methanol	7	Perrich (1981)
methyl butyl keytone	159	Perrich (1981)
methyl isoamyl keytone	169	Perrich (1981)
methyl isobutyl keytone	169	Perrich (1981)
methyl propyl keytone	139	Perrich (1981)
methylene chloride	1.9/1.16	Dobbs and Cohen (1980)
methylethyl keytone	94	Perrich (1981)
morpholine	0.	Dobbs and Cohen (1980)
n-amyl alcohol	155	Perrich (1981)
n-butylphthalate	220/0.37	Perrich (1981)
n-hexanol	191	Perrich (1981)
naphthalene	123/0.41	Mathews (1981)
naphthalene	132/0.42	Dobbs and Cohen (1980)
naphthalene	62.8/0.302	Feehtman and Dobbs (1981)
nitrobenzene	135/0.43	Weber et. al. (1980)
nitrobenzene	68/0.43	Dobbs and Cohen (1980)
o-anisidine	50/0.34	Dobbs and Cohen (1980)
o-xylene	120/0.22	El Dib and Broadway (1980)
p-chlorometacresol	124/0.16	Dobbs and Cohen (1980)
p-nitroaniline	140/0.27	Dobbs and Cohen (1980)
p-nonylphenol	250/0.37	Dobbs and Cohen (1980)
p-xylene	85/0.16	Dobbs and Cohen (1980)
paraldehyde	148	Perrich (1981)
pentachlorophenol	150/0.42	Dobbs and Cohen (1980)
phenanthrene	135/0.45	Mathews (1981)
phenanthrene	215/0.44	Dobbs and Cohen (1980)
phenol	234/0.0878	Singer and Tan (1981)
phenol	21/0.54	Dobbs and Cohen (1980)
phenol	86/0.14	Weber et. al. (1977)
phenylmercuric acetate	270/0.44	Dobbs and Cohen (1980)
phenylmercuric acetate(pH=9)	130/0.54	Dobbs and Cohen (1980)
propanol	38	Perrich (1981)
propionaldehyde	18	Perrich (1981)
propionic acid	65	Perrich (1981)
propylene dichloride	183	Perrich (1981)

propylene glycol	116	Perrich (1981)
propylene oxide	52	Perrich (1981)
pyrene	66/0.24	Mathews (1981)
styrene	120/0.56	Dobbs and Cohen (1980)
styrene oxide	190	Perrich (1981)
t-butanol	59	Perrich (1981)
tetrachloroethene	276/0.54	Weber et. al. (1977)
tetrachloroethene	51/0.56	Dobbs and Cohen (1980)
tetraethylene glycol	116	Perrich (1981)
thymine	27/0.51	Dobbs and Cohen (1980)
toluene	26/0.44	Dobbs and Cohen (1980)
toluene	40/0.35	Mathews (1981)
toluene	90/0.30	El Dib and Broadway (1980)
trichloroethene	28/0.62	Dobbs and Cohen (1980)
trichloroethene	92/0.45	Weber et. al. (1980)
trichloroethylene	21/0.50	Perrich (1981)
trichlorofluoromethane	5.6/0.24	Dobbs and Cohen (1980)
triethanolamine	0.	Dobbs and Cohen (1980)
triethylene glycol	105	Perrich (1981)
uracil	11/0.63	Dobbs and Cohen (1980)
valeric acid	159	Perrich (1981)
vinylacetate	129	Perrich (1981)

* The adsorption parameter is mg/l of compound adsorbed per gram of activated carbon at 1 mg/l equilibrium concentration, unless otherwise noted. When the adsorption parameter includes a slash, the paramter corresponds to the Langmuir or Freundlich isotherm parameters, Q and b (mg adsorbed compound/g carbon, and mg/l), or K and n^{-1} (mg adsorbed compound/g carbon, and dimensionless), respectively. Many of the references cited are review summaries and are not the original references for the adsorption data. See each reference for additional notes.

EXPERIMENTAL METHODS

Experimental Apparatus

Reactors and Associated Experimental Equipment

Five identical "Eckenfelder-type" continuous flow activated sludge reactors were operated in parallel and used as a source of microorganisms for experimentation. Of the five reactors, three received refinery wastewater (RW) as feed while the remaining two reactors received a glucose based synthetic substrate. Among the RW fed reactors, PAC additions were made in one and bentonite additions in another, with the third acting as a control. For the 2 glucose fed reactors, PAC additions were made in one and the other served as a control. The operating processes for the reactors is shown below:

	Reactor Number				
	Glucose Fed		Refinery Wastewater Fed		
	1	2	3	4	5
Additions	PAC	none	PAC	none	Bentonite

Each reactor, as shown in Figure 7, was constructed of 1/4" plexiglass with a working volume of 12.2 liters in the aeration section and 1.5 liters in the solids-liquid separator section. The two sections was separated by a sliding baffle which fitted snugly into slots to both sides of the reactor wall. Several holes were provided in the lid of

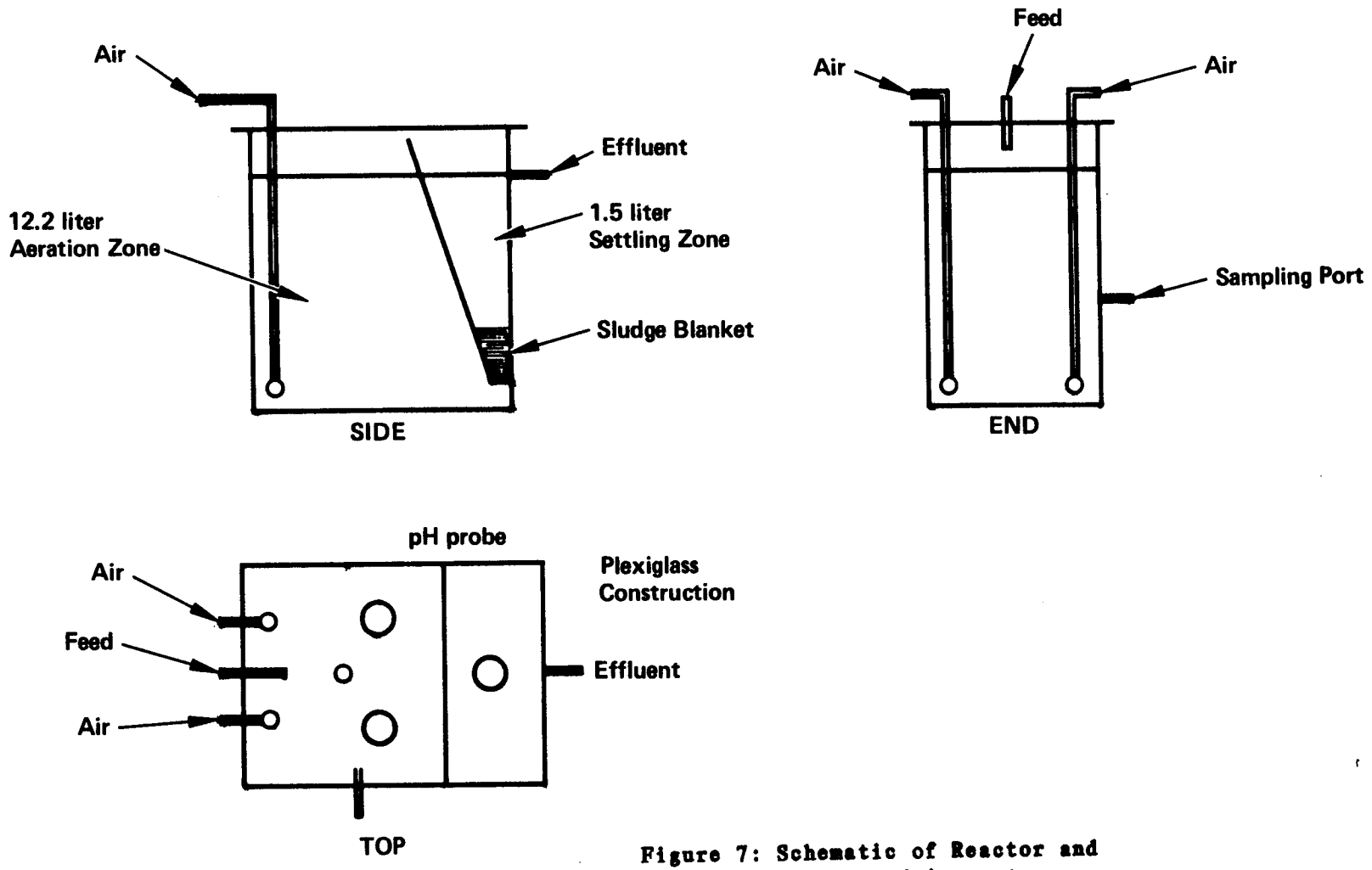


Figure 7: Schematic of Reactor and Assoicated Apparatus

the reactor; larger holes for a pH probe and access for maintenance purposes and smaller holes for influent feed, base addition and air lines. A port hole on the side of the aeration section was used to withdraw mixed liquor for analysis and for the control of sludge age.

Air, which was added through diffuser stones located near the bottom of the mixed liquor aeration section, provided oxygen for microbial activity and growth as well as turbulence for mixing purposes. Airflow rates, which ranged from 5 to 10 SCFH, were monitored independently in each reactor by routing the air through a rotameter prior to its introduction into the mixed liquor. Lower airflow rates were necessary at times to control reactor foaming and higher flow rates were occasionally required to provide adequate mixing. Even with the lowest airflow rates used, the dissolved oxygen concentration in the mixed liquor was always above a level (i.e., 3 mg/l) that would be expected to limit the growth of either heterotrophs or nitrifiers at this high MCRT environment.

The pH was maintained independently in each reactor at a range of 7.0 to 7.2 by means of either an industrial grade pH control unit manufactured by Lees and Northrup or by a similar type unit manufactured by Horizons, Inc. (Model 5997-20). Each pH control system consisted of a combination pH electrode (Orion model 34), immersed in the mixed liquor, and a pH control unit with set point dials to control the action of a pump for base addition. Whenever the pH would fall below a set value, the base pump was actuated, delivering NaOH (1.0 -2.0 N) directly into the aeration section to bring the pH back up to its predetermined operating value.

Feed Systems and Substrates

Glucose-Based Feed Dilution System

Due to the large quantity of glucose-based substrate required by reactors 1 and 2, a dilution system was used whereby concentrated substrate was automatically diluted before being pumped into the reactors. The concentrated feed and a mixing reservoir was contained in a refrigerator at 10^{o} C. A schematic of the dilution system is shown in Figure 8.

The liquid level in the mixing reservoir was electronically sensed by two float switches which controlled both the concentrate feed pumps to the reservoir and an external solenoid valve for the flow of dilution tap water. The diluted substrate was pumped directly from the mixing reservoir into the reactors using a separate pump system.

Glucose Substrate Composition

The substrate feed was composed of glucose, ammonia and other nutrients required to support the growth of heterotrophs and nitrifiers. The composition of the concentrated feed solution is shown in Table 13. The $CaCl_2$-$MgCl_2$ solution was separately pumped into the reservoir at each dilution cycle. This was done to prevent the formation of calcium phosphate precipitates in the concentrate. The pH of freshly prepared feed concentrate was adjusted from 7.9 to 7.0 with concentrated HCl prior to use. Fresh concentrate was prepared when required (usually 4-5 days) or when, in rare occasions, the concentrate appeared cloudy. This

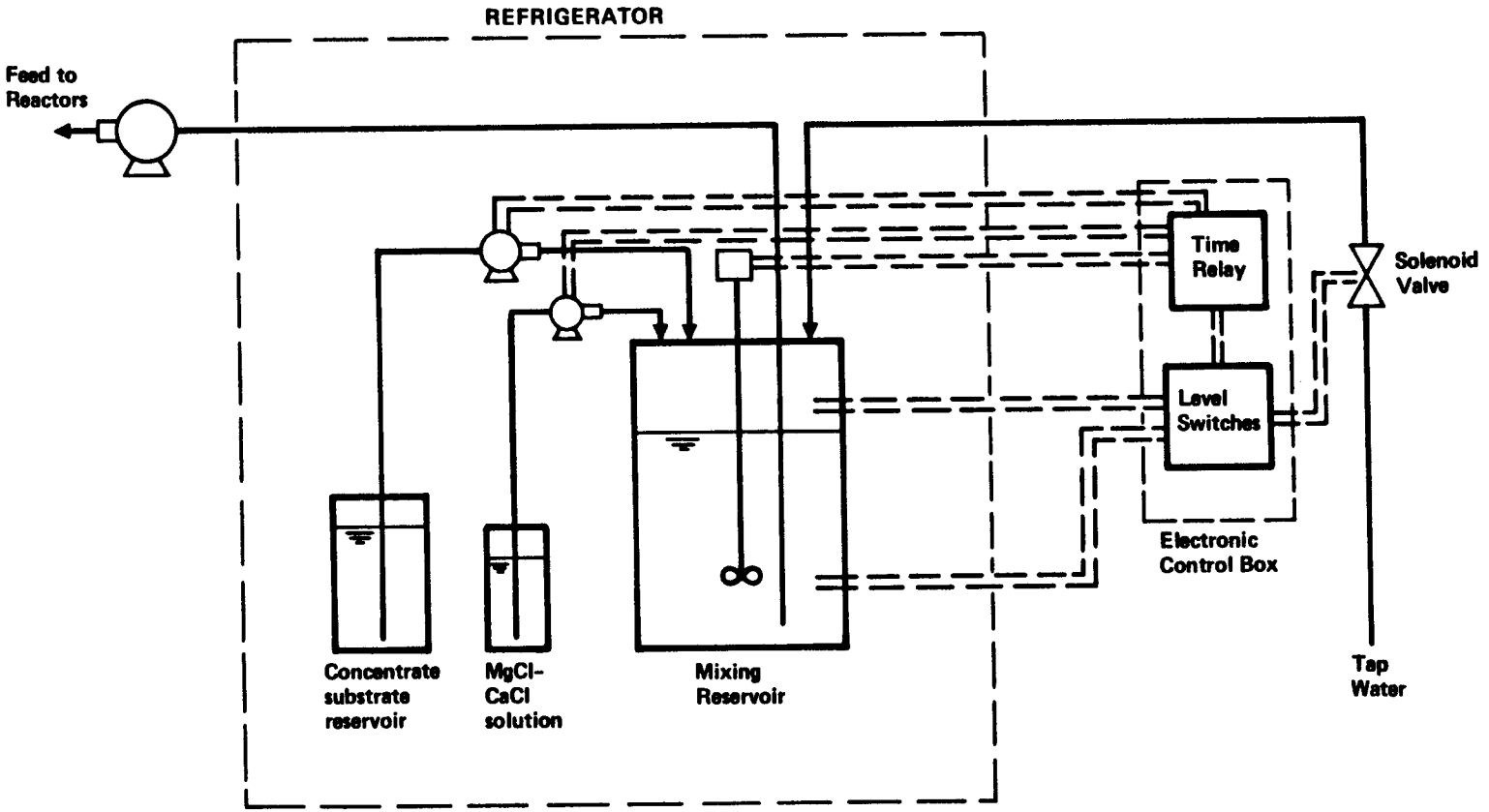


Figure 8: Schematic of Substrate Dilution System

Table 13: Concentrate Feed and Mineral Solutions

Concentrated Feed (add to 1.0 liter Distilled Water)

Component	Amount *
Trace Mineral Solution	2 ml.
$CaCl_2$-$MgCl_2$ Solution	8ml.
K_2HPO_4	25.0
Yeast Extract	10.0
Glucose	103.5
$(NH_4)_2SO_4$	10.0
NH_4Cl	45.0

Trace Mineral Solution (to 0.5 liter Distilled Water)

$FeCl_3$	19.5
$MnCl_2$-4 H_2O	4.75
$ZnCl_2$	3.30
$CuCl_2$-2 H_2O	2.05
$CoCl_2$-6 H_2O	2.90
$(NH_4)Mo_7O_{24}$-4 H_2O	2.10
Na_3 citrate	176.5
$Na_2B_4O_7$- 10 H_2O	1.20

$CaCl_2$-$MgCl_2$ Solution (to 0.2 liter Distilled Water)

$CaCl_2$	2.30
$MgCl_2$-6 H_2O	4.10

* grams added unless otherwise noted

cloudiness indicated excessive microbial contamination. The concentrate was diluted approximately 250 times during each cycle, resulting the the calculated influent concentrations shown in Table 14. The total steady state influent ammonia-N concentration was calculated and measured to be 50 mg/l NH_4^+-N.

The following steps were taken to minimize microbial growth in the feed lines and in the mixing reservoir:

1. The reservoir was thoroughly rinsed with hot water at least once a week.

2. The entire length of the feed lines (Tygon tubing) was manually squeezed daily and its contents disposed of.

3. The feed lines were changed every 3 weeks.

4. The temperature in the refrigerator was maintained at 10^o C at all times.

Refinery Wastewater Feed

Three of the five reactors were fed refinery wastewater obtained from a nearby oil refinery. The wastewater, drawn from the effluent side of dissolved air flotation units (DAF), was trucked from the refinery to the UCLA campus in seven 55 gallon polyethylene drums and stored in a walk-in refrigerator at 4^o C. From there, the wastewater was automatically pumped, using an electronic filling/refilling system, into a refrigerated reservoir in the laboratory. From the reservoir,

Table 14: Influent Substrate Concentrations

Substance	Concentration (mg/l)
Glucose	371.3
K_2HPO_4	88.1
NH_4Cl	158.7
$(NH_4)_2SO_4$	35.3
Yeast extract	35.3
$CaCl_2$	5.9
$MgCl_2-6H_2O$	10.5
(Trace Salts)	
$FeCl_3$	0.275
$MnCl_2-4H_2$	0.067
$ZnCl_2$	0.047
$CuCl_2-2H_2O$	0.029
$CoCl_2-6H_2O$	0.041
$(NH_4)Mo_7O_{24}-4H_2O$	0.029
$Na_2B_4O_7-10H_2O$	0.016
Na_3 citrate	2.49

the wastewater was pumped into the activated sludge reactors by a separate pump system. Since refinery wastewaters are generally lacking in phosphorus, an essential nutrient for microbial growth, phosphoric acid (1 mg/l feed) was manually added to the RW reactors on a daily basis. No other nutrient was supplemented.

For each batch of wastewater received from the refinery, determinations of ammonia-N, nitrate-N and TOC were made. A GC-MS analysis was performed for base/neutral/acidic extractable organics in three separate batches of wastewater in order to screen for potential inhibitors of nitrification and to assess the general composition of the wastewater. This work was done by James Montgomery Consulting Engineers, Inc. in Pasadena, California, using a Finnigan 4000 GC/MS. In addition, a heavy metal analysis on a single batch of wastewater was performed by the SOHIO Research Center, Cleveland, Ohio.

Reactor Start-Up and Operation

The powdered activated carbon (PAC) used in this study was Westvaco Nuchar SA-15 and the bentonite clay used was obtained from Westwood Ceramic Co., Los Angeles. Prior to reactor seeding, the following steps were taken to prepare the PAC reactors:

1. PAC was dried at 150° C for 3 hours and stored in a dessicator before use.

2. The steady state carbon concentration to be used, based on initial operating variables, was calculated using the following mass

balance equation (Grieves and Stenstrom, 1977);

3.

$$C = (Ci)(\theta_c)/(\theta_H).....(41)$$

where C = equilibrium mixed liquor carbon concentration, mg/l

Ci = Influent carbon concentration based on feed rate, mg/l

= 25 mg/l

θ_c = sludge age, in days

= 20 days

θ_H = hydraulic retention time, days

= 0.5 days

4. Based upon the initial steady state reactor carbon concentration calculated (C=1000 mg/l) and the volume of the aeration section, an appropriate amount of dried PAC was weighed and placed into the reactor.

5. The reactor was then filled with tap water and the air turned on, allowing the carbon to wet.

6. After 2 days, the air was turned off and the carbon allowed to settle.

7. The supernatant was decanted from the reactors and the reactor was seeded with activated sludge.

Similar steps were taken to prepare the bentonite reactors; however, because the bentonite particles tended to agglomerate when wetted, it was necessary to slowly suspend the particles in an agitated beaker containing tap water before adding the suspension to the reactor.

All reactors were seeded with mixed liquor obtained from the aeration tanks of the Hyperion Wastewater Treatment Plant, Los Angeles, California. The initial MLSS concentration was measured at 2450 mg/l. Due to delays in obtaining the refinery wastewater feed, the initial feed used for all reactors was diluted Sego, a complex carbonaceous substrate, supplemented with potassium phosphate and ammonium chloride. The TOC, NH_4^+-N and $PO_4^=$-P concentrations in the influent was maintained at approximately 160 mg/l, 25 mg/l and 10 mg/l, respectively during the start-up period. Appropriate volumes of PAC and bentonite were added as a slurry to the aeration section of the reactors on a daily basis.

Following one month of steady state operation, the sludge age for all reactors were increased to 60 days in anticipation of the final operating conditions to be used during experimentation. Two months after the initial reactor start-up, the feed was switched from Sego to the refinery wastewater in reactors 3, 4 and 5. At this time, the PAC and bentonite dosage and the hydraulic retention time for these reactors were increased to the final operating values of 50 mg/l feed and 24 hours, respectively. Due to the presence of organic nitrogen in the Sego substrate, reactors 1 and 2 were switched to the glucose-based feed. This change was necessary to facilitate the measurement of nitrogenous species in the influent and to have better control of nitrogen

input.

The final operating conditions used during the experimental period were as follows:

Reactor feed	HRT (days)	Sludge Age (days)	Ci (mg/l)	C (mg/l)
Glucose based substrate	0.5	60	25	3000
Refinery wastewater	1.0	60	50	3000

To ensure steady state conditions existed during the experimental period, all reactors were operated at the parameters specified above for the period of 2 sludge ages prior to the start of experiments. With the exception of initial foaming problems encountered in the bentonite reactor and occasional technical problems associated with pH control, all the reactors operating smoothly during this period, regularly achieving TOC removal efficiencies of > 95% for glucose reactors and > 80% for the refinery reactors. Slightly higher TOC removal efficiences were noted for the PAC reactors in both the glucose and refinery wastewater units. Consistently high NH_4^+-N removal efficiencies were observed for the glucose fed reactors, however the NH_4^+-N removal efficiencies for all refinery reactors were erratic (19-90%) during the first 6 weeks of operation. This may have been due to the presence of inhibitors in the wastewater. In the following 3 weeks, the refinery reactors consistently removed ammonia at efficiencies greater than 99 %. It was after this period of stable operation that experimentation began.

Experimental Procedures

Isotherm Studies

Two sets of adsorption isotherm studies were performed. One to assess the relative adsorptive capacities of PAC and bentonite using the refinery wastewater as an adsorbate and another to determine the adsorptive characteristics of aniline using PAC and bentonite as the adsorbent. The later isotherm was done because aniline was considered to be an "ideal" inhibitor for experimental use in this study. Its effects on nitrification are well documented in the literature and it is known to be both biodegradable and adsorbable, although the extent of its adsorptive properties have not been well established.

1) Refinery Wastewater Isotherm: The procedure for this isotherm consisted of the following steps;

a) PAC and bentonite were dried at 150° C for 3 hours and placed in a dessicator before use.

b) Appropriate amounts of PAC and bentonite were analytically weighed and placed into 150 ml Pyrex square bottles. Two bottles containing neither PAC or bentonite was retained as controls.

c) 125 ml of refinery wastewater was added into each bottle, resulting in PAC or bentonite concentrations of 0.1, 0.2, 0.4, 1.0, 2.0, 5.0 and 10.0 grams/l refinery wastewater and 2 controls.

d) All bottles were stoppered; placed in a horizontal position on an orbital shaker (Labline, Model 3590) and agitated at 130 RPM for 3 hours (room temperature = 27-29° C).

e) At the end of the agitation period, the contents of each flask were vacuum filtered through a 0.45 micron membrane filter and analyzed for TOC.

2) Aniline Isotherm: The procedure used for this isotherm was similar to that just described, except that 100 ml of a test solution containing 100 mg/l (pH adjusted to 7.0) aniline was used in lieu of the refinery wastewater. Also, a slightly wider range of carbon and bentonite doses were evaluated (0.1, 0.2, 0.6, 1.0, 2.0, 5.0, 8.0, 10.0, 15.0 and 20.0 grams/l solution). The agitation period was 9 hours which was representative of a typical batch inhibition experiment. Residual liquid phase aniline concentrations were determined by gas-liquid chromatography after centrifugation.

Batch Experiments with Specific Inhibitors of Nitrification

A series of batch experiments was run to examine the short-term influences of PAC, bentonite and sludge acclimation on activated sludge nitrification rates in the presence of selectively spiked compounds. The selection of the compounds used in these experiments was based primarily on the information available in the literature on the inhibition and adsorption characteristics of the specific compound. Other considerations included the solubility and the industrial significance of the compound. The selected compounds at the concentrations used in

experiments are summarized as follows:

adsorbable-inhibitory:	aniline (10, 10 mg/l)
	phenol (10, 20 mg/l)
adsorbable-noninhibitory:	toluene (10 mg/l)
nonadsorbable-inhibitory:	cyanide (1, 3, 3 mg/l)
nonadsorbable-noninhibitory:	acrylonitrile (10 mg/l)

Unless otherwise noted, the following procedure was used for all batch experiments:

a. Prior to each test, the NH_4^+-N and NO_3^--N effluent concentrations were determined for each activated sludge reactor and recorded.

b. 430 ml of mixed liquor was withdrawn from each reactor into 500 ml flasks and an appropriate volume (1 to 3 ml) of a NH_4Cl solution was mixed into each flask to bring the concentration of NH_4^+-N to a predetermined level (i.e., 75 mg-N/l).

c. The volume of mixed liquor in each flask was divided equally into two 250 ml Erlenmeyer flasks containing mixing beads. For each pair of flasks, an appropriate volume of the test solution compound (e.g., sodium cyanide) was added into one of the flask to bring its concentration to the desired level, while the other flask was retained as a control.

d. All flasks (250 ml) were placed under a hood and aerated for a

specific period of time. The air was supplied through a disposable Disposo pipet at a flow rate of 3.5 SCFH to ensure adequate dissolved oxygen concentrations and mixing at all times.

e. Shortly after the start of aeration (typically 1 to 2 minutes), two separate 5 or 10 ml samples of mixed liquor was withdrawn from each flask with volumetric pipets. The mixed-liquor samples were diluted to volume with an appropriate dilution solution in 100 ml volumetric flasks, capped, and stored for the analysis of ammonia and nitrate at the end of the aeration period. These samples were designated as t=0. All samples were preserved according to the procedures prescribed by Standard Methods (1975). See also Analytical Methods.

f. Additional samples were taken throughout the aeration period. In the first two experiments with 10 mg/l aniline and 1 mg/l cyanide, the aeration period was 24 hours with sampling intervals ranging from 1.5 to 9 hours. In all the following experiments, the aeration period was 9 hours with samples taken at t = 1, 3, 5 and 9 hours.

g. Every hour throughout the aeration period, the pH was checked and adjusted, if necessary, to the range of 7.2 to 7.4 (using 0.05 or 0.1N NaOH). The volume of caustic use was noted and recorded after each pH adjustment.

Chronic Experiments with Aniline

Two consecutive tests were conducted to determine the long-term influences of PAC and bentonite additions on activated sludge nitrification rates in the presence of aniline, a known adsorbable and inhibitory compound. The two tests were carried out over a period of 2 months using the three continuous flow RW fed reactors as experimental units. Consistent ammonia oxidation efficiencies of > 99% over a period of one week or longer were a necessary prerequisite prior to the start of each test. This was to ensure that the observed inhibitory effect could be attributed to the presence of aniline rather than to poor nitrification in general.

In the initial test, all three reactors were subjected to a simultaneous step and pulse input of 30 mg/l aniline and a pulse input of 75 mg/l NH_4^+-N. Immediately following this, the concentrations of ammonia and nitrate in the mixed liquor were sampled with time at regular intervals (every 2 to 8 hours) for 265 hours and then once a day for the next 3 weeks.

In the second test, the reactors, which had been operating with a continuous input of 30 mg/l aniline in the RW feed, were subjected to another simultaneous step and pulse input of aniline and a pulse spike of 75 mg/l NH_4^+-N. The step input resulted in the increase of aniline concentration from 30 to 60 mg/l in the feed, while the pulse input consisted of a spike of 60 mg/l aniline directly into the reactors. The concentrations of ammonia, nitrite and nitrate was monitored with time using sampling intervals of 2 to 3 hours up to the first 100 hours and

once a day thereafter.

The general procedure for these tests consisted of the following steps:

1. Prior to the chronic experiments, a separate pump system was installed for the continuous addition of aniline to the RW reservoir. This system consisted of a time-controlled pump operating via liquid level switches in the feed reservoir. At each reservoir filling cycle, a predetermined volume of aniline was pumped; diluted and mixed with the incoming stream of refinery wastewater. The aniline was pumped from a prepared concentrated stock solution contained in a flask adjacent to the reservoir. The amount of concentrated aniline pumped gave an incoming feed concentration of 30 and 60 mg/l aniline for the first and second test, respectively.

2. Prior to the start of each experiment, the RW reservoir was thoroughly cleaned by scrubbing and rinsing with hot water. Each test began with the reservoir at the start of a new filling cycle.

3. The influent and effluent ammonia concentrations were measured for each RW fed activated sludge unit.

4. The RW feed pumps were turned on to give the step input of aniline.

5. 10 ml of a concentrated aniline solution was pipeted directly into the aeration section of each reactor to give an instantaneous

(pulse) input of aniline.

6. 20 ml of a concentrated stock solution of ammonium chloride was pipeted into each reactor to give an instantaneous concentration of 75 mg/1 NH_4^+-N.

7. Two 10 ml mixed liquor samples were taken directly from the aeration section of each reactor at every sampling period. The samples were diluted to volume in a 100 ml volumetric flask with distilled water (for the analysis of NH_4^+-N) or with a dilution solution (for the analysis of NO_3^--N). For nitrite samples, 100 mls. of mixed liquor from each test reactor was withdrawn and allow to settle. Using a volumetric pipet, 1 or 2 mls. of the resulting supernatant was withdrawn and diluted with distilled water into a 200 ml. volumetric flask. The settle mixed liquor and supernatant was then promptly returned into aeration section of each corresponding reactor.

8. Throughout the entire test period, the pH was maintained at 7.0 to 7.2. Cumulative caustic (1.0 N NaOH) usage was recorded for each reactor.

9. Usual daily sludge wastage and carbon or bentonite additions were maintained throughout the tests.

Carbon Dose Experiments

A series of batch experiments using variable carbon doses was performed to study PAC-activated sludge nitrification enchancement in the presence of a known nitrification inhibitors with different adsorption characteristics. The general procedure consisted of the following steps:

1. PAC was dried at 150° C for a minimum of 3 hours and stored in a dessicator until use.

2. For the initial experiment, a weighed amount of dried PAC was put into solution and mixed into a volumetric flask.

3. The NH_4^+-N and NO_3^--N concentrations in the effluent of the non-carbon glucose fed reactor were measured.

4. A measured volume of mixed liquor was withdrawn from reactor and divided equally into Erylenmeyer flasks (250-500 ml volume).

5. In the first experiment, appropriate volumes of the PAC slurry was pipetted into 4 of the flasks to give the desired carbon concentrations. PAC doses tested in the initial experiment was calculated to be 387, 775, 1960, and 4000 mg/l. In the subsequent experiments, the PAC was analytically weighed and placed dry into the designated empty flasks prior to the introduction of mixed liquor. PAC doses tested in here were 500, 1000, 2000, and 4000 mg/l. In all experiments, a

minimum of two flasks were retained as controls and did not recieve any PAC.

6. An exogenous source of NH_4^+-N in the form of a solution of ammonium chloride was pipetted into each flask to bring the NH_4^+-N concentration from <0.1 to 40-50-N mg/1.

7. 1.0 ml. of test compound from a concentration stock solution was pipetted into the test flasks to give the desired calculated concentration.

8. It should be noted that the changes in volumes brought about by the additions of PAC, NH_4Cl, and the aniline were accounted for in the calculation of the final concentrations used.

9. All flasks were placed under a hood and aerated throughout the experiment. In the initial experiment, air was supplied through a disposable Disposo pipet at a flow rate of 3.5 SCFH to ensure adequate dissolved oxygen concentrations and mixing at all times. In subsequent experiments, air was supplied through disposable, plastic aquarium diffuser stones at the same flowrate.

10. One or two minutes after the start of aeration (designated t=0), 2 separate 5-10 ml portions of the mixed liquor were withdrawn from each flask, using volumetric pipets, and added into a 100 ml volumetric flask half filled with distilled water and an appropriate preservative. The flasks were then

diluted to volume, capped, shaken, and stored for the analysis of ammonia and nitrate at the end of the experiment.

11. Step 10 was repeated for t = 2,4,6, and 8 or 9 hours.

12. Every hour throughout the aeration period, the pH was checked and manually adjusted, if necessary, to the range of 7.2 to 7.4 using 0.1 N NaOH.

Activated sludge mixed liquor used in the initial carbon dose experiment was obtained from a continuously fed non-carbon glucose reactor operating at a sludge age of 60 days, hydraulic retention time of 24 hours and an average influent ammonia concentration of 45 mg/l ammonia-N. Automated control of pH in the reactor was achieved by adding NaOH. Subsequent carbon dose experiments, performed 9 to 12 months after the initial experiment, utilized mixed liquor from the reactor now operating at a sludge age of 9.0 days, hydraulic retention time of 8 hours, and influent ammonia-N concentrations of approximately 50 mg/l. Automated control of pH in the reactor was achieved using a saturated solution of sodium bicarbonate.

Analytical Procedures

Analysis of Ammonia-N

An Orion Model 95-10 ammonia specific ion electrode in conjunction with an Orion ionanalyzer (Model 407A) was used to directly measure ammonia concentrations in the influent, mixed liquor and effluent samples. The probe was calibrated at least once, using laboratory prepared standards, prior to and during each analytical run. All samples were preserved with 1M HCl (0.1 ml/0.1 liter of sample) and stored in capped volumetric flasks. Because the probe is only sensitive to dissolved ammonia, the pH of the sample was raised to 11-13 with 10 M NaOH immediately prior to analysis. The probe method was found to give accurate and repeatable readings for the concentrations encountered in both glucose and RW reactor derived samples. Sample volume analyzed were 100 ml or aliquots diluted to 100 ml.

Analysis of Nitrite-N

Nitrite nitrogen was determined by a wet chemical technique as described in Standard Methods, (1975, method 418C, page 370). All nitrite samples were preserved with 4 mg $HgCl_2$/100 ml sample, filtered, and diluted with distilled water to be cover the applicable range of the method (0.01 to 1.0 mg/l NO_2^--N). Briefly, the method consisted of measuring NO_2^- in the sample through the formation of a reddish purple azo dye produced by the coupling of diazotized sulfanic acid with NED dihydrochloride. Photometric determinations was accomplished by using

Bausch and Lomb Spectronic 20 (1 cm light path) at 543 nm. Standard curves were obtained for each analytical run using serially diluted nitrite standards ($NaNO_2$) prepared in the laboratory.

Analysis of Nitrate-N

A calibrated Orion 93-07 nitrate electrode with an Orion Model 407A ionanalyzer was used for the determination of nitrate in all samples. Initially the cadmium-reduction method (Standard Methods, 1975, pp. 370) was used. However, after an evaluation of the two methods for analyzing nitrates in refinery wastewater, it was decided that the probe method was the better choice, based on the following observations:

1. Although both methods suffered from unknown interferences present in RW MLSS, the electrode method was judged to be at least as accurate as the Cd-reduction method.

2. The electrode method was a much faster and a more economical technique than the Cd-reduction method.

3. The efficiency of the Cd-reduction column decreased rapidly and eventually exhausted completely after only a few sample runs (approximately 8-9 samples). The degree of the reduction in column efficiency or the point of exhaustion was difficult to detect or predict during analytical runs. This made it difficult to establish the proper time for corrective measures, such as Cd regeneration or cadmium granule replacement. Often times, the reduction in column efficiency was detected only after samples had

already been analyzed.

Other colorimetric techniques for the analysis of nitrate, such as the Chromotropic acid and the Brucine methods, were also considered for potential application. These methods were eventually abandoned in light of the fact that these methods also suffered in one way or another from serious interferences, poor reproducibility, poor sensitivity or excessively tedious procedures with undesirable reagents (Jenkins, 1977). It was recognized that although significant experimental errors may result with the use of the electrode method, it was considered that this method was best suited for the particular wastewater to be analyzed.

In using the electrode method for the determination of nitrate, a constant ionic strength background must be maintained. This is accomplished by the addition of an ionic strength adjuster (ISA) solution directly into the sample to be analyzed. The recommended ISA for water and domestic wastewater samples is 2 ml of 2M ammonium sulfate/100 ml sample, resulting in an ionic strength background of 0.12 M. This ISA gave good accuracy and reproducibility for glucose fed influent, MLSS, and effluent samples, but poor to adequate results were obtained with refinery wastewater derived samples. An alternate ISA (Milham, 1970; Orion Methods Manual, 1976) normally used for the analysis for nitrates in soils, was tested and found to provide more acceptable results for RW samples (see Table 15). The modified ISA consisted of the following: 16.66 g $Al_2(SO_4)_3 \cdot 18H_2O$, 4.67 g Ag_2SO_4 and 2.43 g NH_2SO_3 in one liter of distilled water. The ionic strength background for this ISA was calculated to be 0.49 M. According to Milham (1970), the aluminum sulfate

Table 15: Comparative Results of Different ISAS in Analysis of Nitrates in Refinery Wastewater Samples (diluted 1:20) Using Known Addition Method

Intially Measured NO_3 (ppm-N)	Added NO_3	Calculated Total NO_3	Measured NO_3 Using regular ISA	% Error
76.0	4.9	80.9	85.4	5.6
	9.6	85.6	106.9*	24.9
	14.2	90.0	95.3*	5.9
	18.5	94.5	106.5*	12.7

Initially Measured NO_3 (ppm-N)	Added NO_3	Calculated Total NO_3	Measured NO_3 Using Modified ISA	% Error
76.5	4.9	81.4	85.0	5.3
	9.6	86.1	85.5	0.6
	14.2	90.7	92.5	1.9
	18.5	95.0	112.5	18.4

* = Unstable Readings on Meter

removes organic anions; the silver sulfate precipitates chlorides and the sulfamic acid destroys excessive nitrites that may interfere with the analysis. In this study, the modified ISA solution was used as a dilution liquid for the analysis of nitrates in RW derived samples, while the 2M ammonium sulfate ISA was used for nitrate analysis in glucose substrate derived samples. The dilution liquid used in the later cases was distilled water. All samples were preserved with 1 M boric acid (1 ml/100 ml sample).

Analysis of Organic Nitrogen

Organic nitrogen was analyzed for using a modification of the method described by Scheiner (1976). The method is essentially the micro-Kjeldahl technique described in Standard Methods (1976). Sample volumes of 5 to 10 ml were digested in 100 ml Kjeldahl flasks for at least 2 hours. The digestive solution was composed of 134 g potassium sulfate, 200 ml concentrated sulfuric acid and 5 ml selenium oxychloride (catalyst) per liter. Following digestion, the mixture was diluted with distilled water to volume in a 200 ml volumetric flask. The resulting solution was analyzed for ammonia nitrogen using the selective ion electrode described earlier, using a 10 N NaOH and NaI (30 g/0.1) solution in lieu of 10 N NaOH for pH adjustment to > 11.0. Calculation of ammonia nitrogen concentration was made according to the Orion Methods Manual (1976) using the concentration ratio, Q (unknown/known) for Kjeldahl digests. Standard known ammonia concentrations ranged from 1.0 to 5.0 mg/l ammonia-N. Differences in ammonia concentration before and after digestion was attributed to organic nitrogen. Accuracy of the

overall method was determined by analyzing a number of amino acids and ammonia chloride solutions of known concentrations. Results of these preliminary tests indicated measured values were within 5 % or better of known values (see Appendix IV).

Gas Liquid Chromatography Analysis

Liquid phase aniline concentrations for the adsorption isotherm and carbon dose experiments were measured by direct aqueous injection using a Varian Vista series 6000 gas chromatograph and a Hewitt Packard 3300 integrator. The column used was a 22 meter SP2100 operated isothermally at 110° C. Optimal operating conditions for the analysis of aniline was found to be as follows;

- Injection temperature = 200° C.
- Detector (Flame Ionization) temperature = 300° C.
- Carrier gas (He) flowrate = 2.0 ml./minute.
- Detector make-up gas flowrate = 30.0 ml./minute.
- Air flowrate = 300 ml./minute.
- Hydrogen flowrate = 30 ml./minute.
- Method of injection: splitless at 1.0 microliter sample size.
- Purge vent: opened after 0.9 minutes at 100 ml He/minute.
- Sensitivity = 4 to 32 x 10^{-11}.

Reproducibility for a given injection was found to be within 5% for peak area response and 7% for peak height response. All calculations were based on peak area response. A standard area curve was found to be more reliable than an absolute response factor (i.e., concentration/area counts) for determining unknown aniline concentration. Using the standard curve and at the lowest sensitivity used, it was possible to detect aniline at a concentration of approximately 0.3 mg/l by direct aqueous injection. According to the definition of the limits of detection (defined a three times the baseline noise), it is possible to detect aniline to less that 0.1 mg/l at the sensitivity used.

For the carbon dose experiments, a preliminary test was conducted to determine whether aniline could be recovered from the liquid phase of mixed liquor and accurately analyzed for by gas chromatography. The nature of the substrate used for reactor feed suggested that there would be little interference in the analysis of aniline by direct aqueous injection. The procedure used for the test was as follows;

1. 1.35 liters of mixed liquor (MLSS= 1250 mg/l) was withdrawn from the glucose non-carbon reactor and divided equally into 3 flasks with magnetic stirrers.

2. An appropriate volume of aniline stock solution was added via volumetric pipet into each flask while mixing in vortex, resulting in calculated aniline concentrations of 1.0, 5.0, and 10.0 mg/l aniline.

3. After mixing the contents in each flask for 2 minutes, 10.0 ml. of mixed liquor was withdrawn from each flask and centrifuged for 3 minutes or when a clear supernantant was visible. The resulting supernantant was then pipetted into 7.7 ml glass vials with teflon lined caps and stored at 4° C until analysis. These steps were necessary to preserve the stability of aniline and have been found to be satisfactory for subsequent analysis of gas chromatography.

Results of this test are shown below;

Calculated Aniline Concentration	Area Counts (HP Integrator)	Aniline Conc. (ave.)	Measured % Difference
1.0	3208,3600	0.86	14.0
5.0	15402,14128	4.34	13.2
10.0	30991,33156	9.64	3.6

The largest differences were associated with the analysis of the lower aniline concentrations. It is likely that part of the difference between the calculated and measured aniline concentrations was due to inherent experimental error and/or due to adsorption of aniline onto the biomass. The latter explanation is reasonable since all measured concentrations were found to be less than the cooresponding calculated values. The largest difference (14%) in this test was substantially less than that of an extraction step for the analysis of aniline wastewaters. Riggins, et. al. (1983), using a SP2100 glass capillary column to quantify aniline and its derivatives in wastewaters (by extraction with methylene

chloride) reported aniline recoveries of 75% or better. Given that the aniline recoveries at the concentrations of interest for these particular tests were greater than 75% by direct aqueous injection, an extraction step was deemed unnecessary.

TOC analysis

All total organic carbon (TOC) determinations were made with an Ionics TOD and TOC analyzer (Model 1270) which employed a combustion-infrared method for the analysis of soluble and purgable TOC. Samples were collected in glass bottles; preserved with concentrated HCl (pH < 2) and stored at 4^{o} C, if analysis was not immediate. All samples were either filtered (0.45 micron) or centrifuged and purged of inorganic carbon using HCl prior to analysis. A minimum of 3 consecutive replicates per sample was analyzed with the last two replicates averaged and recorded. The calibration solution standard was acetic acid and a new standard curve was obtained for each analytical run.

Analysis of MLSS and MLVSS

Total mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) concentrations for each reactor were determined at various times throughout the entire experimental program. For the non-carbon reactors, the solids were determined by the following procedure;

1) 2.1 cm glass microfiber filters (Whatman 934-AH) were placed into porcelain Gooch crucibles (Coors C8450-4) and washed by vacuum

filtration of 3 successive 20 ml portions of distilled water. The crucibles were ignited in a muffle furnace at 550 + 50°C for 15 minutes; allowed to cool in dessicator; and analytically weighed (constant weight) immediately before use.

2) A well mixed 5 ml portion of mixed liquor was passed through the filter in the crucible. The entire crucible was dried at 103°C in an oven for one hour; allowed to cool in a dessicator and weighed for the determination of MLSS. Two replicates were made for each sample with the averaged value recorded.

3) For volatile solids determinations, the crucibles from the preceding analysis were ignited in a muffle furnace at 550 + 50°C for 15 minutes, allowed to cool in a dessicator and weighed.

For the carbon and bentonite reactors, the MLSS concentrations may be determined using the above procedure and the following equation based on the steady state reactor concentration of carbon or bentonite;

$$MLSS_{(biomass)} = MLSS_{(measured)} - C$$

where $MLSS_{(biomass)}$ = the total suspended biological solids in the reactor

$MLSS_{(measured)}$ = the MLSS measured using the proceeding technique

C = the steady state carbon or bentonite concentration in the reactor. This is defined by the variables in equation 41.

The MLVSS concentration can be determined by a similar steady state equation;

$$MLVSS_{(biomass)} = MLVSS - C * MLSS * X$$

where X = the fraction of PAC or bentonite volatilized in MLVSS tests (assumed to be equal to the fraction of PAC or bentonite volatilized in a separate test with PAC or bentonite)

Alternately, Arbuckle, et al. (1982) proposed a method that can be used for the determination of biological solids concentrations in PAC sludges operating under nonsteady state conditions. Briefly, the procedure involves the determination of MLSS, MLVSS at 400° C, and MLVSS at 550° C for PAC sludges and PAC by itself. The following equation was developed by Arbuckle calculate $MLVSS_{(biomass)}$,

$$\frac{MLVSS_{(biomass)}}{MLSS} = V\ (550) - CAR * X\ (550)$$

where V (550) = fraction of MLSS volatilized at 550° C

X (550) = fraction of PAC volatilized at 550° C

CAR = fraction of MLSS which is PAC

Based on studies with different PAC sludges, Arbuckle used the following empirical equation to define CAR;

$$CAR = \frac{V(400)-0.9*V(550)}{X(400)-0.9*X(550)}$$

where V(400), V(550) = fraction of MLSS volatilized at 400° C and 500° C, respectively

X(400), X(550) = fraction of PAC volatilized at 400° C and 500° C, respectively

According to Arbuckle, the average error of 5 tests using this method was 4.4 % with a high of 16.7 % . In this study, the steady state

method was used to determine the MLSS and MLVSS concentrations in the bentonite reactors, while Arbuckle's method was used for PAC reactors.

RESULTS AND DISCUSSION

PRELIMINARY EXPERIMENTS

GC/MS Analysis

GC/MS analysis of the untreated refinery wastewater was performed three times during the beginning of the study. The purpose of the analysis was to determine the variability of the wastewater and to screen the wastewater for specific nitrification inhibitors. As mentioned previously, it was desired to use inhibitors in the later experiments which are typically found in refinery wastewater. Table 16 show the results of the GC/MS analysis conducted on September, 1982, December, 1982, and February, 1983. The tables show the Merk index number in parenthesis after the compound, in many instances. The tests are similar in that they show the presence of a large number of phenolic compounds and straight chain hydrocarbons. All tests detected aniline. Organic acids and ring compounds, such as anthracene, were found at low concentrations. Compounds were selected for further evaluation in the batch and chronic tests from this data and from the results of the literature review of nitrification inhibitors and adsorption characteristics.

Table 16: Results and Comparisions of GC/MS Analysis

Compound	Set 1 (Sept 24, 1982)	Set 2 Dec 28, 1982	Set 3 Feb 2, 1983
a a dimethylbenzenemethanol			0.12
1,1-dimethylcyclopentane			5.6
1,2,3,4-tetrahydronaphthalene			0.038
1,2-dimethylpiperidine	0.12		
1,4-dimethylnaphthalene	0.04		
1,7-dimethylnaphthalene	0.014		
1-butoxy-2-propanol			0.42
1-methyl-2,3-dihydroindene			0.1
1-methyl-2-piperidinone			0.26
1-methyl-2-pyrrolidinine (nicotine)	2.3	0.34	
1-methylethyl benzene			0.028
1-methylnaphthalene	0.05	0.04	
phenol	4.1	2.3	10.
2-methylphenol (o-cresol,o-hydroxytoluene,2571)	7.	3.6	10.
3-methylphenol (m-cresol,2570)	2.6	6.6	11.
2,3 dimethylphenol (vic-o-xylenol,9744)	2.6	1.1	3.2
2,4-dimethylphenol (m-xylenol,9744)	1.6	3.3	3.0
2,5 dimethylphenol (p-xylenol,9744)	0.94	0.46	0.96
2-ethyl-4-methylphenol	0.38	0.15	
2-ethyl-5-methylphenol	0.25	0.47	
2,4,5 trimethylphenol	0.180		
2,4,6 trimethylphenol	0.31		
C_3 phenols			3.7
3,4 dimethylphenol (as-o-xylenol,9744)	0.93	0.47	1.2
4-ethyl-2-methylphenol	0.25	0.17	
2,6-dimethylpiperidine	0.36	0.04	
2-butoxyethanol (butyl cellosolve,ethylmonobutylether)	0.12		
2-chlorotoluene		0.08	0.07
2-ethylpyridine		0.2	0.41
2-methyl-2-butanone		0.08	
2-methyl-2-cyclopenten-1-one		0.11	
2-methyl-2-hepten-4-one			0.16
2-methyl-2-pentanone		0.02	
2-methyl-cyclopentanone		0.06	
2-methylbutanoic acid			0.71
2-methylcyclopentanone			0.05
2-methylfuran	0.05		
2-methylnaphthalene	0.17	0.04	
2-methylpropanoic acid (isobutyric A,methylisobutyrate)	1.3	1.3	

2-methylpyridine (α-picoline)	0.11		0.041
3-ethylcyclopentene			0.072
3-methyl-2-cyclohexen-1-one		0.12	0.14
3-methyl-2-cyclopenten-1-one	0.49	0.5	0.46
3-methylbutanoic acid (isovaleric A,delphinic A)	1.9	2.2	0.88
3-methylcyclopentanone			0.024
3-methylthiophene		0.04	0.02
3-pentanone		0.06	0.04
4-ethylpyridine		0.1	
4-methyl-2,3-dihydroindene			0.1
4-methyl-2-pentanol			0.008
4-methyl-2-pentanone		0.01	0.008
4-methylaniline (monomethylamine,monomethylaniline)	2.3	0.45	
4-octyne		0.39	0.78
aniline (benzenamine,phenylamine)	4.3	1.9	0.91
anthracene * (718)	0.003	0.001	
benzene		0.21	1.7
benzoanthracene			0.0028
butanoic acid (butyric A,ethylacetic A)	1.2	2.1	1.9
chrysene			0.0019
cyclohexanone (ketohexamethylene, adipic keytone)	0.06	0.06	
cyclopentanone (ketocyclopentane, adipic ketone)	0.1	0.140	0.09
dimethyldisulfide (thiodiglycolic A, mercaptoacetic A)	0.25	0.24	0.12
dimethylnaphthalenes		0.01	0.11
dimethylphthalate * (DMP,methylphathalate,3244)	0.15		
docosane ($C_{22}H_{46}$)	0.02	0.01	0.05
dodecane ($C_{12}H_{28}$)	0.12	0.05	0.45
eicosane ($C_{20}H_{42}$)	0.023	0.01	0.05
ethyl benzene * (3695)	0.13	0.18	0.23
heneicosane ($C_{21}H_{44}$)	0.023	0.01	0.05
heptacosane ($C_{27}H_{56}$)	0.007	0.003	0.02
heptadecane ($C_{17}H_{36}$)	0.03	0.02	0.14
heptanoic acid (enanthic A,oenanthic A,4522)	4.	5.2	0.59
hexacosane ($C_{26}H_{54}$)	0.011	0.005	0.02
hexadecane ($C_{16}H_{34}$)	0.07	0.03	0.16
hexanoic acid (n-caproic A, 9368)	5.1	6.7	2.5
m,p xylene * (dimethylbenzene,9743)	0.36	0.52	0.51
naphthalene * (tar camphor,6194)	0.08	0.03	0.1
nonacosane			0.01
nonadecane			0.06
o-xylene * (dimethylbenzene,9743)	0.2	0.33	0.39
octacosane ($C_{28}H_{58}$)	0.005	0.003	0.15
octadecane ($C_{18}H_{38}$)	0.03	0.02	0.07
pentacosane ($C_{25}H_{52}$)	0.015	0.01	0.04
pentadecane ($C_{15}H_{32}$)	0.09	0.03	0.28
pentanoic acid (valeric A,propylacetic A,5093)	4.8	2.3	2.2

phenanthrene + (iso-anthracene,6996)	0.007	0.002	
pyrene		0.003	0.0044
pyridine		0.08	0.14
pyrrole		0.07	
tetracosane ($C_{24}H_{50}$)	0.017	0.01	0.04
tetradecane ($C_{14}H_{30}$)	0.13	0.05	0.44
tetrahydrothiophene		0.04	0.02
toluene * (methylbenzene,9225)	0.67	0.82	0.8
triacontane			0.007
tricosane ($C_{23}H_{48}$)	0.02	0.01	0.04
tridecane		0.04	0.39
trimethylbenzenes (mesitylene)	0.17	0.4	0.710
undecanal			0.013
undecane ($C_{11}H_{24}$)	0.085	0.03	0.25

* volatile organic priority pollutant

+ organic priority pollutant

Compounds in parenthesis are alternate names; the number in parenthesis is the Merck Index (1976) registry number.

Refinery Wastewater Isotherms

Refinery Wastewater Isotherms: Two isotherms were performed with refinery wastewater as the adsorbate. Westvaco PAC (Aqua-Nuchar SA) was used as the adsorbent in one test and bentonite clay in the other. Results from the isotherms, which were conducted using the same batch of wastewater, are shown in Table 17 and Figures (9-14). Data for carbon demonstrated less-than-favorable adsorption equilibria for this particular wastewater, and in the case of bentonite, little or no adsorption capacity was demonstrated. For PAC results, the equilibrium adsorption data correlated well with the Freundlich (r^2 = 0.8) isotherm model.

Using the calculated parameters for the Freundlich adsorption model, the reactor equilibrium conditions (i.e., when the effluent TOC = 100 mg/l; see Fig. 15) suggest that at a carbon dose of 50 mg/l, the steady state reduction in TOC due to carbon adsorption would be less than 1.0 mg/l. However, steady state operational data on effluent TOC (see Fig. 16) indicates that the refinery PAC and bentonite units consistently achieved greater TOC reduction efficiencies than that of the corresponding control unit. Also increased organic removal due to PAC addition is consistent with observations cited in a number of studies.

Several mechanisms have been proposed to explain this common observation. Among those are adsorption of influent substrate, enhanced biological assimilation, bioregeneration of adsorbed organics and metabolic end product adsorption.

Table 17: Refinery Wastewater Isotherm Results

PAC Dose(mg)	STOC C_e (mg/1)	q	ln q	ln C_e	1/q	1/C_e
12.5	420.0	0.300	-1.20	6.04	3.33	0.00240
50.0	400.0	0.125	-2.80	5.99	8.00	0.00250
125.0	345.0	0.105	-2.25	5.84	9.52	0.00289
250.0	292.0	0.079	-2.54	5.67	12.65	0.00342
625.0	235.0	0.043	-3.15	5.45	23.25	0.00458
1250.0	218.0	0.023	-3.76	5.38	43.10	0.00458
2500.0	165.0	0.014	-4.25	5.10	70.20	0.00606
Bentonite Dose(mg)						
12.5	400.0	0.340	-1.80	5.99	2.94	0.00250
50.0	425.0	0.025	-3.69	6.04	40.00	0.00235
125.0	398.0	0.036	-3.32	5.98	27.80	0.00251
250.0	408.0	0.013	-4.34	6.01	16.90	0.00245
625.0	398.0	0.0072	-4.93	5.98	138.90	0.00251
1250.0	-	-	-	-	-	-
2500.0	-	-	-	-	-	-

Note: At bentonite doses greater than 1250 mg, it was not possible to filter samples to obtain a consistent value of C_e.

Blank TOC= 450 mg/1

Volume of sample = 125 ml.

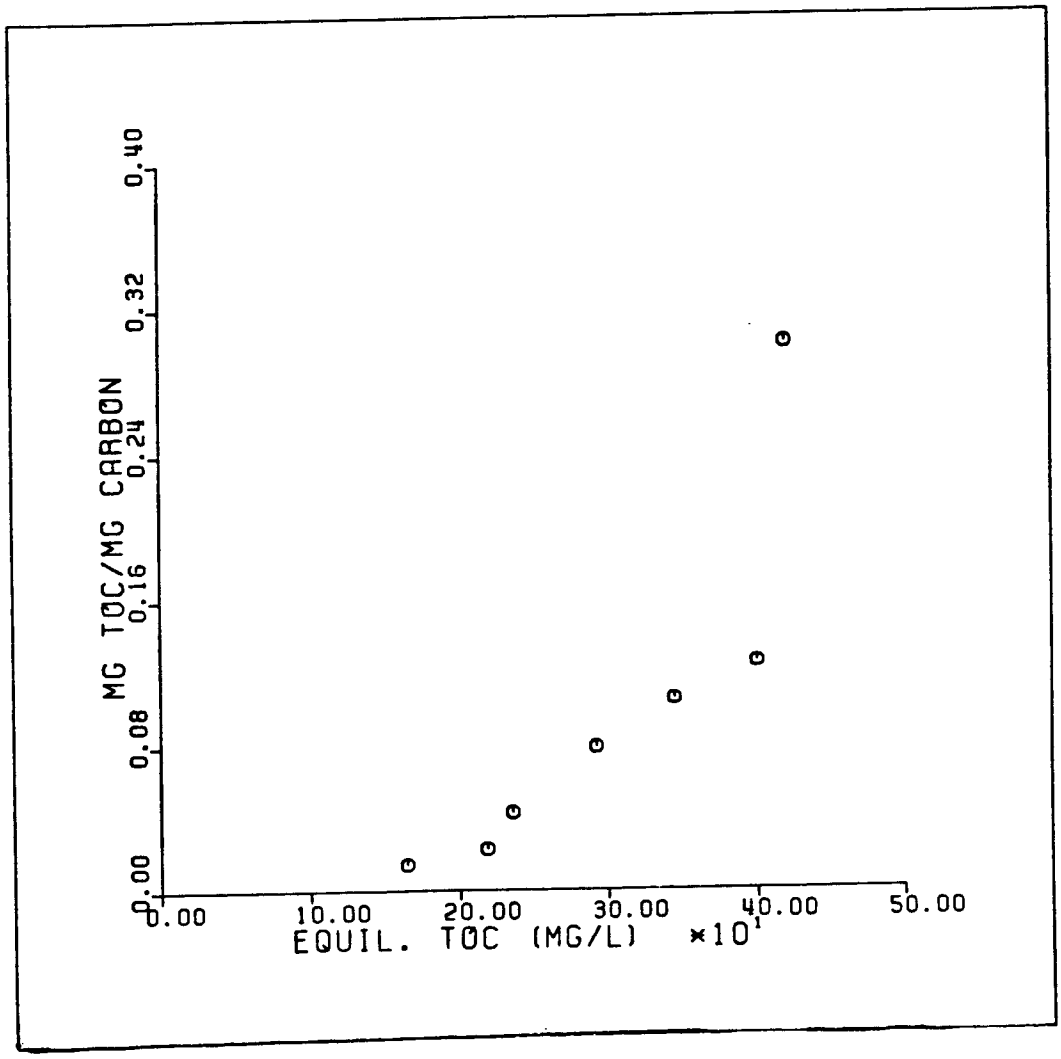


Figure 9: Refinery Wastewater Isotherm: q_e vs C_e for PAC

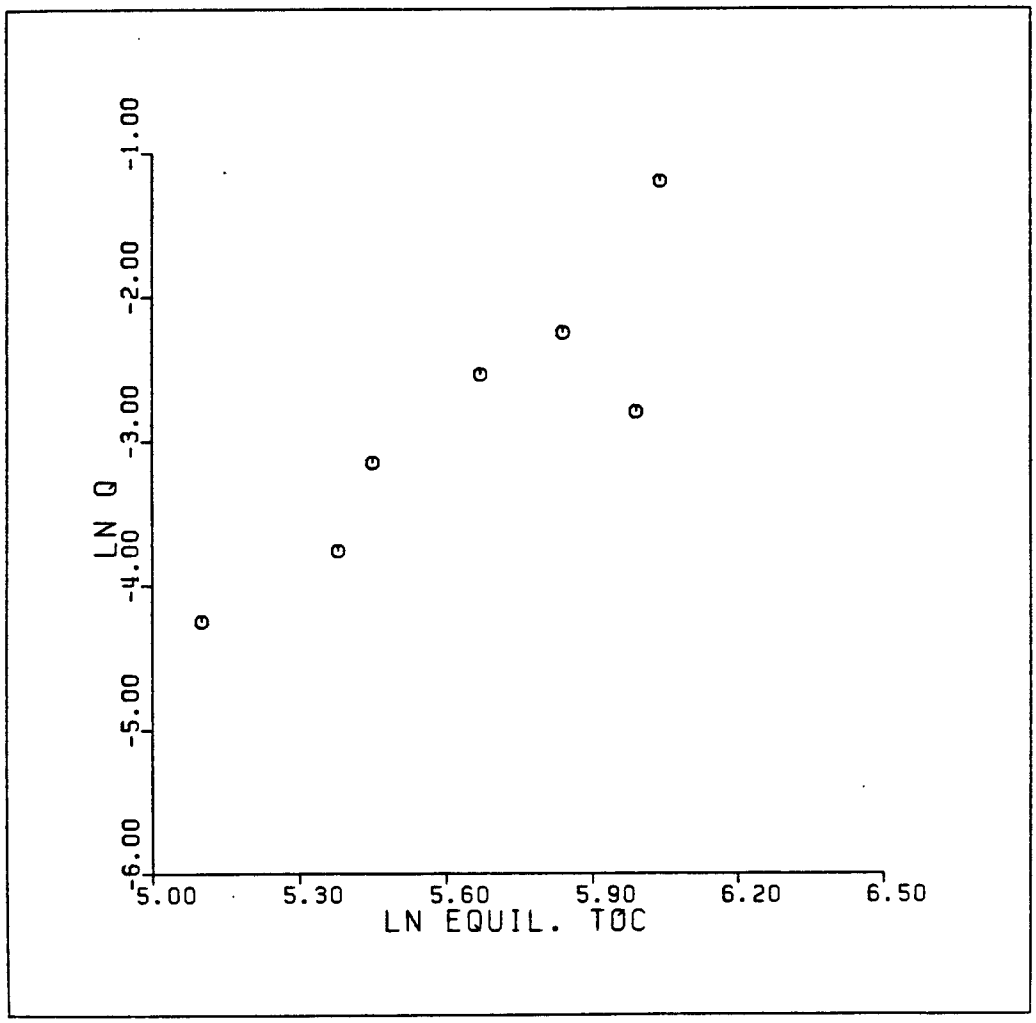


Figure 10: Refinery Wastewater Isotherm: ln q_e vs ln C_e for PAC

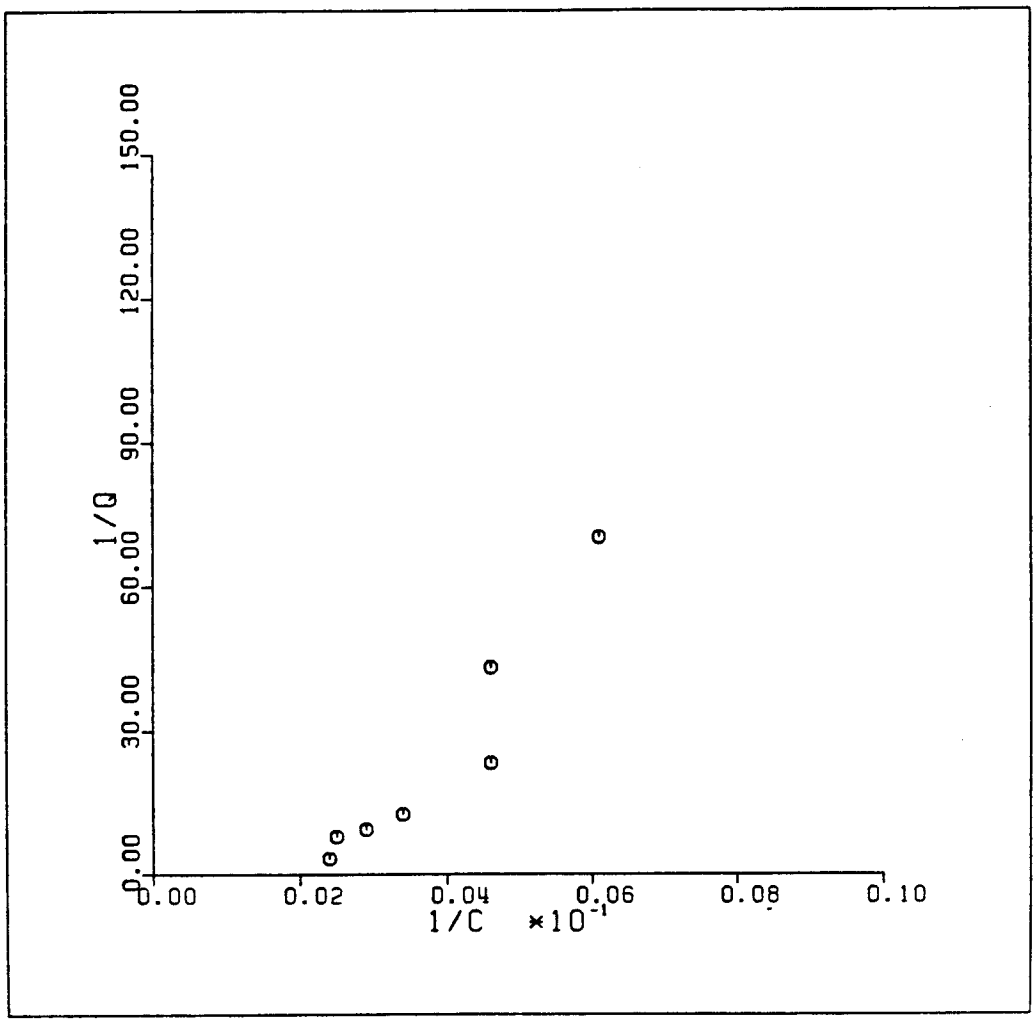


Figure 11: Refinery Wastewater Isotherm: 1/q vs 1/C for PAC

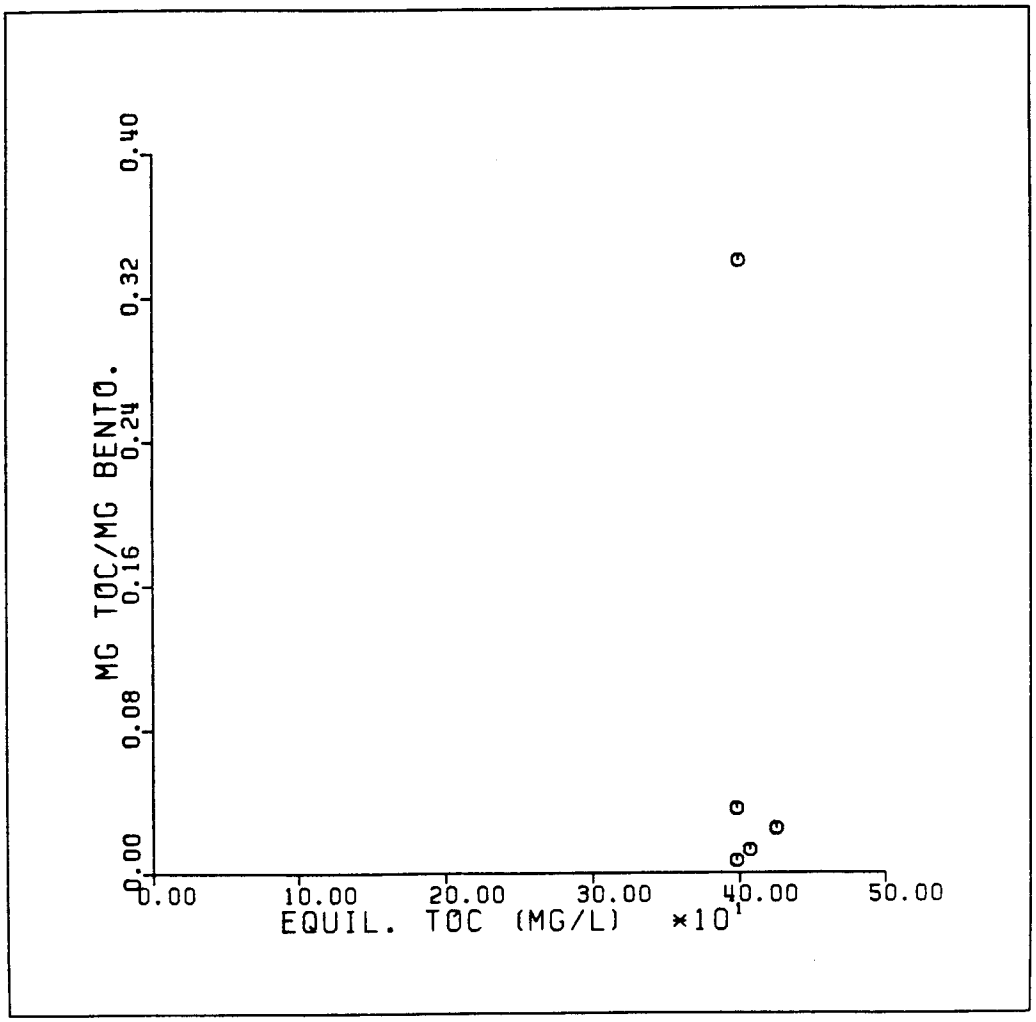


Figure 12: Refinery Wastewater Isotherm: q_e vs C_e for Bentonite

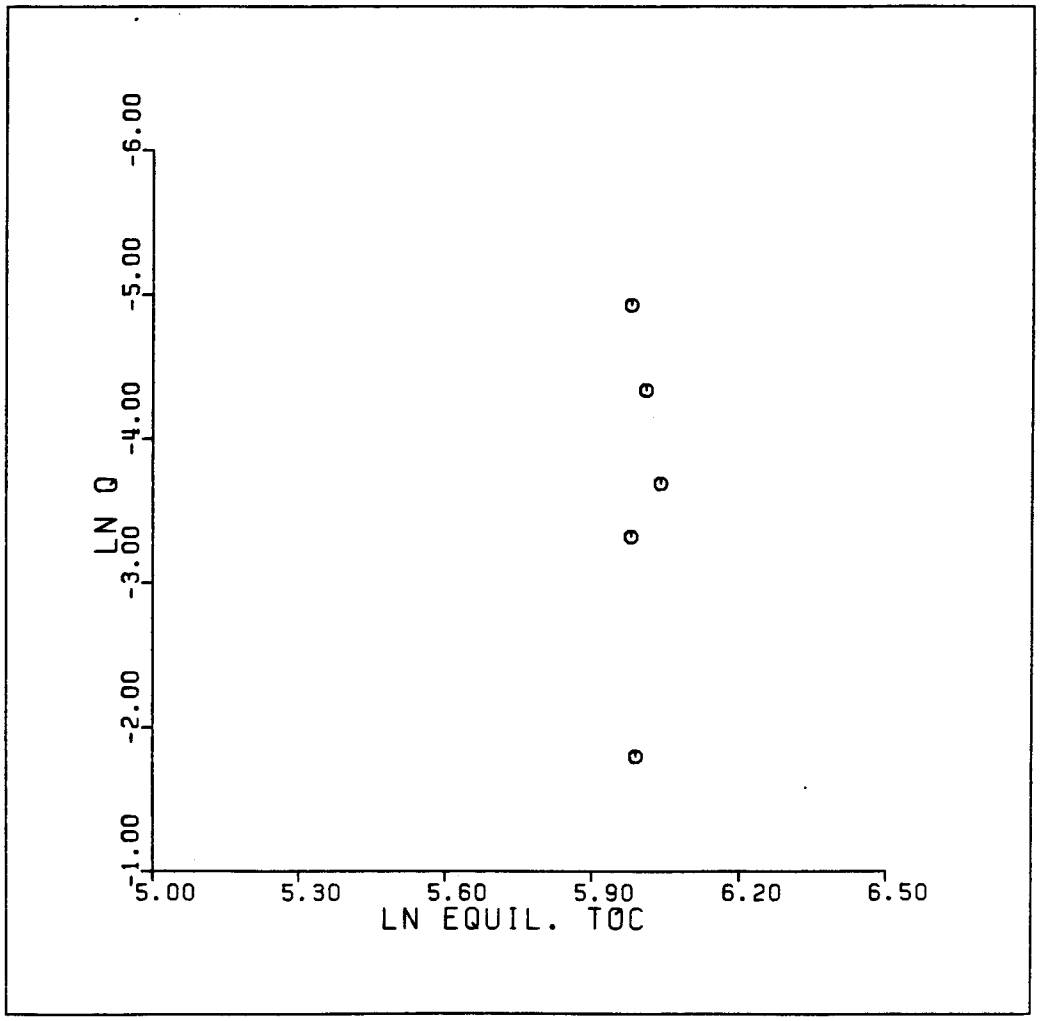


Figure 13: Refinery Wastewater Isotherm: ln q_e vs ln C_e for Bentonite

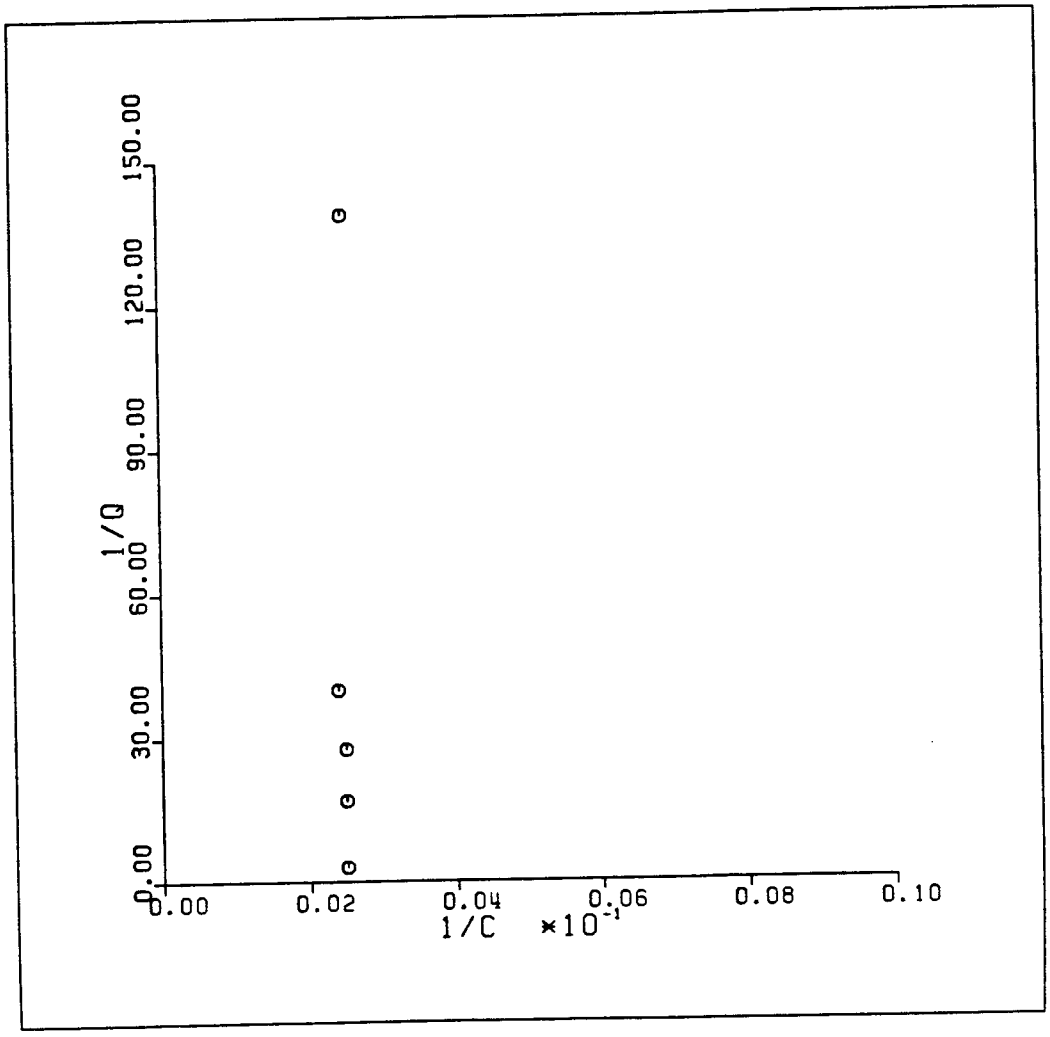


Figure 14: Refinery Wastewater Isotherm: 1/q vs 1/C for Bentonite

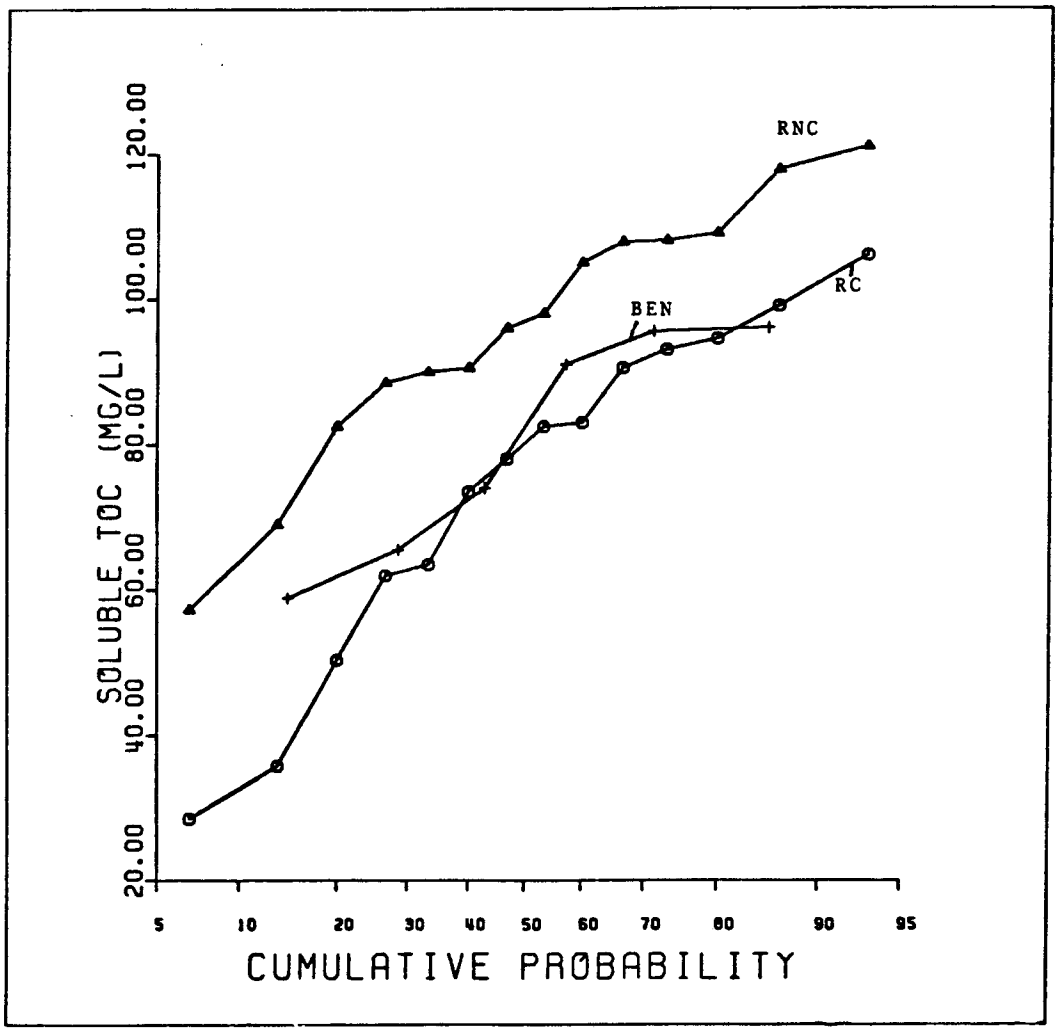


Figure 15: Cumulative SOC Probability For Refinery Reactors (BEN=Bentonite Clay, RNC=Refinery Non-Carbon, RC=Refinery Carbon)

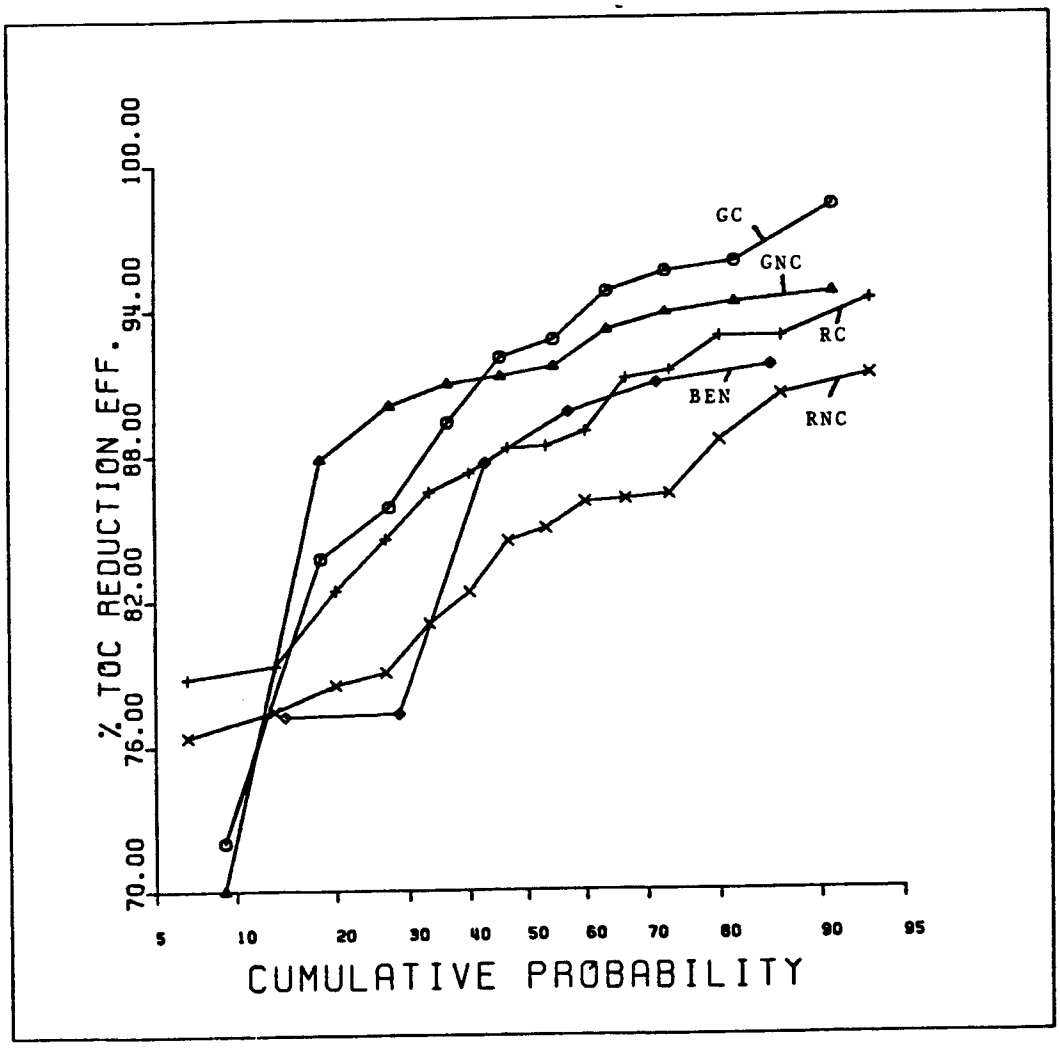


Figure 16: Cumulative TOC Reduction Efficiency Probability (GC=Glucose Carbon, GNC=Glucose Non-Carbon, BEN=Bentonite Clay, RC=Refinery Carbon, RNC=Refinery Non-Carbon)

The results from the isotherm study suggest that the increased TOC removals in the PAC unit are due to some mechanism(s) other than the steady state adsorption of the influent substrate. This is supported, although less dramatically, by the steady state effluent TOC data for the glucose-fed reactors (see Fig. 17). Although glucose is a non-adsorbable substrate, the PAC reactor generally outperformed the non-PAC reactor in TOC reduction. Therefore it appears that in this study, influent substrate adsorption is not important under steady state conditions because PAC is at equilibrium with the treated effluent compounds and concentration rather than the influent compounds and concentration.

Other mechanisms such as enhanced biological assimilation (EBA) can be considered an explanation for increased organics removal. In other studies, increased steady state oxygen uptake rates and/or increased MLVSS in PAC units over non-PAC units were taken as evidence for EBA. In this study steady state data on oxygen uptake rates and MLVSS concentration (see Table 18) taken at various intervals throughout the entire study period provides no definite evidence to support the existence of EBA. In fact, the MLVSS concentrations in the PAC and bentonite clay reactors were generally less than in the corresponding control reactor.

One possible mechanism that may account for the observed TOC reduction by PAC is metabolic end product (MEP) adsorption. MEP are organics of microbial origin (i.e., metabolites, slime or cellular components from lysed organisms). Recent studies by Schultz (1982) with radioactively labeled phenol substrates showed that 75% of the MEP pro-

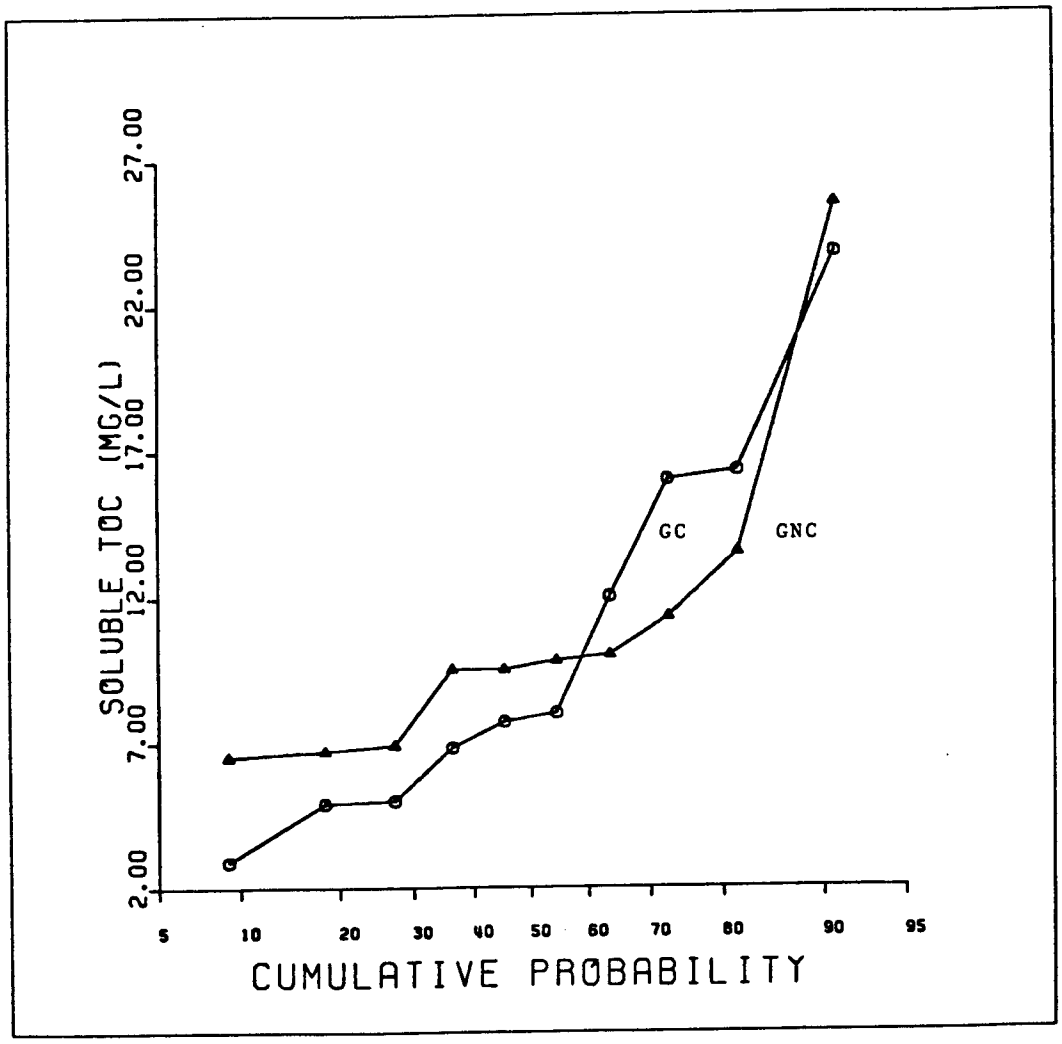


Figure 17: Cumulative SOC Probability For Glucose Reactors
(GC=Glucose Carbon, GNC=Glucose Non-Carbon)

Table 18: Oxygen Uptake Rate and MLVSS Data

Date	Oxygen uptake rates (mg O_2/1 hr)				
	GC	GNC	RC	RNC	BEN
12-6-82	10.6	10.0	16.0	14.4	14.0
2-18-83	11.2	11.3	15.7	15.0	14.2
3-15-83	11.1	10.8	15.6	15.1	15.0
	MLVSS (mg/1)				
	GC	GNC	RC	RNC	BEN
12-18-82	3060	2520	2836	3059	2918
1-19-82	-	4440	-	-	-
2-8-83	4404	5262	4000	5998	3754
2-28-83	3021	4998	5634	6000	3770
6-7-83	4782	6296	6517	4844	3292
7-27-83	-	-	8396	5313	4443

Note: Arbuckle's method (1982) was used to calculate MLVSS for the PAC reactors. Values for X_{400}=0.0784 and X_{550} = 0.74 were determined experimentally. MVSS for the bentonite reactors was measured and calculated by the steady-state method. Oxygen uptake rates were measured with an immersed DO probe in BOD bottles.

duced from the biodegradation of phenol was irreversibly adsorbed by PAC. Other studies (as cited in Schultz (1982)) suggested that the adsorption of MEP may play an important role in PAC systems, especially if the wastewater to be treated contains a low amount of adsorbable compounds.

The less pronounced differences in TOC removal efficiencies noted for the glucose reactors (see Fig. 17) may be attributed to the difference in substrate fed. Glucose is a very simple and easily biodegradable substrate, whereas the refinery wastewater contains a wide variety of very complex compounds, many of which biodegrade very slowly (see Table 16). The differences in substrate no doubt influence the microbial population in the respective reactors -- thus influencing the nature and quantity of MEP produced. It should be noted that the bentonite unit performed as effectively as the PAC unit with respect to TOC removal efficiency. If indeed the MEP adsorption theory is valid in this situation, a MEP isotherm study with bentonite should be undertaken.

Aniline Isotherms

Two isotherms were performed using aniline as the adsorbate with PAC and bentonite as the adsorbents. The results, shown in Tables 19-20 and Figures (18-21), indicate that aniline is nonadsorbable on bentonite and adsorbable on PAC. Aniline was not adsorbed at bentonite doses of less than 1000 mg/l, consequently these dosages were excluded in the calculation of isotherm parameters. The equilibrium data for the

Table 19: Powdered Activated Carbon Isotherm Results

PAC Dose(g/l)	Aniline C_e(mg/l)	q (mg/mg)	log q	1/q	$1/C_e$	log C_e
20.0	0.25	0.00498	-2.302	200.5	4.000	-0.602
15.0	0.38	0.00664	-2.178	150.6	2.630	-0.420
10.0	0.50	0.00995	-2.002	100.5	2.000	-0.301
8.0	1.27	0.01234	-1.908	81.0	0.790	-0.104
5.0	1.32	0.01974	-1.705	50.7	0.750	-0.120
2.0	13.5	0.04325	-1.364	23.1	0.074	1.13
1.0	30.1	0.06989	-1.155	14.3	0.033	1.47
0.6	41.1	0.09817	-1.008	10.2	0.024	1.61
0.2	76.8	0.11600	-0.9355	8.6	0.013	1.88
0.1	88.6	0.11399	-0.9431	8.8	0.011	1.94
Bentonite Dose (g/l)						
20.0	53.5	0.00232	-2.6335	430.1	0.019	1.72
15.0	71.9	0.00187	-2.7273	533.8	0.014	1.85
10.0	73.0	0.00270	-2.5686	370.4	0.013	1.86
8.0	77.2	0.00285	-2.5451	350.9	0.013	1.89
5.0	78.0	0.00439	-2.3565	227.3	0.013	1.89
2.0	81.8	0.00989	-2.0409	109.9	0.012	1.91
1.0	94.2	0.00580	-2.2365	172.4	0.011	1.97

Initial aniline = 100.0 mg/l
Volume of sample =100 ml.

Table 20: Calculated Adsorption Parameters for PAC

Freundlich Model	r^2	1/n	K_f at C_e = 1.0 mg/l
Adsorbate			
Refinery Wastewater	0.8	2.58	2.8×10^{-3} mg/g PAC
Aniline	0.98	0.52	12.2 mg/g PAC
Langmiur Model	r^2	$\frac{1}{bQ^o}$	$\frac{1}{Q^o}$
Adsorbate			
Refinery Wastewater	*	*	*
Aniline	0.97	47.5	15.4

Note: For RW: At C_e = 100 mg/l TOC, q_e = 4.1 mg/g PAC according to the Freundlich model.

For aniline: At C_e = 1.0 mg/l, q_e = 12.2 mg/g PAC according to the Freundlich model and 15.8 mg/g PAC according to the Langmuir model.

*: Data does not fit model

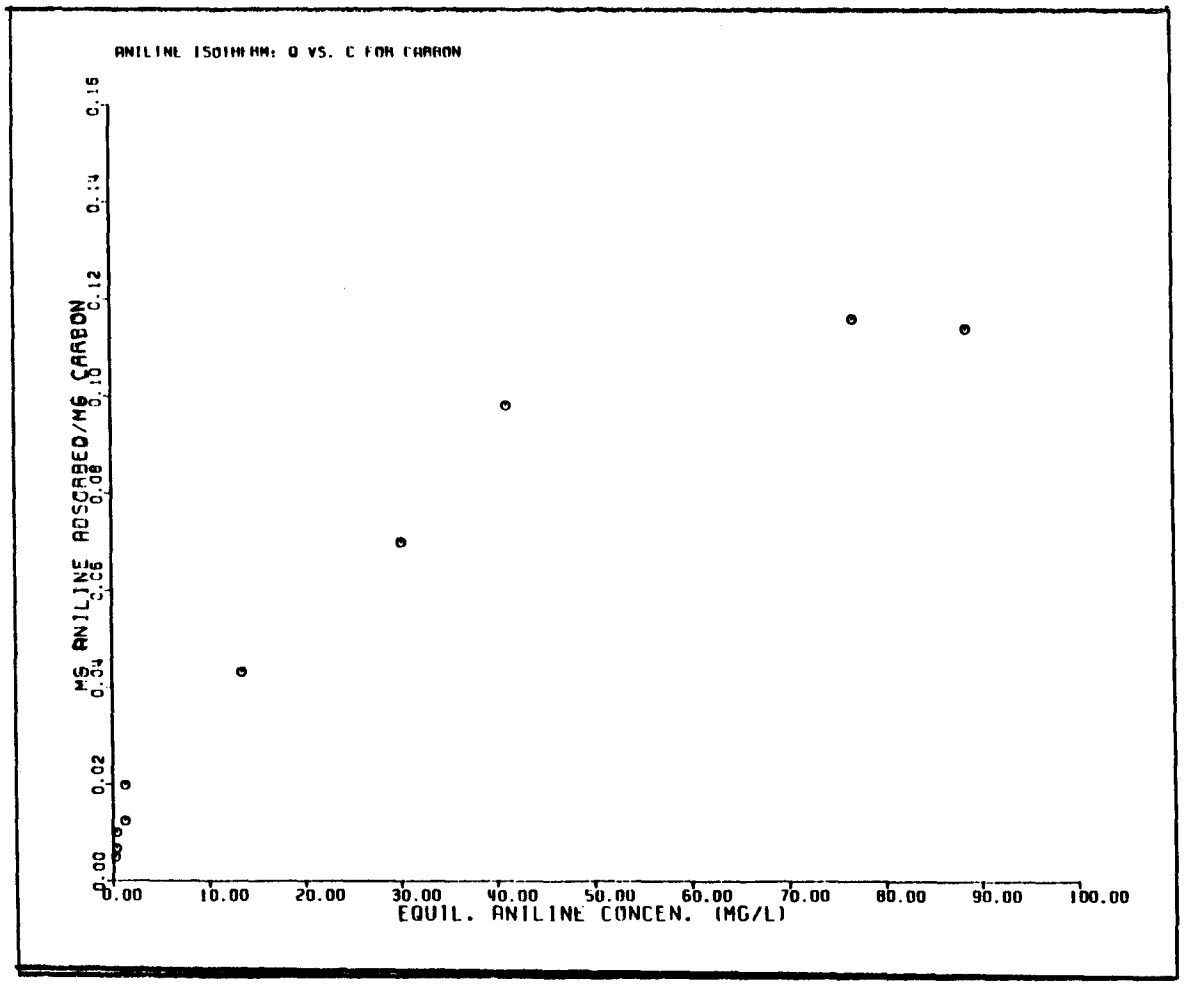


Figure 18: Aniline Isotherm: q_e vs. C_e for Carbon

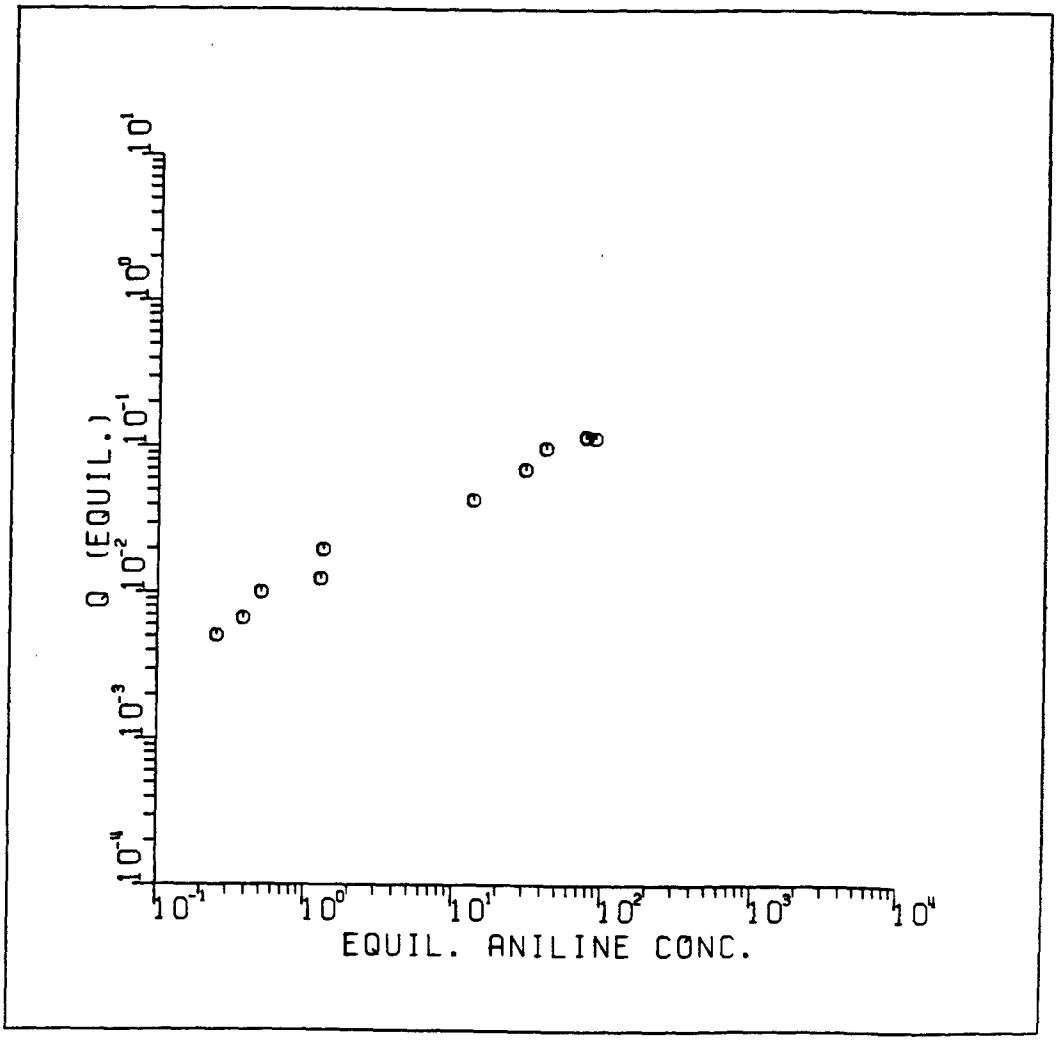


Figure 19: Aniline Isotherm: log q_e vs. log C_e for Carbon

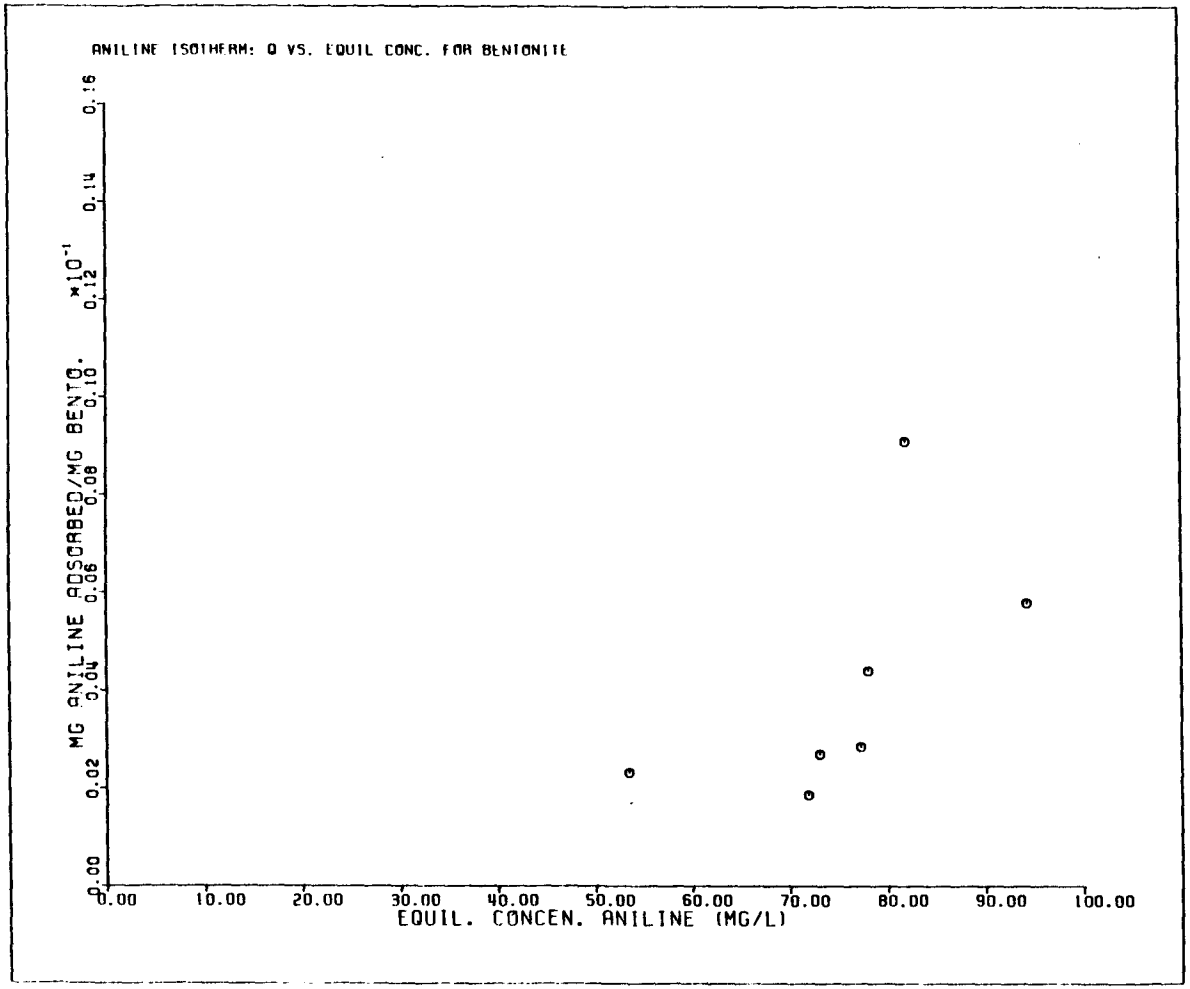


Figure 20: Aniline Isotherm: q_e vs C_e for Bentonite

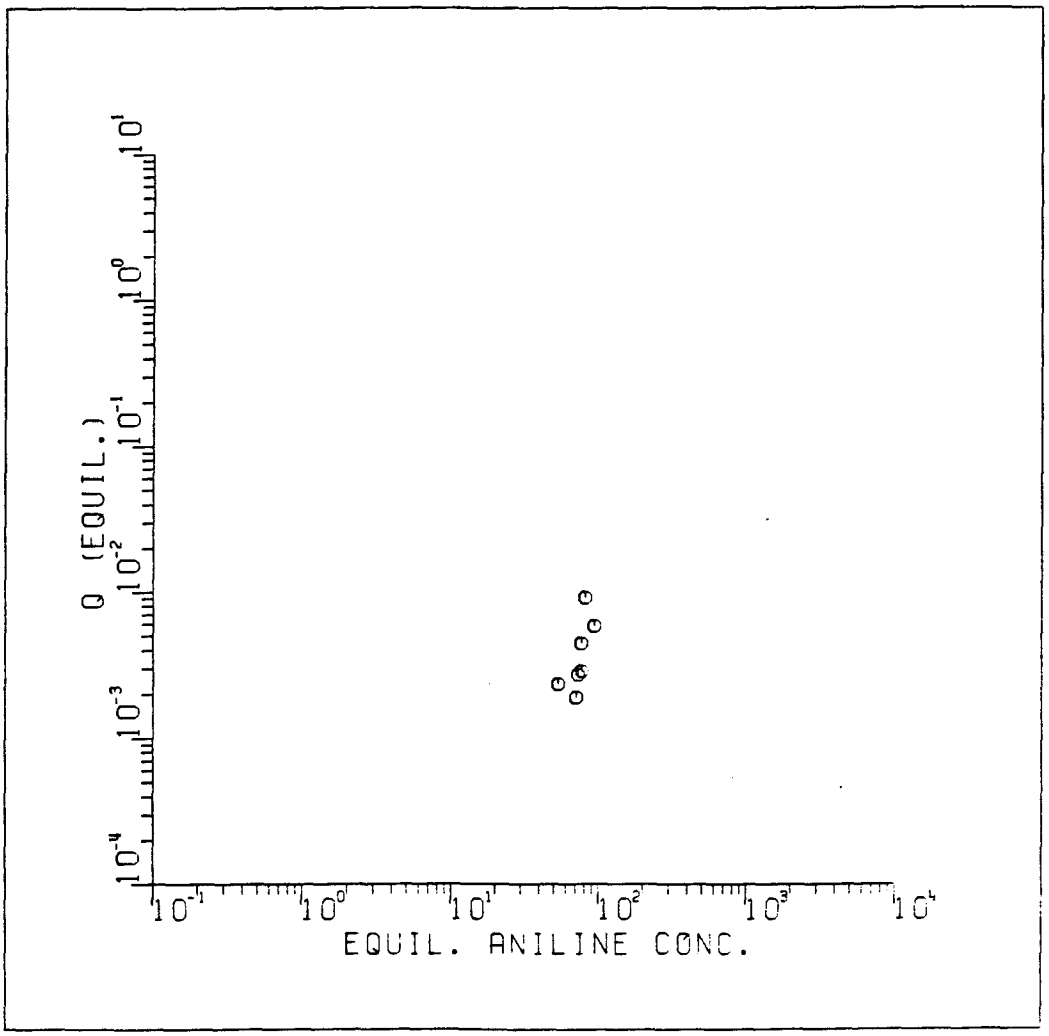


Figure 21: Aniline Isotherm: log q_e vs log C_e for Bentonite

adsorption of aniline on carbon fitted both the Freundlich (r^2 = 0.98) and the Langmuir (r^2 = 0.97) reasonably well. Figure 18 shows that the PAC isotherm resembles that typical of an isotherm described by the Langmuir model. This suggests monolayer solute disposition and reversible adsorption sites on the carbon surface. Equilibrium considerations suggest that approximately 12.2 mg of aniline per gram of carbon is adsorbed at a equilibrium aniline concentration of 1.0 mg/l. Calculated adsorption parameters for both the refinery and aniline isotherms are shown in Table 20.

BATCH INHIBITION EXPERIMENTS

A series of batch experiments was performed for the various inhibitors used in the study. In order to interpret observed nitrification rates, some quantitative method of determining a reaction parameter was needed. Using knowledge of the half-saturation Monod coefficients, a zero-order kinetic method can be used.

It has been shown that the K_S value for nitrification is in the range of 0.5 to 2.0 mg/l; therefore, for ammonia concentrations above 2.0 mg/l one can use zero-order kinetics to describe ammonia oxidation. The kinetic expression for ammonia oxidation in a batch reactor becomes:

$$\frac{dNH_4^+}{dt} = -K \qquad (48)$$

If ammonia oxidation is rate limiting, the nitrate production can be similarly modeled:

$$\frac{dNO_3^-}{dt} = K \qquad (49)$$

Therefore the rate constant, K, can be determined using simple linear regression when the of ammonia concentration is above a few milligrams per liter.

Data analysis included linear regression for the evaluation of the ammonia and nitrate reaction constants from observed zero order kinetics and covariance analysis using the Generalized Linear Model (GLM) procedure of the Statistical Analysis System (SAS, 1981). Covariance analysis tests for the heterogeneity of the slopes between the treatment group (i.e., in the presence of the added compound) and the control group. This analysis was restricted to comparisons between test and control groups since they differ only in the presence or absence of the added inhibitory compound.

The calculated reaction rate constants, K for each experimental flask are shown in Table 21. In general, for RW derived flasks, the K values for ammonia oxidation and nitrate production were not found to be in stoichiometric agreement; nitrate production rates were usually higher than the corresponding ammonia oxidation rates. This was probably due to excessive cellular lysis releasing nitrogen from the addition of the inhibitory compound or because of the inherent analytical difficulties associated with the measurement of nitrate in this particular type of wastewater (see Analytical Methods). For this reason the ammonia data, which was considered to be more reliable than the nitrate data, was selected for further analysis.

Table 21: Ammonia Oxidation Constants, K and Regression Coefficients, r

Spiked Compound	Type		GC	GCC	GNC	GNCC	RC	RCC	RNC	RNCC	BEN	BENC
Acrylonitrile 10 mg/l (5/5)	NANI	r	0.96	0.99	0.96	0.99	0.95	0.97	0.92	0.97	0.92	0.98
		-K	3.47	3.33	3.35	3.28	2.39	2.49	1.25	1.48	1.07	1.13
Aniline 10 mg/l (6/14)	AI	r	0.95	0.98	0.72	0.99	0.94	0.99	0.96	0.99	0.89	0.99
		-K	5.12	6.98	1.87	5.92	4.76	5.47	4.17	4.82	3.77	4.14
	Duplicate Test	r	0.98	0.99								
		-K	4.82	6.98								
Aniline 10 mg/l (2/28)	AI	r	0.88	0.094	0.66	0.582	0.97	0.96	0.94	0.98	0.81	0.95
		-K	0.9	0.03	-0.39	-0.269	1.44	1.83	0.84	1.75	0.49	1.42
Cyanide 3 mg/l (4/28)	NAI	r	0.81	0.99	0.99	0.97	0.97	0.93	0.97	0.93	0.99	0.98
		-K	0.66	2.9	1.39	3.76	1.25	2.46	1.3	2.15	0.752	1.78
Cyanide 3 mg/l (6/7)	NAI	r	0.12	0.99	0.49	0.97	0.78	0.96	0.88	0.95	0.89	0.94
		-K	-0.16	4.51	0.72	3.88	2.17	4.86	1.33	2.37	1.77	2.5
Phenol 10 mg/l (5/13)	AI	r	0.69	0.95	0.93	0.94	0.96	0.94	0.99	0.98	0.97	0.96
		-K	1.26	2.89	2.69	3.65	2.46	2.51	1.84	1.83	1.82	1.77
Phenol 20 mg/l (5/25)	AI	r	0.98	0.99	0.67	0.97	.099	0.97	0.98	0.91	0.98	0.96
		-K	4.36	5.42	1.59	4.42	3.28	3.46	2.65	2.44	2.03	2.1
Toluene 10 mg/l (6/10)	ANI	r	0.99	0.99	0.99	0.99	0.98	0.95	0.96	0.95	0.97	0.93
		-K	5.38	5.44	5.24	5.19	4.22	4.02	3.15	3.13	4.24	3.29
	Duplicate Test	r					0.97	0.99				
		-K					3.88	4.05				

NANI= non-adsorbable, non-inhibitory compound
AI= adsorbable, inhibitory compound
NAI= non-adsorbable, inhibitory compound
ANI= adsorbable, non-inhibitory compound
K= ammonia oxidation rate constant, mg NH_4^+/hour
r= linear regression correlation coefficient

GC = glucose carbon reactor
GNC = glucose non-carbon reactor
RC = refinery carbon reactor
RNC = refinery non-carbon reactor
BEN = refinery bentonite
Appended C denotes corresponding control

Table 22 presents the ammonia oxidation rate constant in terms of an inhibition coefficient, "I". I is defined as the ratio of K in the presence of the added compound to K of the control flask. This is done to facilitate comparisons among the different reactor types and the degrees of inhibition observed. An I value of 1.0, or less than 1.0 would indicate, respectively, no inhibition, or an inhibitory effect on nitrification, due to the added inhibitory compound.

Inspection of Table 22 reveals that the addition of acrylonitrile (non-adsorbable) and toluene (adsorbable), which are both known to be non-inhibitory to nitrifiers, did not significantly affect the rate of nitrification. With the exception of two cases (toluene in the bentonite reactor, I=1.29, and acrylonitrile in the refinery wastewater non-carbon reactor, I=0.85), all I values for all flasks are close to unity. Duplicate tests on the same reactor type in two separate test runs (i.e., aniline and toluene) indicated that the reproducibility of I within an experimental run is approximately 7% or less.

The original intent of performing experiments with known non-inhibitory compounds was not only to provide for the evaluation of a full spectrum of compounds, but also to provide a means of determining the precision experimental technique. In this respect, the results with the non-inhibitory compounds show that these objectives are met.

Table 22 shows that for adsorbable/inhibitory compounds such as aniline or phenol, the benefits of PAC addition are pronounced for unacclimated activated sludges (i.e., glucose fed reactors). In two cases out of three, the PAC reactor was able to handle a shock load of the

Table 22: Nitrification Inhibition Coefficients, I

Spiked Compound	Type	GC	GNC	RC	RNC	RBEN
Acrylonitrile 10 mg/1	NANI	1.04	1.02	0.96	0.85	0.95
Aniline 10 mg/1 (2-28-83)	AI	*	*	0.787	0.48	0.345
Aniline 10 mg/1 (6-14-83)	AI	0.733,0.691	0.316	0.87	0.87	0.91
Cyanide 1 mg/1+	NAI	0.15	0.183	0.592	0.542	0.444
Cyanide 3 mg/1	NAI	0.228	0.369	0.508	0.607	0.422
Cyanide 3 mg/1	NAI	0.153	0.186	0.446	0.56	0.708
Phenol 10 mg/1	AI	0.435	0.736	0.98	1.0	1.02
Phenol 20 mg/1	AI	0.804	0.36	0.95	1.09	0.97
Toluene 10 mg/1	ANI	0.99	1.0	1.05,0.96	1.0	1.29

* The reactors were not nitrifying prior to the experiment. Therefore valid comparisons could not be made.
+ Because ammonia concentration was greater at the end of the test period than at the start for the test flasks, K(test)+1/K(control)+1 was used to calculate I.

inhibitory compound much more readily than the corresponding non-PAC reactor. In the case where the non-PAC unit outperformed the PAC unit (according to I values), the difference is not statistically significant, and will be discussed further later.

As seen in Table 22, the differences among the refinery wastewater fed reactors subjected to adsorbable inhibitors are less pronounced than that of the glucose fed reactors. This is attributed to the ability of the microorganisms to acclimate to a particular type of wastewater feed. Since the microorganisms in these reactors were routinely exposed to a wide variety of inhibitory compounds in the refinery wastewater for an extended period of time (including aniline and phenol), it is speculated that the adsorption due to the relatively low PAC or bentonite dose (i.e., 50 mg/l feed) used was not sufficient to provided any further improvement beyond the benefits of acclimation.

It should be noted however, that in the first batch experimental run conducted (i.e., 10 mg/l aniline), the PAC reactor outperformed the corresponding non-PAC and bentonite reactors. It is conceivable that the refinery reactors, at this earliest stage of experimentation, were not yet fully acclimated to the wastewater. This then, would provide a more sensitive experiment for the detection of any beneficial effect of the added carbon. A similar experiment with 10 mg/l aniline which was run 3 1/2 months later showed no appreciable benefit of PAC for the refinery reactors.

Perhaps the strongest evidence for microbial acclimation ability in the refinery wastewater is the experiments when cyanide was spiked. Table 22 shows that cyanide, a non-adsorbable inhibitor, had detrimental effect on all measured nitrification rates. Although the results are inconclusive with respect to any observable benefit of added PAC or bentonite, the benefits of acclimation are evident in all three runs in which cyanide was tested (refinery wastewaters usually have some small cyanide concentration).

Table 23 presents a more rigorous analysis of the batch inhibition experiments with a pre-established criteria for judging the benefit of PAC or bentonite addition. The results of covariance analysis (SAS, 1981) on ammonia oxidation rate data for all experimental runs are shown in this table. Covariance analysis can be described as combining the methods of linear regression and analysis of variance. In this study, the null hypothesis (i.e., no difference between the test and control flasks) is tested, and either accepted or rejected according to a prescribed level of probability, using the F statistic. The F statistic is defined as the ratio of the variance between oxidation rates in the test flask and control flask to the variance within each flask;

$$F = \frac{\sigma^2 \text{ (between)}}{\sigma^2 \text{ (within)}} \qquad (51)$$

Theoretically, the two variance estimates should be equal if the null hypothesis is true, however due to sampling fluctuations F may differ substantially from 1.0.

Table 23: Regression Results (F statistics) for Ammonia Data
(Test flask versus control flask)

Reactor type	Acrylonitrile (10 mg/1) (NANI)	Aniline (10 mg/1) (AI)	Aniline (10 mg/1) (AI)	Cyanide (1 mg/1) (NAI)	Cyanide (3 mg/1) (NAI)	Cyanide (3 mg/1) (NAI)	Phenol (10 mg/1) (AI)	Phenol (20 mg/1) (AI)	Toluene (20 mg/1) (ANI)
Glucose Carbon	0.07/0.866 (no)	Note 1	2.23/0.186 (no)	16.25/0.007 (yes)	45.3/0.005 (yes)	32.24/0.001 (yes)	3.2/0.123 (no)	2.13/0.195 (no)	0.01/0.934 (no)
Glucose Non-Carbon	0.01/0.913 (no)	Note 1	13.82/0.01 (yes)	25.1/0.002 (yes)	5.69/0.054 (no)	12.23/0.129 (yes)	0.04/0.85 (no)	6.02/0.0495 (yes)	0.02/0.88 (no)
Refinery Carbon	0.03/0.866 (no)	1.64/0.237 (no)	0.42/0.541 (no)	14.65/0.009 (yes)	4.4/0.081 (no)	4.23/0.085 (no)	0.16/0.705 (no)	0.08/0.781 (no)	0.05/0.837 (no)
Refinery Non-Carbon	0.39/0.55 (no)	15.74/0.004 (yes)	0.68/0.44 (no)	0.86/0.39 (no)	3.12/0.126 (no)	2.31/0.179 (no)	0.0/0.99 (no)	0.08/0.786 (no)	0.0/0.988 (no)
Refinery Bentonite	0.05/0.828 (no)	10.2/0.013 (yes)	0.1/0.77 (no)	10.76/0.17 (yes)	26.8/0.002 (yes)	1.1/0.336 (no)	0.01/0.922 (no)	0.02/0.884 (no)	0.92/0.375 (no)

Notes:

1. Reactors were not nitrifying prior to experiment.

2. Ammonia data points less that 2.0 mg/1 were deleted.

3. Ammonia data points taken after 10.5 hours were deleted.

KEY: Boxes read as follows:
F value/ probability > F
significantly different at $\alpha = 0.05$
(yes or no)

For a given sample size and level of significance, the larger the F value, the more significant the difference between the variates. The prob > F is the observed level of significance of F or the probability of accepting the null hypothesis if true. Thus, the larger the prob>F value, the greater the credibility associated with the accepting the null hypothesis. The level of significance typically used to reject the null hypothesis is either 0.01, 0.05, or 0.1. In this study, the preselected level of significance is 0.05. Thus, if the null hypothesis is rejected at a 0.05 level of significance, it may be said with 95% confidence that the rates of ammonia oxidation between the test and control units differ and that this difference is attributed to the differences between the control and experiment, which for this case is only the presence of the inhibitory compound.

The results of the statistical analysis confirm the generalizations drawn from the table of inhibition coefficients. No differences are observed for the non-inhibitory compounds regardless of adsorption characteristics. The statistical results for cyanide, a non-adsorbable inhibitor, show no definite advantage in adding either PAC or bentonite. In fact, the non-PAC units performed as well or better than the PAC or bentonite units when spiked with cyanide. For the adsorbable inhibitors, the benefit of PAC is clearly demonstrated in unacclimated activated sludge. For the refinery reactors, the benefits of PAC is statistically significant only in the earliest experimental run. This is presumably because the refinery reactors were not yet fully acclimated.

The raw data generated from the series of batch inhibition experiments are presented in Appendix I.

CHRONIC EXPERIMENTS

The results of the chronic experiments are presented in Appendix 2 and plotted in Figures (22-29). In the initial test (Figures 22-24), each refinery reactor was subjected to a simultaneous pulse and step addition of 30 mg/1 aniline, an adsorbable nitrification inhibitor. Figure 22 shows surprising results in that nitrification was completely inhibited in the PAC reactor, whereas the control and bentonite reactors resumed nitrification to completion following a lag period of 6 (control) to 14 hours (bentonite) of continuous operation. Further evidence for the complete inhibition of nitrification in the PAC reactor is provided by the concurrent observations of negligible sodium hydroxide consumption for pH maintenance (see Figure 23) and a continuous decrease in reactor nitrate concentration (see Figure 24).

The reason(s) for the lack of nitrification in the PAC unit were quite perplexing and it was decided (on the 75th hour of the experiment) to add 1000 mg/1 of PAC to the inhibited reactor. Figures 22-24 shows that the biological response to the added PAC was dramatic with an immediate resumption of nitrification, sodium hydroxide uptake and nitrate production. Although Figure 23 indicates a short lag in sodium hydroxide uptake, it is not indicative of true consumption, since the aniline spike had raised the reactor pH from 7.0 to 8.3. The caustic pump was set to actuate when the pH dropped below 7.0; thus the lag

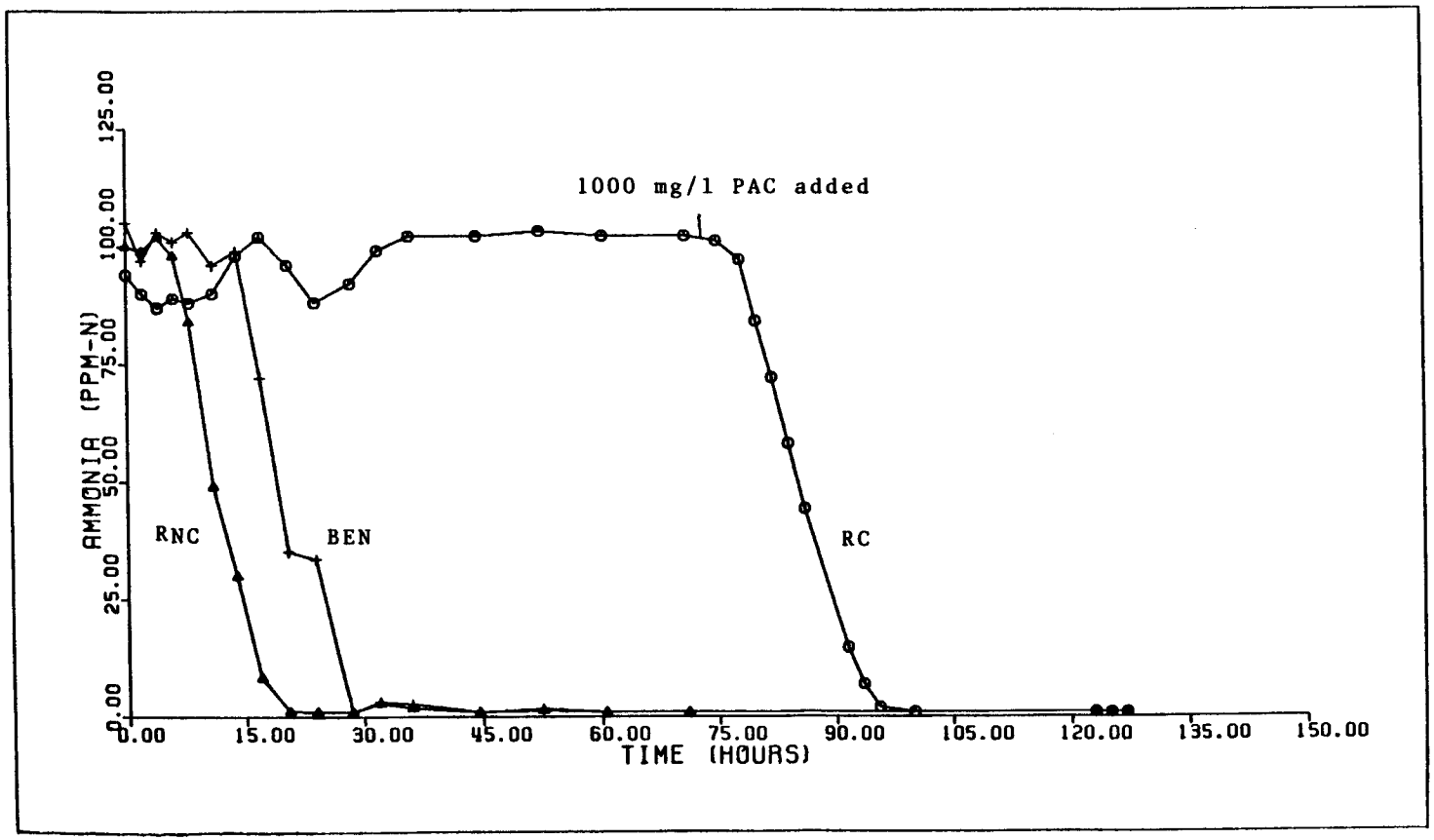


Figure 22: First Chronic Experiment: Ammonia vs Time
(BEN=Bentonite Clay, RC=Refinery Carbon, RNC=Refinery Non-Carbon)

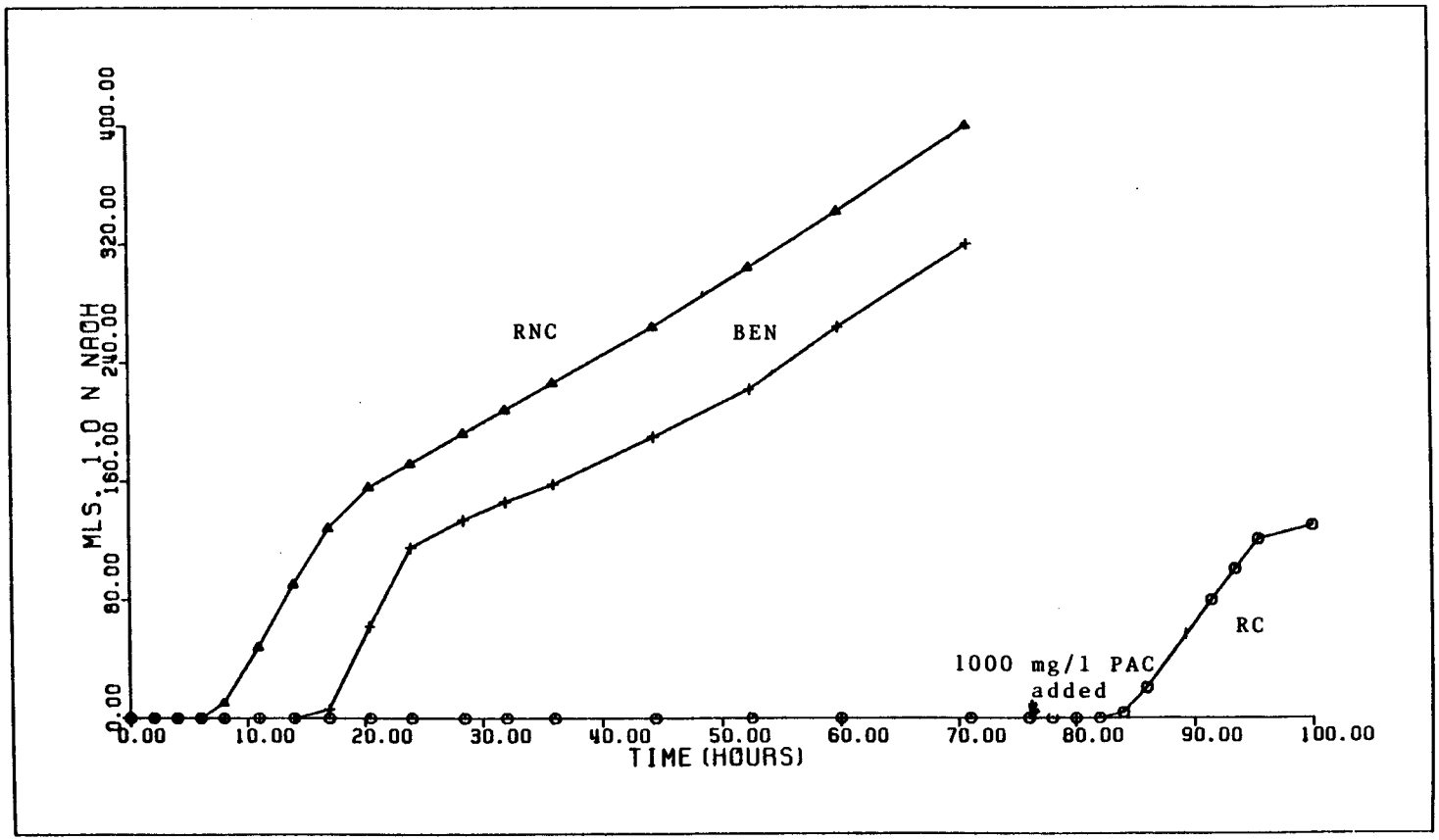


Figure 23: First Chronic Experiment: Caustic Uptake vs Time

(BEN=Bentonite Clay, RC=Refinery Carbon, RNC=Refinery Non-Carbon)

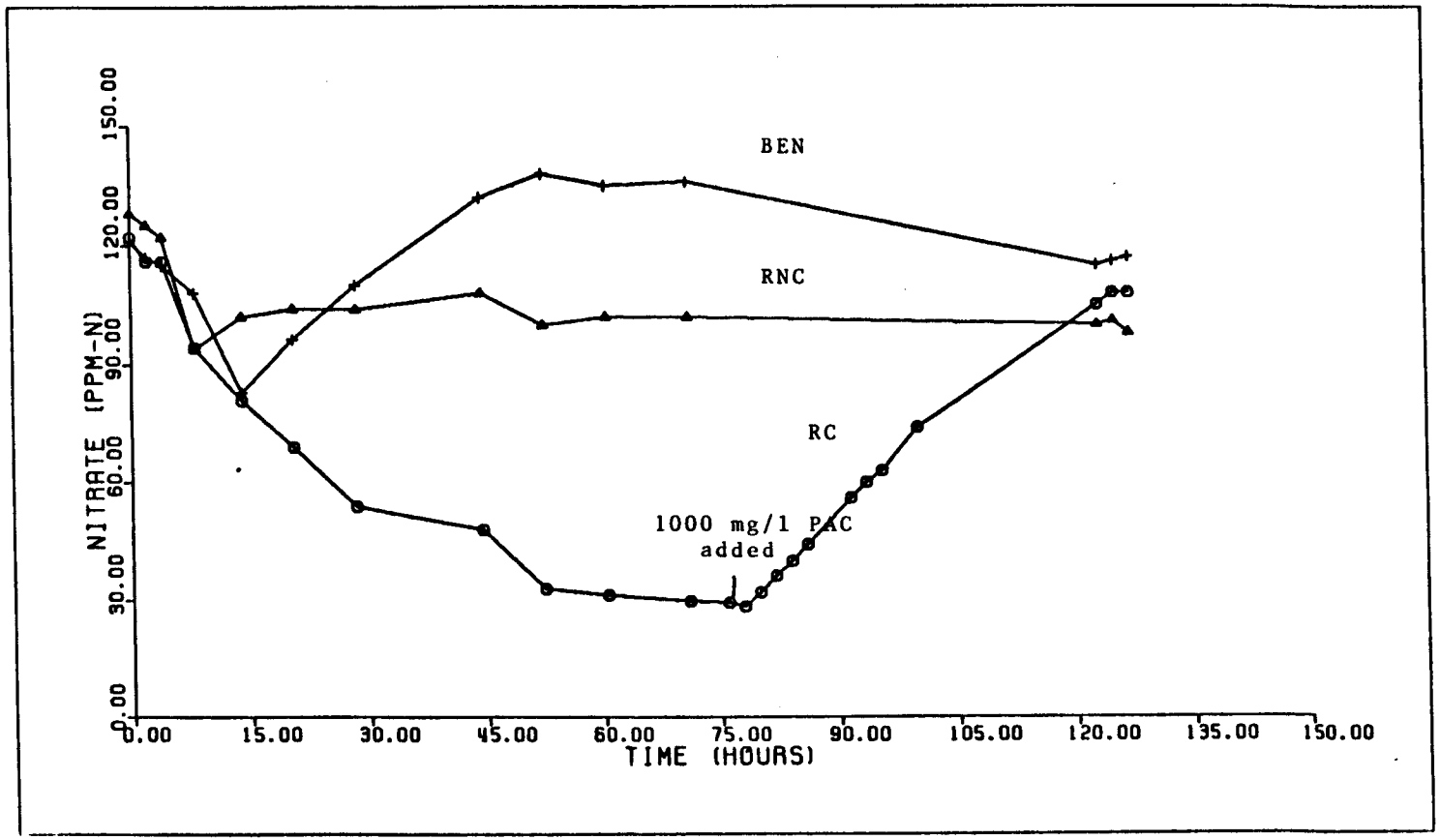


Figure 24: First Chronic Experiment: Nitrate vs Time

(BEN=Bentonite Clay, RC=Refinery Carbon, RNC=Refinery Non-Carbon)

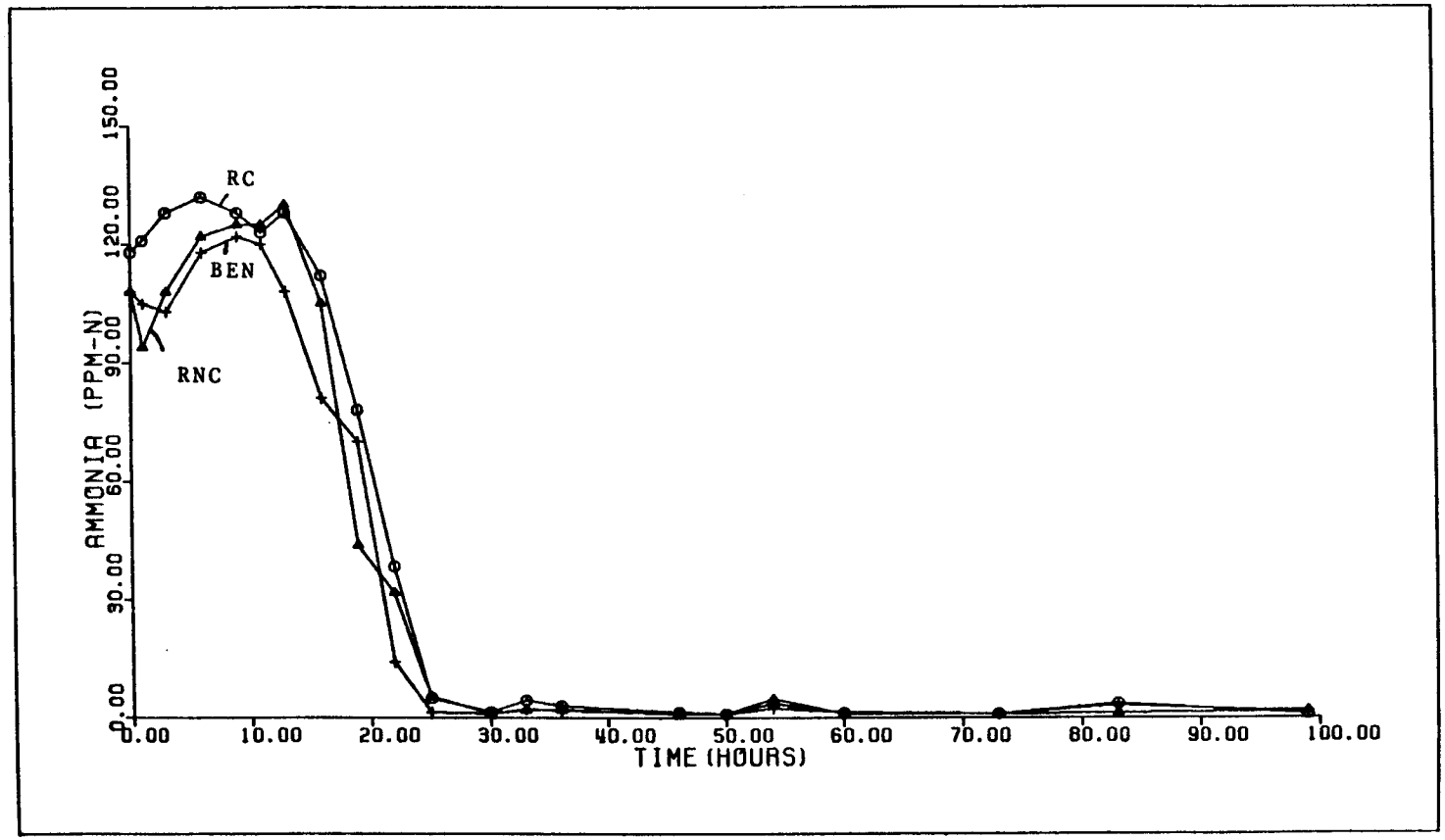


Figure 25: Second Chronic Experiment: Ammonia vs Time
(BEN=Bentonite Clay, RC=Refinery Carbon, RNC=Refinery Non-Carbon)

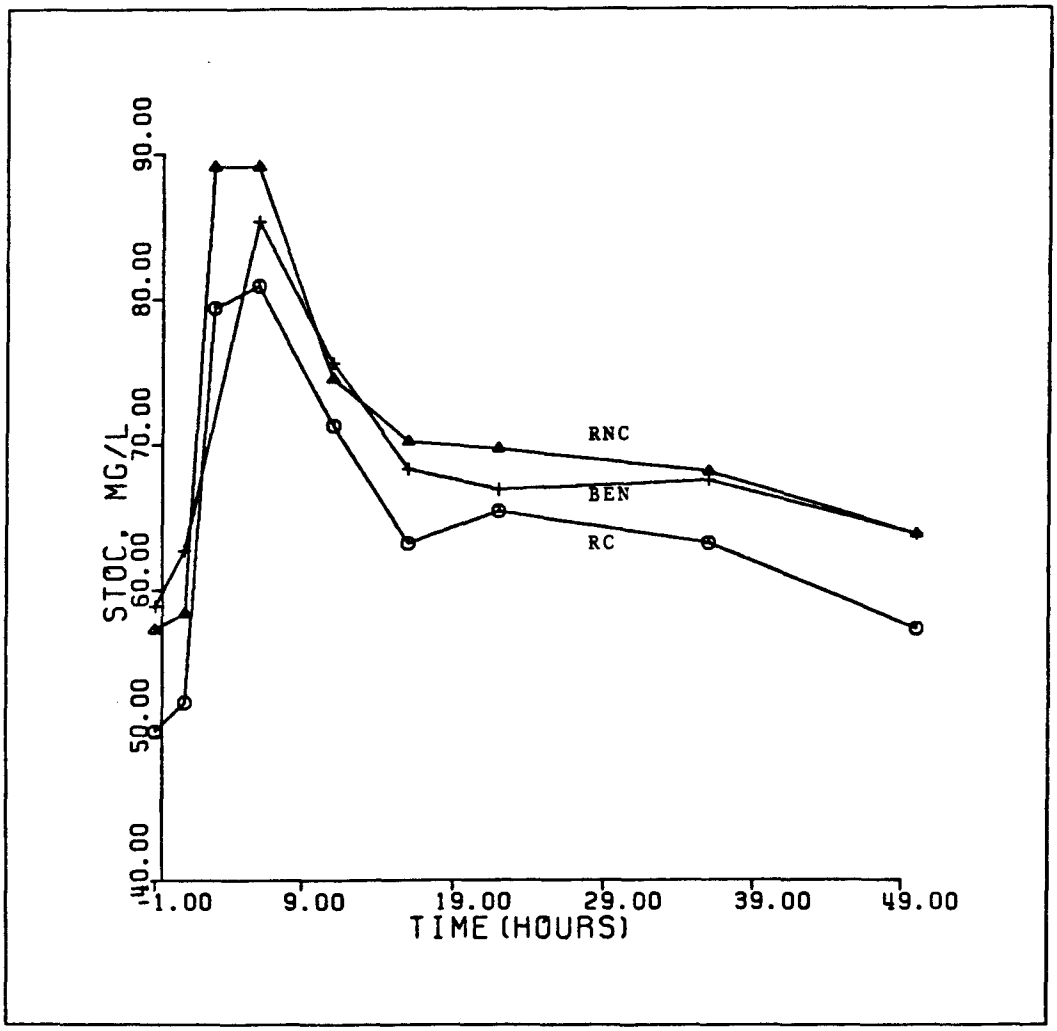


Figure 26: Second Chronic Experiment: SOC vs Time
(BEN=Bentonite Clay, RC=Refinery Carbon, RNC=Refinery Non-Carbon)

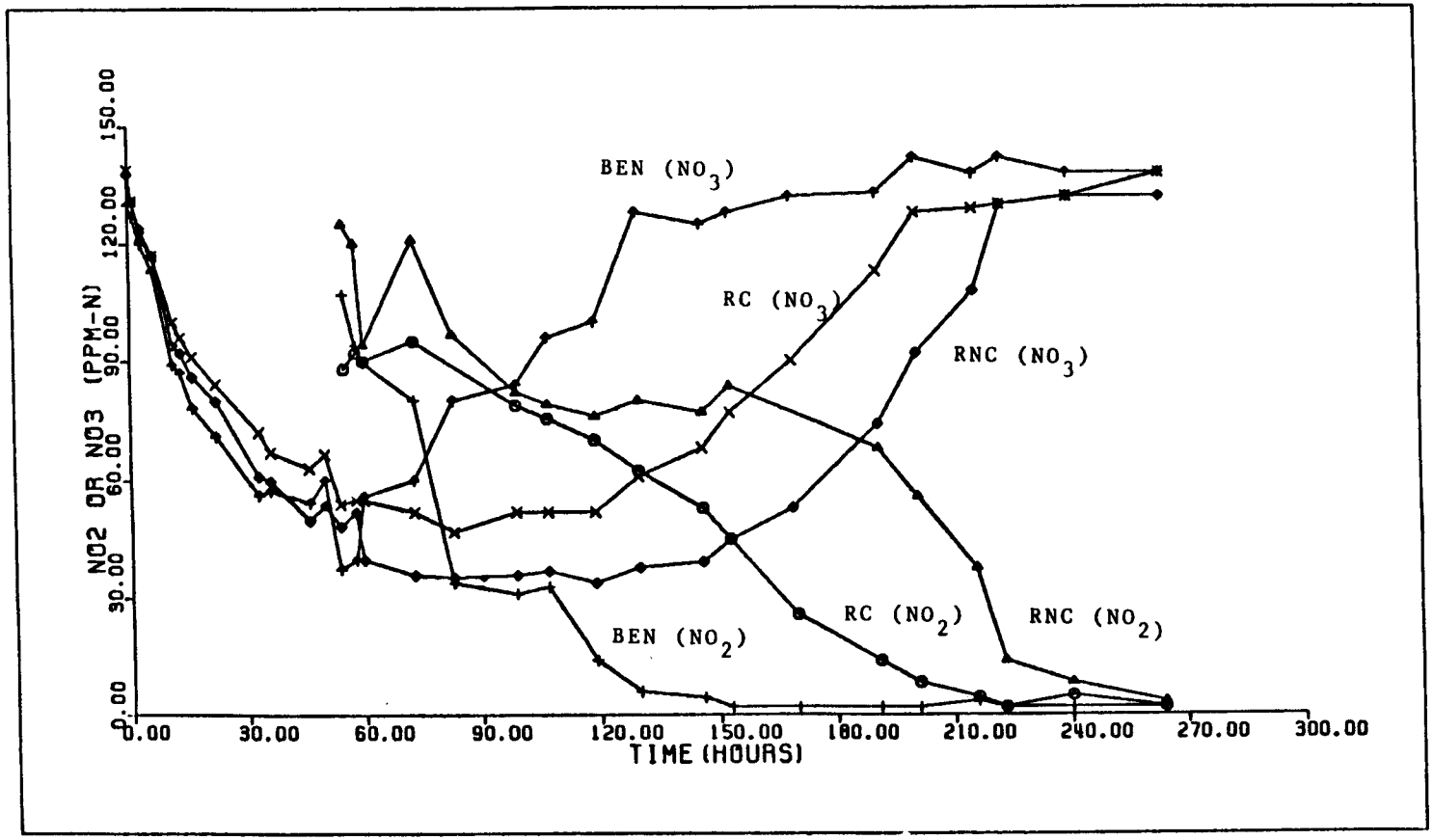


Figure 27: Second Chronic Experiment: NO_2 or NO_3 vs Time
(BEN=Bentonite Clay, RC=Refinery Carbon, RNC=Refinery Non-Carbon)

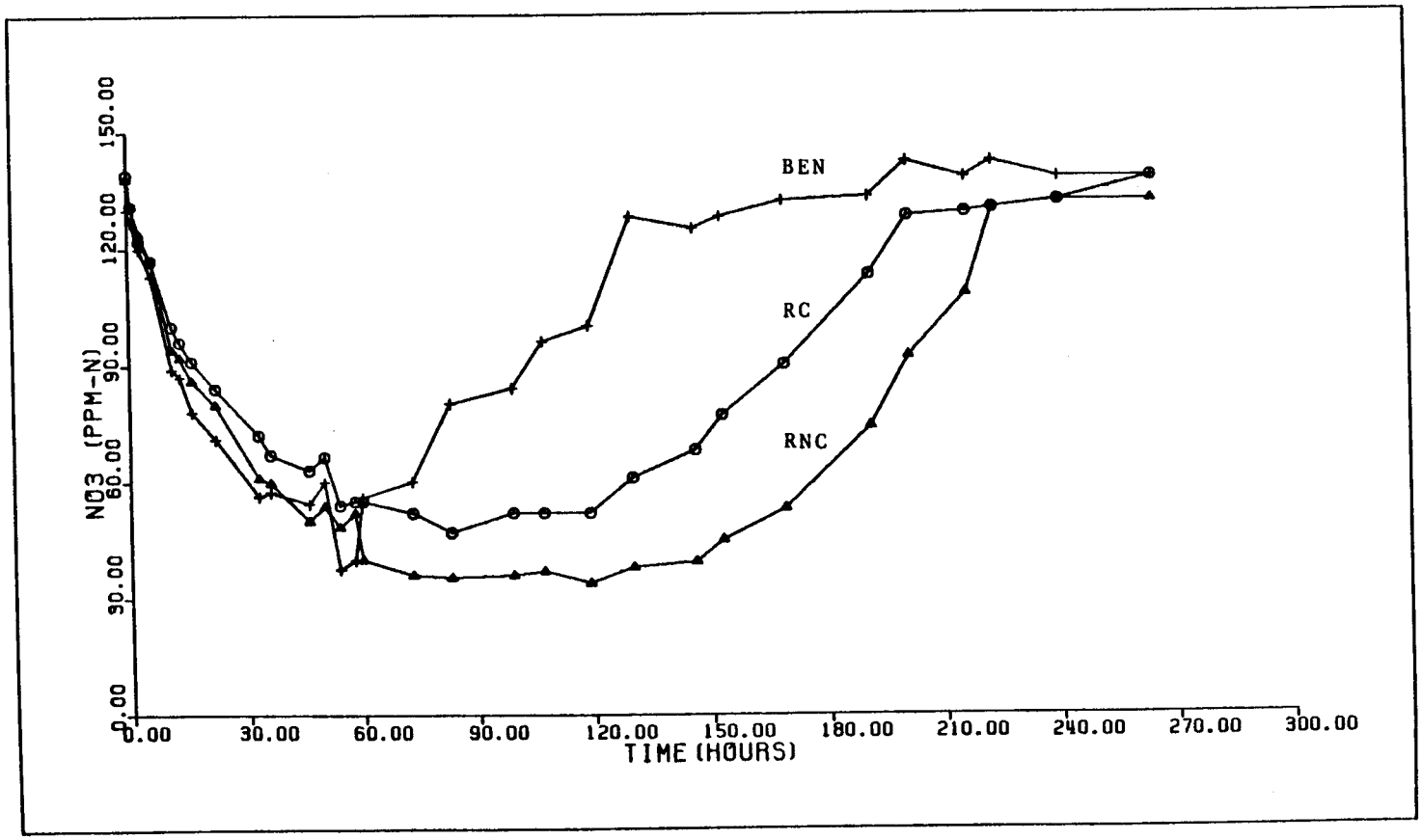


Figure 28: Second Chronic Experiment: Nitrate vs Time

(BEN=Bentonite Clay, RC=Refinery Carbon, RNC=Refinery Non-Carbon)

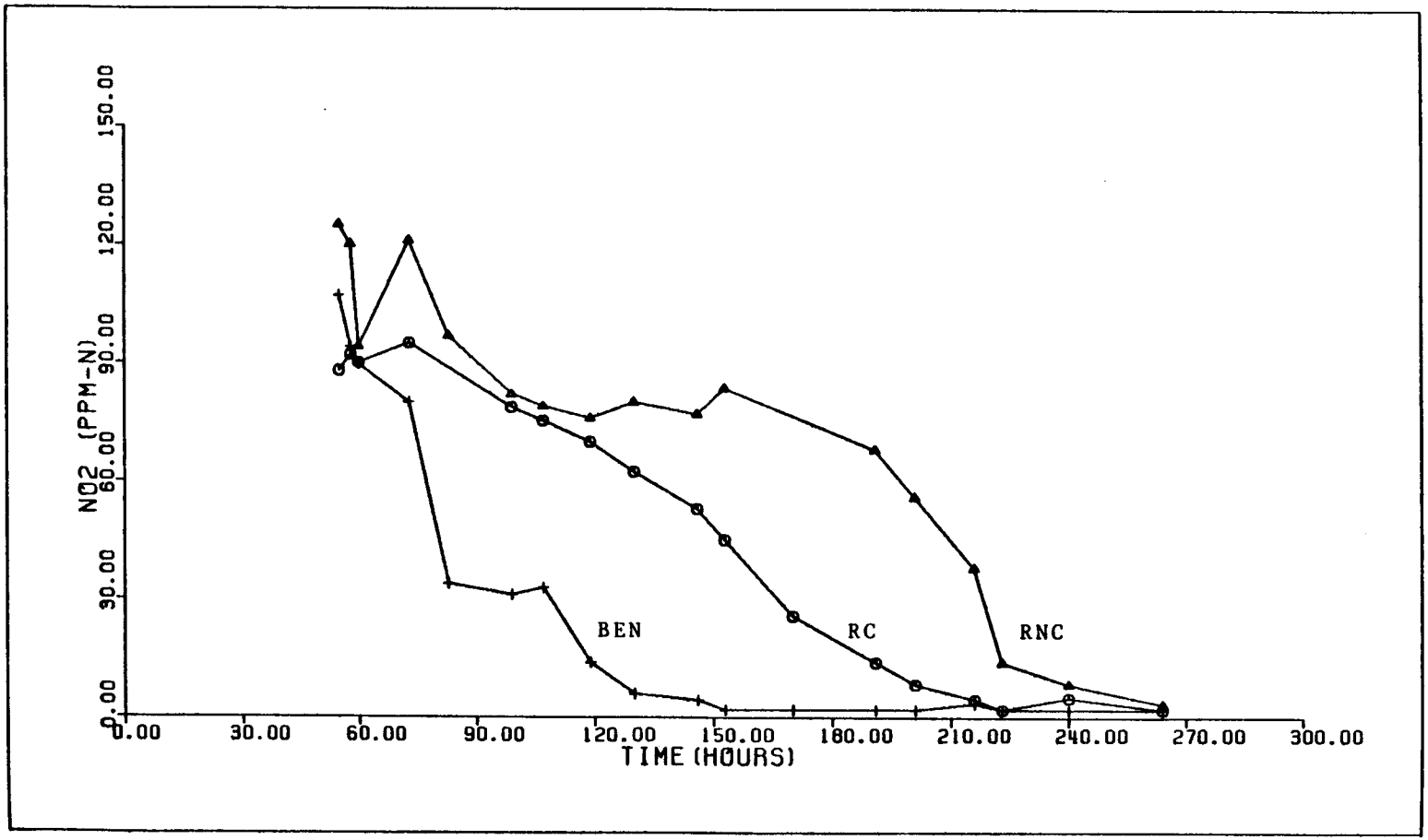


Figure 29: Second Chronic Experiment: Nitrite vs Time

(BEN=Bentonite Clay, RC=Refinery Carbon, RNC=Refinery Non-Carbon)

noted in Figure 23 actually represents the period of time in which the reactor pH dropped from 8.3 to 7.0.

Following the experiment, daily reactor effluent samples indicated that nitrification proceeded in all reactors at efficiencies greater that 99 % (with 30 mg/l aniline in the feed) for the next three weeks until the second chronic experiment commenced.

It is difficult to interpret the results of the initial chronic experiment, given that aniline is an adsorbable inhibitor and that the PAC unit was the only reactor type severely inhibited. One plausible explanation for the observed results is desorption. Desorption involves the displacement of a previously adsorbed compound by another whose adsorption affinity for the carbon is greater. It is possible that such a compound existed in the refinery wastewater and when displaced by the spiked aniline into the liquid phase, even in minute quantities, resulted in the observed inhibition. In effect, the PAC had sequestered this potential inhibitor from the microorganisms in the bulk solution. If acclimation to the compound was possible, it was effectively prevented by reduced exposure. Desorption of the compound may have allowed the microbes an opportunity to slowly acclimate for a short period of time (i.e., 75 hours) until the virgin PAC was added. For this explanation to be feasible, the unknown compound of interest must be both a powerful inhibitor of nitrification and less adsorbable than aniline. Inspection of Tables 16-18 on the GC/MS results of the refinery wastewater and Table 10 on nitrification inhibitors shows many potential candidates.

In the second chronic experiment, (Figures 25-29) the reactors were subjected to a step increase aniline concentration from 30 to 60 mg/1 along with a pulse addition of 60 mg/1 aniline. It should be noted that all reactor conditions were similar to that of the first chronic experiment except that the PAC reactor was now running at a steady state PAC concentration of 4000 mg/1. This was because of the additional 1000 mg/1 PAC spiked in the initial experiment. The daily carbon dose was adjusted appropriately to maintain this PAC reactor concentration (i.e., 66.7 mg/1 feed).

Figure 25 shows that all three reactor types responded similarly with respect to ammonia oxidation with no apparent advantage of PAC or bentonite addition. Ammonia oxidation was typically inhibited for 15 hours before the effects of the aniline dissipated. This observation is consistent with the results of Joel and Grady (1977) who suggested that heterotrophic aniline degradation and nitrification was sequential with nitrification commencing as soon as the aniline was reduced to non-inhibitory concentrations. Concurrent data on soluble TOC further supports this theory. Figure 26 shows that the filtered mixed liquor SOC in each reactor sharply increases approximately 30 to 40 mg/1 when the spiked aniline was added. An aniline concentration of 60 mg/1 corresponds roughly to 48 mg/1 TOC. After approximately 16 hours, the initial increase in SOC was reduced by more than 50% in all reactors. Whether this decrease was primarily due to microbial degradation or to the "washout" of aniline (i.e., θ_H = 24 hours) or a combination thereof is unknown, however, it appears that as soon as the aniline reaches some threshold concentration, nitrification begins.

Figure 26 also suggests at a carbon dose of 66.6 mg/l feed, little of the aniline is adsorbed from the liquid phase. This is evident from the differences noted among the increases in SOC in each reactor. If the SOC increases are due wholly to the addition of aniline, it would appear that the PAC reactor adsorbed no more than 5 mg/l SOC (6.5 mg/l aniline). This, in turn, would suggest that the adsorptive capacity of the PAC in the reactor had been effectively exhausted under prior steady state conditions. Thus, for the PAC dose and aniline concentration used in this experiment, the results indicate that the acclimation period of activated sludge to an inhibitory compound is more important in mediating the toxic effects to nitrifiers than the addition of PAC under steady state conditions.

Data on nitrate production (see Figures 27-28) shows, unexpectedly, that Nitrobacter activity was inhibited. Prior batch experiments and the initial chronic experiment with aniline demonstrated that the inhibition of Nitrosomonas is the primarily mechanism; however, it is apparent that at higher concentrations, Nitrobacter is severely inhibited. Figure 29 points to the interesting observation that nitrite uptake and nitrate production recovered most rapidly in the bentonite and PAC reactors. This logically suggests that of the nitrifying organisms, Nitrobacter would be more apt to be associated with surface growth attachment. Although this theory could account for some of the discrepancies reported in the literature regarding the role of suspended solids on nitrification, much more research is needed to substantiate this hypothesis.

Following the chronic experiments, daily effluent samples indicated that all reactors consistently achieved nitrification efficiencies greater than 99% in the presence of 60 mg/l aniline in the feed.

CARBON DOSE EXPERIMENTS

Preliminary Discussion

Results of the initial carbon dose experiment using 10 mg/l aniline, shown in Table 24 and Figures 30-32, demonstrate that adsorption of inhibitory compounds, without other mechanisms, can enhance nitrification. Table 24 also indicates that ammonia oxidation and nitrate production was not in stoichiometric agreement; nitrate production was up to 60% higher than that expected for an inorganic nitrogen balance. Non-stoichiometric nitrification, although not as pronounced, had been noted in earlier experimental runs involving refinery wastewaters and was attributed to a number of possible factors. Among these factors were nitrogen release from excessive cell lysis due to the addition of the inhibitory compound; endogenous decay; analytical difficulties associated with the measurement of nitrate in refinery wastewaters; or a combination of these factors.

Other investigators (Anderson, 1964; Hopper and Nason, 1965; Painter, 1970; Wood, et. al., 1981) have noted "non-quantitative" nitrification in activated sludges, however, a loss of inorganic nitrogen rather than a gain was cited in these previous studies.

To address this apparent discrepancy, an experiment was attempted to assess and differentiate nitrogen release due either to endogenous decay or to excess cell lysis from the addition of aniline. The procedure was to follow all measurable nitrogenous species with time in 3 activated sludge cultures differing only in the addition of thiourea

Table 24: Effect of 10 ppm Aniline on Nitrification under Variable Powdered Activated Carbon Doses

Ammonia Concentration (mg/l-N)						
Hours	1*	2	3	4	5	6
0.0	45.8	46.2	44.0	43.8	45.7	46.0
2.0	35.0	44.8	36.0	37.0	38.8	42.2
4.0	22.7	44.2	23.5	26.5	29.5	38.0
6.0	16.1	42.2	12.4	16.0	19.9	30.0
9.0	2.9	42.5	1.4	1.6	5.6	18.2
Nitrate Concentration (mg/l-N)						
0.0	23.8	25.0	24.2	26.2	26.2	27.5
2.0	37.0	30.0	42.5	43.0	40.5	38.0
4.0	47.5	34.5	56.5	54.2	54.0	44.0
6.0	53.8	40.0	71.0	67.0	63.0	54.0
9.0	66.0	46.0	107.0	90.0	89.6	67.0

1= control: no carbon, no aniline addition
2= aniline control: no carbon, 10 mg/l aniline
3= carbon dose: 4000 mg/l; 10 mg/l aniline
4= carbon dose: 1960 mg/l; 10 mg/l aniline
5= carbon dose: 775 mg/l; 10 mg/l aniline
6= carbon dose: 387 mg/l; 10 mg/l aniline

(MLSS concentration= 3300 mg/l)

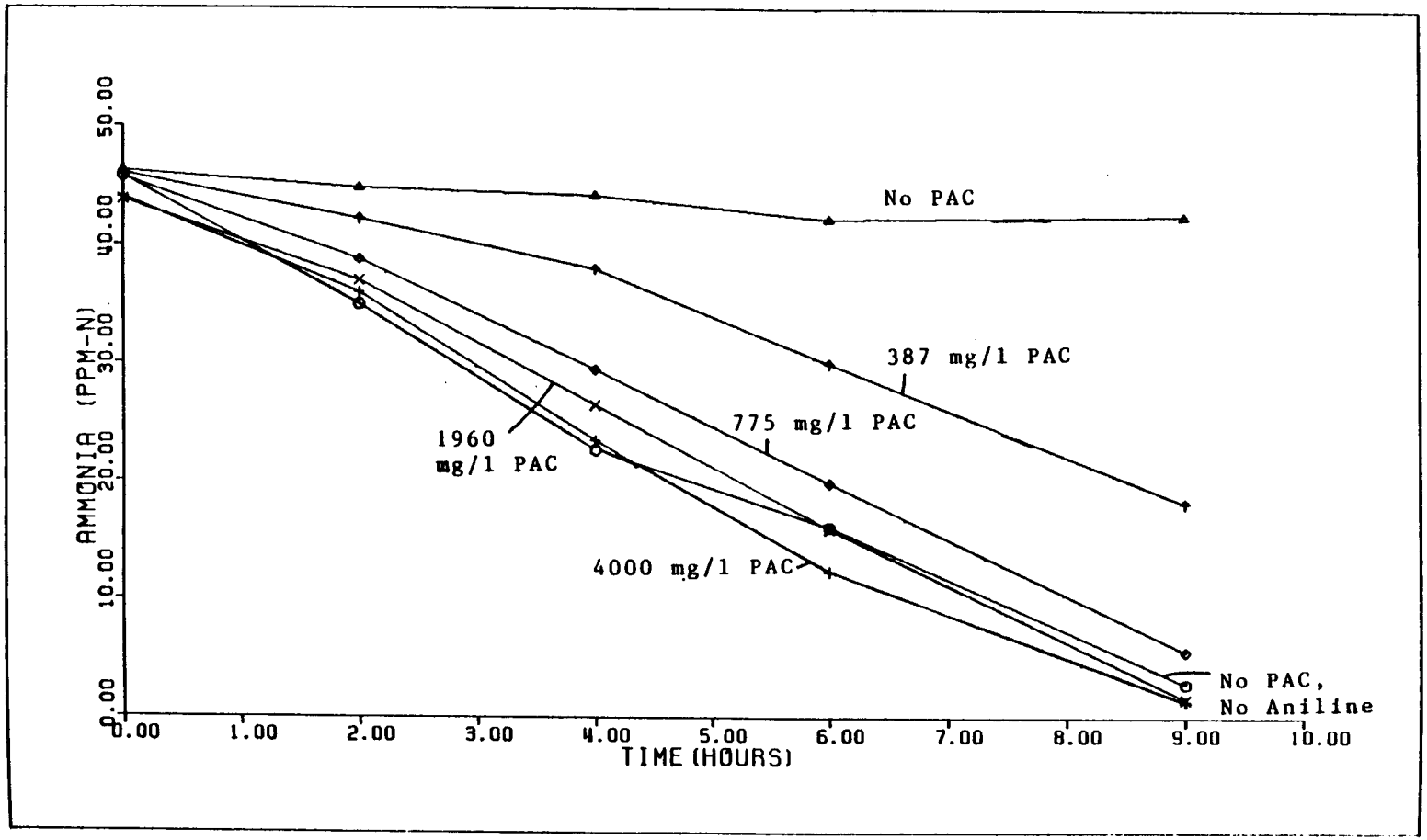


Figure 30: Initial Carbon Dose Experiment: Ammonia vs Time

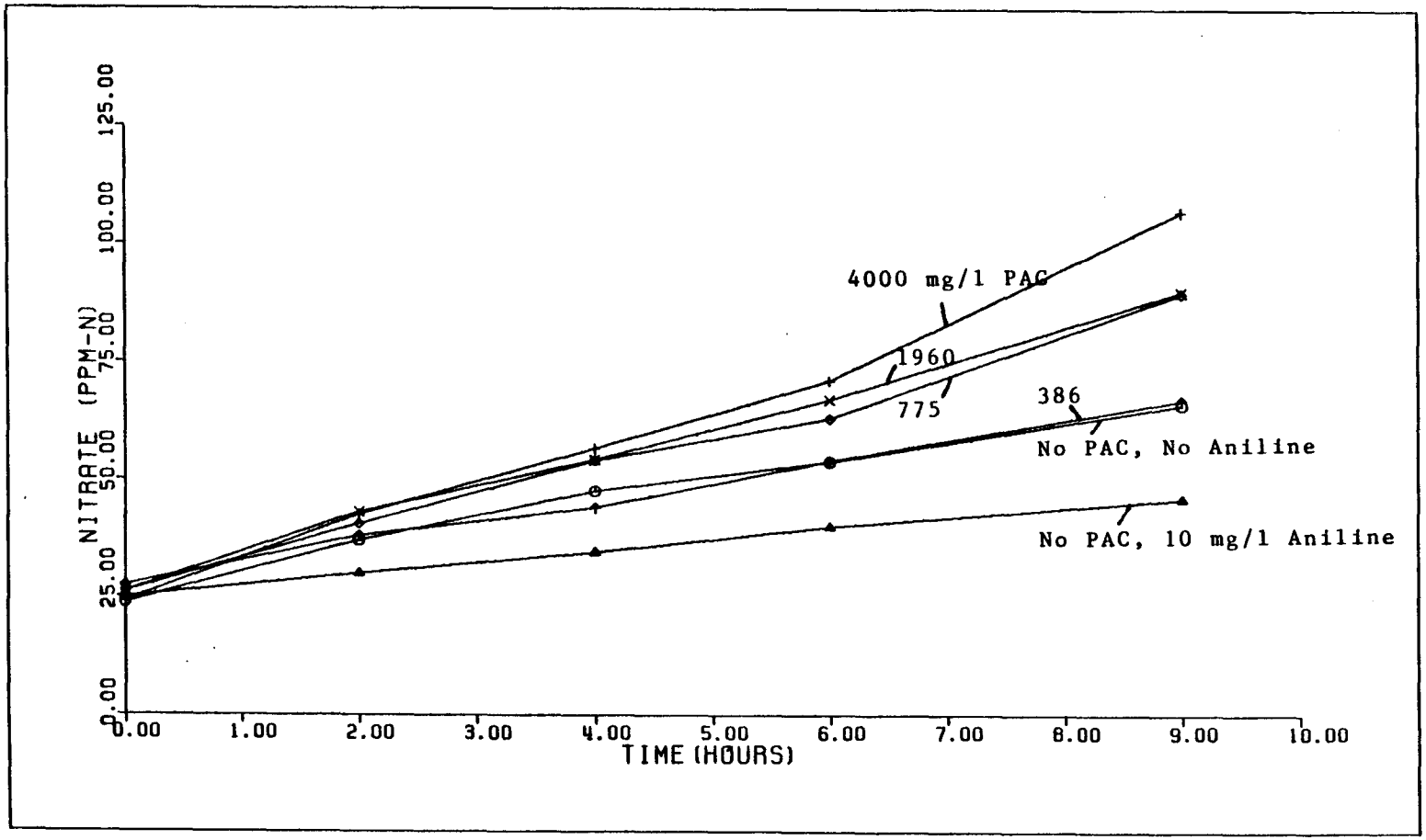


Figure 31: Initial Carbon Dose Experiment: Nitrate vs Time

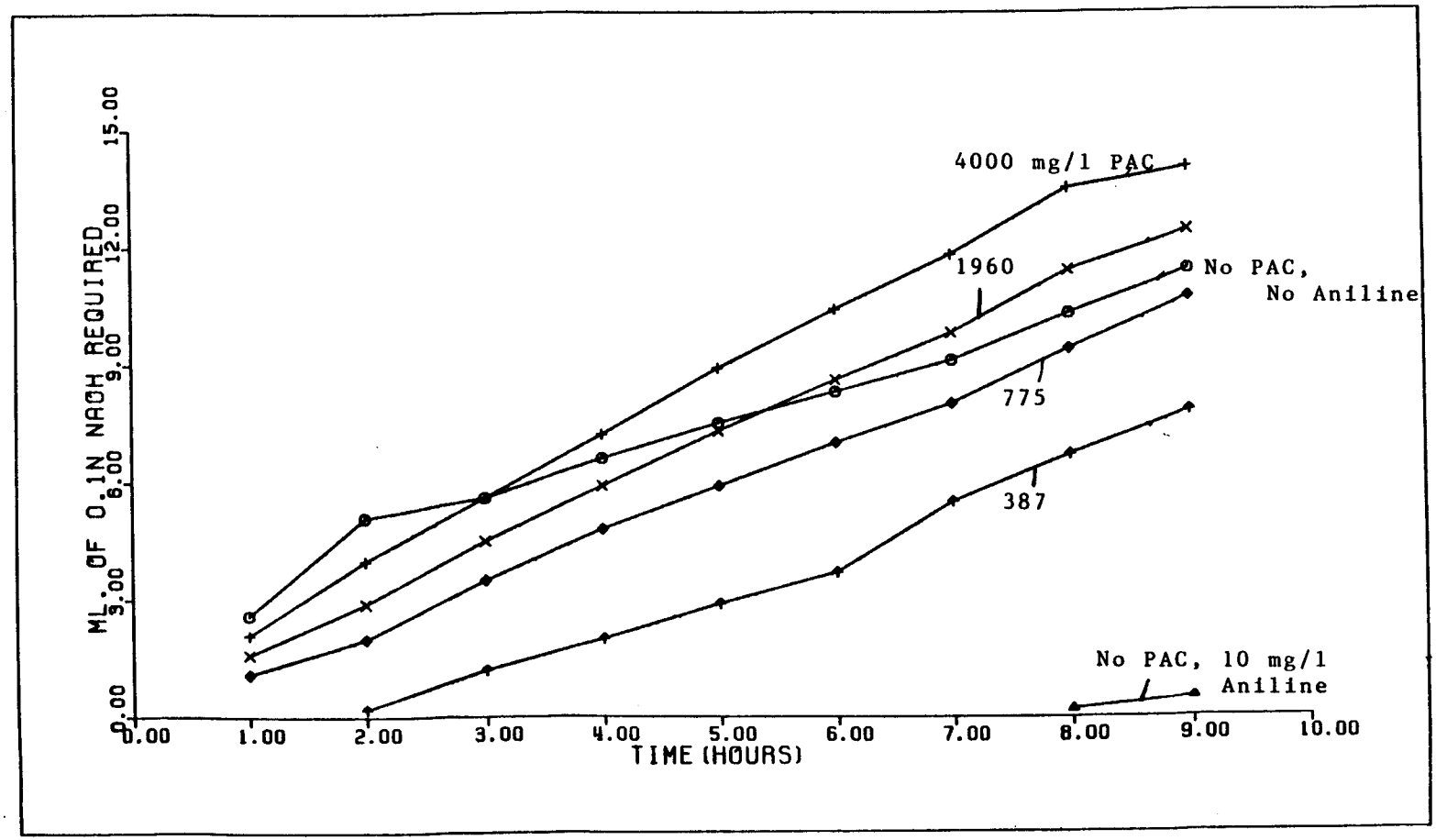


Figure 32: Initial Carbon Dose Experiment: Caustic Uptake vs Time

(1.0 mg/1); thiourea (1.0 mg/1) and aniline (10.0 mg/1); or no additions at all. Thiourea is a known potent inhibitor of ammonia oxidation (0.075 mg/1 produces 75% inhibition, Tomilinson, 1966) and is commonly used to inhibit nitrification during BOD tests. Nitrogen release due to endogenous decay was followed in the culture with no added compounds. Cell lysis due to aniline addition was taken to be the difference in inorganic nitrogen production (after adjusting for endogenous decay) between the cultures with and without aniline addition.

It should be noted that nitrate in the initial carbon dose experiment was analyzed for by the same nitrate sensing module used in the earlier experiments involving refinery wastewaters. To avoid the possible introduction of probe error associated with its prior use in refinery wastewaters, a new sensing module was obtained and used for this and subsequent experiments.

Results of the nitrogen balance experiment are shown in Figures 33-35. Initial nitrogen measurements for the activated sludge used were 1.0 mg/1 ammonia-N, 0.26 mg/1 nitrite-N, 43.5 mg/1 nitrate-N, and 260.0 mg/1-N Total Kjeldahl Nitrogen (TKN). Mixed liquor suspended solids was measured to be 2150 mg/1 and filtered TKN was 0.94 mg/1-N. Figure 33, corrected for initial nitrate-N, indicates that for the culture with no additions, practically all inorganic nitrogen was in the form of nitrate-N with only a trace of nitrite-N (0.1 mg/1) present. Over the 8 hour aeration period, approximately 6 mg/1 nitrate-N (corrected for initial ammonia-N) was formed. Using initial Kjeldahl Nitrogen (KN) as an indication of microbial mass and neglecting heterotrophic ammonia uptake

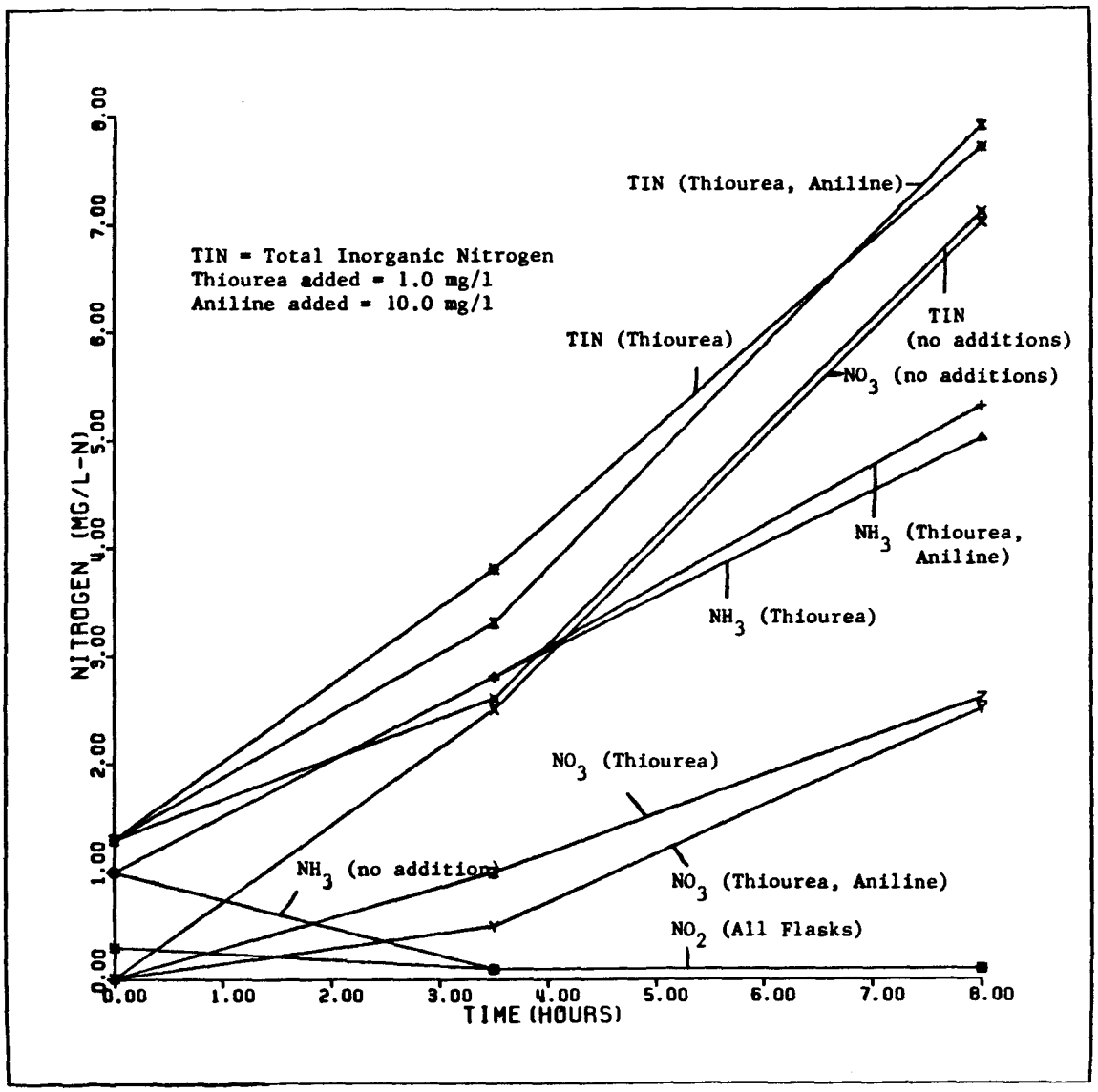


Figure 33: Inorganic N Production with Time

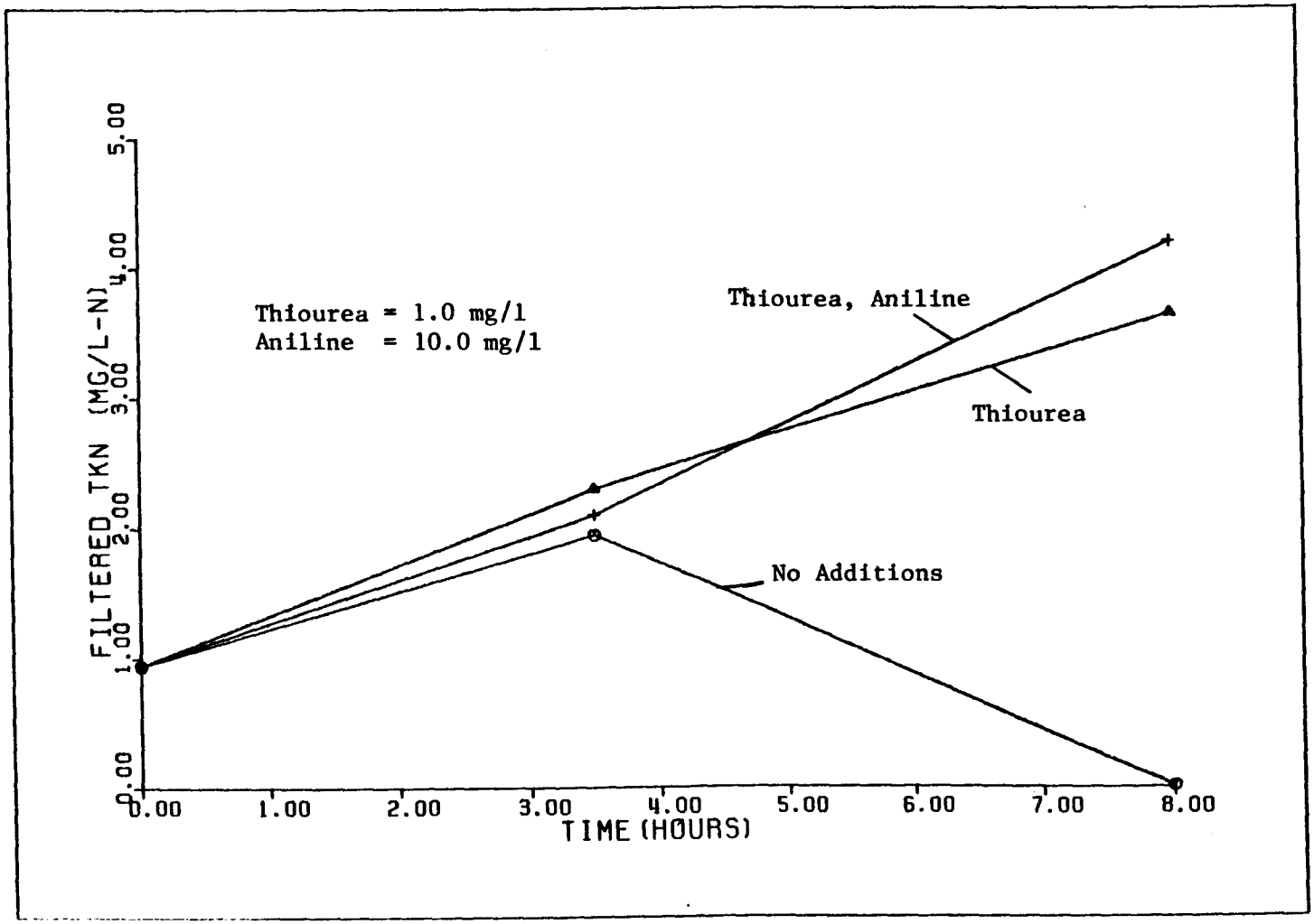


Figure 34: Filtered TKN with Time

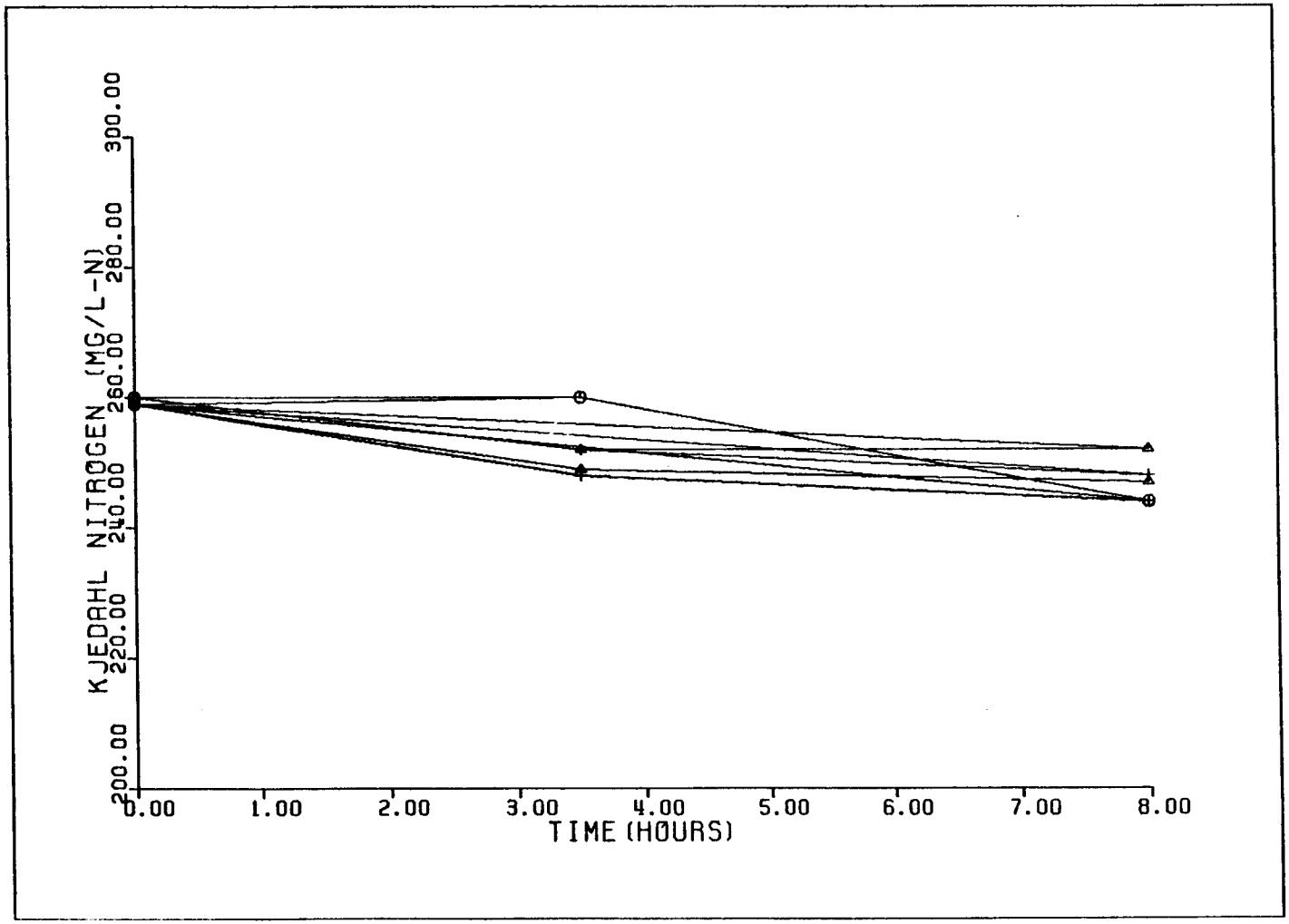


Figure 35: Unfiltered TKN and KN with Time

(i.e., effluent TOC < 6.0 mg/1), the endogenous decay coefficient, K_d, can be approximated to be 0.07 day^{-1}. This value is typical for activated sludge systems operating under the conditions specified (θ_c = 9 days, θ_H = 8 hours).

Figure 33 also shows that while ammonia oxidation was inhibited in cultures with added compounds, nitrite oxidation did occur. Both thiourea and aniline are specific inhibitors of ammonia oxidation at the concentrations used; however, it appears that complete nitrification inhibition did not occur since the total nitrate produced in these cultures exceeded the initial concentration of nitrite. After correcting for the initial ammonia-N concentration, the total inorganic nitrogen produced after 8 hours for cultures spiked with thiourea and thiourea & aniline was 6.7 and 6.9 mg/1-N, respectively. In comparison of inorganic nitrogen production in all three test cultures, there appeared to be no significant difference in the amount of nitrogen release due to the addition of aniline and/or thiourea over the duration of the test.

The purpose of including TKN analysis on filtered and unfiltered samples in this experiment was to obtain an overall nitrogen balance. Unfortunately, KN determinations on unfiltered samples were found not to be sensitive enough to detect the expected corresponding decrease in organic N (i.e., 6-7 mg/1-N). Figure 35 shows that measured KN values to be within the limits of analytical error inherent in the Kjeldahl method used (5%, see Appendix IV). It was anticipated that filtered KN determinations (Figure 34) would provide for a more sensitive technique by which the organic N loss could be detected. However, with the excep-

tion of one sample, the TKN detected in the filtrate samples corresponded to the measured ammonia-N for any given sampling period. After correcting for the ammonia-N present in the TKN determinations, KN (organic N) was detected in only one filtrate sample.

The results of the nitrogen balance experiment suggested that the total amount of nitrogen released through either endogenous decay or through excess cell lysis was not sufficient to account for the non-stoichiometry of nitrification observed in the initial carbon dose experiment. Therefore, the pronounced inorganic nitrogen inbalance resulting in the initial experiment was attributed to a faulty nitrate sensing module in the selective ion probe. It should be noted that the normal lifetime of the sensing module, depending on use, varies from a few months to 1 year. Although probe performance was routinely monitored through calibration checks prior to all experiments, it is conceivable that the probe had begun to fail during analysis of nitrate in the initial carbon dose experiment.

Nitrate was analyzed for with a new sensing module in all subsequent experiments reported. Carbon dose experiments using aniline (10 mg/l) were repeated twice. In addition, nitrification inhibitors with different adsorption characteristics including phenol (20 mg/l), cyanide (0.7 and 1.4 mg/l) and ethanol (2500 mg/l) were also evaluated. Nitrification inhibition and adsorptive characteristics for these compounds are shown in Table 25. Data from Table 25 were largely extracted from the literature and hence are subject to interpretation due to differing sets of conditions under which the values were obtained. Nonetheless,

Table 25: Adsorption and Nitrification Inhibitory Characteristics of Compounds Tested In Carbon Dose Experiments

Compound	Freundlich Parameters	Langmuir Parameters	Concentration Required for 75% Inhibition
Aniline	K=12.2 mg/g n^{-1} = 0.52	Q=0.065 b= 0.324	7.7 mg/1
Phenol	K=21.0 mg/g n^{-1} = 0.54	Q=0.158 b=0.176	5.6 mg/1
Cyanide	*	max. absorp.= 2.0 mg/g at C_o = 20.0 mg/1	0.65 mg/1
Ethyl Alcohol	K = 0 mg/g	max adsorp.= 20 mg/g at C_o = 1000 mg/1	2500 mg/1

* = no data available
See table 12 for references on adsorption data
Inhibition data from Tomilinson (1966)

table 25 does provide an indication of the relative adsorptive and inhibitory characteristics of the compounds under evaluation. For non-adsorbable compounds, inhibitor concentrations to be tested were chosen to give approximately 75% inhibition in control units without PAC addition. This was done to ensure that nitrification would continue in any given experimental unit and that any benefit, due to PAC addition, would be detected.

Results, presented in the order in which the experiments were performed, are plotted on Figures 36-37 for 10 mg/l aniline; Figures 38-39 for 20 mg/l phenol; Figures 40-41 for 0.7 mg/l cyanide; Figures 42-43 for 1.4 mg/l cyanide; Figures 44-45 for 2500 mg/l ethanol; and 46-49 for 10 mg/l aniline. In the latter experiment, nitrite and liquid phase aniline was measured by gas chromatography at specific sampling intervals. Raw data for all experimental runs are presented in Appendix III.

Calculated ammonia and associated nitrate production constants K, for each experiment are presented on Table 26. Ammonia oxidation reaction constants were determined by simple linear regression on observed zero-ordered reaction rates (under non-limiting substrate conditions, i.e., < 2.0 mg/l ammonia-N). Also included in table 26 are coefficients of determination r^2, which indicates the accuracy of fit between the data and the linear regression equation. When necessary, the nitrate production rate constant was estimated using only the corresponding data points from which the ammonia oxidation rate constant was determined. This was done because, in certain cases towards the end of an experiment, nitrate production rates were observed to level off to a plateau

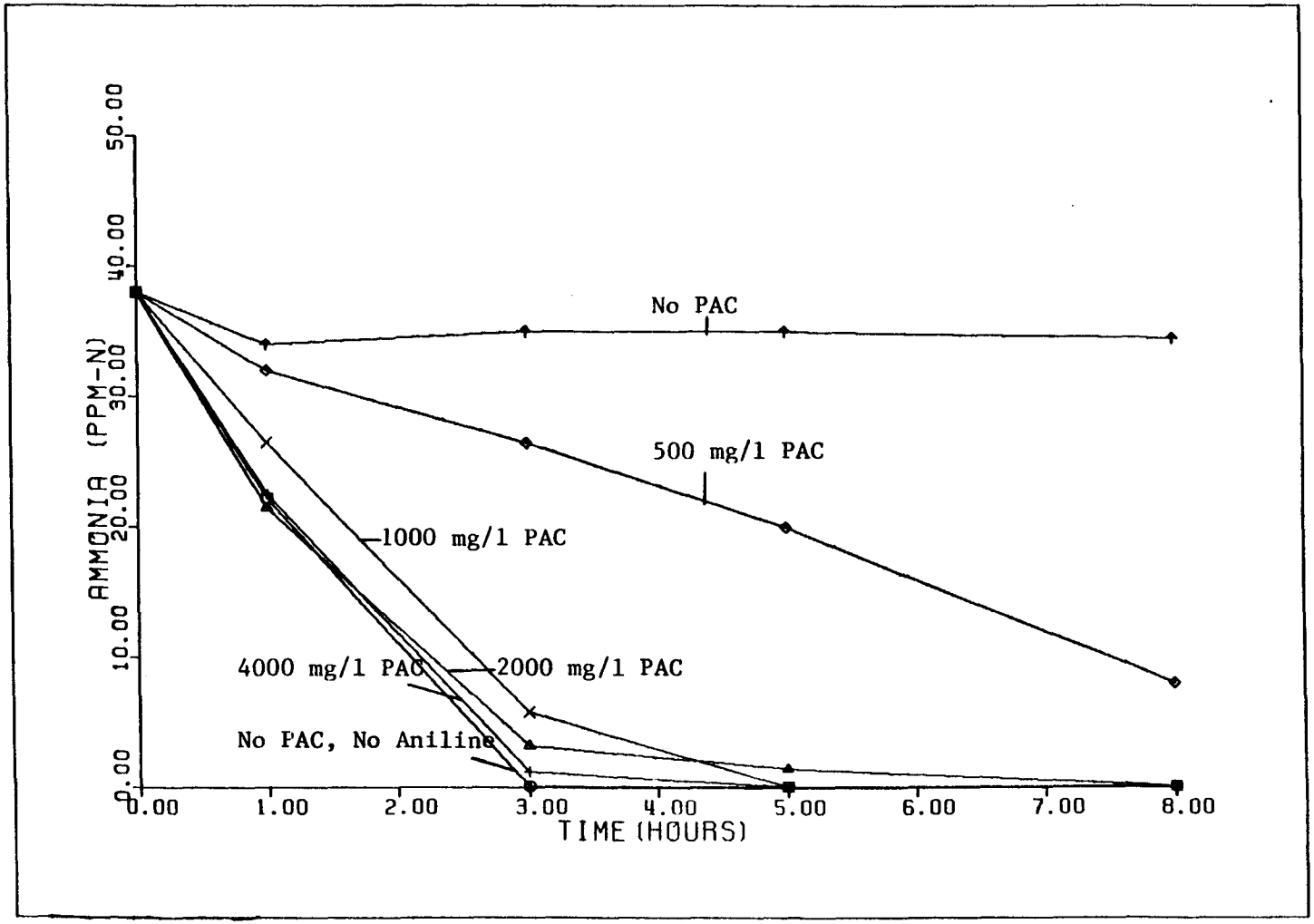


Figure 36: NH_3 vs Time for Variable Carbon Dose (10 mg/l Aniline)

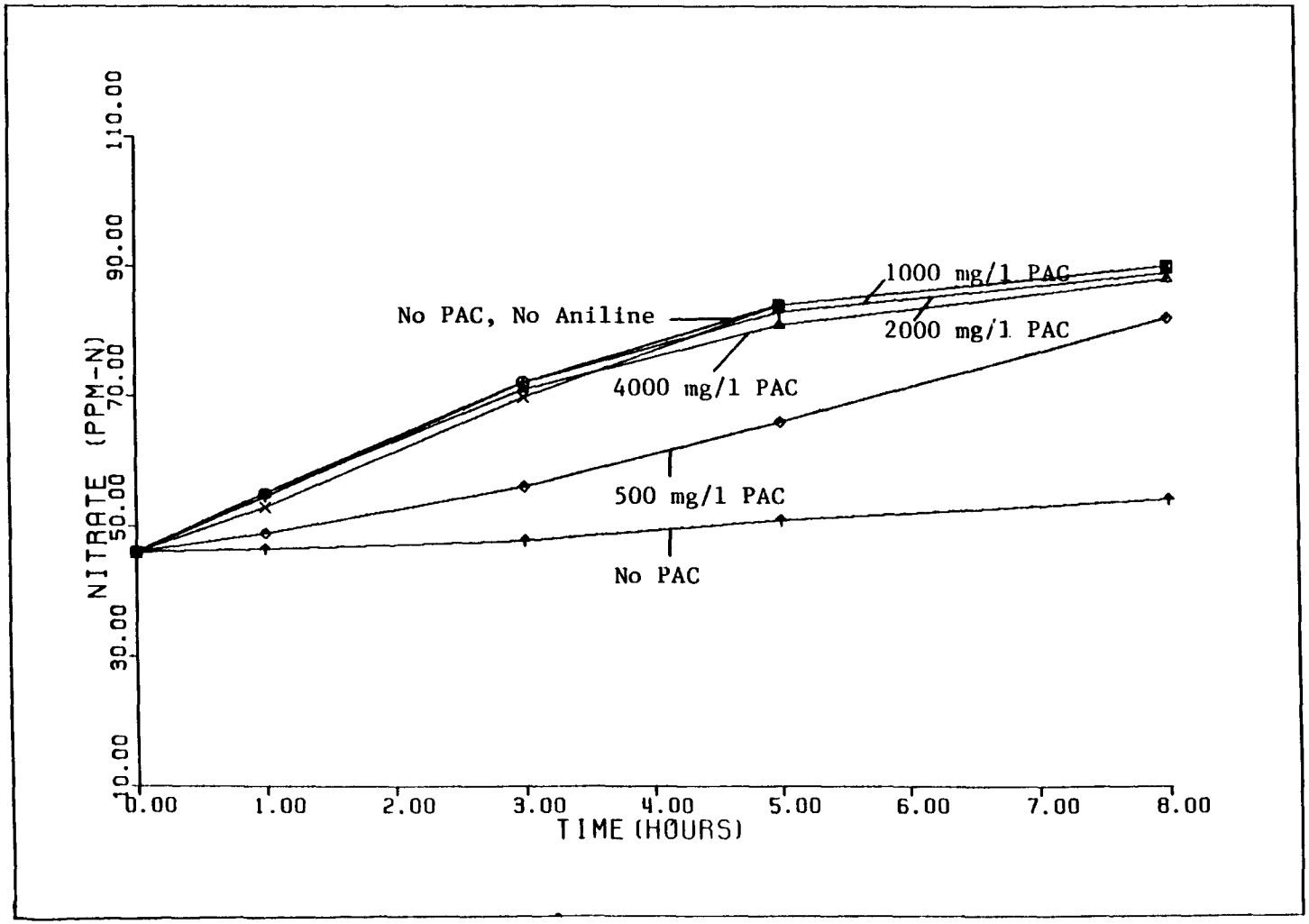


Figure 37: NO_3 vs Time for Variable Carbon Dose (10 mg/1 Aniline)

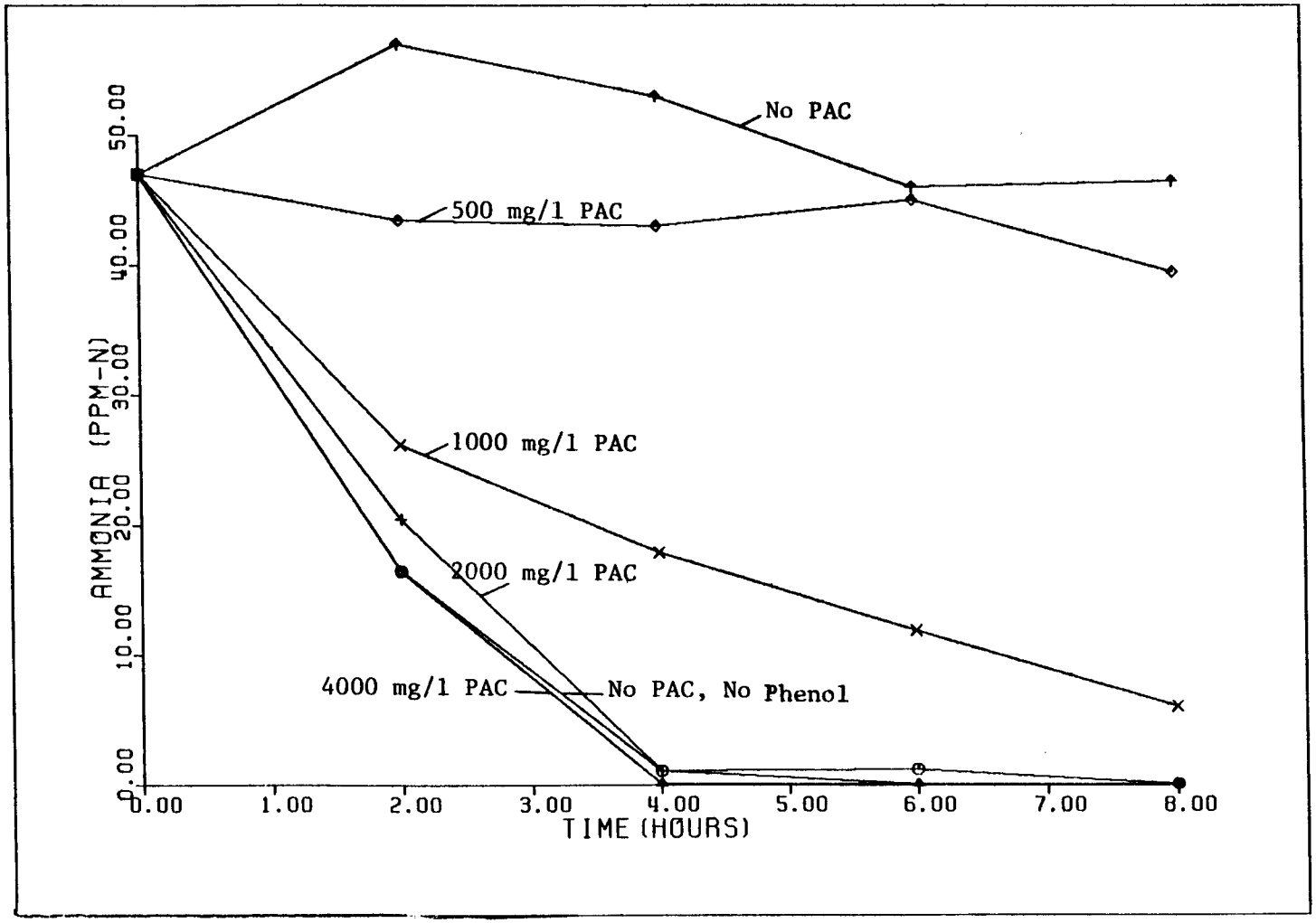


Figure 38: NH_3 vs Time for Variable Carbon Dose (20 mg/1 Phenol)

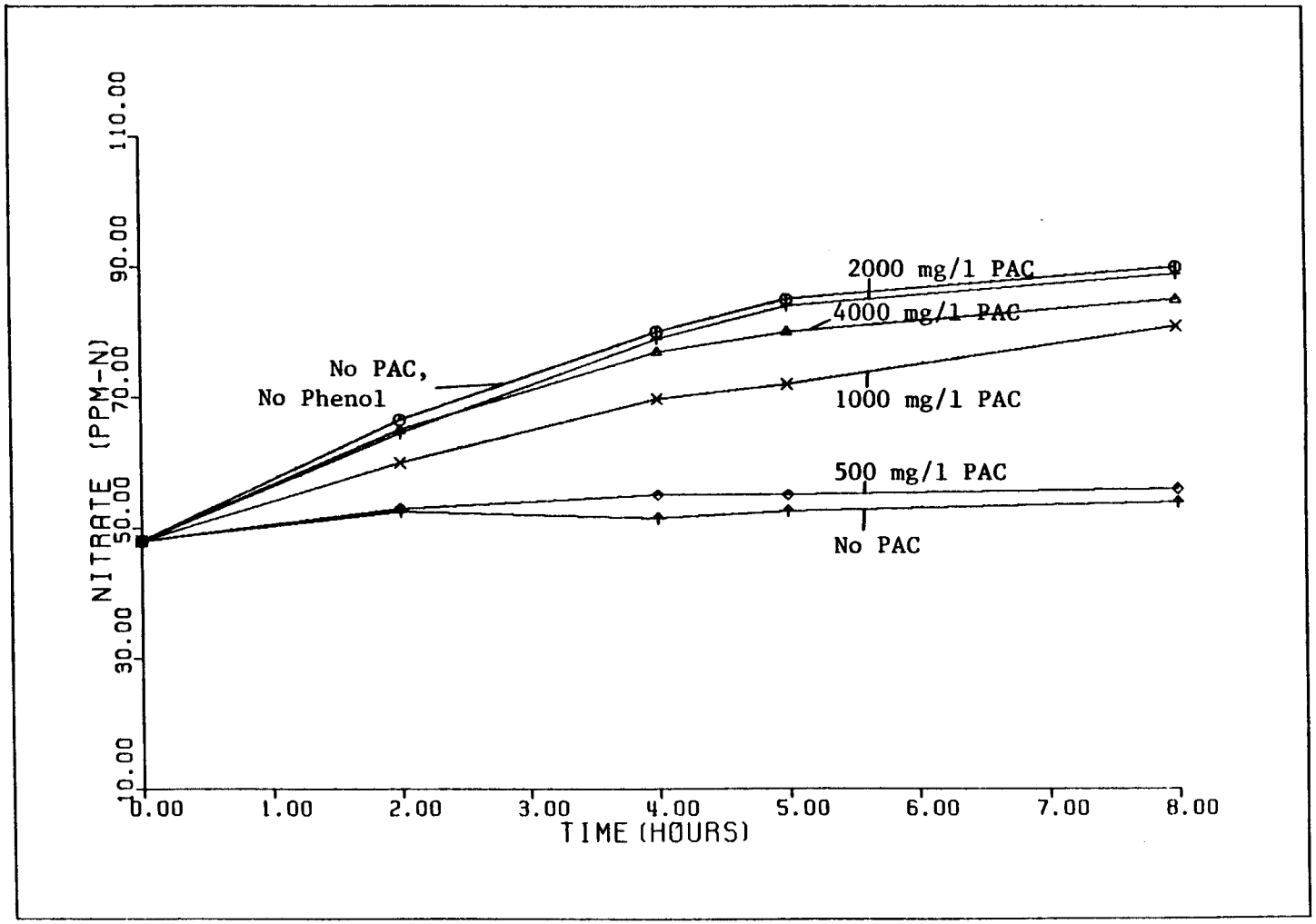


Figure 39: NO_3 vs Time for Variable Carbon Dose (20 mg/1 Phenol)

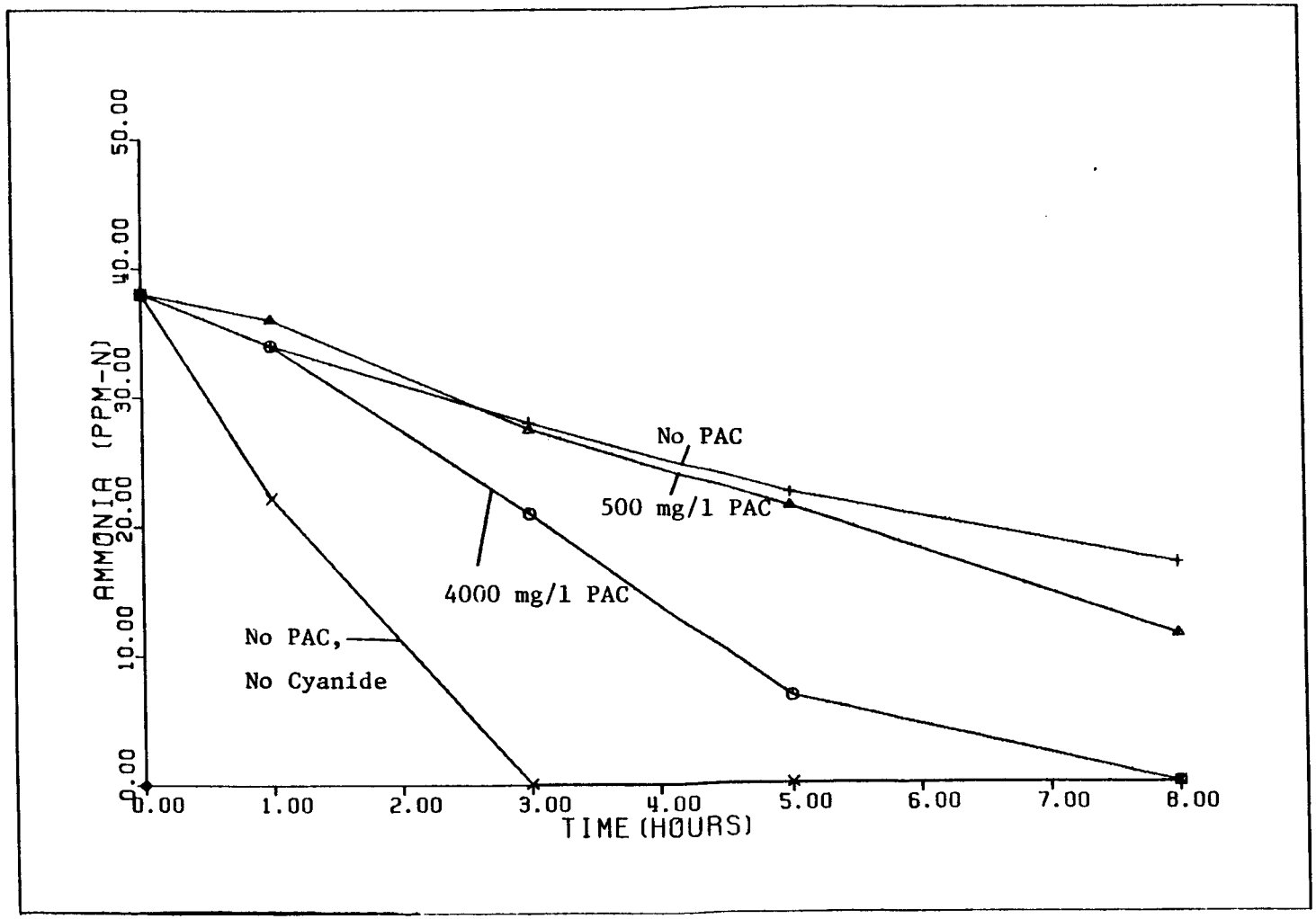


Figure 40: NH_3 vs Time for Variable Carbon Dose (0.7 mg/l Cyanide)

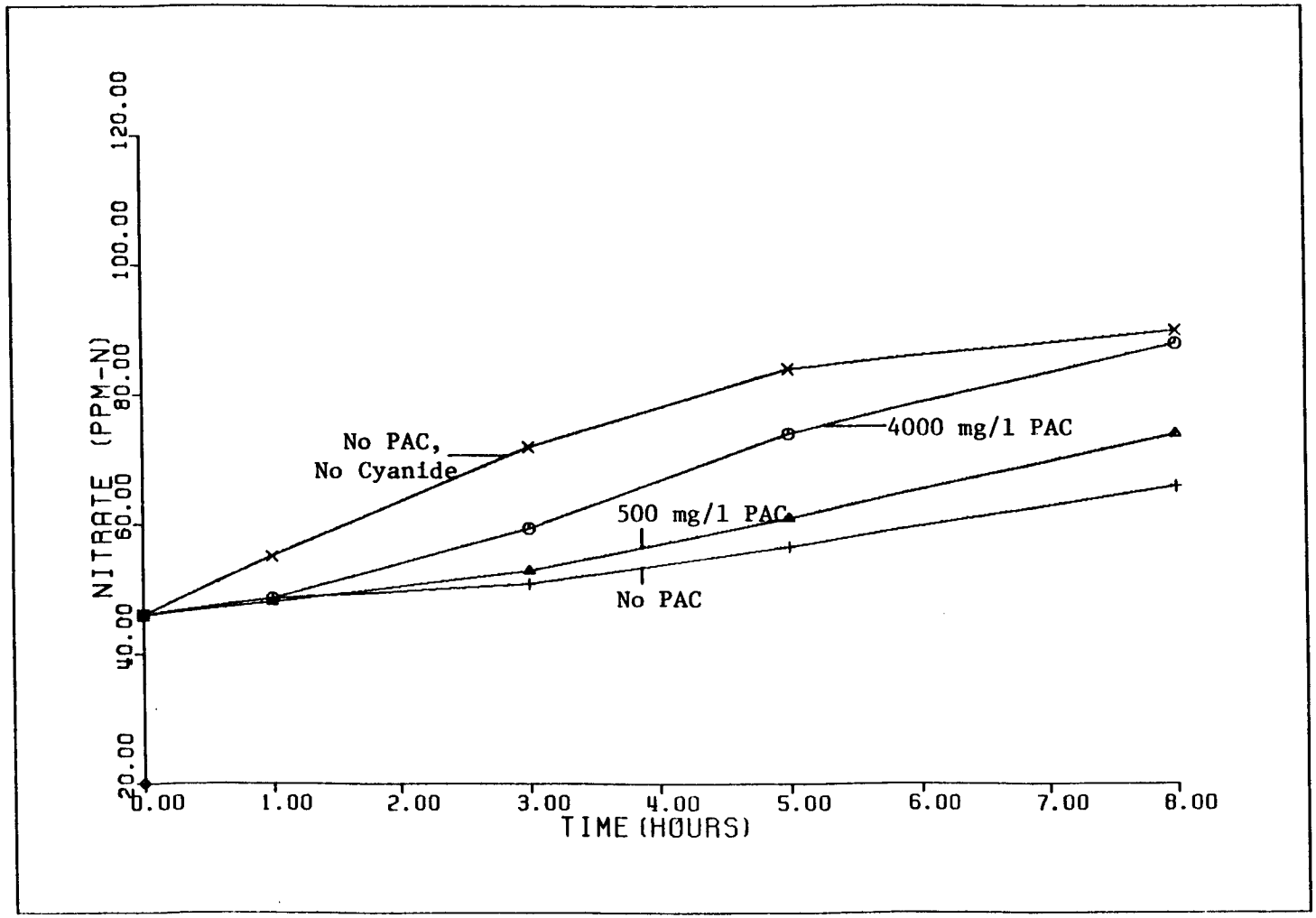


Figure 41: NO_3 vs Time for Variable Carbon Dose (0.7 mg/1 Cyanide)

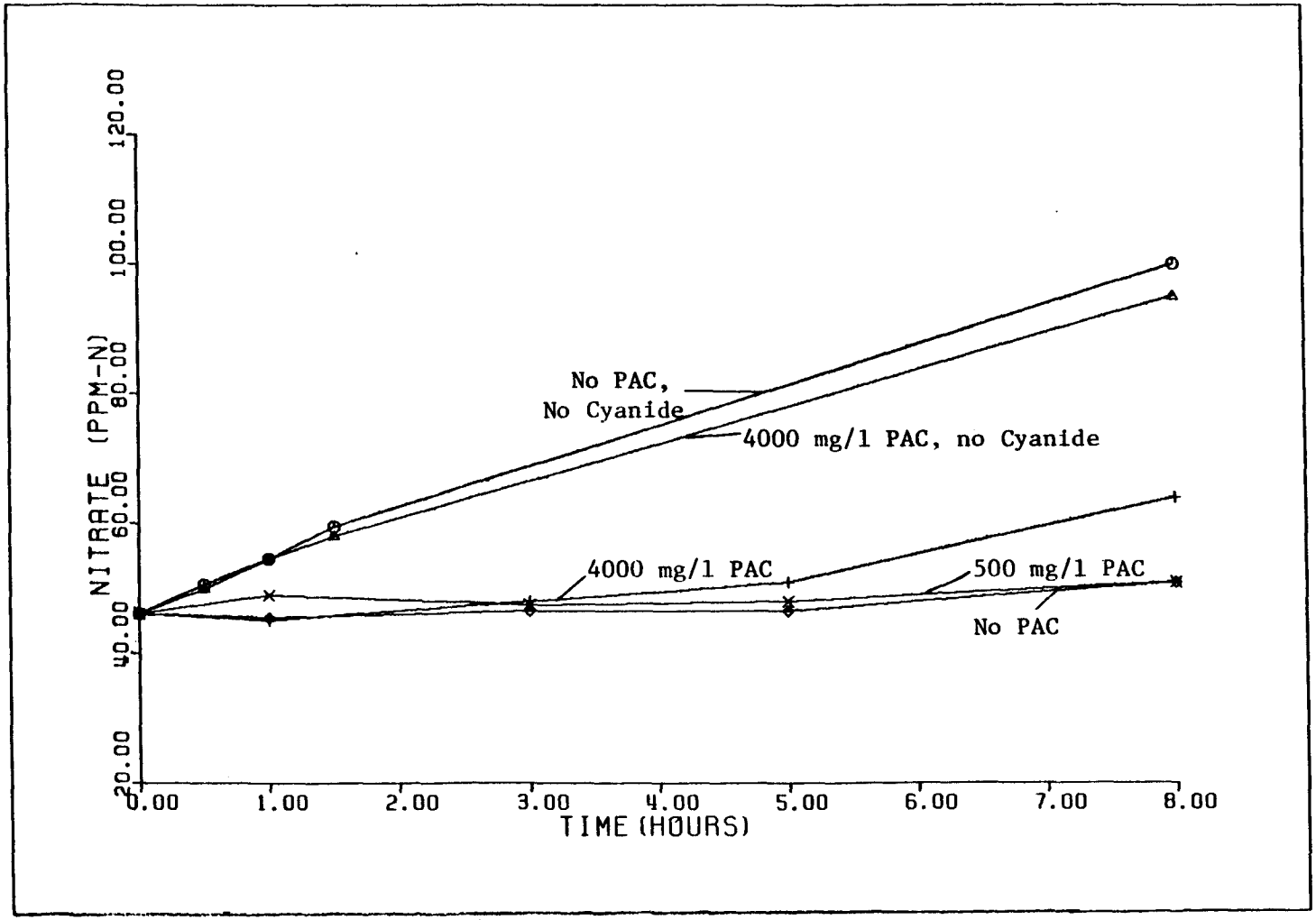


Figure 42: NH_3 vs Time for Variable Carbon Dose (1.4 mg/l Cyanide

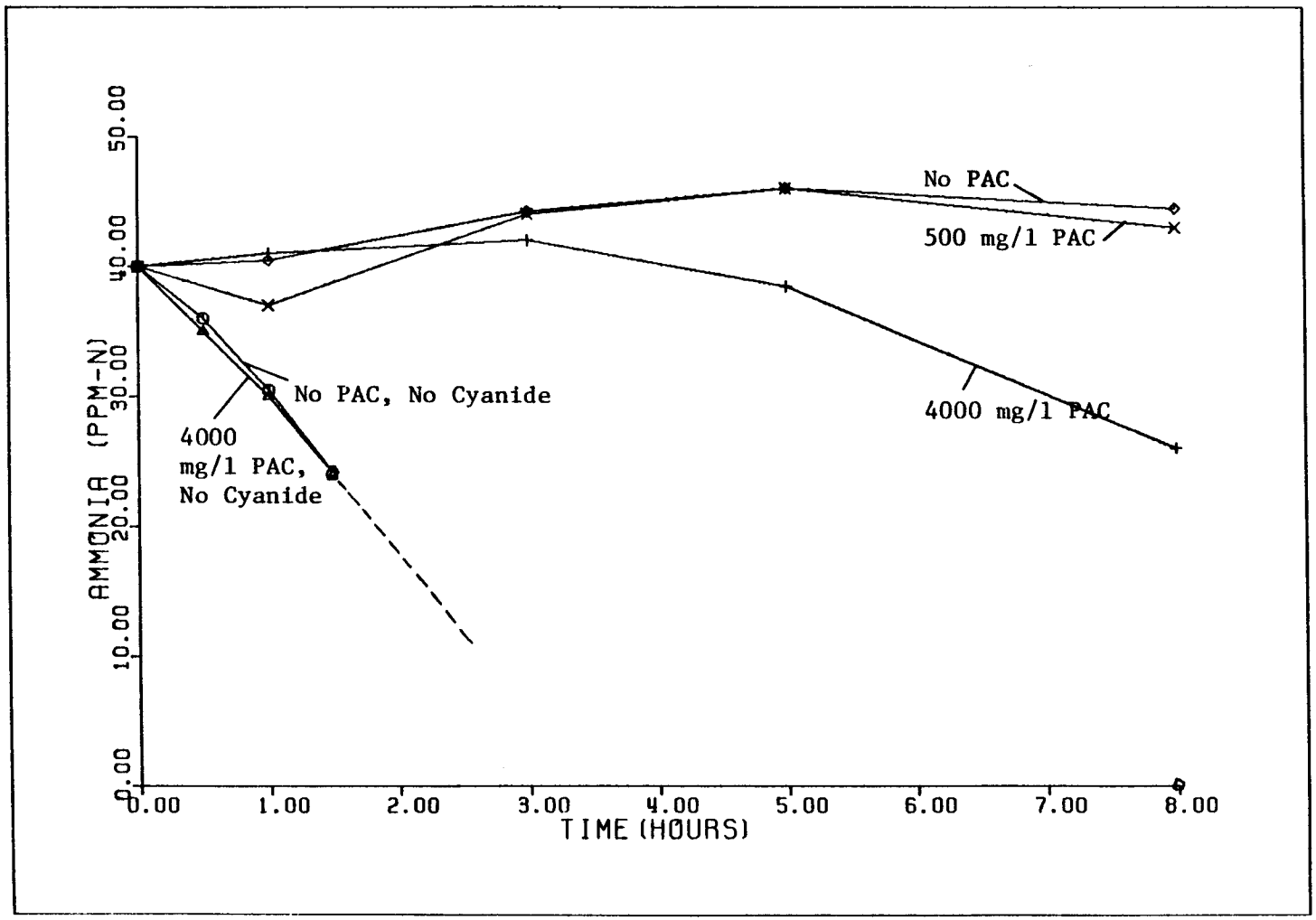


Figure 43: NO_3 vs Time for Variable Carbon Dose (1.4 mg/1 Cyanide)

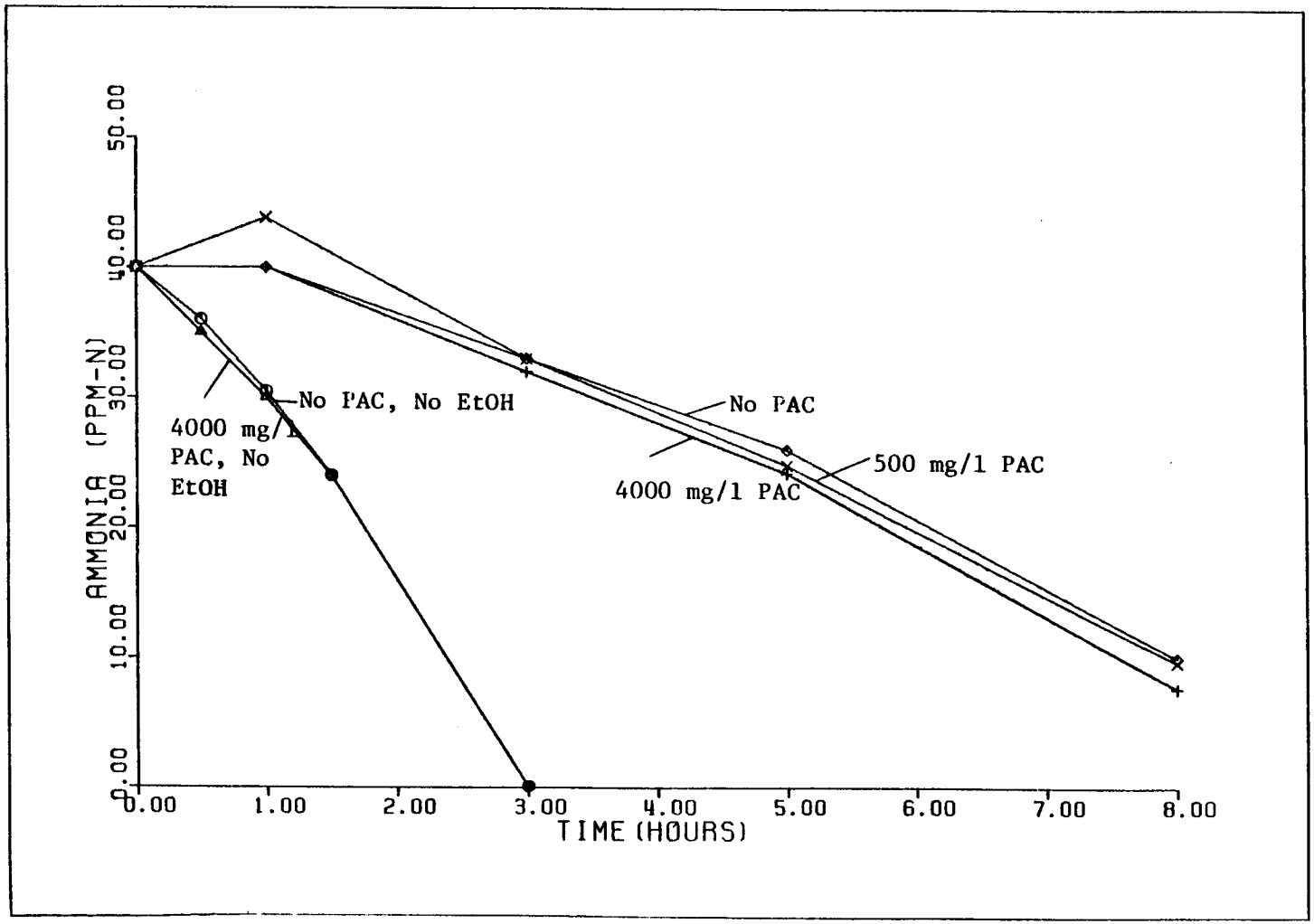


Figure 44: NH_3 vs Time for Variable Carbon Dose (Ethanol, 2500 mg/1)

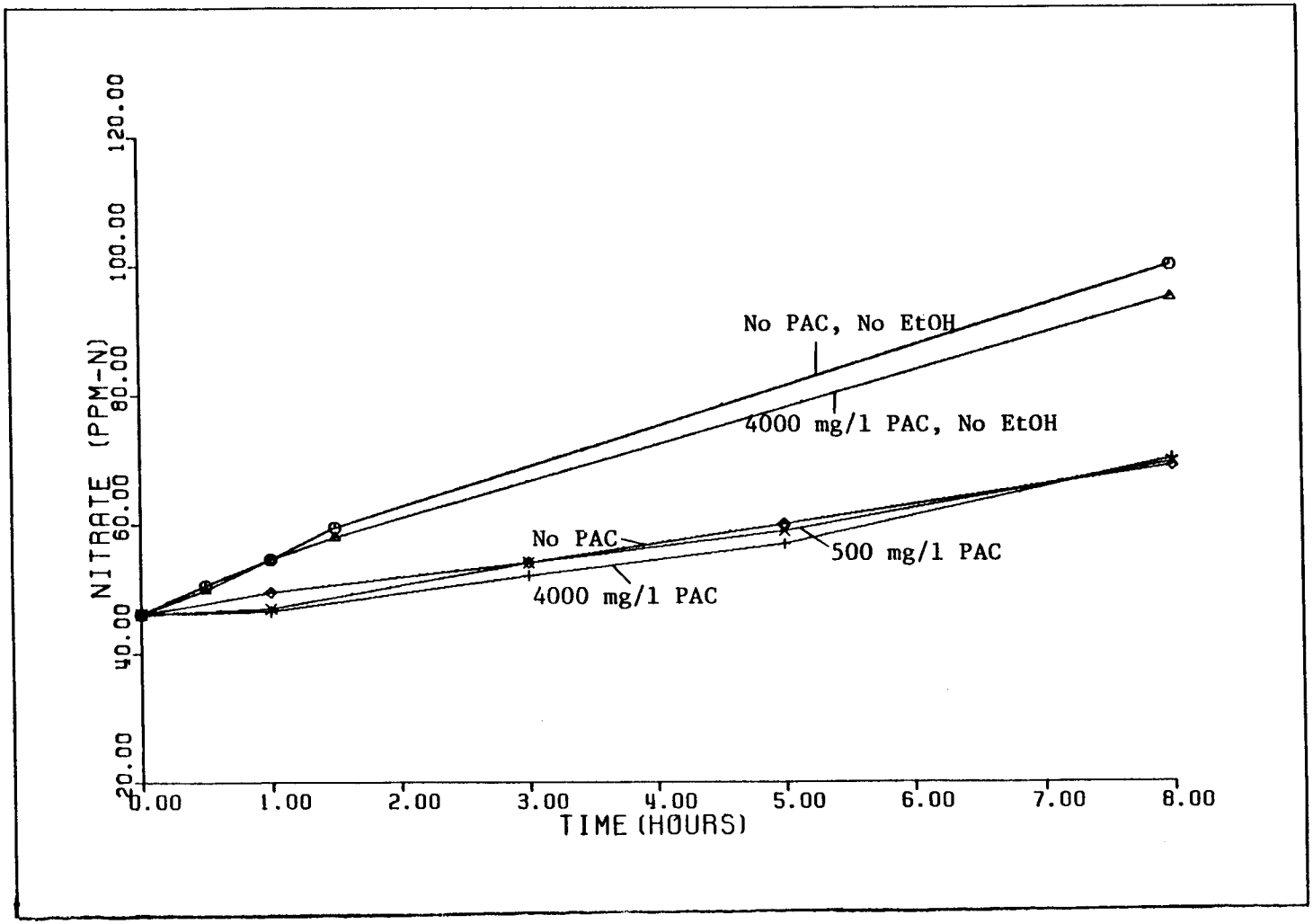


Figure 45: NO_3 vs Time for Variable Carbon Dose (Ethanol, 2500 mg/1)

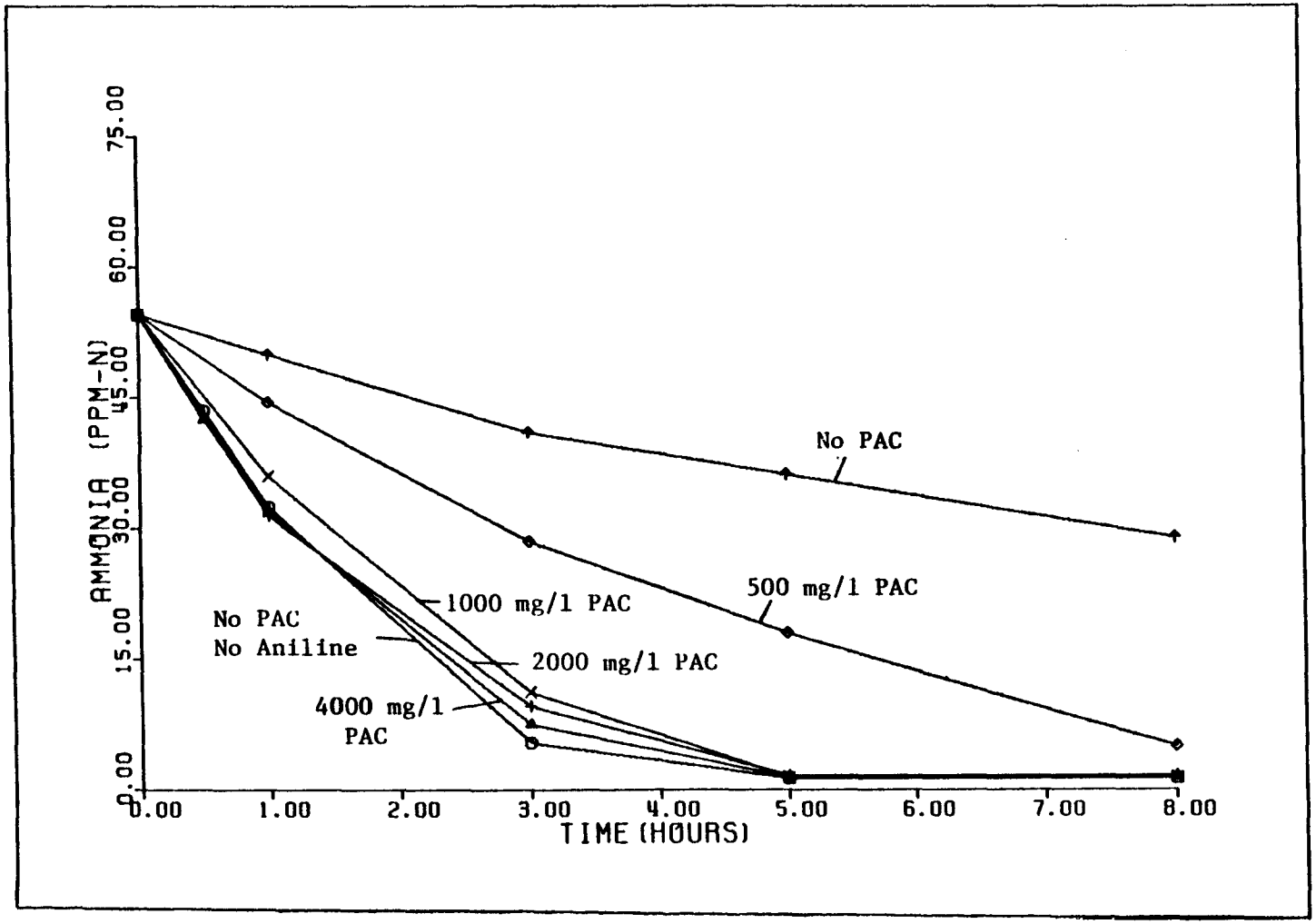


Figure 46: NH_3 vs Time for Variable Carbon Dose (Aniline, 10 mg/1)

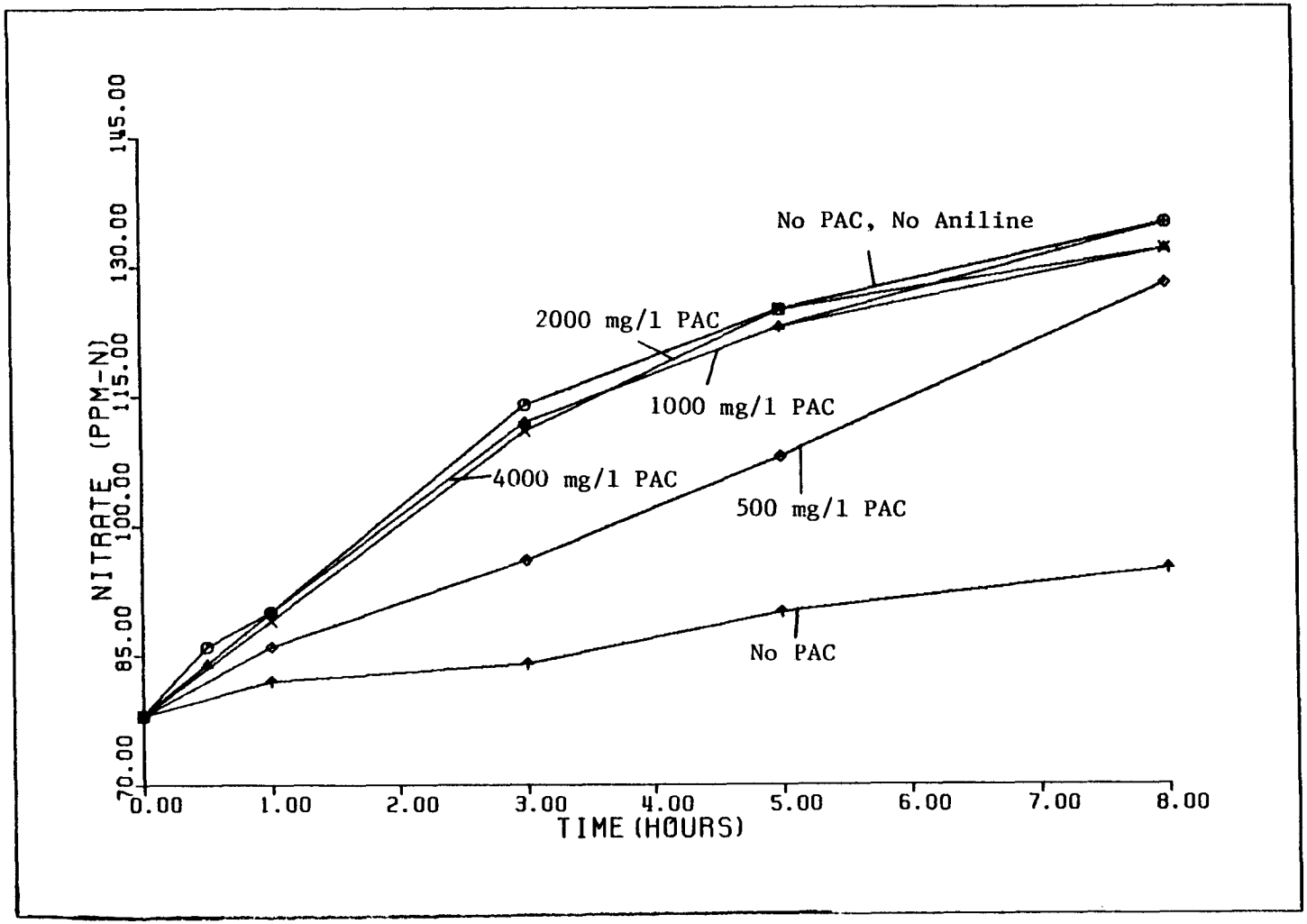


Figure 47: NO_3 vs Time for Variable Carbon Dose (10 mg/1 Aniline)

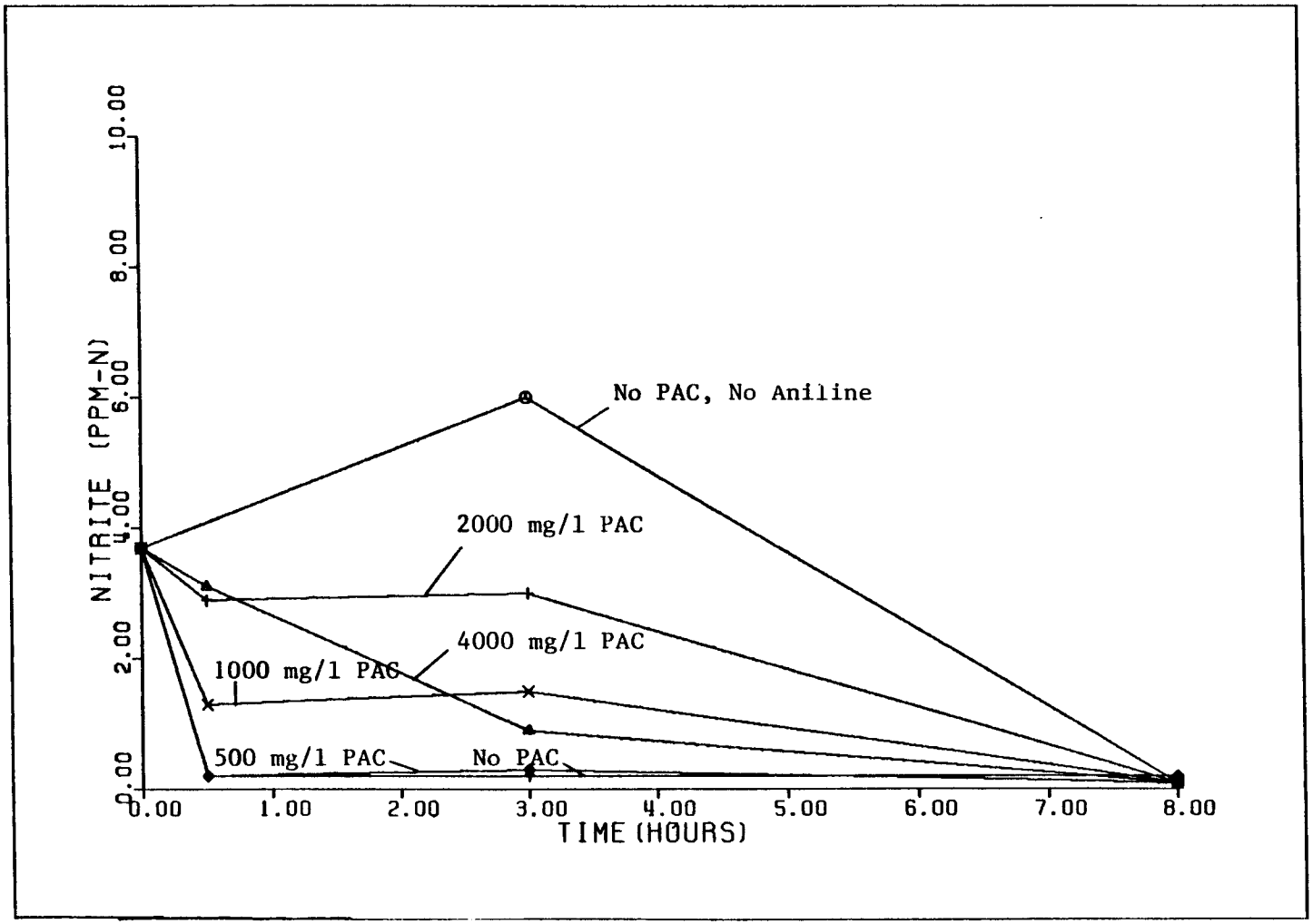


Figure 48: NO_2 vs Time for Variable Carbon Dose (10 mg/1 Aniline)

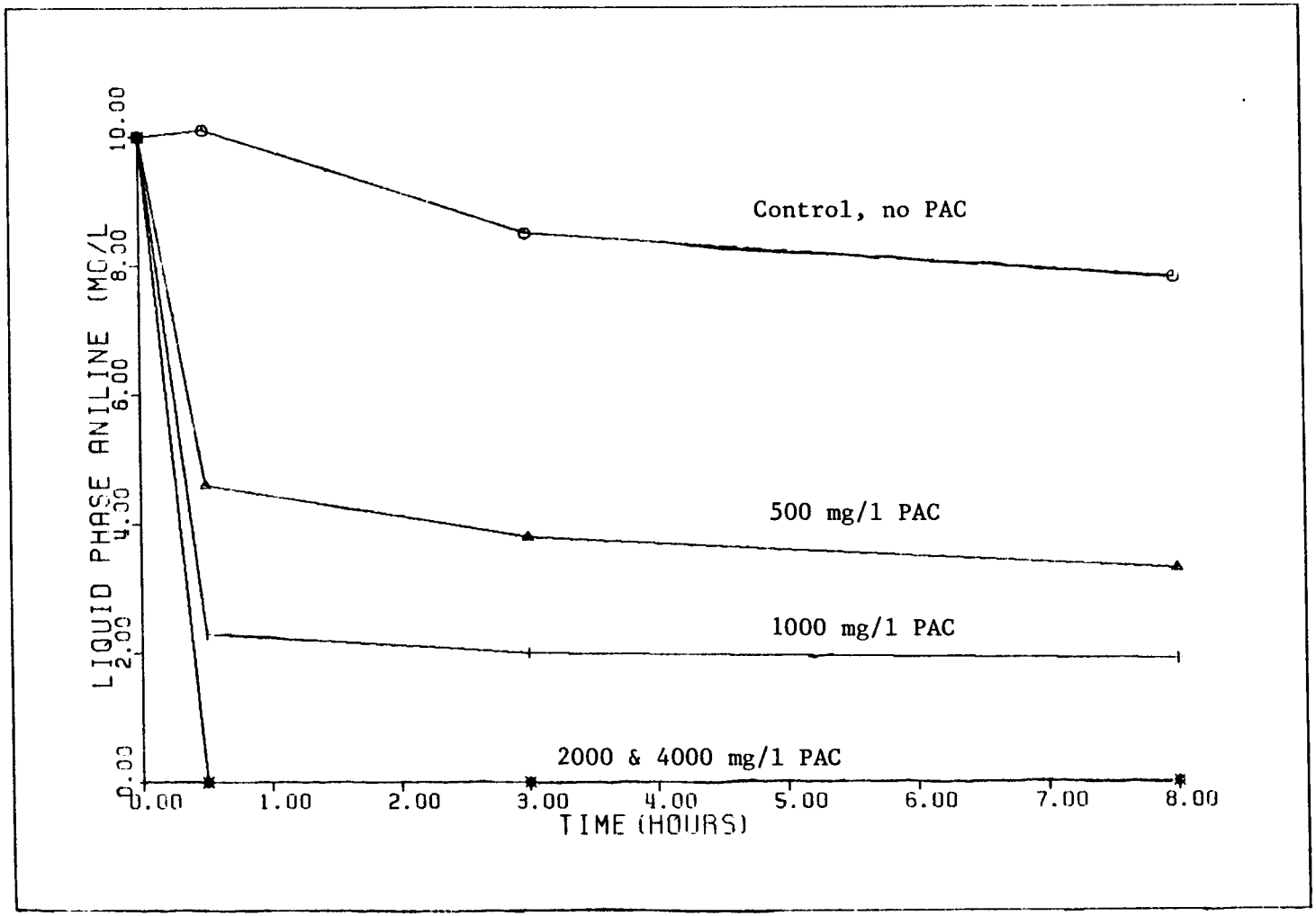


Figure 49: Liquid Phase Aniline Concentration vs. Time

Table 26: NH_3-N and NO_3-N Reaction Constants and r^2 for Carbon Dose Experiments

Spiked Compound	Type		control NA	control A	4000 A	2000 A	1000 A	500 A
Aniline 10 mg/l (1-85)	AI	r^2	0.99	0.99	0.97	0.99	0.99	0.99
		$-K(NH_3)$	12.1	0.25	12.0	11.3	10.7	3.6
		r^2	0.99	0.99	0.99	0.99	0.99	0.99
		$K(NO_3)$	8.6	0.6	8.3	8.7	8.0	3.4
Phenol 20 mg/l (1-85)	AI	r^2	0.96	0.23	0.96	0.99	0.87	0.2
		$-K(NH_3)$	11.7	0.65	11.75	11.73	4.35	0.78
		r^2	0.99	0.7	0.99	0.99	0.95	0.74
		$K(NO_3)$	8.0	0.54	7.2	7.8	3.5	0.79
Cyanide 0.7 mg/l (1-85)	NAI	r^2	0.99	0.99	0.99	•	•	0.97
		$-K(NH_3)$	12.1	2.6	6.3	•	•	4.5
		r^2	0.99	0.98	0.99	•	•	0.98
		$K(NO_3)$	8.6	2.44	5.5	•	•	3.5
Cyanide 1.4 mg/l (2-85)	NAI	r^2	0.99	0.65	0.69	•	•	0.4
		$-K(NH_3)$	10.7	-0.67	1.69	•	•	-0.7
		r^2	0.99	0.74	0.86	•	•	0.6
		$K(NO_3)$	8.84	0.59	2.2	•	•	0.43
Ethanol 2500 mg/l (2-85)	NAI	r^2	0.99	0.96	0.97	•	•	0.95
		$-K(NH_3)$	10.7	3.82	4.14	•	•	4.12
		r^2	0.99	0.99	0.96	•	•	0.99
		$K(NO_3)$	8.8	2.82	3.0	•	•	2.98
Aniline 10 mg/l (2-85)	AI	r^2	0.98	0.97	0.97	0.96	0.99	0.97
		$-K(NH_3)$	15.9	3.16	15.0	14.4	14.1	6.1
		r^2	0.99	0.98	0.99	0.99	0.97	0.99
		$K(NO_3)$	11.8	2.07	11.3	11.3	11.0	6.1

AI = Adsorbable inhibitor; NAI = Non-adsorbable inhibitor
Control NA = Control with no activated carbon or inhibitor added
Control A = Control with inhibitor added but no activated carbon
4000 A = Inhibitor and 4000 mg/l carbon added
500 A = Inhibitor and 500 mg/l carbon added
• = Carbon doses not evaluated in these experimental runs

following depletion of ammonia; thus obscuring the inherent linearity of the reaction.

In general, nitrate production rate constants were observed to range from 60 to 100% of the corresponding ammonia oxidation rate constants. However, at the end of each experiment, measured nitrate concentrations were all within 14% of expected values (based on the stoichiometry of nitrification and excluding ammonia release due to endogenous decay). The reason(s) for the low nitrate production rates, relative to the corresponding ammonia oxidation rates, observed especially in the early stages of the experiments are unknown; although similar findings have been reported in published studies. Hall and Murphy (1980) reported data from batch activated sludge nitrification studies, indicating nitrate production rates 85% of corresponding ammonia oxidation rates. They offered no explanation for these observations. One possible explanation is the accumulation of nitrites, implying that Nitobacter had limited overall nitrification. In the final carbon dose experiment with 10 mg/l aniline, nitrite was measured with time. These results, shown in Figure 48, indicate that nitrite oxidation limiting conditions may explain the lag in nitrate production rates.

Another possible explanation is that offered by Wood, et. al. (1980), who reported inorganic nitrogen losses of up to 43% in batch nitrification inhibition studies with activated sludge cultures. After exploring a number of possible explanations to account for their results, the investigators attributed the losses to the reduction of nitrite by hydroxylamine-nitrite reductase in Nitrosomonas when "suffi-

cient" nitrite is present. The enzyme hydroxylamine-nitrite reductase, isolated by Hopper (1968), reduces nitrite in the presence of hydroxylamine to give nitrous (N_2O) and nitric oxides (NO) under aerobic conditions. The authors did not define what constituted a "sufficient" nitrite concentration but the prerequisite for this reaction to occur is the presence of nitrite. However, if the reaction kinetics for this form of aerobic denitrification is zero-ordered, then nitrite need not be detected during nitrification. Also, it should also be pointed out that the mixed liquor used in the carbon dose experiments was withdrawn from continuously fed reactors operating under steady state conditions (i.e., effluent < 1.0 mg/l NH_3). Subjecting nitrifiers to a pulse addition of a relatively high concentration of ammonia creates conditions whereby, at least initially, high nitrite concentrations may result.

For Woods' explanation to be plausible, the degree of inorganic nitrogen loss observed in the carbon dose experiments should be inversely related to the degree of nitrification inhibition observed; since the function of hydroxylamine-nitrite reductase in Nitrosomonas is also assumed to be subjected to the inhibitory effects of the added compound. Inspection of Figure 50, which represents the observed % inhibition and % difference in K between ammonia oxidation and nitrate production for all carbon dose experiments, reveals a suggestive trend in that nitrate production relative to ammonia oxidation decreases with decreasing degree of inhibition. This trend is more pronounced if data from each experimental run are considered separately (see Table 27). Figures 51-56 are plots of the data in Table 27. The relationships between % inhibition and % N loss in Figures 51-56 appear to be compound specific.

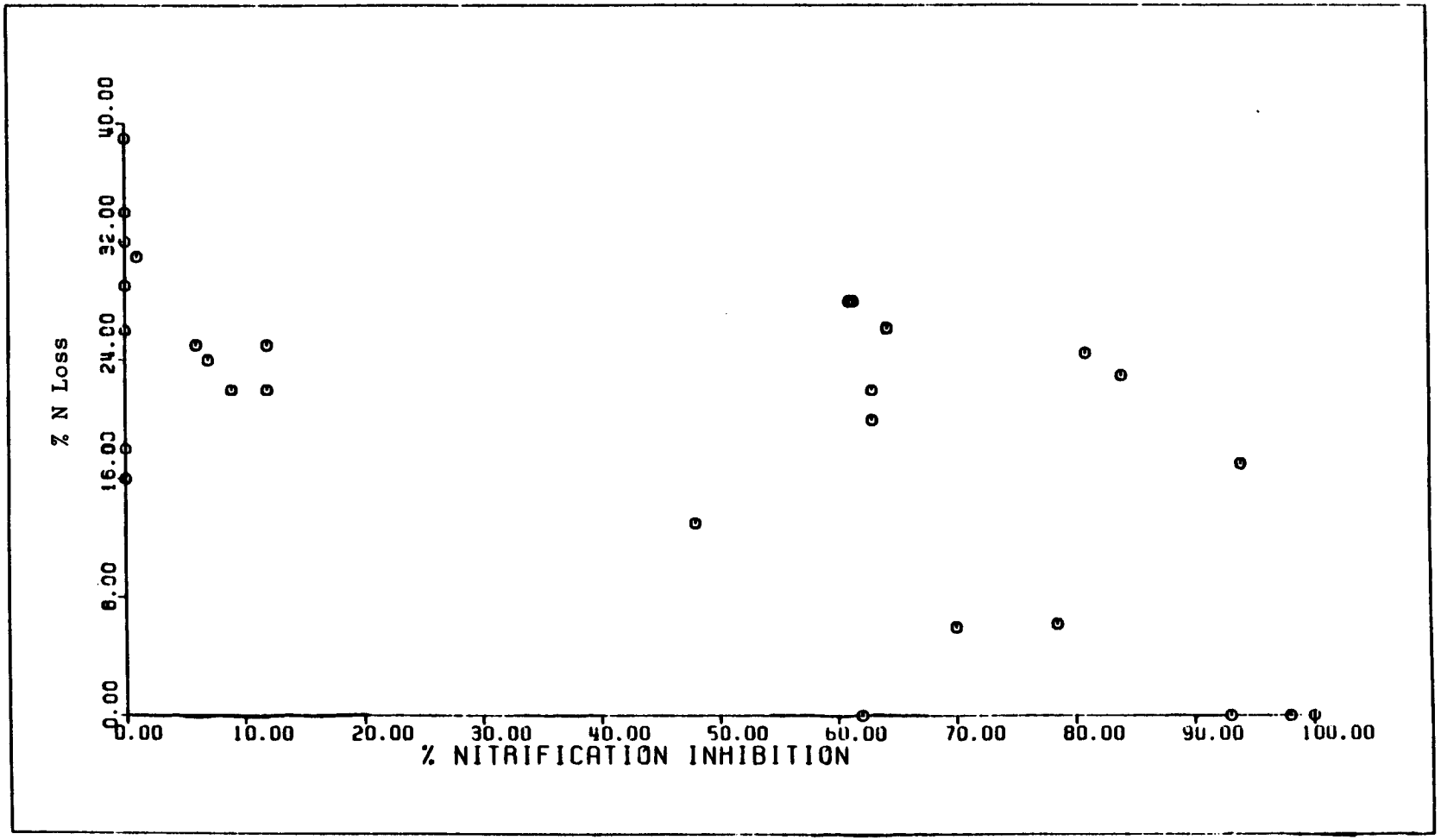


Figure 50: % Inhibition vs % N Loss for All Carbon Dose Experiments

Table 27: Percent Inhibition and Percent Inorganic Nitrogen Loss Observed for Carbon Dose Experiments

Compound	Control NA	4000 A	2000 A	1000 A	500 A	Control A
Aniline (10 mg/1)						
% Inhibition	0.0	1.0	7.0	12.0	70.0	98.0
% N Loss	29.0	31.0	24.0	25.0	6.0	0.0
Aniline (10 mg/1)						
% Inhibition	0.0	6.0	9.0	12.0	62.0	81.0
% N Loss	26.0	25.0	22.0	22.0	0.0	29.5
Phenol (20 mg/1)						
% Inhibition	0.0	0.0	0.0	63.0	93.0	94.0
% N Loss	32.0	39.0	34.0	20.0	0.0	17.0
Cyanide (1.4 mg/1)						
% Inhibition	0.0	84.0	*	*	100.0	100.0
% N Loss	16.0	23.0	*	*	0.0	0.0
Cyanide (0.7 mg/1)						
% Inhibition	0.0	48.0	*	*	63.0	78.5
% N Loss	29.0	13.0	*	*	22.0	6.2
Ethanol (2500 mg/1)						
% Inhibition	0.0	61.1	*	*	61.5	64.3
% N Loss	18.0	28.0	*	*	28.0	26.2

Control NA = No inhibitor or PAC added
Control A = inhibitor added, no PAC
4000, 2000, 1000 and 500 A are PAC doses used with the added inhibitor.
% N Loss = 100 - ($K(NO_3)/-K(NH_3)$ * 100)

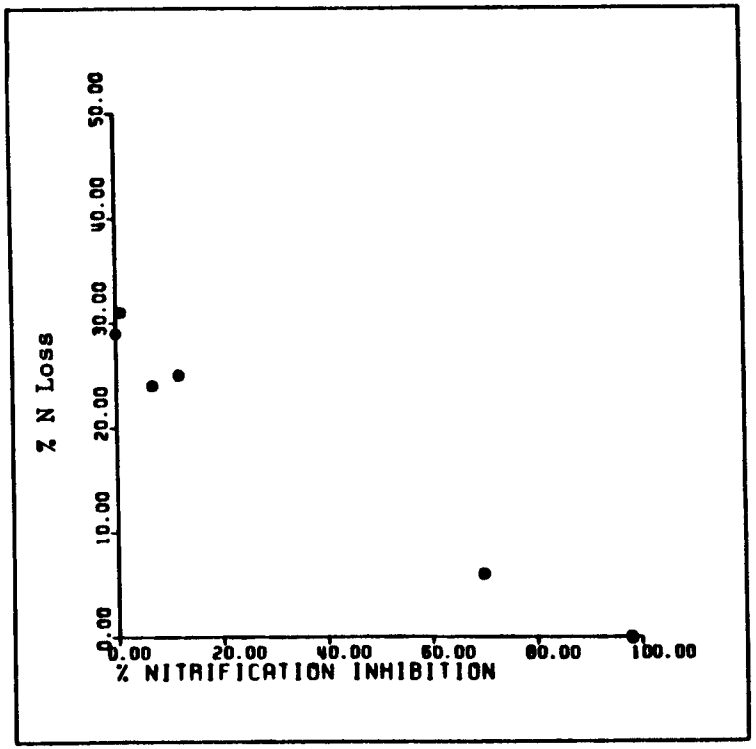


Figure 51: % Inhib. vs % N Loss (Aniline, 10 mg/l)

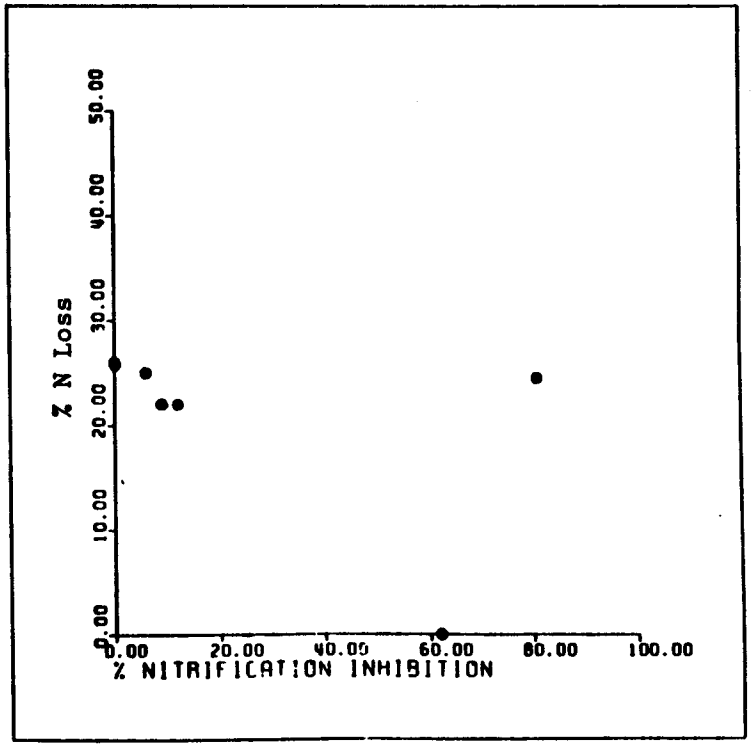


Figure 52: % Inhib. vs % N Loss (Aniline, 10 mg/l)

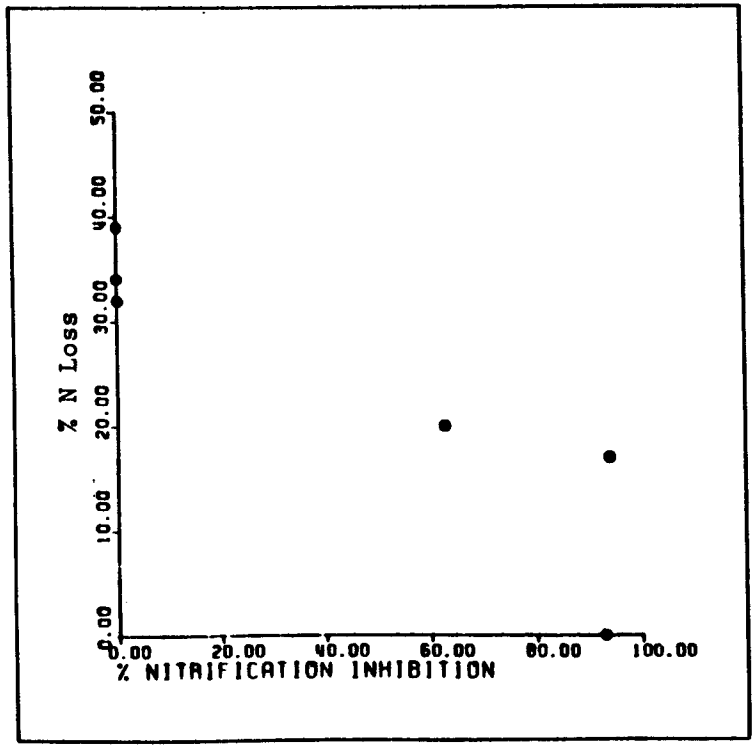


Figure 53: % Inhib. vs % N Loss (Phenol, 20 mg/1)

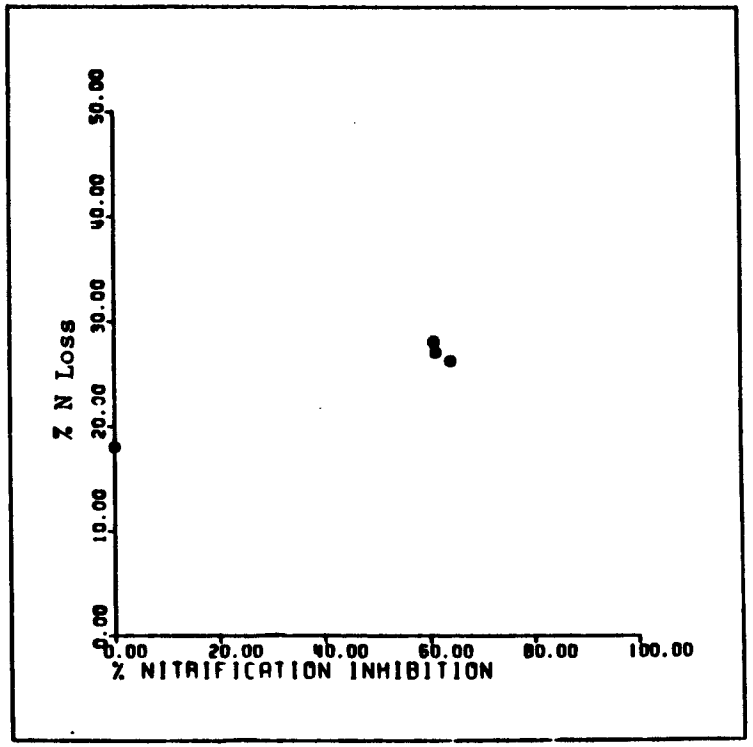


Figure 54: % Inhib. vs % N Loss (Ethanol, 2500 mg/1)

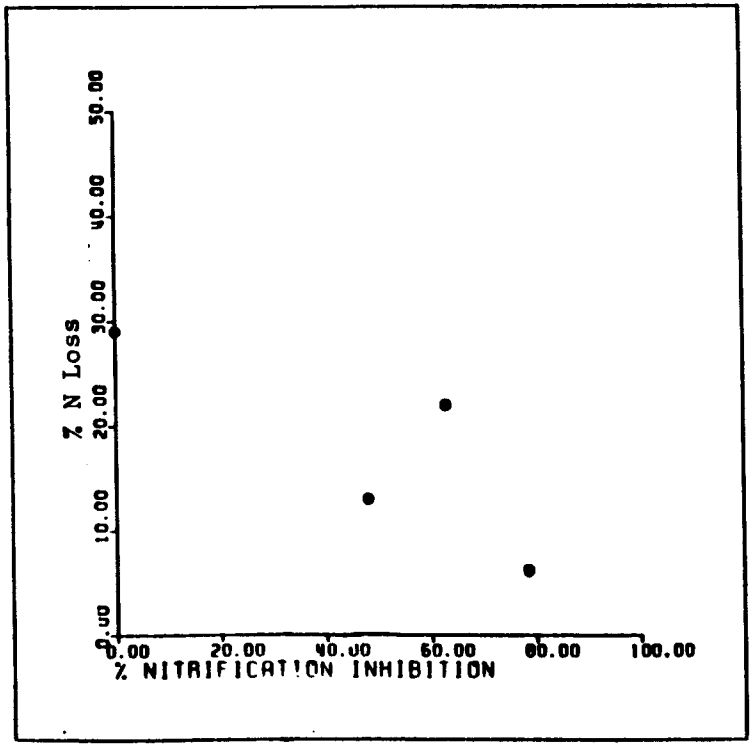


Figure 55: % Inhib. vs % N Loss (Cyanide, 0.7 mg/l)

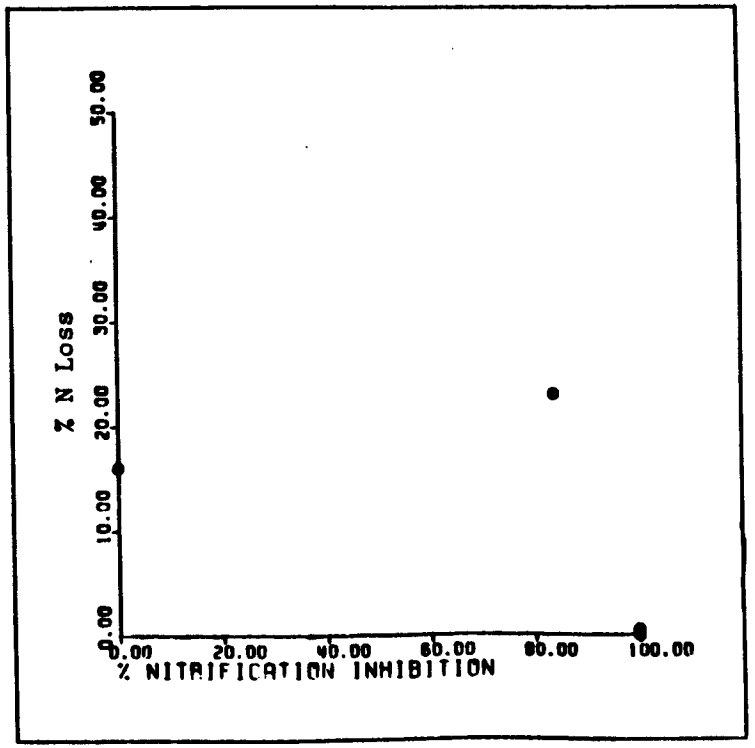


Figure 56: % Inhib. vs % N Loss (Cyanide, 1.4 mg/l)

For the adsorbable inhibitors, aniline and phenol, Woods' explanation for aerobic denitrification is supported. The limited data available for non-adsorbable inhibitors, cyanide and ethanol, is less convincing support for aerobic denitrification. If Figures 51-56 are to be taken as evidence for the inhibition of hydroxylamine-nitrite reductase activity, then the observed disparities between initial ammonia and nitrate reaction rates in the carbon dose experiments can be explained by aerobic denitrification.

Discussion on Carbon Dose Experiments

The results for experiments performed using adsorbable inhibitors (i.e., phenol and aniline) clearly demonstrate enhancement of nitrification rates due to the addition of powdered activated carbon.

In the final carbon dose experiment with 10 mg/l aniline, liquid phase aniline concentrations were measured by gas chromatography at specific sampling periods throughout the experiment. Results, plotted on Figure 49, show that liquid phase aniline concentration is directly related to nitrification inhibition (see Figures 46-47) and inversely related to PAC concentration. It appears that for cultures with no PAC addition, approximately 2 mg/l of aniline was either metabolized and/or adsorbed onto the biological mass. Figure 49 also indicates that equilibrium conditions for aniline adsorption were rapidly established with 80 to 95 % of the total adsorption occurring within 0.5 hour.

Using the previously determined adsorption isotherm parameters for aniline, the expected liquid phase aniline concentration at any given carbon dosage may be determined. Expected concentrations can then be compared with measured concentrations at the carbon dosages used in the experiment. The following equation, derived from the Freundlich adsorption model, was used to calculate expected liquid phase aniline concentrations;

$$\frac{C_i - C_f}{C_f^{1/n}} = e^{(\ln K_f + \ln M)} \qquad (52)$$

where C_i = initial aniline concentration, 10 mg/l

C_f = final equilibrium liquid phase concentration , mg/l

$1/n$ = experimentally determined Freundlich parameter, = 0.52

$\ln K_f$ = experimentally determined Freundlich parameter, = -4.402; K_f = 12.9 mg/g at C_e=1.0 mg/l

M = carbon dose, g/l

C_e, the only unknown, can be solved for by trial and error or by a programmable calculator. Results of expected and measured C_e at the PAC concentrations used in the experiment are shown below;

PAC dose (mg/l)	Expected Liquid Phase Aniline (mg/l)	Measured Aniline (mg/l)
500.0	1.8	3.3
1000.0	0.6	1.9
2000.0	0.17	not detected
4000.0	0.05	not detected

The limit of detection for the gas chromatography method used to analyzed aniline was 0.3 mg/l. The reasons for the differences between

the expected and observed values of liquid phase aniline are speculative. Aside from the different mixing conditions used in the experiment and the isotherm, the presence of biological solids may have interferred with carbon adsorption sites. Martin and Iwugo (1982) recently reported on the effects of inorganic and biological solids on the adsorption of organics by activated carbon. They concluded that suspended solids, particularly organic suspended solids, could interfere with the adsorption process, both in terms of adsorption capacity and adsorption rate. They found that organic suspended solids beginning at 500 mg/l appeared to interfere with the adsorption process for single solute solutions and that adsorption was significantly reduced.

By interpolation of Figure 57 (to be discussed) for the final aniline experiment of 2-85, it can be estimated that the PAC concentration at which 75% nitrification occurred was 103 mg/l. The corresponding liquid phase aniline concentration for this PAC dosage, based on the isotherm parameters, is 6.8 mg/l. For comparative purposes, Tomilinson (1966) observed, under similar experimental conditions, that the concentration of aniline causing 75% nitrification inhibition in nitrifying activated sludge was 7.7 mg/l.

The foregoing discussion suggests, at least for aniline, that nitrification inhibition is caused by liquid phase inhibitor concentration as opposed to total inhibitor concentration in a given PAC-activated sludge system.

Results from experiments using non-adsorbable inhibitors (i.e. cyanide, ethanol), at concentrations below that producing complete inhibition, indicate little or no significant nitrification enhancement due to the addition of powdered activated carbon. The results of the ethanol experiment (i.e., Figures 44-45) are in dramatic contrast to experiments with adsorbable inhibitors where the degree of nitrification enhancement was directly related to PAC dosage. In using ethanol, the least adsorbable inhibitor of all compounds evaluated, there was found to be no significant difference among nitrification rates in all test flasks. It should be noted that dissolved oxygen (DO) levels in each flask was measured during and after the carbon dose experiment with 2500 mg/l ethanol addition. This was done in recognition that potential oxygen limiting conditions might be created through increased heterotrophic oxygen uptake due to the addition of high concentrations of carbonaceous substrate. All DO levels were measured to be greater than 5 mg/l.

Data (Figures 40-43) from the experimental runs using cyanide as an inhibitor of nitrification showed a measurable enhancement effect due to PAC addition. The degree of nitrification enhancement noted was significantly less than those observed for adsorbable compounds and is consistent with other observations suggesting that the degree of nitrification enhancement is directly related to adsorptivity of the inhibitor. However, the degree of nitrification enhancement exceeded that expected from adsorption since cyanide, as it exists in solution, in highly ionized (CN-).

A subsequent literature search on methods of cyanide waste treatment revealed that the adsorptive characteristics of cyanide could be altered under a given set of circumstances. Bernardin (1973) and Hoffman (1973), in investigations involving the detoxification of cyanide-bearing wastewaters, demonstrated that granular activated carbon will catalyze the oxidation of cyanide to cyanate (CNO^-) in the presence of dissolved oxygen. According to Hoffman, the resulting cyanate ion can be up to 26 times more adsorbable than cyanide when complexed with copper or any other divalent metal (except for iron) present. The studies showed that a 1:1 ratio by weight of divalent metal to cyanide was optimal for adsorption onto granular activated carbon. Hoffman also suggested, depending on contact time, that the presence of activated carbon and DO can promote the subsequent hydrolysis of cyanate to bicarbonate and ammonia. Reactor feed contained a number of divalent metals including copper, cobalt, zinc and manganese at trace concentrations. The total concentration of divalent metals, excluding iron, in the feed was calculated to be approximately 0.062 mg/l. Assuming that 10% of this is in free form, the resulting ratios of cyanide to available divalent metal in the experiments involving cyanide was approximately 0.001 to 0.002. These ratios would result in little enhanced adsorptivity for cyanide for the case of granular activated carbon, however, it is probable that powdered activated carbon, which is a much more efficient adsorbent in terms of external surface area, can provide a means for increased adsorption of cyanide-metal complexes.

To form a more quantitative basis for comparing the degree of nitrification enhancement among different experimental runs, the inhibition coefficient (I) can be calculated to express the degree of inhibition observed at each carbon dosage for each inhibitor evaluated. I, which was defined and used earlier, is the ratio of calculated reaction rate constant K in the presence of the added compound to K in the control (i.e., no added compound). Calculated I's for ammonia oxidation and nitrate production rate constants for all experimental runs are presented on Tables 28 and 29. Inspection of both tables reveals that the degree of nitrification enhancement relative to the corresponding control (no added compound) at any given carbon dose is related to the adsorptivity of the inhibitory compound under evaluation. For nonadsorbable compounds such as cyanide, nitrification enhancement above that in the control with no PAC addition was approximately 16 and 30% at the highest PAC dose tested (4000 mg/1), for 0.7 and 1.4 mg/1, respectively. For ethanol, the least adsorbable of the compounds tested, degree of nitrification enhancement was only 3 % at the highest PAC tested. In contrast, nitrification enhancements of 75, 97 and 94 % over the corresponding controls with no PAC addition were noted for aniline; aniline; and phenol, respectively, at 4000 mg/1 PAC. These results provide further evidence that the adsorption is the major mechanism of nitrification enhancement in activated sludges.

Figures 57 and 58 show, respectively, I values for ammonia and nitrate reaction constants plotted against PAC concentrations for all carbon dose experiments. Both figures show a general relationship between the degree of nitrification enhancement and PAC concentration

Table 28: Nitrification Inhibition Coefficients, I
Based on Ammonia Reaction Constants
For Carbon Dose Experiments

Spiked Compound	Type	Adsorption Parameters	Control A (No PAC)	4000 A mg/l	2000 A mg/l	1000 A mg/l	500 A mg/l
Aniline 10 mg/l (2-85)	AI	12.2 mg/g 0.52	0.19	0.94	0.91	0.88	0.38
Aniline 10 mg/l (1-85)	AI	12.2 mg/g 0.52	0.02	0.99	0.93	0.88	0.3
Phenol 20 mg/l (1-85)	AI	21.0 mg/g 0.54	0.06	1.0	1.0	0.37	0.07
Cyanide 1.4 mg/l (2-85)	NAI	2.0 mg/g	0.0	0.158	*	*	0.0
Cyanide 0.7 mg/l (1-85)	NAI	2.0 mg/g	0.22	0.52	*	*	0.37
Ethanol 2500 mg/l (2-85)	NAI	0.0 mg/g	0.357	0.387	*	*	0.385

* Carbon doses not evaluated in these experiments
AI, NAI = Adsorbable and non-adsorbable nitrification inhibitor.
Adsorption parameters are Freunlich parameters, K and 1/n for aniline, phenol, and ethanol based on C sub e# = 1.0 mg/l.
For cyanide, the adsorption parameter represents the maximum adsorption observed at an initial concentration of 20 mg/l.

Table 29: Nitrification Inhibition Coefficients, I
Based on Nitrate Production Constants
For Carbon Dose Experiments

Spiked Compound	Type	Adsorption Parameters	Control A (no PAC)	4000 A mg/1	2000 A mg/1	1000 A mg/1	500 A mg/1
Aniline 10 mg/1 (2-85)	AI	12.2 mg/g 0.52	0.18	0.96	0.96	0.93	0.52
Aniline 10 mg/1 (1-85)	AI	12.2 mg/g 0.52	0.07	0.97	1.01	0.93	0.39
Phenol 20 mg/1 (1-85)	AI	21.0 mg/g 0.54	0.07	0.9	0.98	0.44	0.1
Cyanide 1.4 mg/1 (2-85)	NAI	2.0 mg/g	0.07	0.25	*	*	0.05
Cyanide 0.7 mg/1 (1-85)	NAI	2.0 mg/g	0.28	0.64	*	*	0.41
Ethanol 2500 mg/1 (2-85)	NAI	0.0 mg/g	0.32	0.34	*	*	0.34

* Carbon doses not evaluated in these experimental runs
AI, NAI = Adsorbable and non-adsorbable nitrification inhibitor
Adsorption parameters are Freunlich parameters, K and 1/n for aniline, phenol, and ethanol based on C_e = 1.0 mg/1.
For cyanide, the adsorption parameter represents the maximum adsorption observed at an initial concentration of 20 mg/1 CN.

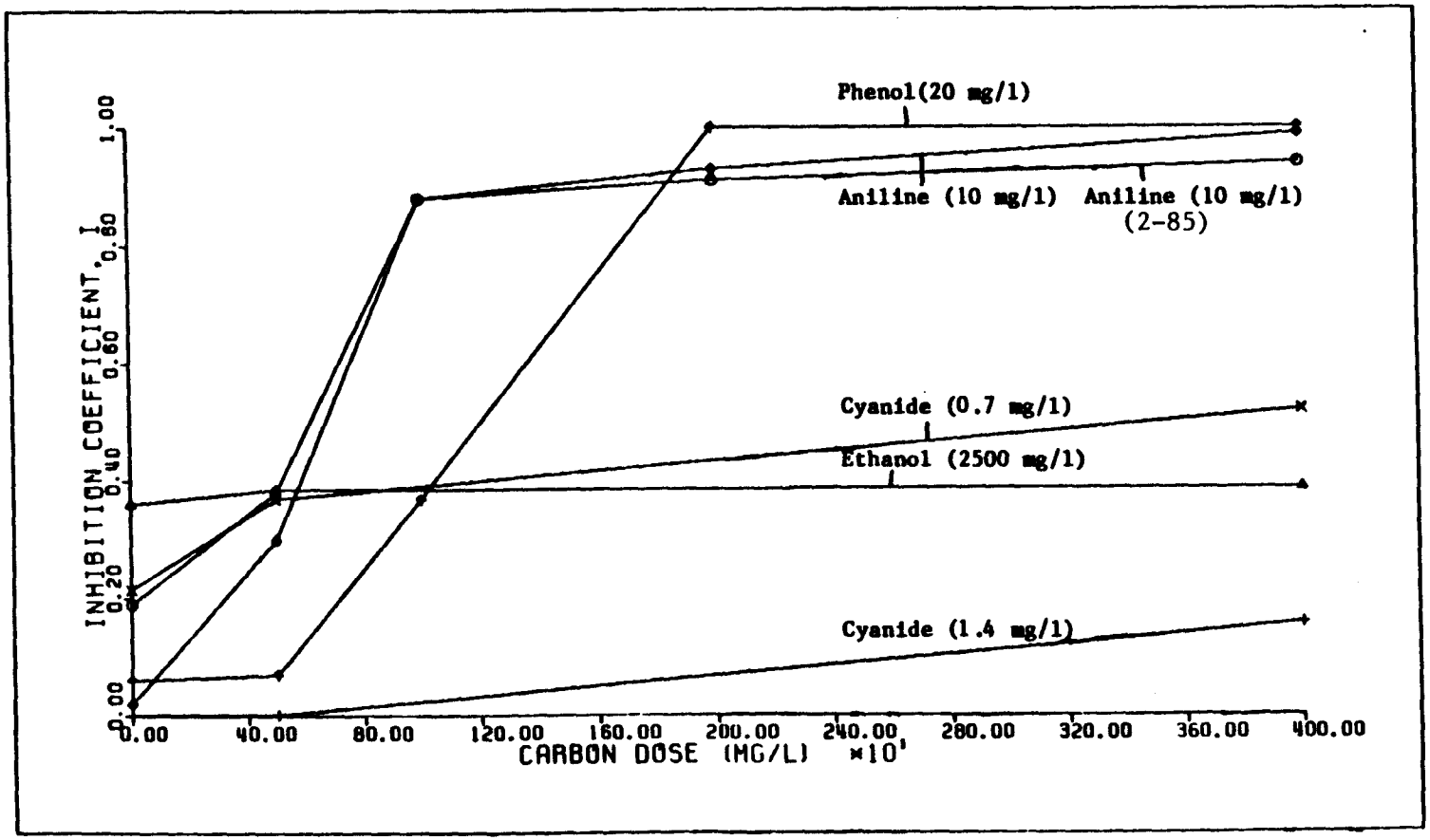


Figure 57: Inhibition Coefficient, I (NH_3) vs PAC Dosage

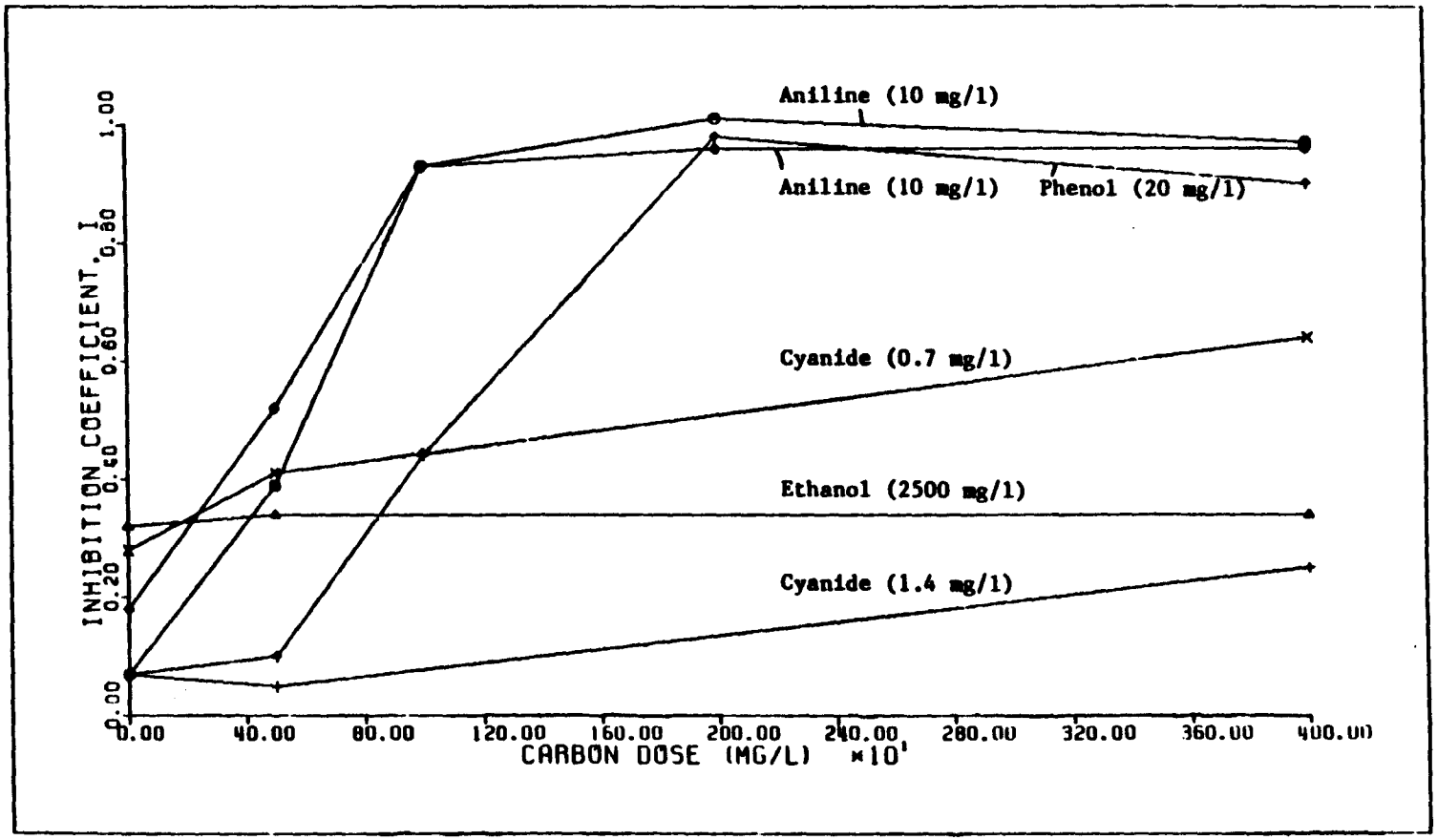


Figure 58: Inhibition Coefficient, I (NO_3) vs PAC Dosage

depending on the adsorptivity of the compound tested. For adsorbable compounds, at the initial concentrations used, the relationship can be characterized by a "S" type curve. This indicates that nitrification was enhanced marginally at low PAC doses and that the rate of nitrification increased steadily with increasing PAC concentration until a plateau was reached (i.e., no further enhancement). From this relationship, it appears that nitrification enhancement depends upon the lowering of inhibitor concentration, through adsorption, to some threshold value before nitrification can proceed at reasonable rates. For nonadsorbable compounds, the enhancement/PAC relationship is characterize by a relatively horizontal curve indicating no or little nitrification enhancement with PAC concentration.

It is important to note that in these experiments, unacclimated activated sludge and virgin PAC was used. For adsorbable inhibitors, the beneficial effects of carbon were observed almost immediately after the beginning of the test (i.e., 2 hours). These results support the adsorption of inhibitory compounds theory of enhancement, and disproves the other theories, at least for this series of experiments. The other theories cannot account for the results of these experiments since there was insufficient time for preferential growth on the carbon surfaces, and the virgin carbon could not have been laden with trace nutrients or have been able to concentrate them over short contact times. Therefore, the role of PAC in mediating the effects of adsorbable inhibitory compounds on nitrification in unacclimated activated sludge has been demonstrated. In addition, these experiments provide strong evidence that a major benefit of PAC addition in nitrifying activated sludge is

adsorption of toxic compounds that may be present in the waste stream.

ADDITIONAL OBSERVATIONS

Several other noteworthy observations can be made with respect to refinery wastewater fed PAC activated sludge. Throughout the duration of this study, the PAC reactor consistently demonstrated the greatest stability.

Operation at an high sludge age (i.e., 60 days) and high organic loading (i.e., influent > 500 mg/l TOC, θ_H = 24 hours) invariably results in high MLSS concentrations and probable settling difficulties in the final clarifiers. There was never a problem in the PAC reactor since PAC served as a weighting agent to improve settling. On the other hand, the control and bentonite units often suffered from settling problems with high sludge blankets; they generally did not demonstrate consistent and desirable settling characteristics.

The difference between batches of wastewater received provided an opportunity to compare the stability of all reactors. The PAC reactor was observed to be the most stable in terms of foam suppression and color removal. With almost every new wastewater batch, the control and, especially, the bentonite reactor, experienced severe foaming problems. No foaming was ever noted in the PAC reactor. Typically it would take one week of stable operation with a new batch of wastewater before foaming problems would subside in the non-PAC units. Increased color removal in the PAC units over the control and bentonite units was also routinely observed. The color of the refinery wastewater was yellowish and the effluent from the PAC reactors was noted to be as clear as the effluent from the glucose reactors. In contrast, the control and

bentonite reactor effluents retained the yellowish characteristic of the influent.

Enhancement of nitrification efficiencies were also evident --- particularly during the initial start-up period for the reactors. The later observation is best supported by Figures 59 and 60 which are cumulative probability plots for effluent ammonia concentration for each reactor type. These figures show that the addition of PAC in the glucose fed reactors made no significant difference in nitrification. However, the addition of PAC to the refinery reactor resulted in significantly more nitrification over the control and bentonite reactors. This was presumably due to a combination the factors noted earlier. It should be noted that these benefits were observed almost exclusively during the start-up period when reactor upsets were common. Following the start-up period, all refinery reactors achieved excellent nitrification and reactor effluent ammonia samples were taken less frequently.

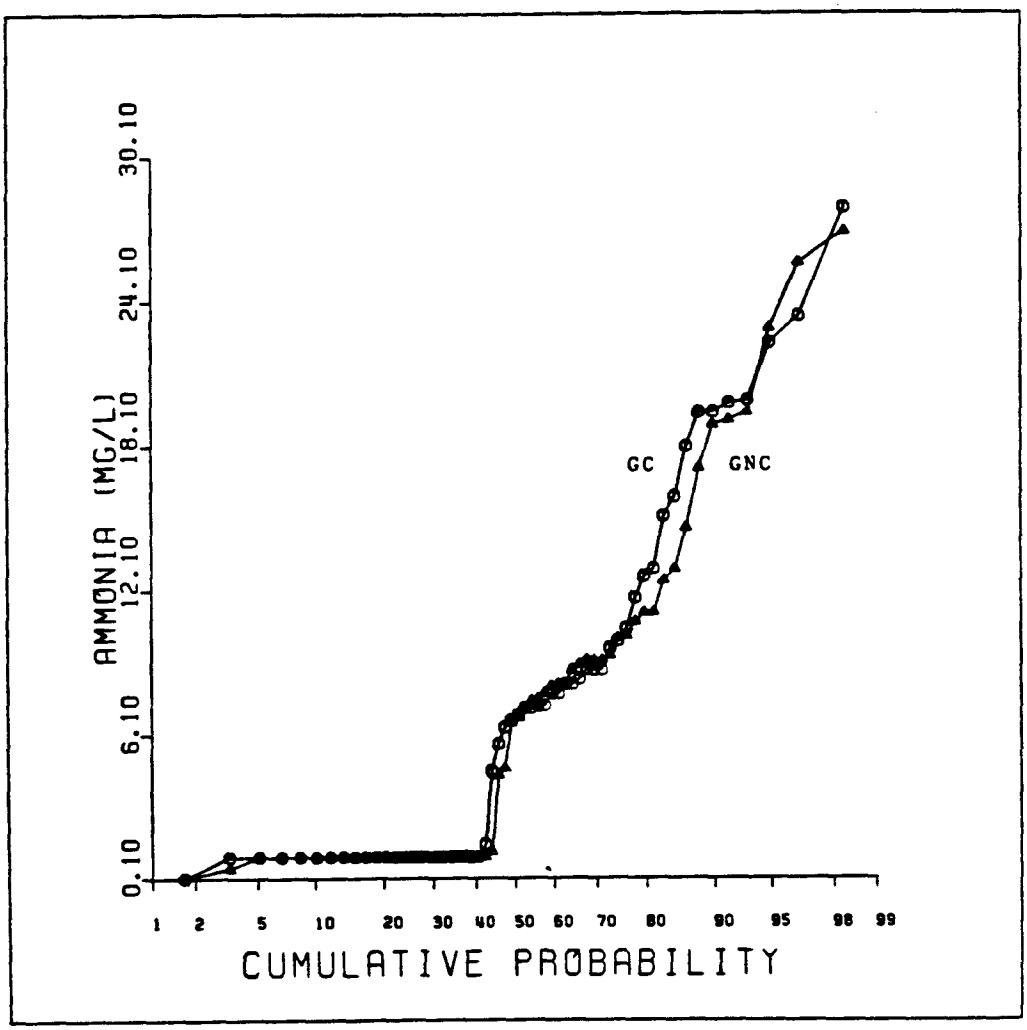


Figure 59: Cumulative Effluent Ammonia Probability for Glucose Reactors

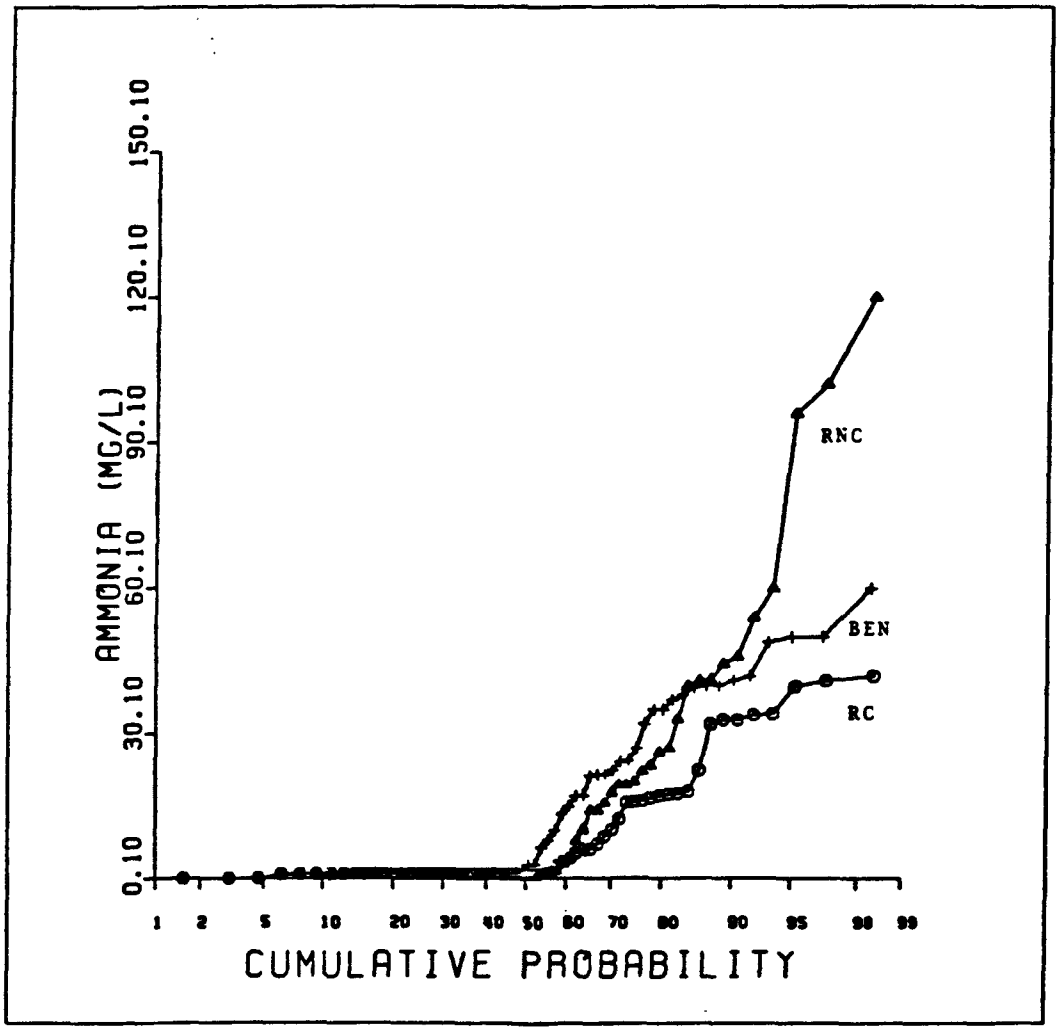


Figure 60: Cumulative Effluent Ammonia Probability for Refinery Reactors

SUMMARY AND CONCLUSIONS

An experimental program was designed to assess the influence of powdered activated carbon (PAC) and bentonite additions on activated sludge nitrification rates in the presence of "spiked" compounds of known inhibitory and adsorptive characteristics. Bentonite (aluminum silicate) is a suspended solid with suitable surface chemistry for microbial attachment but limited ability to adsorb organics. Based on the results reported herein, the findings and conclusions in this study can be summarized as follows:

1. In batch inhibition studies, nitrification enhancement due to PAC addition was demonstrated in unacclimated activated sludge cultures in the presence of adsorbable inhibitors. Enhancement due to the addition of either PAC or bentonite was not evident in any experiments involving a non-adsorbable inhibitor. These results provide evidence that adsorption of inhibitory compounds is a more important mechanism for nitrification enhancement than is enhanced nitrifier growth on the surface of suspended solids.

2. For acclimated activated sludges, nitrification enhancement was much more difficult to demonstrate, presumably because at the low dosages used (50 mg/l influent), the benefits of sludge acclimation were much more pronounced and perhaps obscured any benefit the added carbon could produce.

3. The chronic experiments showed that acclimated sludges are capable of recovering from shock load conditions of very high inhibitor

concentrations to full nitrification with no apparant after effects. At the dosages used (50 mg/l influent), the addition of either PAC or bentonite provided no significant enhancement of nitrification rates under the experimental conditions.

4. The initial chronic experiment gave evidence that the addition of PAC to activated sludge can indirectly inhibit nitrification by virtue of desorption of a previously adsorbed inhibitor. In this same experiment, it was shown that an adequate dose of virgin PAC can dramatically arrest the effect of an adsorbable inhibitor and completely restore nitrification capability. The second chronic experiment demonstrated that at a high concentration of aniline, Nitrobacter activity was inhibited. Previous studies had implicated aniline as an inhibitor only to ammonia oxidation. This experiment also suggested that the Nitrobacter sp. may have an affinity for attachment to suspended solids.

5. Results from the carbon dose experiments indicated that the addition of PAC in the proper amounts can completely nullify the toxic effects of an adsorable inhibitory compound in unacclimated activated sludge cultures. For adsorbable nitrification inhibitors, the addition of PAC resulted in no significant loss of nitrification capability and nitrification enhancements over control units (i.e., no PAC added) of 75 to 100%. For relatively non-adsorbable inhibitors, nitrification enhancements of only 3 to 30% were observed at the same carbon dosage. These results provide convincing evidence in support of the theory that PAC can adsorb

inhibitory compounds, thereby enhancing nitrification rates.

6. There appears to be an optimal dose of carbon required to negate the effects of an inhibitor given that its concentration and isotherm characteristics are known a priori.

7. The results from the carbon dose experiments do not support the theory that concentration of nutrients on the carbon surface is a major mechanism for nitrification enhancement since the benefits to nitrification from adding the virgin PAC was observed to be immediate (i.e., 1 hour or less). Therefore, the effect of concentration of nutrients on the carbon surface over such short contact times could provide little benefit in enhancing nitrifier growth.

8. Over the course of the experiments it became obvious that an important factor for continued high efficiency nitrification was the uniformity of influent fed to the reactors treating refinery wastewater. It appears that providing a highly equalized influent wastewater could provide approximately the same benefits to nitrification as powdered activated carbon addition at dosage evaluated (50 mg/l influent).

CONCLUSIONS

The results presented herein have important implications in the wastewater treatment field, particularly in industrial wastewater treatment, where nitrification inhibitors may be present. This study has shown that the major mechanism by which PAC nitrification occurs is adsorption of inhibitors that may be present. However, the results also show that under steady state conditions, the gradual acclimation of heterotrophic organisms to nitrification inhibitors is equally important in maintaining the highest treatment efficiency possible.

Based on the results of the work reported herein, the major conclusions of this study are threefold;

1. With respect to nitrification enhancement in nitrifying activated sludges, the effects of the addition of powdered activated carbon will be observed only in those wastewaters containing a predominance of adsorbable inhibitors. The addition of inert suspended solid will not enhance nitrification under these conditions.

2. The acclimation ability of heterotrophic microorganisms in activated sludge to nitrification inhibitors present in wastewaters, independent of adsorptive characteristics, can be a major means of preventing loss of nitrification capability. In this regard, the utilization of equalization basins, in situations where the potential for nitrification inhibition exist, may serve to buffer inhibitory effects by allowing ample opportunity for heterotrophic acclimation.

3. If nitrification loss due to adsorbable inhibitors is of primary conceern, the periodic addition of PAC at relatively heavy doses directly into the aeration basin may be a more efficient and economical method of controlling "shock loads" of adsorbable inhibitors than is the steady state addition of PAC at low concentrations (i.e., 50 mg/l influent).

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APPENDICES

Appendix I: Results of Batch Inhibition Experiments
10 mg/l Aniline Spike (6-14-83)

Time	GC	GNC	RC	RNC	BEN	GCC	GNCC	RCC	RNCC	BENC
NH_3										
0.0	62.0	60.0	60.0	63.0	60.0	62.0	60.0	60.0	63.0	60.0
2.0	67.5	72.0	60.0	61.0	68.5	50.0	44.0	50.0	51.0	52.0
4.0	52.0	63.5	56.0	57.0	59.0	27.8	33.0	33.5	42.0	46.0
6.0	35.2	59.0	37.0	40.0	42.5	14.0	20.0	23.2	29.5	32.0
9.0	22.0	47.0	20.0	28.0	32.0	2.1	6.4	12.5	20.2	24.2
NO_3										
0.0	50.5	48.1	120.0	115.0	112.0	50.5	48.1	120.0	115.0	112.0
2.0	49.9	43.0	132.0	112.0	122.0	56.0	54.0	117.0	125.0	114.0
4.0	60.0	48.5	137.0	145.0	141.0	74.0	64.0	150.0	150.0	145.0
6.0	76.5	57.0	162.0	155.0	151.0	89.0	77.0	171.0	158.0	150.0
9.0	93.0	64.5	178.0	169.0	161.0	111.0	95.0	182.0	179.0	165.0

The total volume (mls.) of NaOH required to maintain pH at 7.3 was as follows:

GC:0.25 GNC:0.0 RC:5.7 RNC:4.6 BEN:4.4
GCC:6.6 GNCC:14.3 RCC:10.2 RNCC:7.8 BENC:8.15

In this experiment, the extraction solution (i.e., Orion Methods Manual, 1973) was used for the analysis of nitrates in refinery wastewater mixed liquor samples.

Appendix I: Results of Batch Inhibition Experiments
10 mg/l Toluene Spike (6-10-83)

Time	GC	GNC	RC	RNC	BEN	GCC	GNCC	RCC	RNCC	BENC
NH_3										
0.0	54.2	58.0	53.0	48.0	54.5	54.2	58.0	53.0	48.0	54.5
2.0	47.5	44.5	38.0	36.0	38.0	48.5	44.0	35.5	40.0	42.0
4.0	32.1	36.5	28.5	29.5	35.0	33.0	36.0	26.2	31.0	32.0
6.0	22.1	25.6	24.2	23.0	21.2	21.2	25.1	23.5	22.0	26.0
9.0	7.4	9.8	13.0	19.1	15.5	7.8	10.2	14.0	21.5	25.1
NO_3										
0.0	55.0	51.5	118.0	117.0	113.0	55.0	51.5	118.0	117.0	113.0
2.0	57.0	52.2	120.0	121.0	118.0	57.9	53.0	128.0	123.0	118.0
4.0	73.0	62.5	131.0	127.0	127.0	74.5	62.5	140.0	132.0	135.0
6.0	86.0	73.0	142.0	144.0	150.0	87.0	73.0	145.0	148.0	150.0
9.0	102.0	91.0	171.0	170.0	175.0	105.0	92.0	185.0	185.0	177.0

Note: the initial reactor effluent ammonia concentrations were measured to be as follows prior to ammonia spiking;
GC = <0.1 mg/l GNC = 0.5 mg/l RC, RNC, and BEN= <0.1 mg/l
The total volume (mls.) of NaOH required to maintain pH at 7.3 was as follows:

GC:9.1	GNC:16.2	RC:11.0	RNC:8.65	BEN:9.2
GCC:8.8	GNCC:15.6	RCC:10.8	RNCC:9.25	BENC:8.5

Appendix I: Results of Batch Inhibition Experiments
3.0 mg/l Cyanide Spike (6-7-83)

Time	GC	GNC	RC	RNC	BEN	GCC	GNCC	RCC	RNCC	BENC
NH_3										
0.0	53.0	55.0	51.0	51.0	50.0	53.0	55.0	51.0	51.0	51.0
1.0	65.0	66.0	60.0	55.9	55.0	51.0	60.0	40.0	44.0	42.0
3.0	62.0	62.1	58.0	51.0	50.0	41.0	50.0	24.0	37.0	36.5
5.0	58.0	56.0	53.0	50.0	48.5	30.2	40.5	16.4	31.2	31.0
9.0	57.0	54.0	35.0	41.0	36.0	13.9	23.8	5.6	28.5	26.1
NO_3										
0.0	42.0	38.0	92.0	100.0	94.0	42.0	38.0	92.0	100.0	94.0
1.0	44.0	37.5	98.0	95.0	92.0	49.0	38.0	105.0	110.0	107.0
3.0	50.0	41.9	108.0	104.0	98.0	62.0	47.0	125.0	117.0	113.0
5.0	60.0	47.0	104.0	108.0	107.0	76.0	58.5	139.0	121.0	118.0
9.0	70.0	62.5	120.0	118.0	117.0	101.0	81.0	152.0	132.0	136.0

Note: the initial reactor effluent ammonia concentrations were measured to be as follows prior to ammonia spiking;
GC = <1.0 mg/l GNC = <1.0 mg/l RC, RNC, and BEN= <1.0 mg/l
The total volume (mls.) of NaOH required to maintain pH at 7.3 was as follows:
GC:0.2 GNC:2.75 RC:0.0 RNC:1.6 BEN:0.95
GCC:14.05 GNCC:14.05 RCC:6.15 RNCC:5.6 BENC:5.15
pH of the glucose non-carbon reactor was 5.6 initially and adjusted to 7.2 prior to experiment.

Appendix I: Results of Batch Inhibition Experiments
20 mg/l Phenol Spike (5-25-83)

Time	GC	GNC	RC	RNC	BEN	GCC	GNCC	RCC	RNCC	BENC
NH_3										
0.0	57.2	54.0	51.0	51.0	50.0	57.2	54.0	51.0	51.0	50.0
1.0	57.9	54.0	46.0	46.0	45.0	58.0	56.0	42.0	37.0	44.0
3.0	52.0	57.8	36.0	41.0	44.0	42.0	40.0	32.0	38.0	39.0
6.0	38.0	57.0	30.5	36.5	38.0	31.0	33.9	25.0	29.0	38.0
9.0	19.0	36.5	20.1	25.0	30.0	9.2	14.8	17.8	25.0	28.0
NO_3										
0.0	42.1	78.0	82.0	70.0	69.0	42.1	78.0	82.0	70.0	69.0
1.0	28.5	56.0	79.0	78.0	66.0	38.0	68.0	75.0	66.0	70.0
3.0	36.0	63.0	72.0	65.0	59.0	53.0	81.0	72.0	66.0	60.0
6.0	66.0	73.0	74.0	65.0	58.0	72.0	100.0	78.0	72.8	63.0
9.0	86.5	94.0	98.0	74.0	76.0	94.0	120.0	92.0	82.0	76.0

Note: the initial reactor effluent ammonia concentrations were measured to be as follows prior to ammonia spiking;
GC = 7.2 mg/l GNC = <1.0 mg/l RC, RNC, and BEN= <1.0 mg/l
The total volume (mls.) of NaOH required to maintain pH at 7.3 was as follows:

GC:18.35	GNC:2.7	RC:5.95	RNC:4.65	BEN:4.3
GCC:24.0	GNCC:16.15	RCC:6.65	RNCC:5.75	BENC:4.0

Appendix I: Results of Batch Inhibition Experiments
10 mg/l Phenol Spike (5-13-83)

Time	GC	GNC	RC	RNC	BEN	GCC	GNCC	RCC	RNCC	BENC
NH_3										
0.0	28.0	27.0	25.0	25.1	24.0	28.0	27.0	25.0	25.1	24.0
1.0	22.1	20.1	28.0	22.5	21.0	20.1	15.8	29.1	23.0	23.1
3.0	26.1	21.1	23.9	20.1	21.5	12.4	9.4	26.0	22.2	23.0
6.0	27.1	8.7	*	*	*	4.0	2.8	*	*	*
9.0	11.6	1.0	5.3	8.3	7.3	1.0	1.0	5.4	8.6	8.6
NO_3										
0.0	27.0	28.0	60.0	93.0	92.0	27.0	28.0	60.0	93.0	97.0
1.0	21.5	20.1	110.0	105.0	96.0	25.5	26.0	122.0	115.0	102.0
3.0	25.9	25.2	110.0	98.0	91.0	37.0	39.0	64.0	96.0	92.0
6.0	35.0	44.0	119.0	115.0	90.0	51.0	48.0	118.0	67.0	90.0
9.0	50.0	59.9	135.0	117.0	106.0	64.0	60.0	70.0	105.0	100.0

Note: the initial reactor effluent ammonia concentrations were measured to be as follows prior to ammonia spiking;
GC = 28 mg/1 GNC = 27 mg/1 RC, RNC, and BEN= <1.0 mg/1
The total volume (mls.) of NaOH required to maintain pH at 7.3 was as follows:

GC:4.25	GNC:8.6	RC:9.85	RNC:4.6	BEN:4.5
GCC:10.55	GNCC:9.95	RCC:9.4	RNCC:5.35	BENC:4.35

Appendix I: Results of Batch Inhibition Experiments
10 mg/l Acrylonitrile spike (5-5-83)

Time	GC	GNC	RC	RNC	BEN	GCC	GNCC	RCC	RNCC	BENC
NH_3										
0.0	32.8	31.6	24.5	24.0	22.3	32.8	31.6	24.5	24.0	22.3
1.0	33.0	27.5	21.5	21.2	18.5	28.5	27.0	20.1	22.2	19.5
3.0	17.1	13.3	10.5	17.8	15.5	21.5	18.8	11.5	20.6	17.9
5.0	15.5	10.8	9.8	20.0	17.2	16.5	13.2	8.5	19.0	16.5
9.0	2.7	1.2	2.6	11.1	10.9	2.1	1.6	1.4	10.0	11.2
NO_3										
0.0	27.0	28.0	100.0	103.0	99.0	27.0	28.0	100.0	103.0	99.0
1.0	26.0	28.5	102.0	108.0	98.0	27.9	28.2	115.0	115.0	97.0
3.0	42.0	37.0	113.0	110.0	105.0	42.0	41.0	130.0	121.0	100.0
5.0	56.0	50.0	132.0	138.0	113.0	56.0	47.9	133.0	138.0	115.0

Note: the initial reactor effluent ammonia concentrations were measured to be as follows prior to ammonia spiking;
GC = 7.8 mg/l GNC = 6.6 mg/l RC, RNC, and BEN= <1.0 mg/l
The total volume (mls.) of NaOH required to maintain pH at 7.3 was as follows:

GC:16.85	GNC:17.0	RC:6.35	RNC:6.4	BEN:5.75
GCC:16.9	GNCC:16.55	RCC:6.65	RNCC:6.65	BENC:5.25

Appendix I: Results of Batch Inhibition Experiments
3.0 mg/1 Cyanide Spike (4-28-83)

Time	GC	GNC	RC	RNC	BEN	GCC	GNCC	RCC	RNCC	BENC
NH_3										
0.0	27.0	26.2	27.8	27.8	22.0	27.0	26.2	27.8	27.8	22.0
1.0	31.0	25.0	26.0	25.0	21.5	26.0	18.5	17.8	18.5	18.0
3.0	28.1	23.0	21.5	22.5	20.5	17.0	11.2	12.5	15.5	14.2
5.0	26.1	18.1	21.3	22.0	18.0	12.2	6.6	9.1	12.0	10.5
9.0	23.0	14.2	16.0	15.0	15.5	1.6	1.0	2.8	5.6	5.3
NO_3										
0.0	28.0	26.5	94.0	94.0	92.0	28.0	26.5	94.0	94.0	92.0
1.0	31.0	29.0	96.0	100.0	121.0	34.0	33.5	128.0	115.0	88.0
3.0	33.5	35.0	100.0	138.0	121.0	42.0	41.5	135.0	122.0	92.0
5.0	39.0	40.0	105.0	141.2	125.0	58.0	54.0	140.0	138.0	120.0

Note: the initial reactor effluent ammonia concentrations (mg/1-N) was as follows:
GC: 6.4 GNC: 4.7 RC:<1.0 RNC:<1.0 BEN:<1.0
The total volume (mls.) of NaOH required to maintain pH at 7.3 was as follows:
GC:0.0 GNC:1.60 RC:0.0 RNC:0.5 BEN:1.3
GCC:12.9 GNCC:11.8 RCC:5.6 RNCC:6.20 BENC:6.8

Appendix I: Results of Batch Inhibition Experiments
1.0 mg/l Cyanide Spike (3-25-83)

Time	GC	GNC	RC	RNC	BEN	GCC	GNCC	RCC	RNCC	BENC
NH_3										
0.0	22.4	19.2	25.0	28.0	35.0	22.4	19.2	25.0	28.0	35.0
1.5	21.3	19.2	24.3	27.8	37.2	24.3	19.7	21.3	24.4	36.2
4.0	24.6	25.6	29.1	32.6	41.7	23.6	15.1	13.5	21.6	34.6
7.0	30.0	26.5	29.0	32.0	45.0	21.1	12.0	8.3	23.0	33.5
10.3	28.0	24.0	21.0	25.0	41.0	17.0	7.8	3.5	20.5	28.0
NO_3										
0.0	23.6	28.0	83.6	110.0	58.0	23.6	28.0	83.6	110.0	58.0
1.5	19.2	23.6	98.0	104.0	62.0	35.0	34.2	101.0	108.0	76.0
4.0	22.0	21.8	80.0	90.0	42.0	29.6	40.0	112.0	102.0	81.0
7.0	31.0	27.0	112.0	116.0	58.0	48.0	46.0	122.0	122.0	96.0
10.3	31.0	29.0	111.0	110.0	94.0	50.0	45.8	112.0	114.0	102.0

Note: the initial reactor effluent ammonia concentrations were measured to be as follows prior to ammonia spiking;
GC = 22.1 mg/l GNC = 19.2 mg/l RC= 1.0 mg/l RNC= 15.4 mg/l
BEN = 35.0 mg/l
The total volume (mls.) of NaOH required to maintain pH at 7.3 was as follows:

GC:3.05	GNC:4.5	RC:4.95	RNC:4.4	BEN:1.3
GCC:10.5	GNCC:10.0	RCC:11.1	RNCC:9.45	BENC:11.0

Appendix I: Results of Batch Inhibition Experiments
10 mg/l Aniline Spike (2-23-83)

Time	GC	GNC	RC	RNC	BEN	GCC	GNCC	RCC	RNCC	BENC
NH_3										
0.0	19.8	22.5	22.5	22.5	21.0	19.8	22.5	22.5	22.5	21.0
1.5	24.0	25.0	23.0	21.2	22.0	23.4	24.5	15.2	19.0	17.8
3.0	22.0	22.0	21.5	22.1	20.5	22.5	21.1	12.9	14.2	12.3
6.0	18.2	22.1	14.5	18.0	20.2	22.0	24.2	6.3	9.6	8.4
8.0	15.1	27.0	13.7	17.9	20.2	23.0	26.5	5.1	9.2	8.8
10.5	13.0	27.0	8.3	13.0	15.0	21.0	24.5	1.85	2.7	5.2
0.0	13.8	0.7	73.6	96.8	52.8	13.7	0.7	73.6	96.8	52.8
3.0	24.8	7.4	113.9	107.1	114.8	27.0	18.5	116.9	93.6	74.8

Note: Reactor GC and GNC were not nitrifying
prior to the experiment.
Nitrate analyzed by Cadmium Reduction
Method. pH not adjusted in this experiment

APPENDIX II: Results of Chronic Inhibition Experiments
Simultaneous Pulse-Step Feed of 30 mg/l Aniline (6/23-6/26-83)

Ammonia-N vs. Time (Hours)

Time	RC	RNC	BEN
0.0	94.0	100.0	105.0
2.0	90.0	99.0	97.0
4.0	87.0	102.0	103.0
6.0	89.0	98.0	101.0
8.0	88.0	84.0	103.0
11.0	90.0	49.0	96.0
14.0	98.0	30.0	99.0
17.0	102.0	8.3	72.0
20.5	96.0	1.2	35.2
24.0	88.0	1.0	33.5
28.5	92.0	1.0	1.0
32.0	99.0	2.9	3.1
36.0	102.0	1.9	2.6
44.5	102.0	1.0	1.0
52.5	103.0	1.3	1.6
60.5	102.0	1.0	1.0
71.0	102.0	1.0	1.0
75.0	101.0	*	*
78.0	97.0	*	*
80.0	84.0	*	*
82.0	72.0	*	*
84.0	58.0	*	*
86.0	44.2	*	*
91.5	14.8	*	*
93.5	7.0	*	*
95.5	2.0	*	*
100.0	1.0	*	*
123.0	1.0	1.0	1.0
125.0	1.0	1.0	1.0
127.0	1.0	1.0	1.0

Appendix II: Results of Chronic Inhibition Experiments
Simultaneous Pulse-Step Feed of 30 mg/l Aniline

Nitrate Data (mg/l-N vs. Time)

Time	GC	GNC	RC
0.0	122.0	128.0	121.0
2.0	116.0	125.0	117.0
4.0	116.0	122.0	115.0
8.0	94.5	94.0	108.0
14.0	81.0	102.0	83.0
20.5	69.0	104.0	96.5
28.5	53.8	104.0	110.0
44.5	48.0	108.0	132.0
28.5	53.8	104.0	110.0
44.5	48.0	108.0	132.0
52.5	33.0	100.0	138.0
60.5	31.5	102.0	135.0
71.0	29.9	102.0	136.0
76.0	29.5	*	*
78.0	28.5	*	*
80.0	32.0	*	*
82.0	36.2	*	*
84.0	40.0	*	*
86.0	44.2	*	*
91.5	56.0	*	*
93.5	60.0	*	*
95.5	63.0*	*	
100.0	74.0	*	*
123.0	105.0	100.0	115.0
125.0	108.0	101.0	116.0
127.0	108.0	98.0	117.0

Appendix II: Results of Chronic Inhibition Experiments
Simultaneous Pulse-Step Feed of from 30 to 60 mg/l Aniline

Ammonia Data (mg/l-N vs. time)

Time	GC	GNC	RC
0.0	118.0	108.0	108.0
1.0	121.0	94.0	105.0
3.0	128.0	108.0	103.0
6.0	132.0	122.0	118.0
9.0	128.0	125.0	122.0
11.0	123.0	125.0	120.0
13.0	128.0	130.0	108.0
16.0	112.0	105.0	81.0
19.0	78.0	43.9	70.0
22.0	38.5	31.8	14.2
25.0	5.1	5.4	1.4
30.0	1.4	1.0	1.0
33.0	4.4	2.1	2.3
36.0	3.1	2.0	1.8
46.0	1.5	1.0	1.3
50.0	1.0	1.0	1.0
54.0	3.6	4.8	2.5
60.0	1.3	1.1	1.4
73.0	1.1	1.0	1.0
83.0	3.6	1.2	3.3
99.0	1.0	1.8	1.4

Appendix II: Results of Chronic Inhibition Experiments Simultaneous Pulse-Step Feed of 30 to 60 mg/l Aniline

Nitrate Data (mg/l-N vs. time)

Time	GC	GNC	RC
0.0	139.0	138.0	130.0
1.0	131.0	131.0	127.0
3.0	122.0	124.0	120.0
6.0	117.0	117.0	113.0
11.0	100.0	94.0	89.0
13.0	96.0	92.0	87.0
16.0	91.0	86.0	78.0
22.0	84.0	79.9	71.0
33.0	72.0	61.0	56.5
36.0	67.0	59.9	57.5
46.0	63.0	50.0	54.5
50.0	66.5	53.9	60.0
54.0	54.0	48.5	37.5
58.0	55.0	52.0	40.0
60.0	55.5	40.0	56.0
73.0	52.0	36.0	60.0
83.0	47.0	35.5	80.0
99.0	52.0	36.0	84.0
107.0	52.0	37.0	96.0
119.0	52.0	34.0	100.0
130.0	61.0	38.0	128.0
146.0	68.0	39.5	125.0
153.0	77.0	45.0	128.0
169.0	90.0	53.0	132.0
191.0	113.0	74.0	133.0
201.0	128.0	92.0	142.0
216.0	129.0	108.0	138.0
223.0	130.0	130.0	142.0
240.0	132.0	132.0	138.0
264.0	138.0	132.0	138.0

Appendix II: Results of Chronic Inhibition Experiments Simultaneous Pulse-Step Feed of 30 to 60 mg/l Aniline

Nitrite Data (mg/l-N vs. time)

Time	GC	GNC	RC
55.0	88.0	125.0	107.0
58.0	92.0	120.0	94.0
60.0	90.0	94.0	89.6
73.0	95.0	121.0	80.0
83.0	*	96.9	34.0
99.0	78.8	82.1	31.2
107.0	75.5	79.0	33.0
119.0	70.0	76.0	14.0
130.0	62.5	80.0	6.0
146.0	53.0	77.0	4.4
153.0	45.3	83.7	2.0
170.0	25.8	*	2.0
191.0	14.0	68.0	2.0
201.0	8.4	56.0	2.0
216.0	4.6	38.0	3.6
223.0	2.0	14.0	2.0
240.0	5.0	8.4	2.0
264.0	2.0	3.4	2.0

Mixed Liquor Soluble TOC (mg/l vs. time)

Time	GC	GNC	RC
-1.0	50.3	57.3	58.9
1.0	52.3	58.4	62.7
3.0	79.4	89.1	*
6.0	80.9	89.1	85.3
11.0	71.3	74.5	75.6
16.0	63.2	70.2	68.3
22.0	65.4	69.7	66.9
36.0	63.2	68.1	67.5
50.0	57.3	63.8	63.8

APPENDIX III: Results of Carbon Dose Inhibition Experiments
Experiment with 10 mg/l Aniline Spike (2-17-85)

Time	Control NA	4000 A	2000 A	1000 A	500 A	Control A
NH_3						
0.0	54.5	54.5	54.5	54.5	54.5	54.5
0.5	43.5	42.5	*	*	*	*
1.0	32.5	32.0	31.5	36.0	44.5	50.0
3.0	5.4	7.5	9.6	11.2	28.5	41.0
5.0	1.3	1.4	1.6	1.4	18.0	36.2
8.0	1.3	1.4	1.6	1.4	5.0	29.0
NO_3						
0.0	78.0	78.0	78.0	78.0	78.0	78.0
0.5	86.0	84.0	*	*	*	*
1.0	90.0	90.0	90.0	89.0	86.0	82.0
3.0	114.0	112.0	112.0	111.0	96.0	84.0
5.0	125.0	123.0	123.0	125.0	108.0	90.0
8.0	135.0	132.0	135.0	132.0	128.0	95.0
NO_2						
0.0	3.7	3.7	3.7	3.7	3.7	3.7
0.5	0.0	3.1	2.9	1.3	0.2	0.2
3.0	6.0	0.9	3.0	1.5	0.3	0.2
8.0	0.08	0.1	0.1	0.1	0.1	0.2

APPENDIX III: Results of Liquid Phase Aniline Concentrations With Time in 10 mg/l Aniline Exp. (2-15-85)

Aniline (mg/l vs. time)

Time	4000 A	2000 A	1000 A	500 A	Control A
0.0	10.0	10.0	10.0	10.0	10.0
0.5	n/d	n/d	2.5	6.0	10.1
8.0	n/d	n/d	2.2	5.1	8.6

Note: Gas Chromatograph detection limit = 0.3 mg/l Aniline.
Initial Concentration was calculated.

APPENDIX III: Results of Carbon Dose Inhibition Experiments
Experiment with 10 mg/l Aniline Spike (1-15-85)

Time	Control NA	4000 A	2000 A	1000 A	500 A	Control A
NH_3						
0.0	38.0	38.0	38.0	38.0	38.0	38.0
1.0	22.2	21.5	22.5	26.5	32.0	34.0
3.0	1.0	3.2	1.2	5.8	26.5	35.0
5.0	1.0	1.5	1.0	1.0	20.0	35.0
8.0	1.0	1.0	1.0	1.0	8.0	34.5
NO_3						
0.0	46.0	46.0	46.0	46.0	46.0	46.0
1.0	55.0	55.0	54.5	53.0	49.0	46.5
3.0	72.0	71.0	72.0	69.8	56.0	47.8
5.0	84.0	81.0	83.0	84.0	66.0	51.0
8.0	90.0	88.0	89.0	90.0	82.0	54.2

Control NA = Control, no PAC, no inhibitor
4000 A = 4000 mg/l PAC, inhibitor added
2000 A = 2000 mg/l PAC, inhibitor added
1000 A = 1000 mg/l PAC, inhibitor added
500 A = 500 mg/l PAC, inhibitor added
Control A = Control, no PAC, inhibitor added

APPENDIX III: Results of Carbon Dose Inhibition Experiments
Experiment with 0.7 mg/l Cyanide Spike (1-17-85)

Time	4000 A	500 A	Control A	Control NA
NH_3				
0.0	38.0	38.0	38.0	38.0
1.0	34.0	36.0	34.0	22.2
3.0	21.0	27.5	28.0	1.0
5.0	6.8	21.5	22.5	1.0
8.0	1.0	11.5	17.0	1.0
NO_3				
0.0	46.0	46.0	46.0	46.0
1.0	48.5	48.0	48.5	55.0
3.0	59.5	53.0	51.0	72.0
5.0	74.0	61.0	56.5	84.0
8.0	88.0	74.0	66.0	90.0

APPENDIX III: Results of Carbon Dose Inhibition Experiments
Experiment with 20 mg/1 Phenol Spike (1-19-84)

Time	Control NA	4000 A	2000 A	1000 A	500 A	Control A
NH_3						
0.0	47.0	47.0	47.0	47.0	47.0	47.0
2.0	16.5	16.5	20.5	26.2	43.5	57.0
4.0	1.1	1.0	1.1	17.9	43.0	53.0
6.0	1.2	1.0	1.0	11.9	45.0	46.0
8.0	1.0	1.0	1.0	6.1	39.5	46.5
NO_3						
0.0	48.0	48.0	48.0	48.0	48.0	48.0
2.0	66.5	65.0	64.5	60.0	53.0	52.5
4.0	80.0	77.0	79.0	69.8	55.0	51.5
5.0	85.0	80.0	84.0	72.0	55.0	52.5
8.0	90.0	85.0	89.0	81.0	56.0	54.0

APPENDIX III: Results of Carbon Dose Inhibition Experiments
Experiment with 1.4 mg/l Cyanide Spike (1-22-85)

Time	Control NA	4000 NA	4000 A	500 A	Control A
NH_3					
0.0	40.0	40.0	40.0	40.0	40.0
0.5	36.0	35.0	*	*	*
1.0	30.5	30.0	41.0	37.0	40.5
1.5	24.0	24.0	*	*	*
3.0	*	*	42.0	44.0	44.2
5.0	*	*	38.5	46.0	46.0
8.0	*	*	26.0	43.0	44.5
NO_3					
0.0	46.1	46.1	46.1	46.1	46.1
0.5	50.5	49.9	*	*	*
1.0	54.5	54.5	45.0	49.0	45.5
1.5	59.5	58.0	*	*	*
3.0	*	*	48.0	47.5	46.5
5.0	*	*	51.0	48.0	46.5
8.0	100.0	95.0	64.0	51.0	51.0

APPENDIX III: Results of Carbon Dose Inhibition Experiments
Experiment with 2500 mg/l Ethanol Spike (1-30-85)

Time	Control NA	4000 NA	4000 A	500 A	Control A
NH_3					
0.0	40.0	40.0	40.0	40.0	40.0
0.5	36.0	35.0	*	*	*
1.0	30.5	30.0	40.0	43.8	40.0
1.5	24.0	24.0	*	*	*
3.0	*	*	32.0	33.0	33.0
5.0	*	*	24.2	24.8	26.0
8.0	*	*	7.7	9.7	10.0
NO_3					
0.0	46.1	46.1	46.1	46.1	46.1
0.5	50.5	49.9	*	*	*
1.0	54.5	54.5	46.5	47.0	49.5
1.5	59.5	58.0	*	*	*
3.0	*	*	52.0	54.0	54.0
5.0	*	*	57.0	59.0	60.0
8.0	100.0	95.0	70.0	69.5	69.0

APPENDIX III: Results of Carbon Dose Inhibition Experiments
Intitial Experiment with 10 mg/l Aniline Spike (3-5-84)

Time	Control NA	Control A	4000 A	2000 A	1000 A	500 A
NH_3						
0.0	45.8	46.2	44.0	43.8	45.7	46.0
2.0	35.0	44.8	36.0	37.0	38.8	42.2
4.0	22.7	44.2	23.5	26.5	29.5	38.0
6.0	16.1	42.2	12.4	16.0	19.9	30.0
9.0	2.9	42.5	1.4	1.6	5.6	18.2
NO_3						
0.0	23.8	25.0	24.2	26.2	26.2	27.5
2.0	37.0	30.0	42.5	43.0	40.5	38.0
4.0	47.5	34.5	56.5	54.2	54.0	44.0
6.0	53.8	40.0	71.0	67.0	63.0	54.0
9.0	66.0	46.0	107.0	90.0	89.6	67.0

Initial Carbon Dose Experiment (10 mg/l Aniline)
Caustic Uptake (Mls. of 0.1 N NaOH) with Time

Time	Control NA	Control A	4000 A	2000 A	1000 A	500 A
1.0	2.6	0.0	2.1	1.6	1.1	0.0
2.0	5.1	0.0	4.0	2.9	2.0	0.2
3.0	5.6	0.0	5.6	4.5	3.5	1.2
4.0	6.6	0.0	7.2	5.9	4.8	2.0
5.0	7.5	0.0	8.9	7.3	5.9	2.9
6.0	8.3	0.0	10.4	8.6	7.0	3.7
7.0	9.1	0.0	11.8	9.8	8.0	5.5
8.0	10.3	0.2	13.5	11.4	9.4	6.7
9.0	11.4	0.5	14.0	12.4	10.7	7.8

Appendix IV: Results of Kjeldahl Nitrogen Trial Runs

Standard Compound	Calculated mg/l-N	Expected Concentration	Expected Q	Measured Q	Measured mg/l-N	% Difference
Asparagine	187.0	4.7	2.35	2.4	192.0	2.8
Alanine	157.0	3.9	1.95	1.9	152.0	3.2
Ammonia-N	200.0	5.0	2.5	2.5	200.0	0.0
Asparagine	187.0	4.7	2.35	2.4	192.0	2.8
Ammonia-N[x]	200.0	5.0	1.0	1.0	200.0	0.0
Alanine[x]	157.0	3.9	0.78	0.75	150.0	4.0
Asparagine[x]	187.0	4.7	0.94	0.95	190.0	1.6
Ammonia-N'	10.0	0.5	0.5	0.4	9.0	10.0
Asparagine[x]	187.0	4.7	0.94	0.95	190.0	1.6

* Expected Q based on using 1.0 ml of 200 mg/l ammonia-N as the added standard. mg/l-N = Q*2*0.2/0.005 Vol of digest = 5.0 ml.

' Expected Q based on using 1.0 ml of 100 mg/l ammonia-N as the added standard. Volume of digest= 10.0 ml.

x Expected Q based on using 5.0 ml vol digest. 1.0 ml of 500 mg/l ammonia-N was used as added standard.