THE EVALUATION OF NITRIFICATION INHIBITION SCREENING AS AN INDEX OF TOXICITY IN INDUSTRIAL WASTE STREAMS

By

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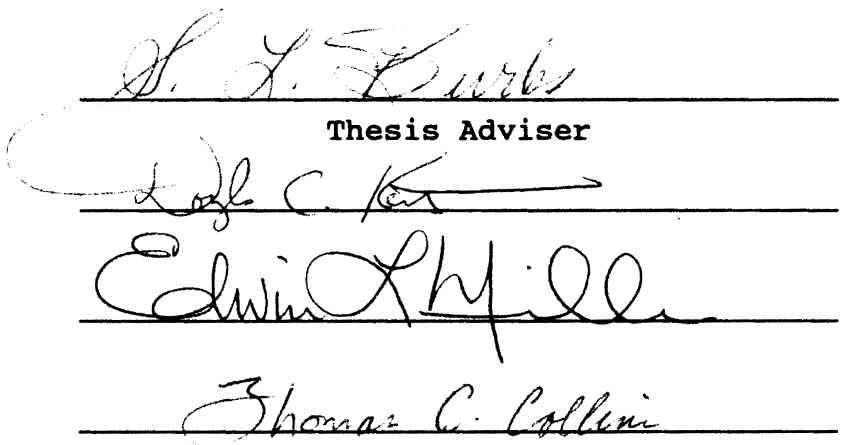

Thesis Adviser

Dean of the Graduate College

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Credits

I wish to thank Dr. S.L. Burks for his patience and understanding, which has helped guide me through the maze. I would also like to extend my appreciation to my friends and cohorts at Stover & Associates for their encouragement and support. I wish to extend my gratitude to Dr. Ed Miller and Dr. Doug Kent for serving on my graduate committee. Last, but definitely not least, I thank Carol, who saw me through this from start to finish. Without her words of encouragement and understanding, I would still be sitting on "go".

Author

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INTRODUCTION

Nitrification Inhibition screening can be used as an expedient and economic indicator of acute toxicity in industrial wastewater. To determine if this statement is true, samples from eight different wastewater streams of a petroleum refinery were screened for nitrification inhibition by measuring the rate of ammonia depletion using enriched nitrifying bacteria added to each raw waste sample. The same 8 samples were also screened for 24-hour acute toxicity by measuring the percent survival of three species of freshwater organisms added to aliquots of the waste stream samples. A comparison of results from both types of toxicity tests is presented in the following paper.

Background

The process wastewater treatment plant at the petroleum refining facility consisted of API gravity oil/water separators, a storm water (oil safety) basin, dissolved air flotation (DAF), a rock trickling filter, an Orbal activated sludge system, a final flocculator clarifier, and an aerobic sludge digester. The treatment plant operated as a two stage biological process, with the first stage trickling-roughing filter removing the easily removable toxic and inhibitory organic constituents and serving as a buffer zone

for the second stage Orbal activated sludge process. Although the two stage system normally performed well, the treatment plant did periodically experience inhibition to nitrification resulting in high levels of ammonia in the discharged effluent. Samples of the eight raw waste streams screened in this study were taken during a period of nitrification inhibition at the refinery's wastewater treatment facility.

LITERATURE REVIEW

Nitrification

Nitrification is the "biological conversion of organic and inorganic nitrogenous compounds from a reduced state to a more oxidized state" as defined by Wetzel (1983). Ammonia-nitrogen, in the reduced state, is oxidized to nitrite-nitrogen, which is further oxidized to nitrate-nitrogen ($NH_3 + O_2 \leftrightarrow NO_2 + O_2 \leftrightarrow NO_3$).

Although there are several known fungi and bacteria, including methane-oxidizing bacteria, capable of nitrification (ammonia depletion), the chemosynthetic bacteria *Nitrosomonas* and *Nitrobacter* are most prevalent. *Nitrosomonas* converts ammonia to nitrite, while *Nitrobacter* completes the oxidation by converting nitrite to nitrate. The energy required for the oxidation steps of nitrification is obtained from the actual chemical breakdown, whereas denitrifying and nitrogen fixing bacteria require energy sources from outside the chemical reaction (Odum,1983). *Nitrosomonas* and *Nitrobacter* are also classified as autotrophic because of the inorganic carbon sources (carbon dioxide or bicarbonate) they require for cell growth, whereas heterotrophs require relatively complex, reduced forms of organic carbon such as glucose (Benefield, 1980).

Importance of Nitrification in Biological Wastewater Treatment Systems

Nitrogen in some form is required by all living organisms for cell synthesis of proteins and nucleic acids. However, only a fraction of the total ammonia-nitrogen in industrial and municipal waste streams is removed by bacterial synthesis. The majority of ammonia-nitrogen is removed by nitrification in the biological wastewater treatment process (Stover, 1979).

One of the most widely used biological treatment processes for wastewater is the activated sludge system. According to Eckenfelder (1979), "Activated sludge treatment is a biological treatment process whereby soluble organic compounds are converted into carbon dioxide, water, and bacterial cells. The main function of the activated sludge process is removal of degradable organics and production of an effluent which is low in both degradable organics and suspended solids." The activated sludge system consists of two parts, the aeration basin and a secondary clarifier. The aeration basin contains the microbial seed culture or mixed liquor which is continuously contacted with organic waste by mixing and aeration. The organic compounds are physically adsorbed by the microorganisms of the mixed liquor. It is in the aeration basin that nitrification takes place through biological oxidation. Suspended solids are settled and concentrated in the secondary clarifier,

with some being returned to the aeration basin as sludge recycle. The treated effluent is then discharged.

Causes of Nitrification Inhibition

Autotrophic nitrifying bacteria are generally more sensitive to changes in environmental conditions than most heterotrophic bacteria responsible for carbonaceous oxidation. Nitrification inhibition can be divided into two categories; inhibition of cell metabolism or inhibition of oxidative reactions. It is difficult to determine which type of inhibition is occurring in an activated sludge system. Some of the more common parameters affecting nitrification in a biological treatment process are listed below (Stover, 1979).

1. Food to microorganism (F/M) ratio
2. Sludge age
3. Temperature
4. Dissolved oxygen concentrations
5. pH
6. Inhibitory and/or toxic compounds

The food to microorganism ratio is an expression of organic loading to mixed liquor concentration, and is usually expressed as pounds of biochemical oxygen demand (BOD) per pound of mixed liquor volatile suspended solids

(MLVSS) per day. Organic loading is one of the more difficult factors to control in a wastewater treatment plant and can have a significant effect on the nitrification process. Low loading rates can cause poor sludge settling performance, while high organic loading can cause incomplete oxidation of organics, resulting in shock loads and toxic buildup in the system. Sometimes, under toxic conditions, complete oxidation can be achieved by extending the hydraulic (liquid) detention time in the biological reactor. This phenomenon of "complete substrate conversion" is called Reactor Resistance to Inhibition (RRI) (Lewandowski, 1985). However, operational control of detention time is often limited in treatment systems because of the lack of control over the hydraulic flow and the volume of the aeration basin.

Sludge age is the major factor in controlling the F/M ratio. By adjustment of the MLVSS concentration, the optimal operating levels for sludge settleablity and effluent quality can be obtained (Eckenfelder, 1979).

The nitrification process is very sensitive to elevated temperatures, with the ideal temperature for stable nitrification being 30°C (Neufeld, 1985). Although the nitrifying bacteria *Nitrosomonas* has a wider tolerance range for temperature (1 to 37°), *Nitrobacter* is less tolerant of low temperatures (Wetzel, 1983). Low temperatures can lead to a breakdown in the nitrite to nitrate step of complete

nitrification, resulting in increased nitrite concentrations in the aeration basin.

Dissolved oxygen concentration is a critical parameter to the nitrifying system due to the increased demand for oxygen during the ammonia oxidation process.

The pH of the mixed liquor in the aeration basin will decrease at a proportional rate to the increase in nitrification due to the formation of nitrous acid during the ammonia to nitrite oxidation step. The optimal pH range for nitrifying bacteria is 7.5 to 8.5 su. This target range can be maintained with the use of a pH controller feeding a solution of sodium bicarbonate to the biological reactor.

There are several compounds known to be toxic to nitrifying microorganisms. Phenol is toxic to nitrification at the 2 mg/L level (Neufeld, 1979) and free cyanide at values greater than 0.2 mg/L (Neufeld, 1985). Other compounds found to be inhibitory to nitrification, based on an IC_{50} value (defined as "the concentration of test chemical at which the respiration rate is 50% of a control respiration rate"), are orthocresol (0.068mg/L); TCMP (12.0 mg/L); pentachlorophenol (15.9 mg/L); parachlorometacresol (20.2 mg/L); 2-methylpyridine (20.4 mg/L); 4-methylpyridine (22.7 mg/L); 4,6-dinitro-o-cresol (24.6 mg/L); 2,4,6-trichlorophenol (39.0 mg/L); pyridine (70.6 mg/L); and 2-chloropyridine (88.1 mg/L) (Kiser, 1989). Also, certain heavy metals are toxic to nitrification, but can be tolerated at concentrations of 10 to 20 mg/L if the pH

remains high (7.5 to 8.5). Precipitated metals, such as hydroxides, can be very toxic if the precipitate dissolves because of low pH (Stover, 1979).

Ammonia Toxicity

Key parameters affecting acute ammonia toxicity include dissolved oxygen concentrations, temperature, pH, previous acclimation to ammonia, carbon dioxide concentrations, total dissolved solids (salinity), and the presence of other toxic compounds (EPA, 1984).

Ammonia is readily found in municipal and industrial wastewater streams in two forms, unionized ammonia (NH_3) and ionized ammonium (NH_4^+). Ammonia toxicity to aquatic life has been demonstrated to be the result of the unionized form (Ruffier, 1981). Municipal wastewater treatment plants, using conventional primary and secondary treatment, remove less than 30% of the total ammonia present. A biological treatment system employing a healthy population of well acclimated nitrifying microorganisms can remove over 90% of the incoming ammonia in a raw wastewater, thus demonstrating the importance of nitrification in reducing ammonia toxicity in aquatic solutions.

Materials and Methods

Enriched nitrification culture and reactor maintenance

A bench-scaled activated sludge system was used to provide nitrifying bacteria used in the inhibition screen test. Alleman (1987a) initially developed the enriched nitrification biomass system. The original nitrifying culture was obtained from the Deer Creek, Oklahoma Wastewater Treatment Plant. The activated sludge system was a 12.35 L aerobic reactor consisting of a 7.5 L aeration basin and a 4.85 L internal clarifier separated by a sliding baffle (Figure 1). The reactor was constructed of 1/4" clear Plexiglass.

The bio-reactor was maintained in a dark room to eliminate possible light induced inhibition (Alleman, 1986). The temperature of the room was kept at 30-35°C to eliminate the possibility of inhibition caused by changes in temperature and pH (Stover, 1979).

A pH controller (Cole-Parmer Model 5656-00) and combination pH electrode were used to maintain a constant pH of 8.0 in the aeration basin. A 5% solution of sodium bicarbonate was selected as the pH controlling buffer because it provided carbon in an inorganic form needed by

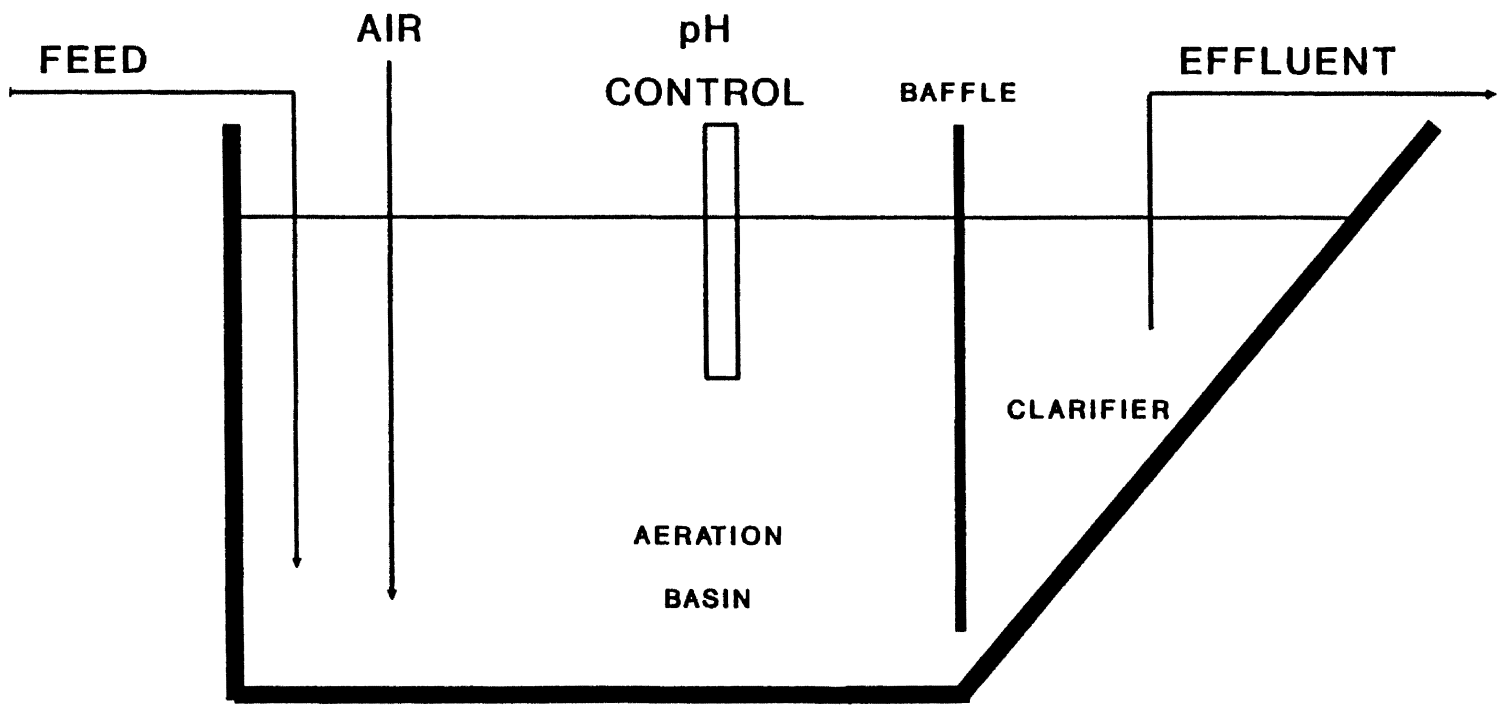

Figure 1. Enriched Nitrification Reactor

the microorganisms to sustain cell growth (Benefield, 1980). Because carbon in the organic form is a limiting nutrient for heterotrophs (Siew Lan, 1983), no organic carbon was fed to the reactor to assure a homogeneous culture of autotrophic nitrifying bacteria.

The mixed liquor was aerated and fed continuously for a period of one year prior to this study. The hydraulic retention time or residence time of the feed through the system was 1.5 days at a flow rate of 5.0 L/day. The biomass was fed an ammonium chloride (NH_4Cl) solution at a concentration of 400 mg/L nitrogen, representing a loading rate of 83.3 mg nitrogen/L/hr. Ammonium chloride was the only form of nitrogen supplied to the nitrification enrichment reactor. The feed was supplemented with BOD dilution water containing ferric chloride (0.25 mg/L), magnesium sulfate (22.5 mg/L), and monobasic potassium phosphate (11.4 mg/L as phosphorus) to provide sufficient quantities of nutrients to sustain microbial life (Standard Methods, 1985).

The inhibition test was performed on subsamples from the mixed liquor nitrification reactor. Aliquots of the subsample were used as a control or spiked with influent from the eight waste streams.

Microbial Activity Calibration (Pre-Test)

Mixed liquor from the enriched nitrification reactor was then analyzed for pH and volatile suspended solids

(MLVSS) just prior to the inhibition screening test, and unspiked and spiked dissolved oxygen uptake rates (DOUR) were measured to determine the health and state of reactivity of the nitrifying biomass. The spiked DOUR was obtained by adding 0.6 ml of a 2% NH_4Cl solution to a 60 ml BOD bottle filled with mixed liquor, approximating an NH_3-N concentration of 50 mg/L, which was the targeted concentration for the inhibition screening test. Several DO readings were then taken at measured time intervals using a YSI Model 57 DO Meter and Series 5720A Probe. The DO concentrations were plotted over time, and the resulting slope was used to determine the DOUR in mg/L/hr. Effluent from the internal clarifier was analyzed for NH_3-N to assure that nitrification (NH_3-N removal) was taking place through the system.

Instrumentation

The ion specific electrode (ISE) and meter has often been the recommended method for analysis of ammonia-nitrogen (NH_3-N) because of its expediency. However, because of potential interferences to the ISE method exhibited by the wastewater streams being screened, the distillation and titration method was selected for this study. The Tecator 1002 Distilling Unit was chosen over the conventional distillation apparatus because of its speed, approximately 5 minutes per sample. All chemical analyses were run in accordance with USEPA Method 350.2 (EPA, 1983).

Nitrification Inhibition

Screening Procedure

Each of the eight raw wastewater streams were first characterized by analyzing for pH, chemical oxygen demand (COD), NH_3-N, and total dissolved solids (TDS).

Four 250 ml erlenmeyer test flasks were prepared with 5, 15, 30, and 50% dilutions each of raw waste sample based on a 200 ml total test volume. One additional test flask with no waste sample was added as a control. Nutrient enriched BOD dilution water and inorganic carbon, in the form of 5% $NaHCO_3$, were added to each test flask. A 2% solution of NH_4Cl was used as needed to adjust each flask to the target NH_3-N concentration of approximately 50 mg/L, taking into account the initial NH_3-N concentration of the waste stream.

All initial pH values were adjusted to the target pH of 8.0 with a weak solution of sulfuric acid (H_2SO_4) or sodium hydroxide (NaOH) before adding mixed liquor from the enrichment reactor. Temperature and pH were monitored throughout each test, and ranged between 22 - 24°C and 8.0 - 8.5, respectively.

The addition of the nitrifying seed represented time zero in the test. Time zero for each flask was staggered in 5 minute intervals for convenience. The general setup is demonstrated in Table 1. The actual setup for each of the waste streams is contained in Appendix A.

TABLE 1

PROTOCOL FOR VOLUMES OF CONSTITUENTS USED IN NITRIFICATION INHIBITION SCREENING TEST

Sample Volume (ml)	Dilution Water (ml)	5% $NaHCO_3$ (ml)	2% NH_4Cl (ml)	Seed Culture (ml)	Total Volume (ml)
0	116.5	1.5	2.0	80	200
10	106.5	1.5	2.0	80	200
30	86.5	1.5	2.0	80	200
60	56.5	1.5	2.0	80	200
100	16.5	1.5	2.0	80	200

The 80 ml volume of mixed liquor or seed culture added to each 250 ml flask was determined as follows:

1. 4.57 mg/L DO is required to oxidize 1 mg/L NH_3-N to NO_3-N.
2. When spiked with 50 mg/L NH_3-N, a DOUR of 108 mg/L/hr was obtained from a mixed liquor sample containing 1,180 mg/L volatile suspended solids (VSS).
3. By dividing 108 mg/L DO by 4.57 mg/L, a theoretical NH_3-N depletion rate of 23.6 mg/L/hr was determined.
4. An arbitrary NH_3-N depletion rate of 10 mg/L/hr was chosen as the target rate for the inhibition tests. Based on the 50 mg/L NH_3-N concentration targeted for each test flask, a sufficient time increment

was developed for data collection, not to exceed 5 hours per waste stream.

5. Because the 23.6 mg/L/hr NH_3-N depletion provided by the 1,180 mg/L MLVSS was 2.36 times greater than the 10 mg/L/hr target, a calculated MLVSS value of 500 mg/L per 200 ml test volume was determined by reducing the original VSS concentration 2.36 times.
6. The target concentration of 500 mg/L MLVSS times the 200 ml total sample volume, divided by the initial 1,180 mg/L MLVSS equaled a calculated seed culture volume of approximately 80 ml.

NH_3-N analyses were run on each flask at time zero and at 30 minute to 1 hour intervals up to a total test time of 3 to 5 hours, depending on the rate of nitrification. The NH_3-N data for each test flask was plotted as concentration of NH_3-N versus time for each concentration of each waste stream. The statistical program in Lotus™ 1-2-3™, version 3.1, was then used to calculate the linear slope of each line. The linear phase of NH_3-N depletion over time represented the NH_3-N removal rate, which was calculated for each waste stream concentration and the control. If the NH_3-N depletion rates of the different dilutions of the refinery samples were approximately 100% of the control, then no inhibition of the nitrifying bacteria was exhibited.

24 Hour Acute Toxicity

Screening Procedure

Three different species of freshwater organisms were chosen for the acute toxicity screening test: Pimephales promelas (fathead minnow), a warm water vertebrate, and Daphnia pulex and Ceriodaphnia dubia, warm water invertebrates. These species have been widely used and are accepted organisms for toxicity testing. Each specie is distributed throughout the United States and is easily cultured in the laboratory (EPA, 1985).

A synthetic hard water was used for the control to best simulate the natural conditions of receiving streams. The reconstituted water had a pH of 7.6, an alkalinity of 116 mg/L, and a hardness of 176 mg/L. Each wastewater was aerated and pH adjusted if initial pH was outside the range of 6.0 - 9.0. A temperature controlled environment of 20°C was maintained throughout the test.

Five test organisms from each of the three species were placed in small vessels containing 100% effluent from each of the eight industrial streams and the control. The acute screening test was set up in duplicate, allowing a total of 10 test organisms per species for each effluent tested. The test vessels were checked and surviving organisms counted at 2, 16, and 24 hours. If the number of live organisms was 90% or greater at the end of 24 hours, the effluent was not considered to be acutely toxic. If survival was less than 90%, the effluent exhibited acute toxicity (EPA, 1985).

RESULTS AND DISCUSSION

Nitrification Seed Efficiency

Seed culture (mixed liquor) and effluent samples taken from the enriched nitrification reactor were characterized to determine the health and NH_3-N removal efficiency of the seed.

TABLE 2

INITIAL MIXED LIQUOR (ML) AND EFFLUENT (Eff) CHARACTERISTICS OF THE ENRICHED NITRIFICATION REACTOR

ML pH (su)	MLVSS (mg/L)	Unspiked DOUR (mg/L/hr)	Spiked DOUR (mg/L/hr)	Eff NH_3-N (mg/L)
8.0	1,180	4.5	108	1.1

The DO uptake rate increased substantially (from 4.5 to 108 mg/L/hr) when spiked with NH_4Cl, indicating a high level of activity (Table 2). The NH_3-N removal or nitrification efficiency was 99.7%, as determined by comparing the incoming feed concentration of 400 mg/L NH_3-N to the outgoing NH_3-N concentration of 1.1 mg/L in the effluent. The results in Table 2 indicated a healthy nitrifying seed

culture in the enriched reactor that would be suitable for the inhibition screening study.

Comparison of Nitrification Inhibition Versus Acute Toxicity

The NH_3-N nitrification rates of each wastewater stream were calculated by linear regression using NH_3-N depletion over time. The degree of inhibition represented by the ammonia nitrogen depletion data was then standardized by comparing the nitrification rates of each set of dilutions to the nitrification rate of its respective control, expressed as percent of the control nitrification rate (percent of nitrifying bacteria to the control).

The overall response of the nitrifying mixed liquor appeared linear with respect to percent volume of the wastewater streams, thus indicating the nitrifying bacteria were inhibited by increasing concentrations of contaminants (Figure 2). Because of this dose response, the nitrification inhibition results for the highest concentration (50% volume) were used for comparison to the conventional 24 hour acute toxicity tests, which were conducted at 100% of wastewater volume.

The percent inhibition of nitrification was 25% when the nitrifying bacteria were exposed to the wastewater from the Cooling Tower Blowdown. The same wastewater stream exhibited no toxicity in the conventional 24 hour acute toxicity tests (Figure 3).

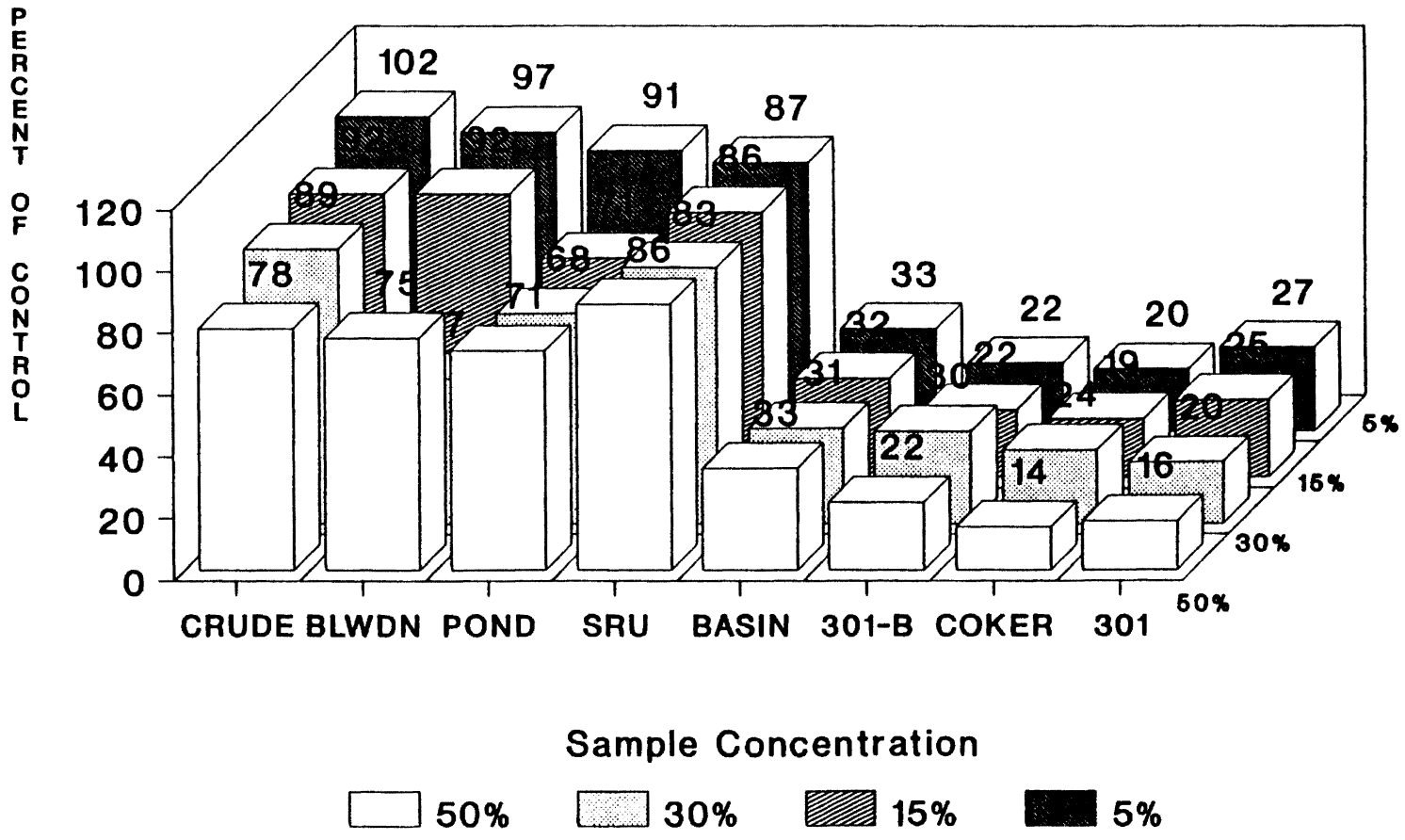

Figure 2. Results of Nitrification Inhibition Screens as Percent of Control Nitrification Rate

The SRU Water, Coker Cooling Water Pond, and Crude Tank Water Streams also caused relatively low levels of nitrification inhibition (Figures 4-6), but these streams caused significant mortality in the conventional acute toxicity tests.

The Safety Basin, D 301-B, Coker Water, and D 301 Streams all caused greater than 67% inhibition of nitrification and also significant mortality in the conventional acute toxicity tests (Figures 7-10).

Using the Linear Interpolation Method (EPA, 1989), the IT_{15} and LT_{15} for each wastewater stream were calculated for further comparison (Figure 11). The IT_{15} represents the time at which 15% nitrification inhibition occurred. The LT_{15} represents the time at which 15% mortality occurred in the acute toxicity tests. The 15% was chosen over the more common 50% or 25% to increase the sensitivity of the method.

The IT_{15} for the Cooling Tower Blowdown Stream was greater than five hours and the LT_{15} for the conventional acute toxicity tests were greater than 24 hours, with all exceeding the time limits of their respective tests.

The SRU Water, Coker Cooling Water Pond, and Crude Tank Water Streams had an IT_{15} of 3 hours or greater and a LT_{15} for the *D. pulex* and *C. dubia* acute toxicity tests of less than one hour. The LT_{15} of the *P. promelas* acute toxicity tests for the first two streams more closely resembled the IT_{15}, with 4.3 hours and 2.9 hours respectively, while the Crude Tank Water Stream had a LT_{15} of less than one hour.

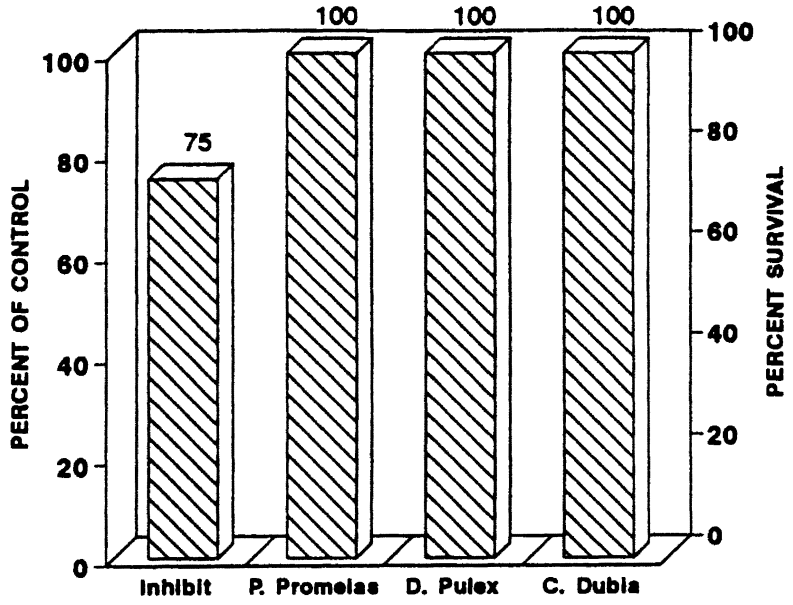

Figure 3. Cooling Tower Blowdown. Nitrification Inhibition at 50% Vol vs 24hr Acute Toxicity.

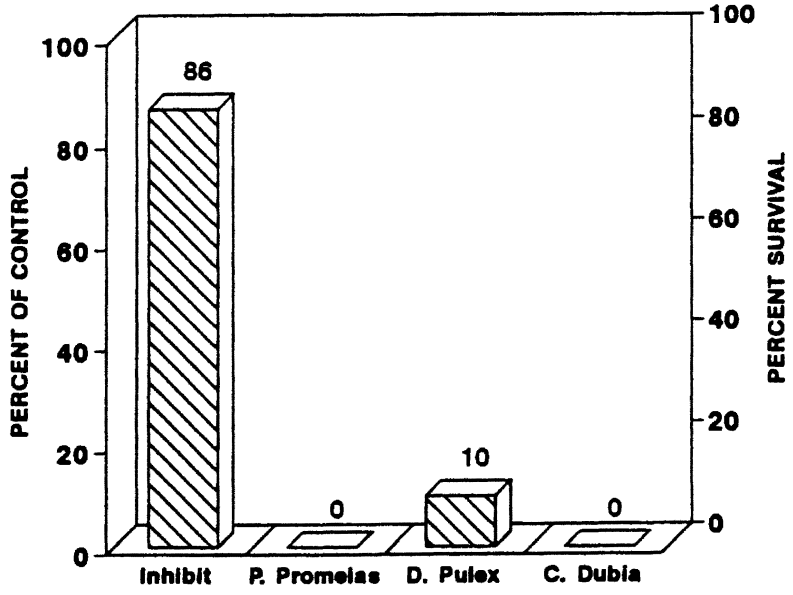

Figure 4. SRU Water. Nitrification Inhibition at 50% Vol vs 24hr Acute Toxicity.

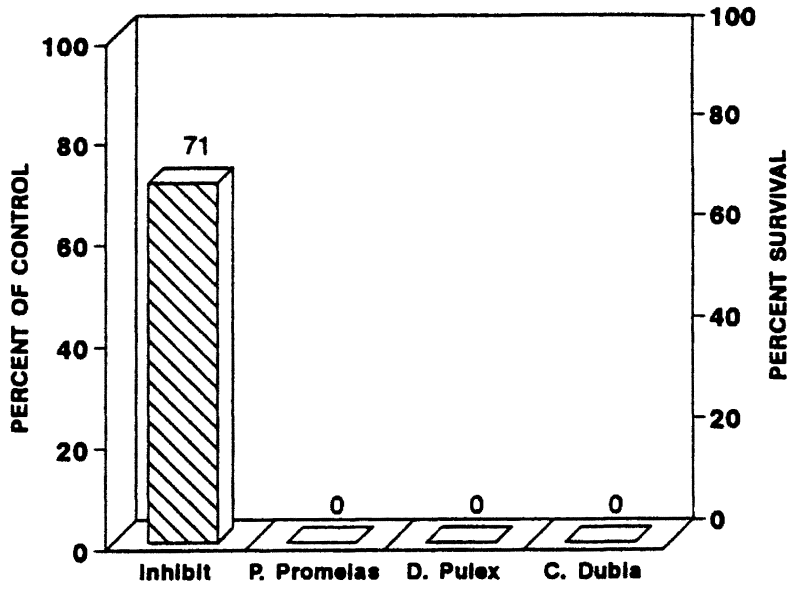

Figure 5. Coker Cooling Water Pond. Nitrification Inhibition at 50% Vol ve 24hr Acute Toxicity.

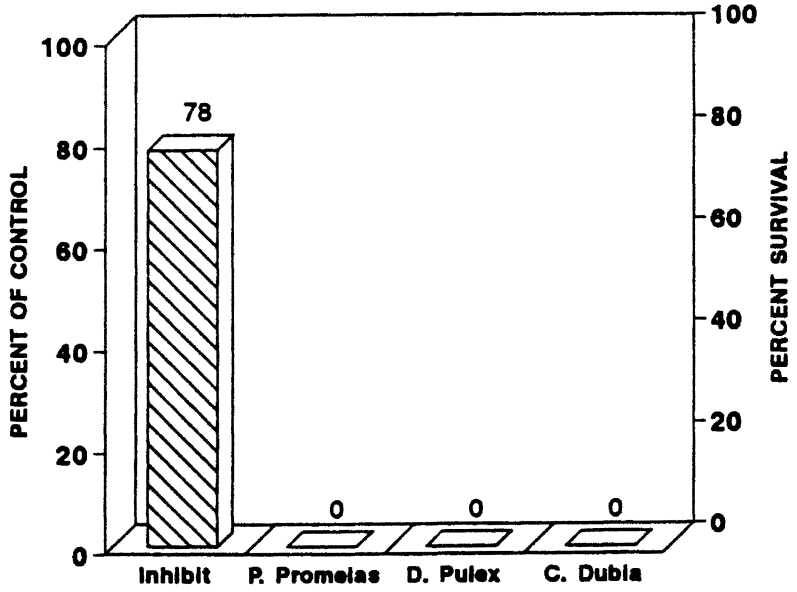

Figure 6. Crude Tank Water Stream. Nitrification Inhibition at 50% Vol ve 24hr Acute Toxicity.

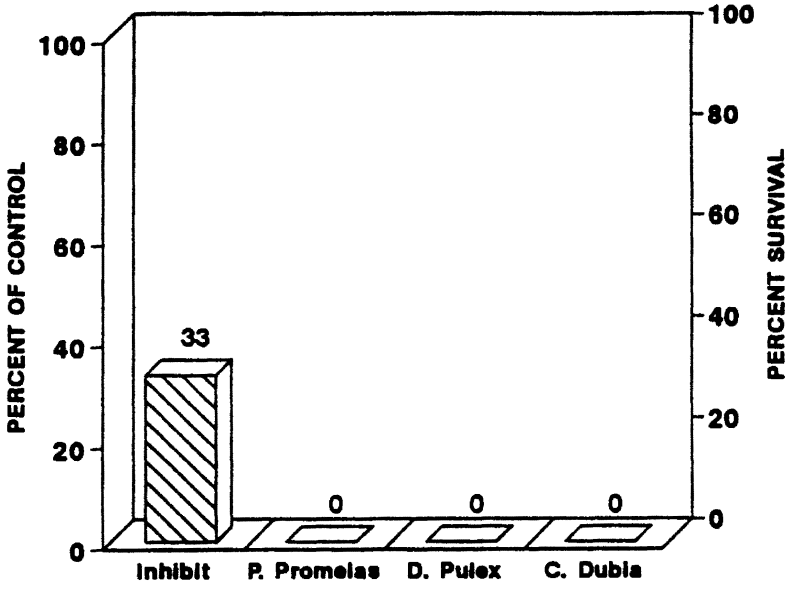

Figure 7. Safety Basin. Nitrification Inhibition at 50% Vol vs 24hr Acute Toxicity.

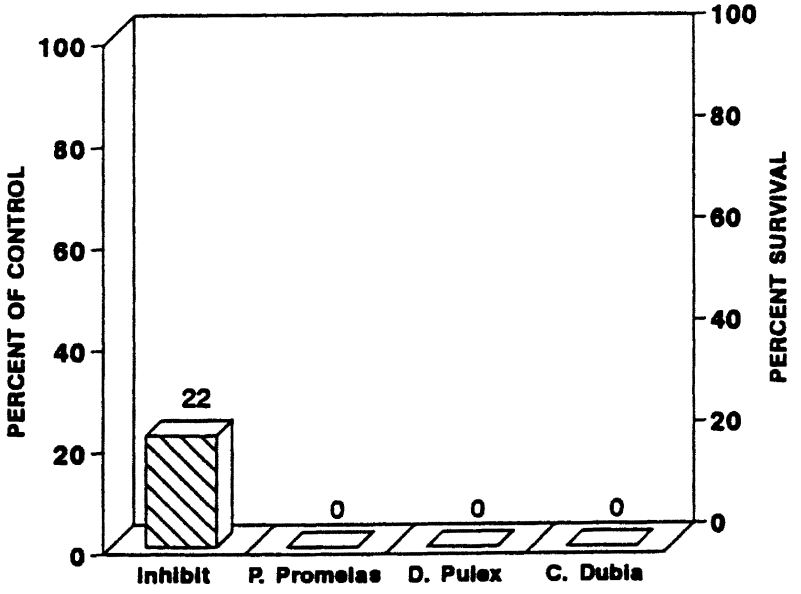

Figure 8. D 301-B Stream. Nitrification Inhibition at 50% Vol vs 24hr Acute Toxicity.

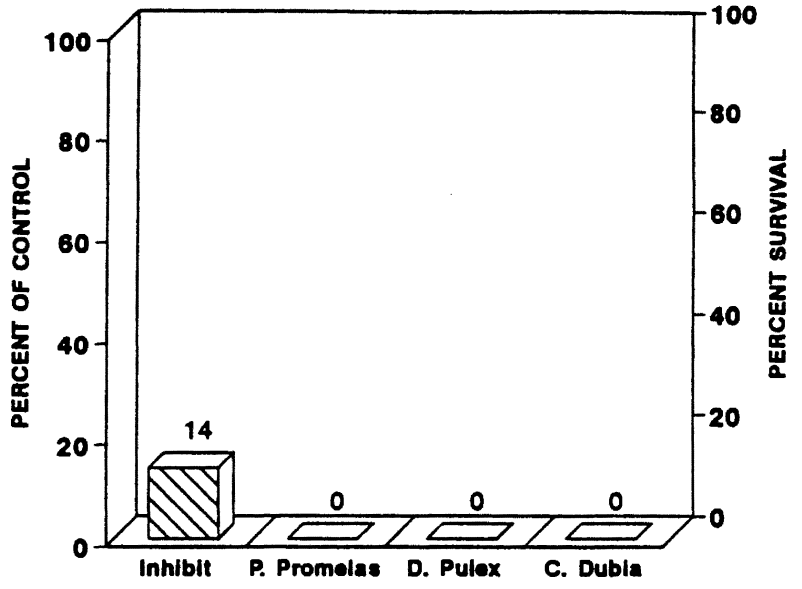

Figure 9. Coker Water. Nitrification Inhibition at 50% Vol vs 24hr Acute Toxicity.

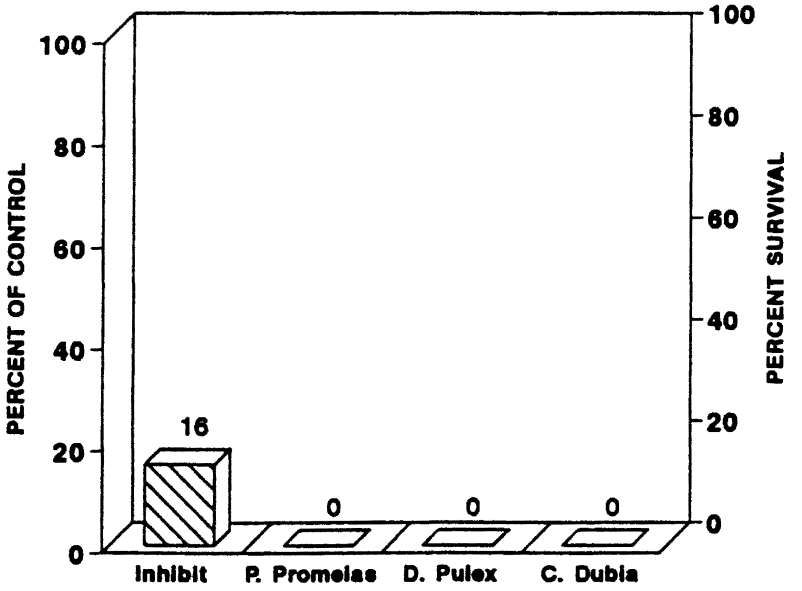

Figure 10. D 301 Stream. Nitrification Inhibition at 50% Vol vs 24hr Acute Toxicity.

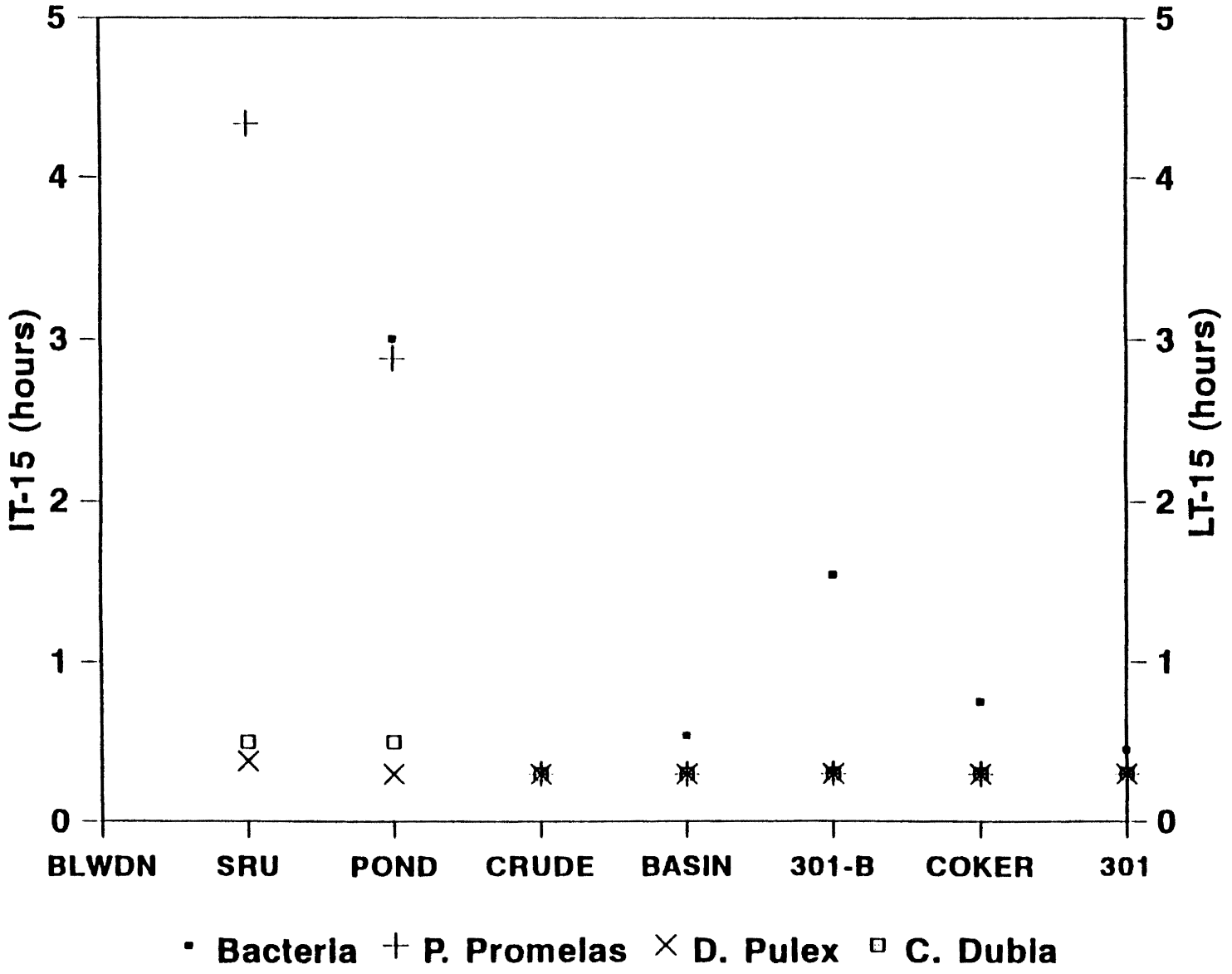

Figure 11. IT-15 vs LT-15

The Safety Basin, D 301-B, Coker Water, and D 301 Streams all had an IT_{15} and LT_{15} of less than 1 hour, with only one exception. The D 301-B Stream had an IT_{15} of 1.5 hours.

These results indicate that a significant inhibition of nitrification (i.e. greater than 50%) might be indicative of acutely toxic effects upon other aquatic organisms. However, low levels of nitrification inhibition were not consistent in predicting toxicity to other aquatic organisms.

Comparison of Inhibition and Toxicity Responses to Known Wastewater Characteristics

Each of the raw wastewater streams was characterized to determine the initial pH, COD, NH_3-N, and TDS prior to the nitrification inhibition and 24 hour acute toxicity tests (Table 3).

The pH of each test aliquot used in the nitrification inhibition and acute toxicity screens was adjusted to 8 s.u. just prior to time zero of the tests and was not a factor in poor performance.

The Crude Tank Water Stream had the highest TDS concentration (17.4 g/L) and 22% nitrification inhibition at 50% volume (Figure 2). The freshwater organisms used in the acute toxicity test had no mortality in TDS concentrations of 3.5 g/L in the Cooling Tower Blowdown Stream, while

showing significant mortality in streams of lesser concentration (Figures 12-14).

TABLE 3

Initial Wastewater Characteristics of the Refinery Streams

Stream Description	pH (su)	COD (mg/L)	NH_3-N (mg/L)	TDS (mg/L)
Crude Tank Water	7.5	13,400	96.0	17,400
Cooling Tower Blowdown	6.8	175	1.4	3,460
Coker Cooling Water Pond	7.3	300	3.0	3,410
SRU Water	9.3	300	2.1	1,440
Safety Basin	8.0	4,500	47.3	2,460
D 301-B	9.7	7,200	42.8	2,190
Coker Water	9.1	6,750	24.4	2,160
D 301	8.9	8,000	24.6	1,600

In the Cooling Tower Blowdown, Coker Cooling Water Pond, and the SRU Water Streams with COD less than 300 mg/L (test samples were diluted 50%), the inhibition of nitrification was less than 29%. As the COD increased in the remaining streams, with the exception of the Crude Tank Water Stream, the percent of nitrification inhibition increased from 67% to 84%. In the acute toxicity tests, the three streams with COD less than 300 mg/L had organisms surviving after two hours, while those streams with COD greater than 4,500-mg/L had 100% mortality at two hours.

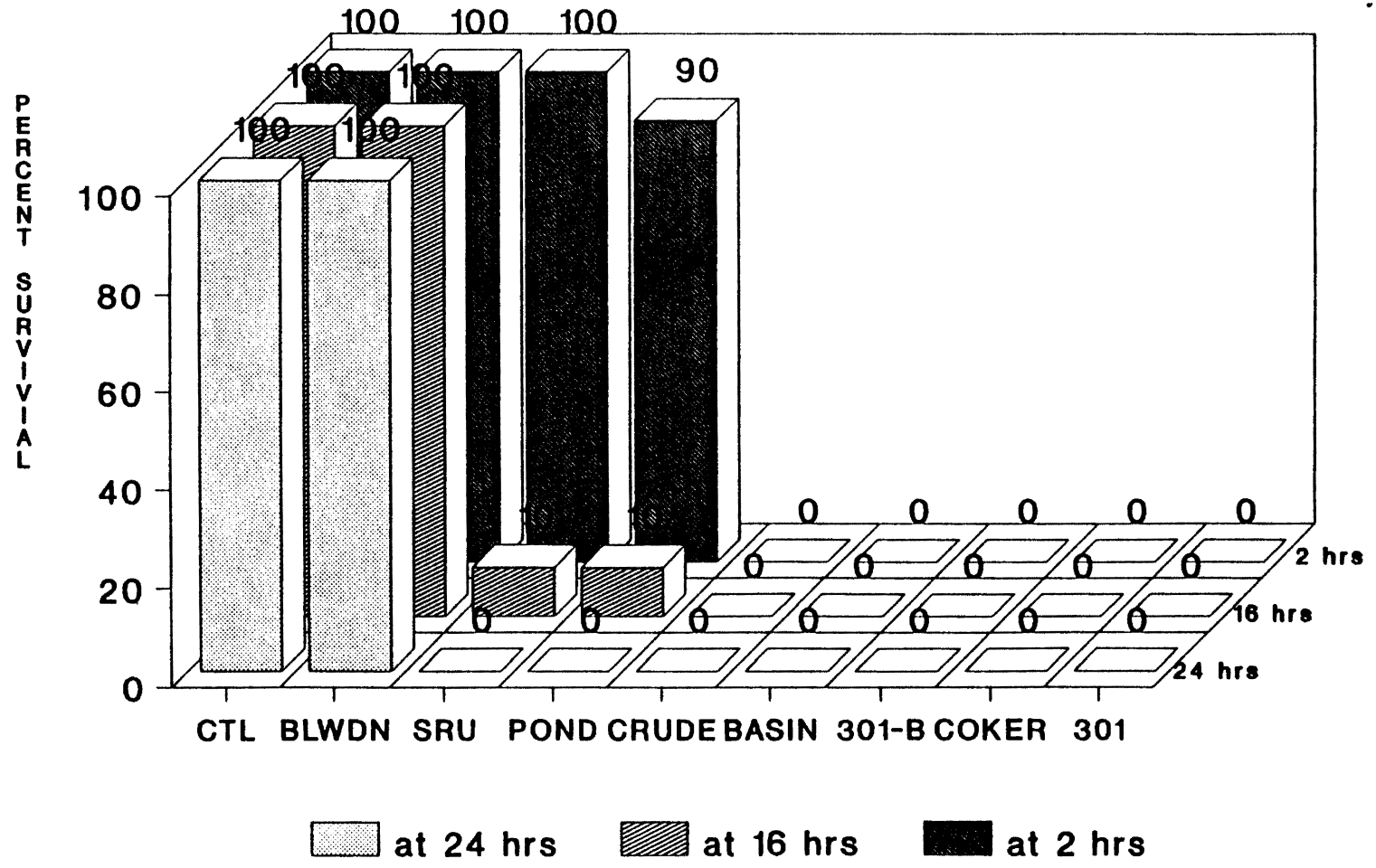

Figure 12. P. Promelas Acute Toxicity Screen. Percent Survival Over 24 hrs.

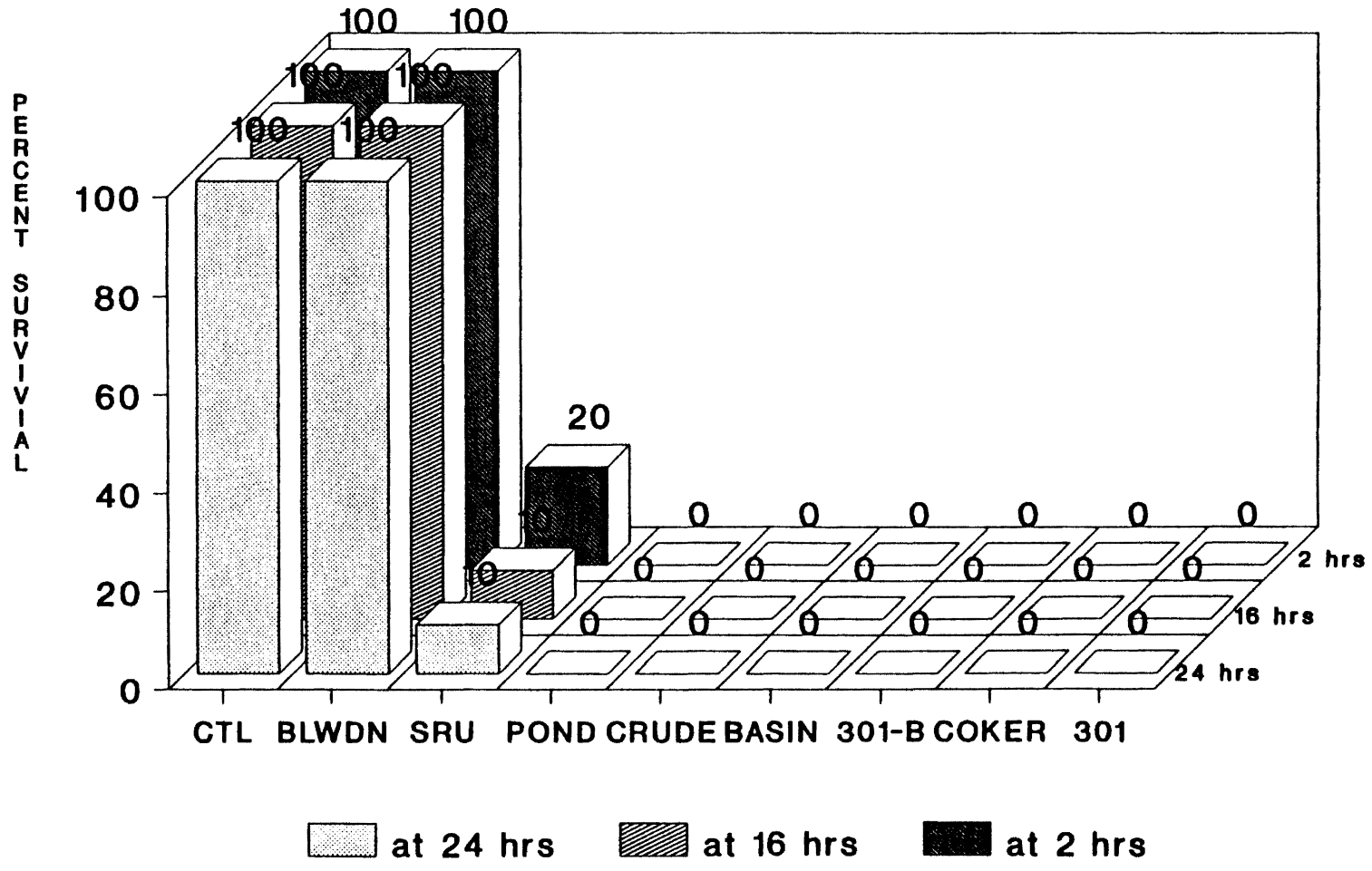

Figure 13. D. Pulex Acute Toxicity Screen. Percent Survival Over 24 hrs.

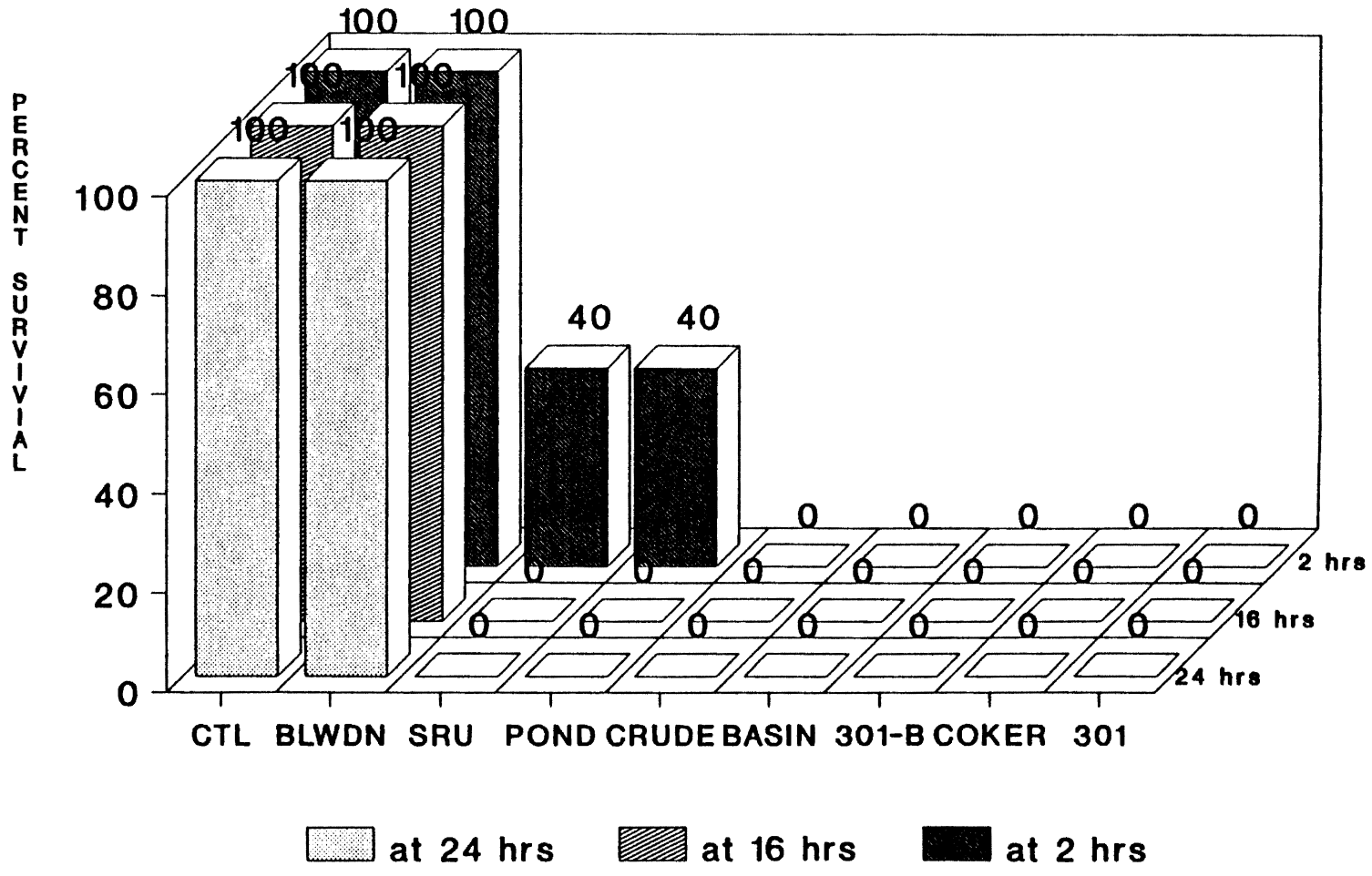

Figure 14. C. Dubia Acute Toxicity Screen. Percent Survival Over 24 hrs.

Ammonia nitrogen concentrations were not a factor in the nitrification inhibition screens because all test samples were adjusted to the target NH_3-N concentration of 50 mg/L prior to testing. The responses to NH_3-N in the conventional acute toxicity tests were identical to those of COD, with some survival after two hours in waste streams containing less than 3.0 mg/L and no survival in streams with more than 24.4 mg/L NH_3-N.

The effect of high TDS concentrations on nitrifying bacteria appeared minimal. Also, the response of the freshwater organisms would indicate that TDS was not a limiting factor in the acute toxicity tests. Ammonia nitrogen concentrations in the five streams which exhibited 100% mortality at 2 hours in the acute toxicity tests had NH_3-N levels high enough to be considered acutely toxic (EPA, 1984). The high COD Concentrations in several of the waste streams could have also contributed to the inhibitory and toxic responses seen in these tests.

CONCLUSIONS

Bacterial bioassays, commonly referred to as "Microtox", have been successful in recent years as an expedient and cost effective alternative to freshwater fish and invertebrate bioassays in the identification of acute toxicity in industrial waste streams going to a municipal wastewater treatment facility (Alleman, 1987b). Nitrification inhibition, a functional response of a microbial nitrifying community, indicated by the disappearance of the substrate ammonia-nitrogen, may also be used as a bacterial bioassay for this purpose.

As shown in figures 3-10 of this study, nitrification inhibition greater than 50% might be an indicator of acute toxicity to freshwater organisms. Low levels of nitrification inhibition were not consistent in predicting toxicity to multi-cellular organisms. However, the nitrification inhibition test would certainly provide valuable data on such parameters as pH control, aeration demands, temperature, etc., for designing an effluent wastewater treatment system.

The results should also be useful in performing waste minimization studies and/or toxicity reduction evaluations, since most industrial and municipal wastewater treatment facilities have the capability of performing nitrification

inhibitation screening tests on site, eliminating the requirement to send samples out for the more expensive and time consuming conventional acute toxicity test.

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APPENDICES

APPENDIX A

RAW DATA FROM NITRIFICATION INHIBITION SCREENING TESTS

TABLE A-1

VOLUMES OF CONSTITUENTS USED IN NITRIFICATION INHIBITION SCREENING TEST FOR CRUDE TANK WATER STREAM

Sample Volume (ml)	Dilution Water (ml)	5% $NaHCO_3$ (ml)	2% NH_4Cl (ml)	Seed Culture (ml)	Total Volume (ml)
0	116.5	1.5	2.0	80	200
10	106.8	1.5	1.7	80	200
30	87.1	1.5	1.4	80	200
60	57.7	1.5	0.8	80	200
100	18.4	1.5	0.1	80	200

TABLE A-2

AMMONIA NITROGEN VALUES AT VARIOUS TIMES DURING NITRIFICATION INHIBITION SCREENING TEST FOR CRUDE TANK WATER STREAM

Time (hr)	Control (mg/L)	5% Vol (mg/L)	15% Vol (mg/L)	30% Vol (mg/L)	50% Vol (mg/L)
0	50.4	47.6	50.4	49.0	49.0
1	39.2	36.4	39.2	37.8	40.6
2	33.6	32.2	35.0	35.0	36.4
3	30.8	29.4	30.8	32.2	33.6

TABLE A-3

VOLUMES OF CONSTITUENTS USED IN NITRIFICATION INHIBITION SCREENING TEST FOR COOLING TOWER BLOWDOWN STREAM

Sample Volume (ml)	Dilution Water (ml)	5% $NaHCO_3$ (ml)	2% NH_4Cl (ml)	Seed Culture (ml)	Total Volume (ml)
0	116.5	1.5	2.0	80	200
10	106.5	1.5	2.0	80	200
30	86.5	1.5	2.0	80	200
60	56.5	1.5	2.0	80	200
100	16.5	1.5	2.0	80	200

TABLE A-4

AMMONIA NITROGEN VALUES AT VARIOUS TIMES DURING NITRIFICATION INHIBITION SCREENING TEST FOR COOLING TOWER BLOWDOWN STREAM

Time (hr)	Control (mg/L)	5% Vol (mg/L)	15% Vol (mg/L)	30% Vol (mg/L)	50% Vol (mg/L)
0	53.2	53.2	53.2	53.2	51.8
0.5	47.6	46.2	49.0	58.8	47.6
1	42.0	42.0	46.2	58.8	46.2
2	37.8	37.8	39.2	49.0	42.0
3	32.2	33.6	35.0	46.2	37.8
4	29.4	29.4	30.8	42.0	33.6
5	28.0	28.0	26.6	37.8	30.8

TABLE A-5

VOLUMES OF CONSTITUENTS USED IN NITRIFICATION INHIBITION SCREENING TEST FOR COKER COOLING WATER POND STREAM

Sample Volume (ml)	Dilution Water (ml)	5% $NaHCO_3$ (ml)	2% NH_4Cl (ml)	Seed Culture (ml)	Total Volume (ml)
0	116.5	1.5	2.0	80	200
10	106.5	1.5	2.0	80	200
30	86.5	1.5	2.0	80	200
60	56.5	1.5	2.0	80	200
100	16.5	1.5	2.0	80	200

TABLE A-6

AMMONIA NITROGEN VALUES AT VARIOUS TIMES DURING NITRIFICATION INHIBITION SCREENING TEST FOR COKER COOLING WATER POND STREAM

Time (hr)	Control (mg/L)	5% Vol (mg/L)	15% Vol (mg/L)	30% Vol (mg/L)	50% Vol (mg/L)
0	50.4	53.2	50.4	51.8	50.4
1	39.2	42.0	43.2	44.8	43.4
2	35.0	37.8	39.2	40.6	39.2
3	32.2	36.3	37.8	39.2	37.8

TABLE A-7

VOLUMES OF CONSTITUENTS USED IN NITRIFICATION INHIBITION SCREENING TEST FOR SRU WATER STREAM

Sample Volume (ml)	Dilution Water (ml)	5% $NaHCO_3$ (ml)	2% NH_4Cl (ml)	Seed Culture (ml)	Total Volume (ml)
0	116.5	1.5	2.0	80	200
10	106.5	1.5	2.0	80	200
30	86.5	1.5	2.0	80	200
60	56.5	1.5	2.0	80	200
100	16.5	1.5	2.0	80	200

TABLE A-8

AMMONIA NITROGEN VALUES AT VARIOUS TIMES DURING NITRIFICATION INHIBITION SCREENING TEST FOR SRU WATER STREAM

Time (hr)	Control (mg/L)	5% Vol (mg/L)	15% Vol (mg/L)	30% Vol (mg/L)	50% Vol (mg/L)
0	56.0	51.8	51.8	50.4	51.8
0.5	42.0	42.0	42.0	42.0	42.0
1	33.6	35.0	36.4	36.4	36.4
2	29.4	30.8	30.8	30.8	30.8
3	28.0	28.0	28.0	29.4	29.4

TABLE A-9

VOLUMES OF CONSTITUENTS USED IN NITRIFICATION INHIBITION SCREENING TEST FOR SAFETY BASIN STREAM

Sample Volume (ml)	Dilution Water (ml)	5% $NaHCO_3$ (ml)	2% NH_4Cl (ml)	Seed Culture (ml)	Total Volume (ml)
0	116.5	1.5	2.0	80	200
10	106.7	1.5	1.8	80	200
30	87.0	1.5	1.6	80	200
60	57.0	1.5	1.4	80	200
100	17.5	1.5	1.0	80	200

TABLE A-10

AMMONIA NITROGEN VALUES AT VARIOUS TIMES DURING NITRIFICATION INHIBITION SCREENING TEST FOR SAFETY BASIN STREAM

Time (hr)	Control (mg/L)	5% Vol (mg/L)	15% Vol (mg/L)	30% Vol (mg/L)	50% Vol (mg/L)
0	49.0	49.0	49.0	49.0	50.4
0.5	40.6	46.2	46.2	49.0	49.0
1	35.0	44.8	44.8	47.6	46.2
2	30.8	42.0	43.4	43.4	43.4
3	28.0	39.2	39.2	40.6	40.6

TABLE A-11

VOLUMES OF CONSTITUENTS USED IN NITRIFICATION INHIBITION SCREENING TEST FOR D 301-B STREAM

Sample Volume (ml)	Dilution Water (ml)	5% $NaHCO_3$ (ml)	2% NH_4Cl (ml)	Seed Culture (ml)	Total Volume (ml)
0	116.5	1.5	2.0	80	200
10	106.7	1.5	1.8	80	200
30	87.0	1.5	1.6	80	200
60	57.0	1.5	1.4	80	200
100	17.5	1.5	1.0	80	200

TABLE A-12

AMMONIA NITROGEN VALUES AT VARIOUS TIMES DURING NITRIFICATION INHIBITION SCREENING TEST FOR D 301-B STREAM

Time (hr)	Control (mg/L)	5% Vol (mg/L)	15% Vol (mg/L)	30% Vol (mg/L)	50% Vol (mg/L)
0	50.4	46.2	46.2	47.6	46.2
1	39.2	43.4	43.4	44.8	43.4
2	35.0	43.4	43.4	43.4	43.4
3	30.8	42.0	42.0	42.0	42.0

TABLE A-13

VOLUMES OF CONSTITUENTS USED IN NITRIFICATION INHIBITION SCREENING TEST FOR COKER WATER STREAM

Sample Volume (ml)	Dilution Water (ml)	5% $NaHCO_3$ (ml)	2% NH_4Cl (ml)	Seed Culture (ml)	Total Volume (ml)
0	116.5	1.5	2.0	80	200
10	106.5	1.5	2.0	80	200
30	86.7	1.5	1.8	80	200
60	56.8	1.5	1.7	80	200
100	17.0	1.5	1.5	80	200

TABLE A-14

AMMONIA NITROGEN VALUES AT VARIOUS TIMES DURING NITRIFICATION INHIBITION SCREENING TEST FOR COKER WATER STREAM

Time (hr)	Control (mg/L)	5% Vol (mg/L)	15% Vol (mg/L)	30% Vol (mg/L)	50% Vol (mg/L)
0	50.4	51.8	53.2	53.2	51.8
1	39.2	49.0	50.4	50.4	49.0
2	33.6	47.6	49.0	49.0	49.0
3	29.4	46.2	47.6	46.2	47.6

TABLE A-15

VOLUMES OF CONSTITUENTS USED IN NITRIFICATION INHIBITION SCREENING TEST FOR D 301 STREAM

Sample Volume (ml)	Dilution Water (ml)	5% $NaHCO_3$ (ml)	2% NH_4Cl (ml)	Seed Culture (ml)	Total Volume (ml)
0	116.5	1.5	2.0	80	200
10	106.5	1.5	2.0	80	200
30	86.7	1.5	1.8	80	200
60	56.8	1.5	1.7	80	200
100	17.0	1.5	1.5	80	200

TABLE A-16

AMMONIA NITROGEN VALUES AT VARIOUS TIMES DURING NITRIFICATION INHIBITION SCREENING TEST D 301 STREAM

Time (hr)	Control (mg/L)	5% Vol (mg/L)	15% Vol (mg/L)	30% Vol (mg/L)	50% Vol (mg/L)
0	51.8	50.4	50.4	50.4	49.0
1	37.8	47.6	49.0	49.0	49.0
2	32.2	46.2	46.2	46.2	46.2
3	28.0	42.0	43.2	44.8	44.8

TABLE A–17

STATISTICAL DATA FOR LINEAR PHASE OF
CRUDE TANK WATER STREAM

STATISTICS FOR CONTROL Regression Output:	
Constant	49.46667
Std Err of Y Est	2.28619
R Squared	0.964286
No. of Observations	3
Degrees of Freedom	1
X Coefficient(s)	−8.4
Std Err of Coef.	1.816581
X INTERCEPT AT Y=20:	3.507937
NITRIFICATION RATE (mg/L/hr):	5.701357

STATISTICS FOR 5% VOL Regression Output:		STATISTICS FOR 15% VOL Regression Output:	
Constant	46.43333	Constant	49.23333
Std Err of Y Est	2.857738	Std Err of Y Est	2.857738
R Squared	0.935567	R Squared	0.935567
No. of Observations	3	No. of Observations	3
Degrees of Freedom	1	Degrees of Freedom	1
X Coefficient(s)	−7.7	X Coefficient(s)	−7.7
Std Err of Coef.	2.020726	Std Err of Coef.	2.020726
X INTERCEPT AT Y=20:	3.4329	X INTERCEPT AT Y=20:	3.796537
NITRIFICATION RATE (mg/L/hr):	5.825977	NITRIFICATION RATE (mg/L/hr):	5.267959
% OF CONTROL	102.1858	% OF CONTROL	92.39833

STATISTICS FOR 30% VOL Regression Output:		STATISTICS FOR 50% VOL Regression Output:	
Constant	47.6	Constant	48.3
Std Err of Y Est	3.429286	Std Err of Y Est	1.714643
R Squared	0.892857	R Squared	0.964286
No. of Observations	3	No. of Observations	3
Degrees of Freedom	1	Degrees of Freedom	1
X Coefficient(s)	−7	X Coefficient(s)	−6.3
Std Err of Coef.	2.424871	Std Err of Coef.	1.212436
X INTERCEPT AT Y=20:	3.942857	X INTERCEPT AT Y=20:	4.492063
NITRIFICATION RATE (mg/L/hr):	5.072464	NITRIFICATION RATE (mg/L/hr):	4.452297
% OF CONTROL	88.9694	% OF CONTROL	78.09187

TABLE A-18

STATISTICAL DATA FOR LINEAR PHASE OF
COOLING TOWER BLOWDOWN STREAM

STATISTICS FOR CONTROL Regression Output:	
Constant	50.42456
Std Err of Y Est	2.289948
R Squared	0.949241
No. of Observations	6
Degrees of Freedom	4
X Coefficient(s)	-5.74737
Std Err of Coef.	0.664521
X INTERCEPT AT Y=20:	5.293651
NITRIFICATION RATE (mg/L/hr):	3.778111

STATISTICS FOR 5% VOL Regression Output:	
Constant	49.90877
Std Err of Y Est	2.289948
R Squared	0.943921
No. of Observations	6
Degrees of Freedom	4
X Coefficient(s)	-5.45263
Std Err of Coef.	0.664521
X INTERCEPT AT Y=20:	5.485199
NITRIFICATION RATE (mg/L/hr):	3.646176
% OF CONTROL	96.5079

STATISTICS FOR 15% VOL Regression Output:	
Constant	51.98175
Std Err of Y Est	1.177181
R Squared	0.98518
No. of Observations	6
Degrees of Freedom	4
X Coefficient(s)	-5.57053
Std Err of Coef.	0.341607
X INTERCEPT AT Y=20:	5.741245
NITRIFICATION RATE (mg/L/hr):	3.483565
% OF CONTROL	92.20389

STATISTICS FOR 30% VOL Regression Output:	
Constant	54.22162
Std Err of Y Est	1.217885
R Squared	0.968985
No. of Observations	5
Degrees of Freedom	3
X Coefficient(s)	-3.06486
Std Err of Coef.	0.316574
X INTERCEPT AT Y=20:	11.16578
NITRIFICATION RATE (mg/L/hr):	1.791188
% OF CONTROL	47.40957

STATISTICS FOR 50% VOL Regression Output:	
Constant	50.74877
Std Err of Y Est	0.734751
R Squared	0.990406
No. of Observations	6
Degrees of Freedom	4
X Coefficient(s)	-4.33263
Std Err of Coef.	0.213218
X INTERCEPT AT Y=20:	7.09702
NITRIFICATION RATE (mg/L/hr):	2.818084
% OF CONTROL	74.58977

TABLE A–19

STATISTICAL DATA FOR LINEAR PHASE OF
COKER COOLING POND STREAM

STATISTICS FOR CONTROL Regression Output:	
Constant	49.23333
Std Err of Y Est	2.857738
R Squared	0.935567
No. of Observations	3
Degrees of Freedom	1
X Coefficient(s)	−7.7
Std Err of Coef.	2.020726
X INTERCEPT AT Y=20:	3.796537
NITRIFICATION RATE (mg/L/hr):	5.267959

STATISTICS FOR 5% VOL Regression Output:		STATISTICS FOR 15% VOL Regression Output:	
Constant	52.03333	Constant	49.86667
Std Err of Y Est	2.857738	Std Err of Y Est	1.306395
R Squared	0.935567	R Squared	0.97351
No. of Observations	3	No. of Observations	3
Degrees of Freedom	1	Degrees of Freedom	1
X Coefficient(s)	−7.7	X Coefficient(s)	−5.6
Std Err of Coef.	2.020726	Std Err of Coef.	0.92376
X INTERCEPT AT Y=20:	4.160173	X INTERCEPT AT Y=20:	5.333333
NITRIFICATION RATE (mg/L/hr):	4.807492	NITRIFICATION RATE (mg/L/hr):	3.75
% OF CONTROL	91.25911	% OF CONTROL	71.18506

STATISTICS FOR 30% VOL Regression Output:		STATISTICS FOR 50% VOL Regression Output:	
Constant	51.33333	Constant	49.93333
Std Err of Y Est	1.143095	Std Err of Y Est	1.143095
R Squared	0.979592	R Squared	0.979592
No. of Observations	3	No. of Observations	3
Degrees of Freedom	1	Degrees of Freedom	1
X Coefficient(s)	−5.6	X Coefficient(s)	−5.6
Std Err of Coef.	0.80829	Std Err of Coef.	0.80829
X INTERCEPT AT Y=20:	5.595238	X INTERCEPT AT Y=20:	5.345238
NITRIFICATION RATE (mg/L/hr):	3.574468	NITRIFICATION RATE (mg/L/hr):	3.741648
% OF CONTROL	67.853	% OF CONTROL	71.02652

TABLE A–20

STATISTICAL DATA FOR LINEAR PHASE
OF SRU WATER STREAM

STATISTICS FOR CONTROL Regression Output:	
Constant	51.24
Std Err of Y Est	5.830266
R Squared	0.835418
No. of Observations	4
Degrees of Freedom	2
X Coefficient(s)	−12.56
Std Err of Coef.	3.941979
X INTERCEPT AT Y=20:	2.487261
NITRIFICATION RATE (mg/L/hr):	8.040973

STATISTICS FOR 5% VOL Regression Output:		STATISTICS FOR 15% VOL Regression Output:	
Constant	48.72	Constant	49
Std Err of Y Est	3.909987	Std Err of Y Est	3.304542
R Squared	0.87907	R Squared	0.909223
No. of Observations	4	No. of Observations	4
Degrees of Freedom	2	Degrees of Freedom	2
X Coefficient(s)	−10.08	X Coefficient(s)	−10
Std Err of Coef.	2.643634	Std Err of Coef.	2.234278
X INTERCEPT AT Y=20:	2.849206	X INTERCEPT AT Y=20:	2.9
NITRIFICATION RATE (mg/L/hr):	7.019499	NITRIFICATION RATE (mg/L/hr):	6.896552
% OF CONTROL	87.29663	% OF CONTROL	85.76763

STATISTICS FOR 30% VOL Regression Output:		STATISTICS FOR 50% VOL Regression Output:	
Constant	48.16	Constant	49
Std Err of Y Est	2.718823	Std Err of Y Est	3.304542
R Squared	0.929506	R Squared	0.909223
No. of Observations	4	No. of Observations	4
Degrees of Freedom	2	Degrees of Freedom	2
X Coefficient(s)	−9.44	X Coefficient(s)	−10
Std Err of Coef.	1.83826	Std Err of Coef.	2.234278
X INTERCEPT AT Y=20:	2.983051	X INTERCEPT AT Y=20:	2.9
NITRIFICATION RATE (mg/L/hr):	6.704545	NITRIFICATION RATE (mg/L/hr):	6.896552
% OF CONTROL	83.37978	% OF CONTROL	85.76763

TABLE A-21

STATISTICAL DATA FOR LINEAR PHASE
OF SAFETY BASIN STREAM

STATISTICS FOR CONTROL Regression Output:	
Constant	46.48
Std Err of Y Est	3.112555
R Squared	0.895665
No. of Observations	4
Degrees of Freedom	2
X Coefficient(s)	-8.72
Std Err of Coef.	2.104471
X INTERCEPT AT Y=20:	3.036697
NITRIFICATION RATE (mg/L/hr):	6.586103

STATISTICS FOR 5% VOL Regression Output:		STATISTICS FOR 15% VOL Regression Output:	
Constant	48.28793	Constant	48.34828
Std Err of Y Est	0.576454	Std Err of Y Est	0.862234
R Squared	0.982581	R Squared	0.95754
No. of Observations	5	No. of Observations	5
Degrees of Freedom	3	Degrees of Freedom	3
X Coefficient(s)	-3.11379	X Coefficient(s)	-2.94483
Std Err of Coef.	0.239359	Std Err of Coef.	0.358023
X INTERCEPT AT Y=20:	9.084718	X INTERCEPT AT Y=20:	9.626464
NITRIFICATION RATE (mg/L/hr):	2.201499	NITRIFICATION RATE (mg/L/hr):	2.077606
% OF CONTROL	33.42644	% OF CONTROL	31.5453

STATISTICS FOR 30% VOL Regression Output:		STATISTICS FOR 50% VOL Regression Output:	
Constant	49.90517	Constant	50.21897
Std Err of Y Est	0.804799	Std Err of Y Est	0.535949
R Squared	0.965577	R Squared	0.986596
No. of Observations	5	No. of Observations	5
Degrees of Freedom	3	Degrees of Freedom	3
X Coefficient(s)	-3.06552	X Coefficient(s)	-3.3069
Std Err of Coef.	0.334175	Std Err of Coef.	0.222541
X INTERCEPT AT Y=20:	9.755343	X INTERCEPT AT Y=20:	9.138165
NITRIFICATION RATE (mg/L/hr):	2.050159	NITRIFICATION RATE (mg/L/hr):	2.188623
% OF CONTROL	31.12855	% OF CONTROL	33.23093

TABLE A-22

STATISTICAL DATA FOR LINEAR PHASE
OF D 301-B STREAM

STATISTICS FOR CONTROL Regression Output:	
Constant	48.3
Std Err of Y Est	2.711088
R Squared	0.931034
No. of Observations	4
Degrees of Freedom	2
X Coefficient(s)	-6.3
Std Err of Coef.	1.212436
X INTERCEPT AT Y=20:	4.492063
NITRIFICATION RATE (mg/L/hr):	4.452297

STATISTICS FOR 5% VOL Regression Output:	
Constant	45.64
Std Err of Y Est	0.828251
R Squared	0.852632
No. of Observations	4
Degrees of Freedom	2
X Coefficient(s)	-1.26
Std Err of Coef.	0.370405
X INTERCEPT AT Y=20:	20.34921
NITRIFICATION RATE (mg/L/hr):	0.982839
% OF CONTROL	22.07488

STATISTICS FOR 15% VOL Regression Output:	
Constant	45.64
Std Err of Y Est	0.828251
R Squared	0.852632
No. of Observations	4
Degrees of Freedom	2
X Coefficient(s)	-1.26
Std Err of Coef.	0.370405
X INTERCEPT AT Y=20:	20.34921
NITRIFICATION RATE (mg/L/hr):	0.982839
% OF CONTROL	22.07488

STATISTICS FOR 30% VOL Regression Output:	
Constant	47.18
Std Err of Y Est	0.542218
R Squared	0.965714
No. of Observations	4
Degrees of Freedom	2
X Coefficient(s)	-1.82
Std Err of Coef.	0.242487
X INTERCEPT AT Y=20:	14.93407
NITRIFICATION RATE (mg/L/hr):	1.33922
% OF CONTROL	30.07931

STATISTICS FOR 50% VOL Regression Output:	
Constant	45.64
Std Err of Y Est	0.828251
R Squared	0.852632
No. of Observations	4
Degrees of Freedom	2
X Coefficient(s)	-1.26
Std Err of Coef.	0.370405
X INTERCEPT AT Y=20:	20.34921
NITRIFICATION RATE (mg/L/hr):	0.982839
% OF CONTROL	22.07488

TABLE A–23

STATISTICAL DATA FOR LINEAR PHASE
OF COKER WATER STREAM

STATISTICS FOR CONTROL Regression Output:	
Constant	49.46667
Std Err of Y Est	2.28619
R Squared	0.964286
No. of Observations	3
Degrees of Freedom	1
X Coefficient(s)	−8.4
Std Err of Coef.	1.616581
X INTERCEPT AT Y=20:	3.507937
NITRIFICATION RATE (mg/L/hr):	5.701357

STATISTICS FOR 5% VOL Regression Output:	
Constant	51.38
Std Err of Y Est	0.542218
R Squared	0.965714
No. of Observations	4
Degrees of Freedom	2
X Coefficient(s)	−1.82
Std Err of Coef.	0.242487
X INTERCEPT AT Y=20:	17.24176
NITRIFICATION RATE (mg/L/hr):	1.159975
% OF CONTROL	20.34558

STATISTICS FOR 15% VOL Regression Output:	
Constant	52.78
Std Err of Y Est	0.542218
R Squared	0.965714
No. of Observations	4
Degrees of Freedom	2
X Coefficient(s)	−1.82
Std Err of Coef.	0.242487
X INTERCEPT AT Y=20:	18.01099
NITRIFICATION RATE (mg/L/hr):	1.110433
% OF CONTROL	19.47665

STATISTICS FOR 30% VOL Regression Output:	
Constant	53.06
Std Err of Y Est	0.442719
R Squared	0.984615
No. of Observations	4
Degrees of Freedom	2
X Coefficient(s)	−2.24
Std Err of Coef.	0.19799
X INTERCEPT AT Y=20:	14.75893
NITRIFICATION RATE (mg/L/hr):	1.355112
% OF CONTROL	23.76823

STATISTICS FOR 50% VOL Regression Output:	
Constant	51.24
Std Err of Y Est	0.828251
R Squared	0.852632
No. of Observations	4
Degrees of Freedom	2
X Coefficient(s)	−1.26
Std Err of Coef.	0.370405
X INTERCEPT AT Y=20:	24.79365
NITRIFICATION RATE (mg/L/hr):	0.806658
% OF CONTROL	14.14853

TABLE A–24

STATISTICAL DATA FOR LINEAR
PHASE OF D 301 STREAM

STATISTICS FOR CONTROL Regression Output:	
Constant	50.4
Std Err of Y Est	3.429286
R Squared	0.942308
No. of Observations	3
Degrees of Freedom	1
X Coefficient(s)	−9.8
Std Err of Coef.	2.424871
X INTERCEPT AT Y=20:	3.102041
NITRIFICATION RATE (mg/L/hr):	6.447368

STATISTICS FOR 5% VOL Regression Output:		STATISTICS FOR 15% VOL Regression Output:	
Constant	50.54	Constant	50.86
Std Err of Y Est	0.828251	Std Err of Y Est	0.596657
R Squared	0.962667	R Squared	0.97664
No. of Observations	4	No. of Observations	4
Degrees of Freedom	2	Degrees of Freedom	2
X Coefficient(s)	−2.66	X Coefficient(s)	−2.44
Std Err of Coef.	0.370405	Std Err of Coef.	0.266833
X INTERCEPT AT Y=20:	11.4812	X INTERCEPT AT Y=20:	12.64754
NITRIFICATION RATE (mg/L/hr):	1.741978	NITRIFICATION RATE (mg/L/hr):	1.581335
% OF CONTROL	27.01843	% OF CONTROL	24.52683

STATISTICS FOR 30% VOL Regression Output:		STATISTICS FOR 50% VOL Regression Output:	
Constant	50.54	Constant	49.56
Std Err of Y Est	0.442719	Std Err of Y Est	0.828251
R Squared	0.98	R Squared	0.896296
No. of Observations	4	No. of Observations	4
Degrees of Freedom	2	Degrees of Freedom	2
X Coefficient(s)	−1.96	X Coefficient(s)	−1.54
Std Err of Coef.	0.19799	Std Err of Coef.	0.370405
X INTERCEPT AT Y=20:	15.58163	X INTERCEPT AT Y=20:	19.19481
NITRIFICATION RATE (mg/L/hr):	1.283563	NITRIFICATION RATE (mg/L/hr):	1.041949
% OF CONTROL	19.90832	% OF CONTROL	16.16084

TABLE A-25

RESULTS OF NITRIFICATION INHIBITION SCREENS
EXPRESSED AS PERCENT OF CONTROL
NITRIFICATION RATE

Sample Description	Percent of Control Nitrification Rate For: 5% Sample Conc	15% Sample Conc	30% Sample Conc	50% Sample Conc
Crude Tank Water	102	92	89	78
Cooling Tower Blowdown	97	92	47	75
Coker Cooling Water Pond	91	71	68	71
SRU Water	87	86	83	86
Safety Basin	33	32	31	33
D 301-B	22	22	30	22
Coker Water	20	19	24	14
D 301	27	25	20	16

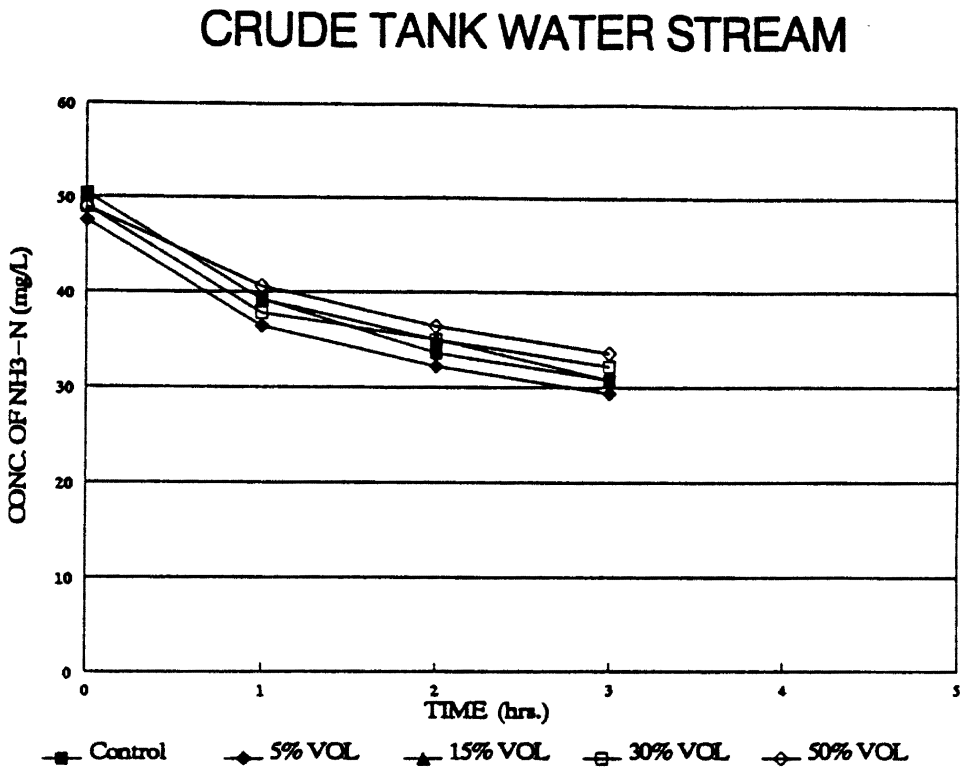

Figure A-1. Ammonia Depletion over Time

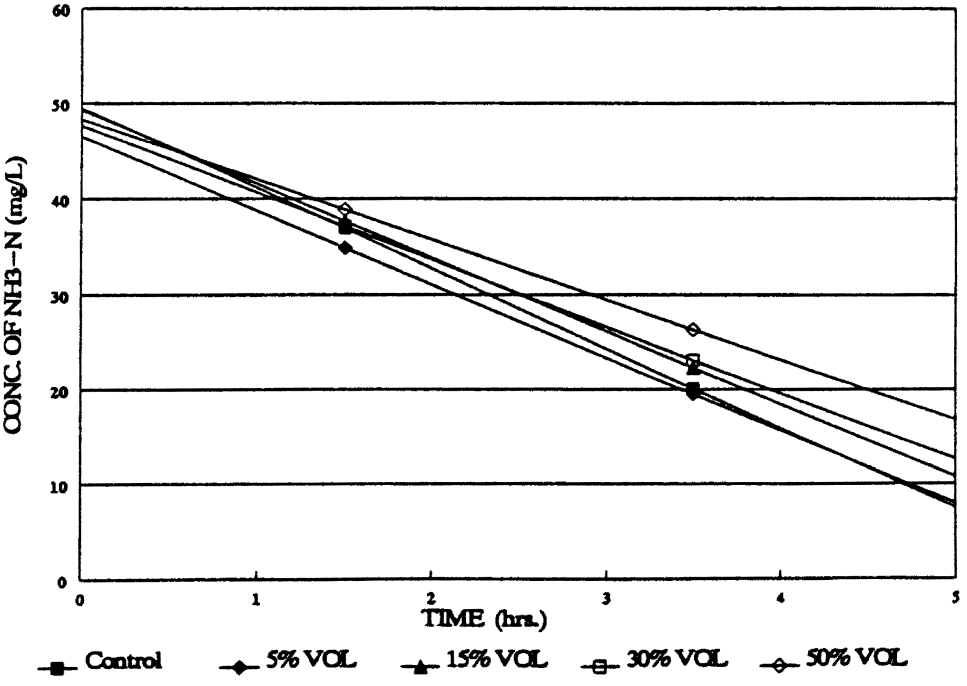

Figure A-2. Linear Phase of Figure A-1 Expressed as Ammonia Nitrification Rates

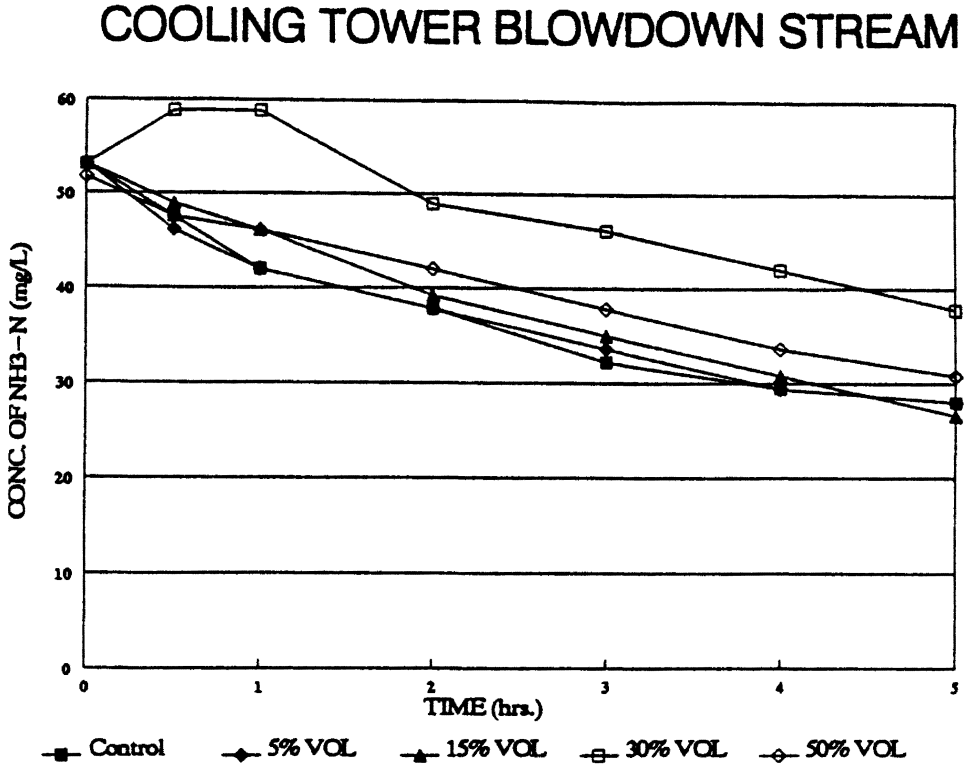

Figure A-3. Ammonia Depletion over Time

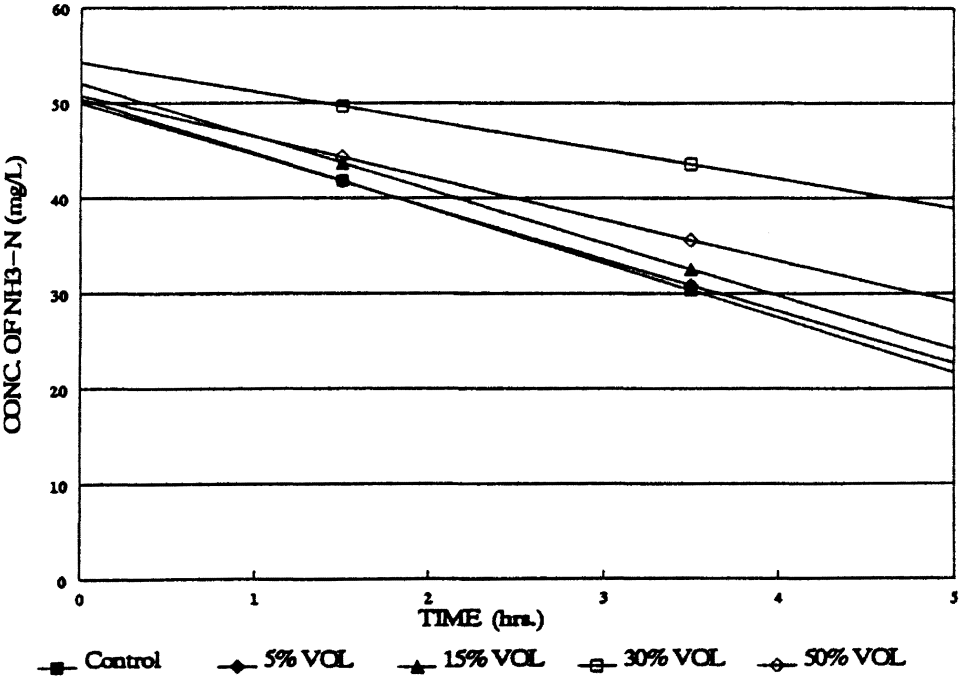

Figure A-4. Linear Phase of Figure A-3 Expressed as Ammonia Nitrification Rates

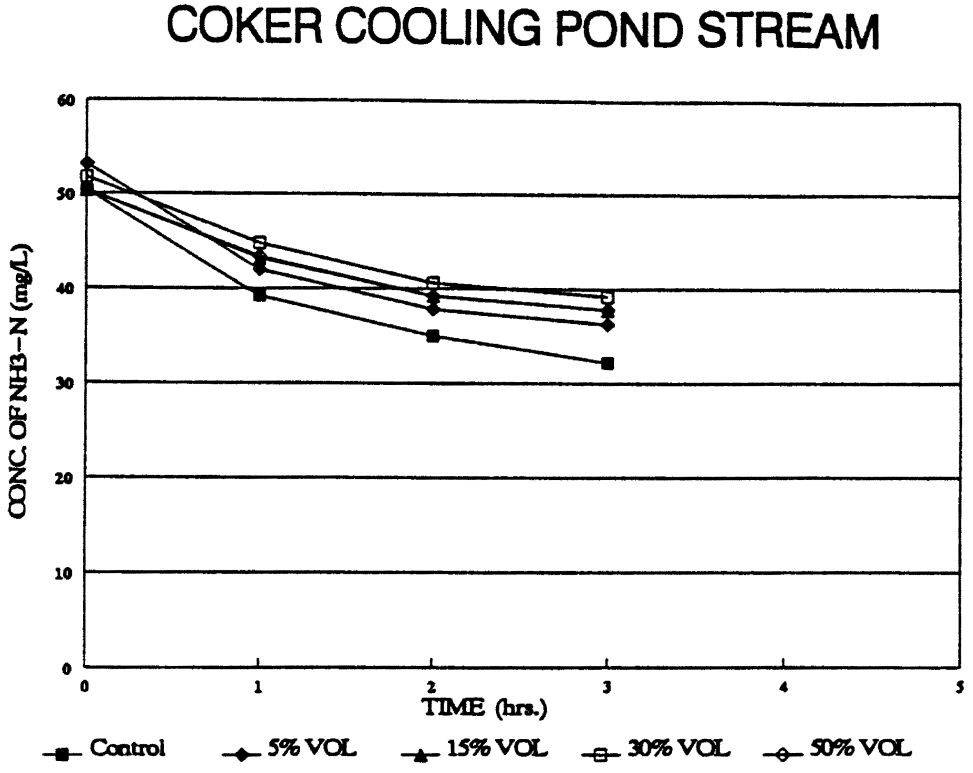

Figure A-5. Ammonia Depletion over Time

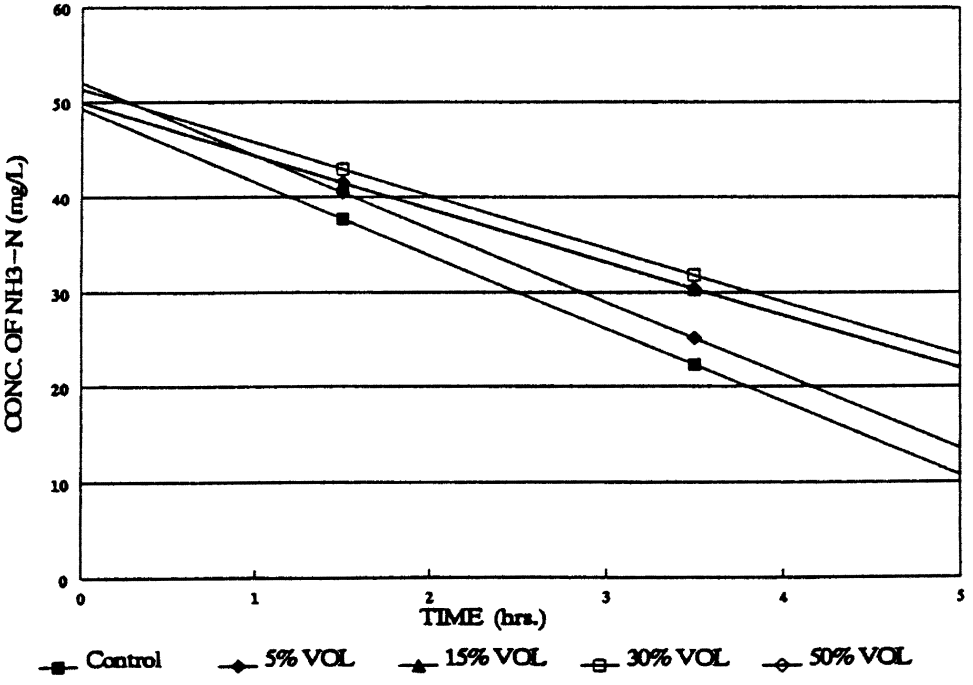

Figure A-6. Linear Phase of Figure A-5 Expressed as Ammonia Nitrification Rates

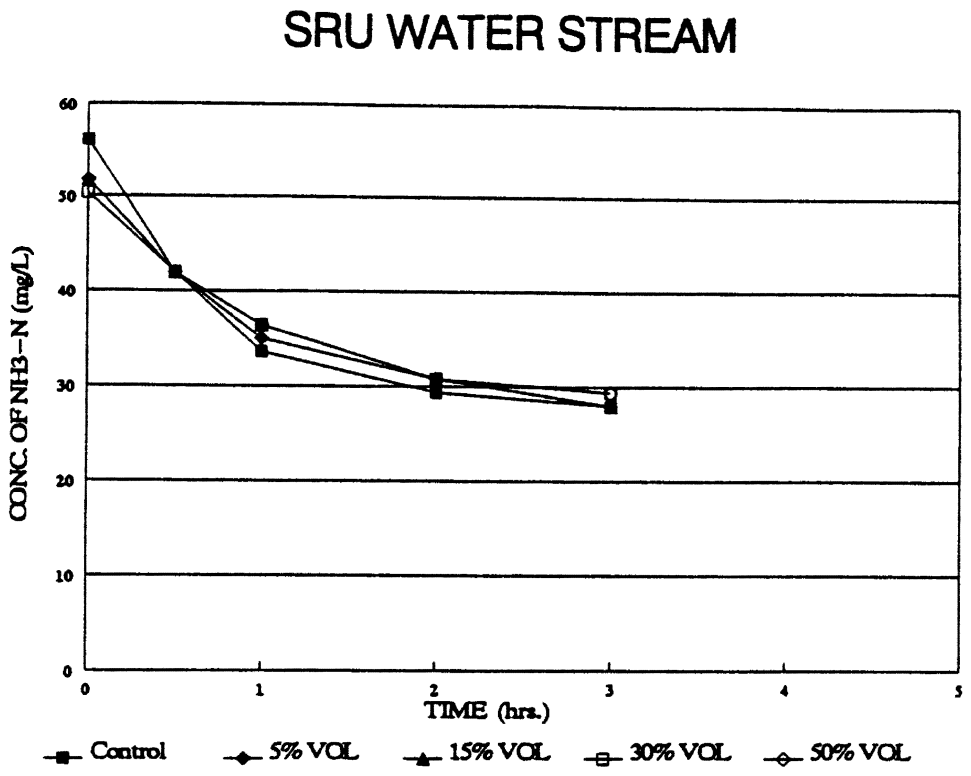

Figure A-7. Ammonia Depletion over Time

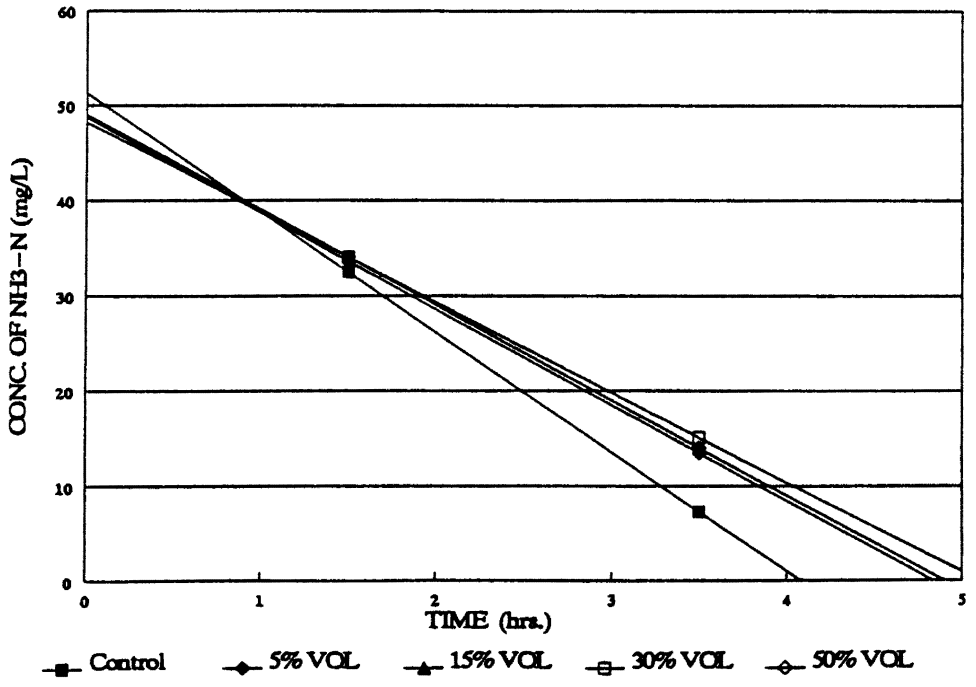

Figure A-8. Linear Phase of Figure A-7 Expressed as Ammonia Nitrification Rates

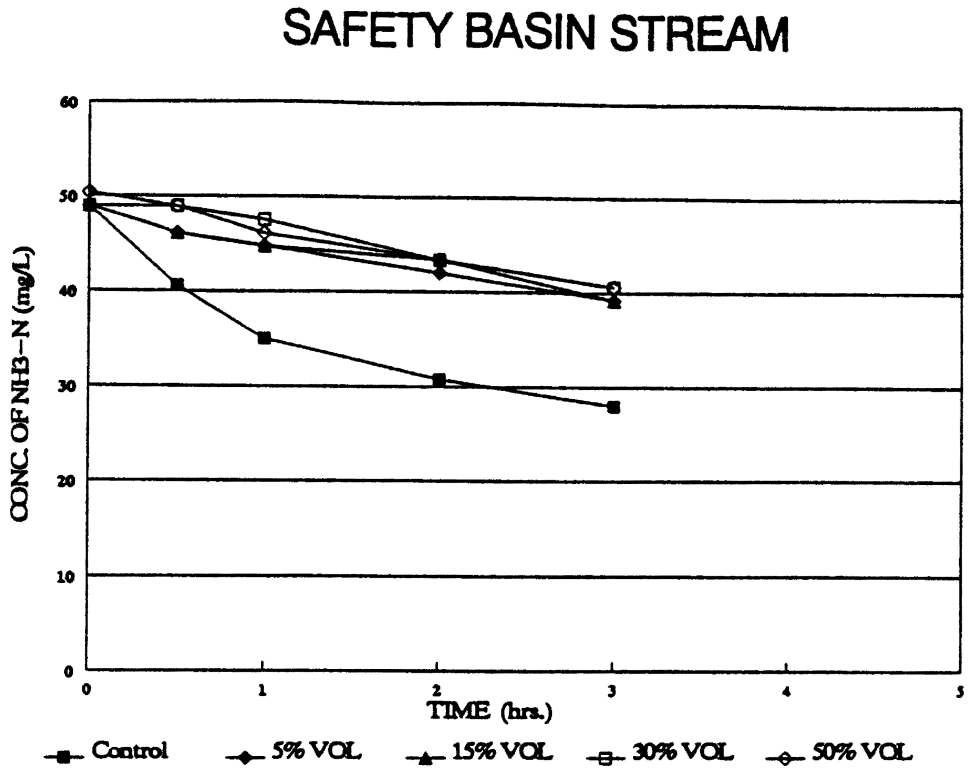

Figure A-9. Ammonia Depletion over Time

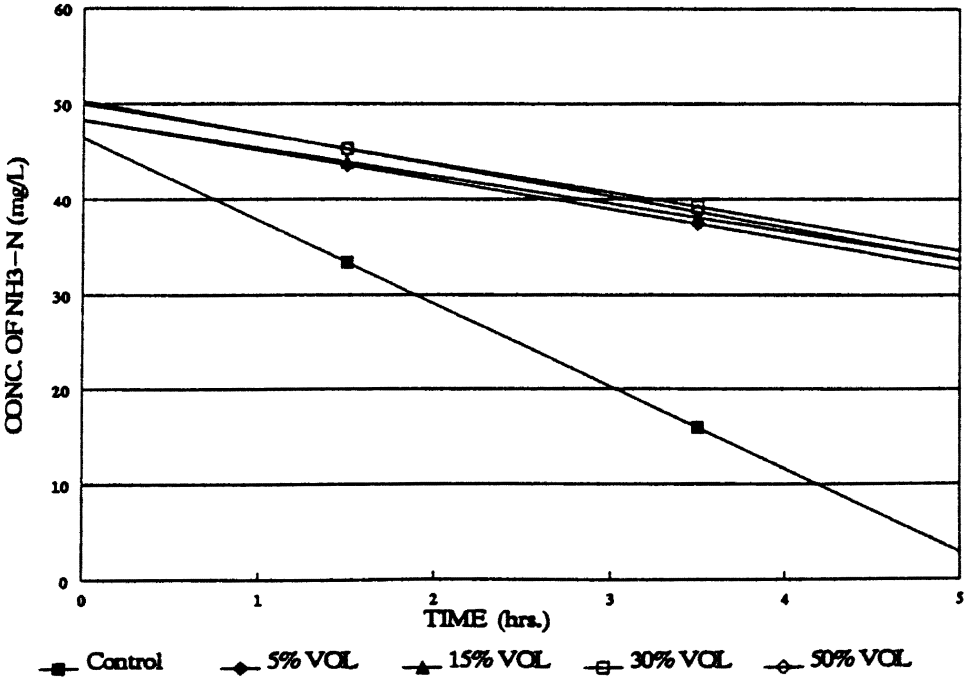

Figure A-10. Linear Phase of Figure A-9 Expressed as Ammonia Nitrification Rates

D 301-B STREAM

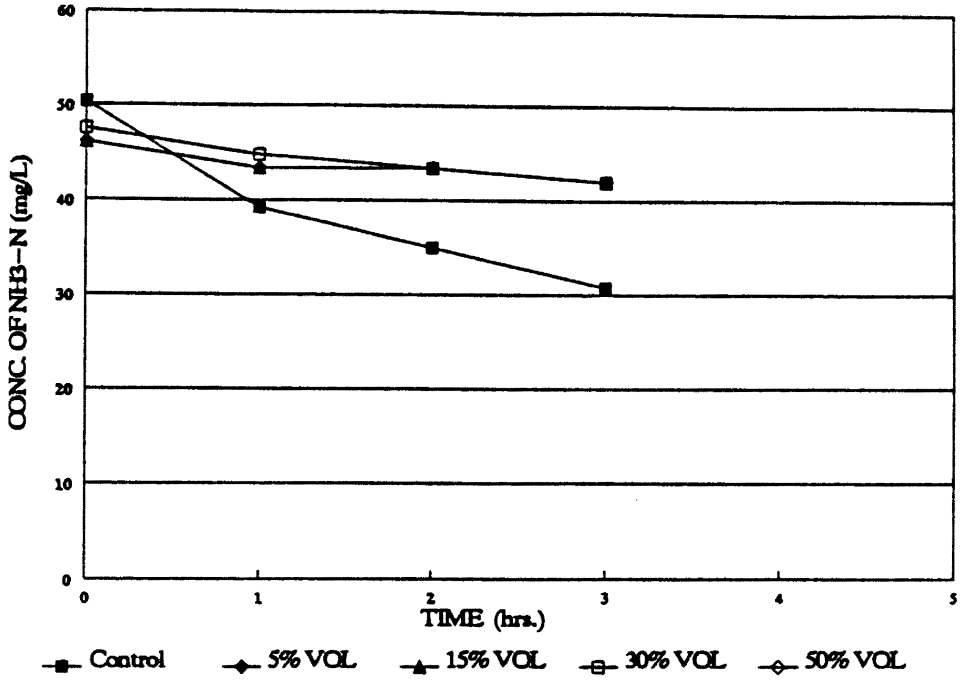

Figure A-11. Ammonia Depletion over Time

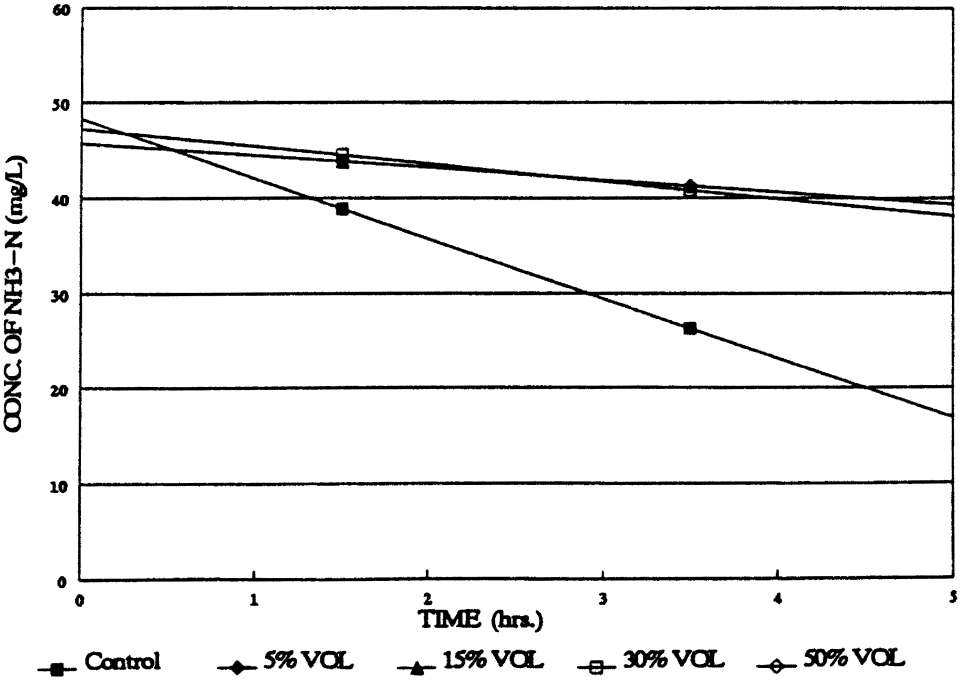

Figure A-12. Linear Phase of Figure A-11 Expressed as Ammonia Nitrification Rates

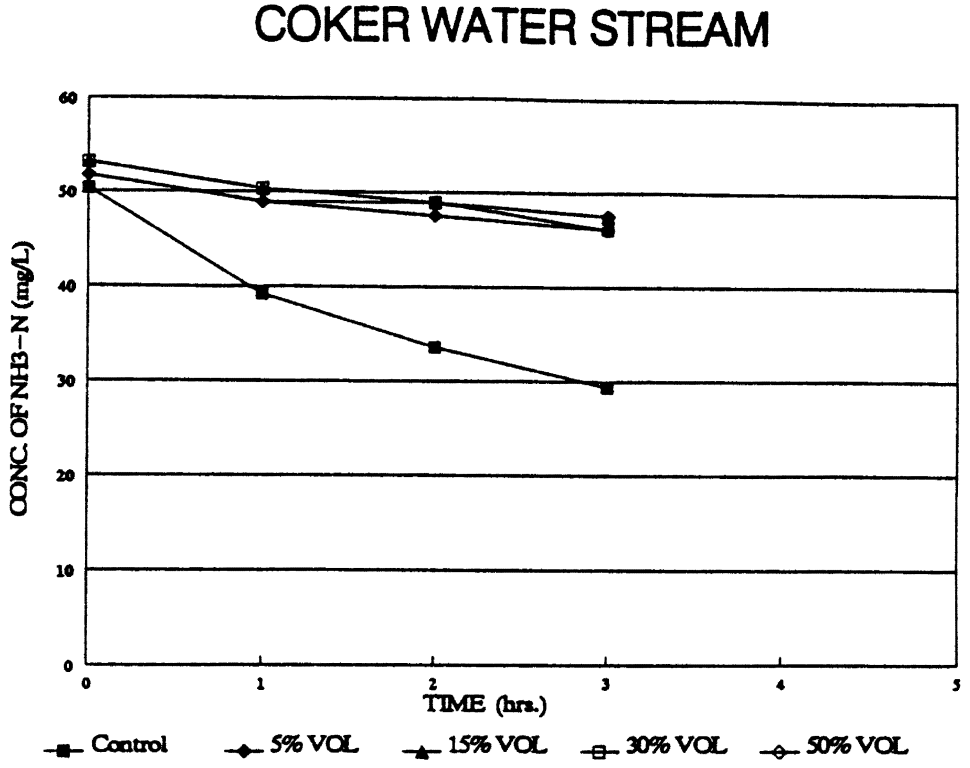

Figure A-13. Ammonia Depletion over Time

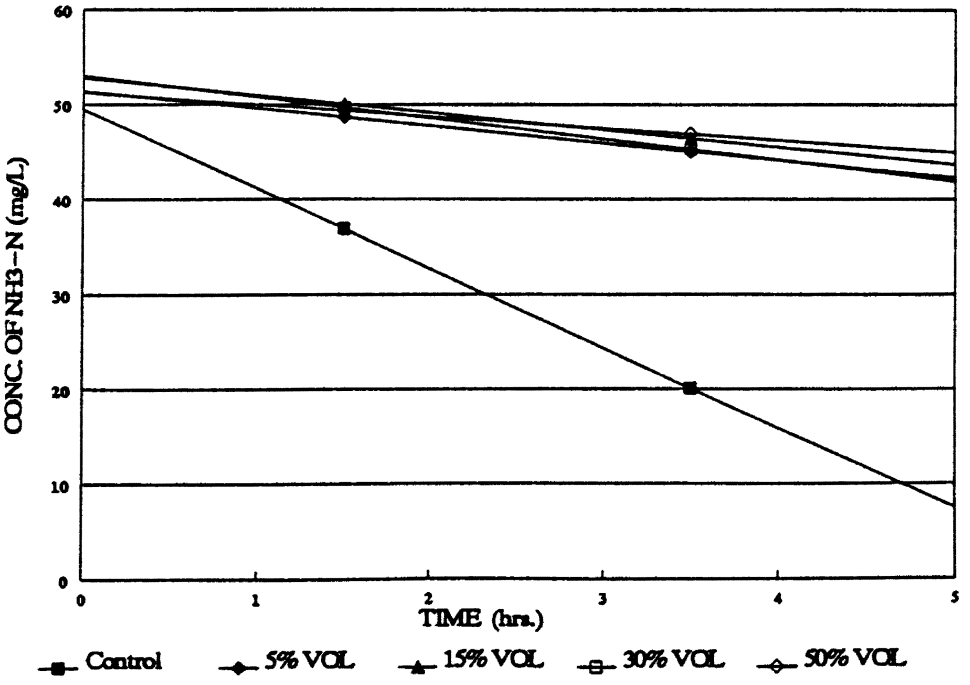

Figure A-14. Linear Phase of Figure A-13 Expressed as Ammonia Nitrification Rates

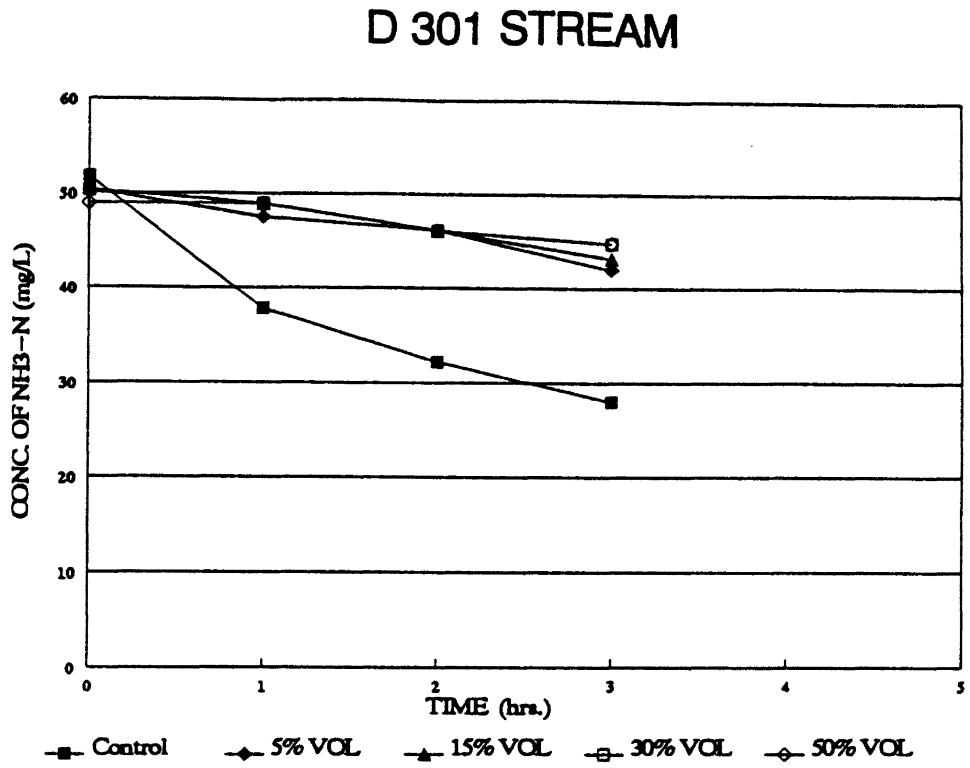

Figure A-15. Ammonia Depletion over Time

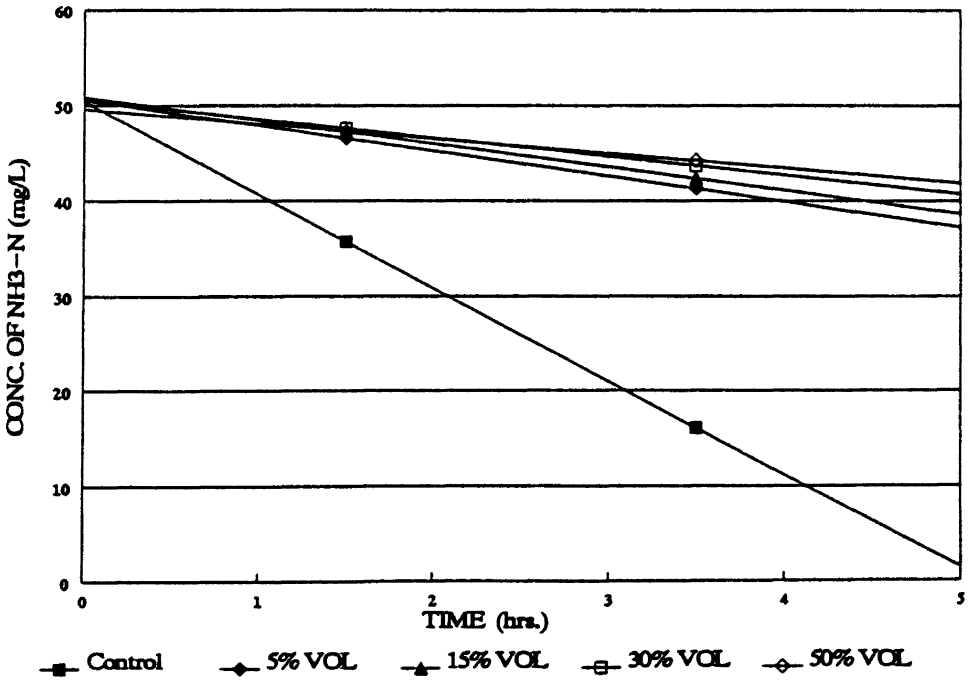

Figure A-16. Linear Phase of Figure A-15 Expressed as Ammonia Nitrification Rates

APPENDIX B

RAW DATA FROM 24 HOUR ACUTE SCREENING TESTS

TABLE B-1

RESULTS OF P. Promelas 24 hr ACUTE TOXICITY TESTS

Stream ID	Organisms Living Over Time (hr) 0hr	2hr	16hr	24hr
Control	10	10	10	10
Cooling Tower Blowdown	10	10	10	10
SRU Water	10	10	1	0
Coker Cooling Pond	10	9	1	0
Crude Tank Water	10	0	-	-
Safety Basin	10	0	-	-
D 301-B	10	0	-	-
Coker Water	10	0	-	-
D 301	10	0	-	-

TABLE B-2

RESULTS OF *D. Pulex* 24 hr ACUTE TOXICITY TESTS

Stream ID	Organisms Living Over Time (hr) 0hr	2hr	16hr	24hr
Control	10	10	10	10
Cooling Tower Blowdown	10	10	10	10
SRU Water	10	2	1	1
Coker Cooling Pond	10	0	-	-
Crude Tank Water	10	0	-	-
Safety Basin	10	0	-	-
D 301-B	10	0	-	-
Coker Water	10	0	-	-
D 301	10	0	-	-

TABLE B-3

RESULTS OF C. Dubia 24 hr ACUTE TOXICITY TESTS

Stream ID	Organisms Living Over Time (hr) 0hr	2hr	16hr	24hr
Control	10	10	10	10
Cooling Tower Blowdown	10	10	10	10
SRU Water	10	4	0	-
Coker Cooling Pond	10	4	0	-
Crude Tank Water	10	0	-	-
Safety Basin	10	0	-	-
D 301-B	10	0	-	-
Coker Water	10	0	-	-
D 301	10	0	-	-

VITA

Robert L. Rogers

Candidate for the Degree of

Master of Science

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