

**Nitrification Inhibition Analysis
For Sanafoam Vaporooter II**

Summary Report

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Introduction

Nitrification is the biological oxidation of ammonia with oxygen into nitrite followed with the oxidation of these nitrites into nitrates. Nitrification plays an important role in the removal of nitrogen from municipal wastewater. Nitrification is a two-step aerobic process, each step facilitated by a different type of bacteria. The oxidation of ammonia (NH_3) to nitrite (NO_2^-) is most often facilitated by *Nitrosomonas* spp. (nitroso = ammonium). Nitrite oxidation to nitrate (NO_3^-), though traditionally believed to be facilitated by *Nitrobacter* spp. (nitro=nitrite), is now known to be facilitated in the environment almost exclusively by *Nitrospira* spp.

Denitrification requires anoxic conditions to encourage the appropriate biological communities to form. It is facilitated by a wide diversity of bacteria. Sand filters, lagooning and reed beds can all be used to reduce nitrogen, but the activated sludge process (if designed well) can do the job the most easily. Since denitrification is the reduction of nitrate to nitrogen gas, an electron donor is needed. This can be, depending on the wastewater, organic matter, sulfide, or an added donor like methanol.

Wastewater treatment plants which nitrify are generally very concerned about any industrial waste which may detrimentally inhibit nitrification. Douglas Products & Packaging, a distributor of the Sanafoam Vaporooter II product, wishes to apply this product within the Hampton Roads Sanitation District (HRSD) in Virginia. HRSD has requested that a test of Vaporooter II (primary active ingredient is metam sodium) be completed to ensure that there will be no detrimental effect on their wastewater treatment processes.

Vaporooter is a combination of herbicides that are placed in sewer lines as foam for the purpose of eliminating roots and preventing further destruction of sewer pipe caused by live tree roots. Only roots within the pipe and a very short distance outside the pipe are affected. Trees and shrubs immediately above the ground are not harmed. Vaporooter is composed of

1) an herbicide which penetrates root cell walls, bursts them and causes the root to die and decay, 2) a root growth inhibitor that attaches to surface-active organic material to inhibit further root cell division or growth for approximately three years, 3) a surfactant blend which strips away grease and slime from the roots permitting penetration of the formulation into the root mass, and 4) a foaming agent which provides a durable, fine-textured "vehicle" to carry Vaporooter into maximum contact with all roots and pipe surfaces (from product website: <http://www.douglasproducts.com>).

HRSD has developed a specific laboratory protocol for nitrification inhibition studies as well as a specific modification of this protocol for testing of metam sodium based products (Appendix A). This report details the results of testing of the Sanafoam Vaporooter II product.

Methods

Treatment Plant Test Material

The nitrification inhibition test protocol specifies that the HRSD's VIP or Nansemond plant mixed liquor be utilized as a source of nitrifying biomass. Because the mixed liquor and raw water influent for the tests must be collected and used the same day as the test, this would preclude using HRSD material as shipping would require at least one day. HRSD allows the use of substitute material as long as it generally conforms to the following operational characteristics of the HRSD raw water influent: BOD of 200-250 ppm, COD of 500-600 ppm, TSS of 125-175 ppm, ammonia of 20-30 ppm and industrial loading of less than 20% (Matt Cox, HRSD, personal communication). Based on these parameters, it was suggested that the Traverse City Wastewater Treatment Plant, located within 3 miles of the GLEC laboratory, would be a viable alternative. The Traverse City WWTP is a nitrifying treatment plant with the following raw water influent characteristics (12 month average): BOD of 280 ppm, TSS of 248 ppm, ammonia of 27.9 ppm, and minimal industrial loading. Traverse City does not monitor for COD. A comparison of the HRSD and Traverse City WWTP (TC WWTP) indicates that the TC WWTP has approximately 15% higher BOD and 40% higher TSS. Conversations with HRSD indicated that the tests could utilize TC WWTP raw water influent diluted approximately 20% with deionized water, and use the undiluted mixed liquor

(Matt Cox, HRSD, personal communication). COD concentrations were measured by GLEC on Traverse City raw water influent diluted 20% with deionized water (as per test protocol) and concentrations ranged from 175 – 200 ppm.

Raw water influent from the TC WWTP was sampled hourly and composited into a 50 liter container. Five liters of influent water was then drawn from this influent composite for use in each days test. The use of a composite influent sample reduced the variability inherent in the raw influent stream from day to day. Eight liters of mixed liquor sample was collected from an aeration basin at the TC WWTP, and this was considered representative based on the expertise of the WWTP operators. These samples were utilized in the analysis described below.

Vaporooter Treatment Levels

HRSD's treatment protocol requires that specific target concentrations of the product are utilized. HRSD states the primary target concentration to be the largest volume of product to be used in any single application project (per day and in units of gallons) divided by 300,000 gallons (lowest hourly flow expected at any HRSD plant at any time of day). This is referred to as target concentration "X". Each nitrification inhibition test was required to be run at a minimum 0.5X, X and 2X concentrations. At the request of Douglas Products, GLEC also ran test concentrations of 0.25X and 0.125 X in addition to the other three. For the purposes of this project, all concentrations were calculated on the basis of complete Vaporooter product, not based on the 30% active ingredient. Discussions with Douglas Products indicated that the maximum amount of Vaporooter applied for any single project, for a day, is approximately 15 gallons. Therefore, given the total Vaporooter maximum product volume and the lowest treatment plant flow rate, that would equate to a concentration of 50 ppm total product (concentration X). The concentrations used for these nitrification inhibition tests are: 2X (100 ppm), X (50 ppm), 0.5X (25 ppm), 0.25X (12.5 ppm) and 0.125X (6.25 ppm).

Nitrification Inhibition Protocol

Approximately eight liters of TC WTTP mixed liquor and five liters of composite raw water influent were collected in a five gallon plastic container with a sealable lid and transported to

the GLEC laboratory. The mixed liquor was allowed to settle for 20 minutes and the liquid was carefully decanted off the top. The solids were immediately aerated after settling in order to bring the dissolved oxygen (D.O.) to a level between 2.0 and 4.0 mg/L. Samples of the aerated solids were collected and submitted for analysis of TSS. During aeration, the specific ammonia electrode (Orion Model 9512 electrode, Orion Model 811 millivolt/pH meter) were calibrated using standard GLEC SOPs. Stock solutions of Vaporooter II in deionized water were made for each day's test, such that the target concentrations (6.25, 12.5, 25, 50, and 100 ppm) were achieved in the final treatment volumes. This consisted of adding the appropriate Vaporooter stock solution to raw influent to reach a volume of 225 mL. The final experimental volumes consisted of 50 mL of aerated mixed liquor solids in a beaker, along with 225 mL of treatment. Three replicate beakers were prepared for each of the five treatment levels. Samples of the raw influent control and treatment concentration X were collected prior to the start of the test run, and after the conclusion of the test for analysis of cyanide (CN). In addition, random treatment samples were collected and analyzed for ammonia by EPA Method 350.2 to compare to the concentrations measured by the Orion ammonia probe.

Immediately after the addition of the treatment concentrations, an aliquot in each of the beakers and the control was removed and starting concentrations of ammonia were measured. The beakers were placed on a shaker table with individual aeration lines for each beaker. This helped to maintain the D.O. between 2.0 and 4.0 mg/L and to keep the solutions well mixed. The time at which each treatment was added to its respective test beaker was recorded. D.O. was monitored during the test period to ensure that the samples were maintained within the desired D.O. range.

The concentration of ammonia was measured in one of the control beakers after two hours to determine if approximately 50% of the starting ammonia concentration had been consumed. The rate of ammonia consumption was calculated and if 50 % had not been consumed, an estimate of the additional test time required for 50% consumption was then calculated. The test was terminated when approximately 50% ammonia in the controls was consumed. Ammonia concentrations were measured in all replicates of each concentration, and the test

time noted. An ammonia consumption rate was calculated for each treatment, and compared to controls to determine if any inhibition of nitrification was present. The observed ammonia consumption readings for each test concentration and companion control was plotted using Axum 6.0 (Mathsoft Corp.) as a basic line plot. Data were expressed as ammonia consumption per hour (mg/L/hr).

Initial nitrification inhibition tests did always show any appreciable ammonia consumption. The Orion ammonia electrode may experience interference from constituents within the test solution. For example, the Orion probe cannot differentiate between monomethylamine (MMA) and ammonia. Because monomethylamine will not nitrify and it is difficult to break down, it was important to determine if the Vaporooter product itself would be a source of MMA or any other source of interference which could potentially bias the ammonia readings. In addition, the surfactants used in the Vaporooter product may also confuse the numbers slightly since they may be made from ammonium salts. An assessment was conducted using the Vaporooter II product at all test concentrations. Deionized water (as a replacement for the influent/mixed liquor) was combined with Vaporooter to achieve the target test concentrations. The ammonia concentration was measured at each of these concentrations, and again after two hours duration to determine if the Vaporooter product itself contributes to the measured ammonia concentration.

Results

Table 1 shows the ammonia consumption rate data as calculated for each test run. There is variability between the test events, as would be expected from differences in both the raw influent and the mixed liquor. In general, it appears that the 0.5X, X and 2X concentrations (25, 50 and 100 ppm Vaporooter II) depress the nitrification process at the TC WWTP, across all five test events. The degree of inhibition differs from each test. A graphical representation of the tests is shown in Figures 1 through 5).

The determination of whether Vaporooter product itself is a source of interference with the ammonia probe does not indicate any specific problem. The data (Table 2) show a low background concentration which is consistent across all treatment levels. In addition,

analytical measurements of ammonia concentrations taken to compare to the Orion ammonia probe with the accepted laboratory determination of ammonia are tabulated in Table 3. In general, the measured concentrations between the probe and analytical tests were very consistent and demonstrated less than 10% difference. Cyanide was not detected in the influent controls or the “X” concentration at the beginning or ends of the test runs (Table 4).

Discussion

The goal of wastewater treatment is the removal of contaminants from the water in order to decrease the possibility of detrimental impacts on humans and the rest of the ecosystem. The term "contaminant" is used here to refer to an undesirable constituent in the water or wastewater that may directly or indirectly affect human or environmental health. Many contaminants, including a wide variety of organic compounds and metals, are toxic to humans and other organisms. Other types of contaminants are not toxic, but nevertheless pose an indirect threat to our well-being. For example, loading of nutrients (e.g., nitrogen and phosphorus) to waterways can result in excessive growth of algae and unwanted vegetation, diminishing the recreational, economic and aesthetic values of lakes, bays and streams.

Nitrogen is found in domestic wastewater mostly in the form of ammonia and organic nitrogen. These can be converted to nitrate nitrogen by bacteria, if the wastewater treatment plant is designed to provide enough oxygen and a long enough "sludge age" to develop these slow-growing types of organisms. The nitrate which is produced may be discharged; it is still usable as a plant nutrient, but it is much less toxic than ammonia. If more complete removal of nitrogen is required, a biological process can be set up which reduces the nitrate to nitrogen gas. Wastewater treatment plants which are optimized to remove ammonia, and perhaps subsequently remove the nitrate as well are cautious about potential industrial waste discharges which may inhibit the nitrification process.

The results for these nitrification inhibition trials do indicate there is a potential threshold for an observed effect. This threshold is somewhere near 25 ppm total Vaporooter product (approximately 7.5 ppm metam sodium, 30% active ingredient). At concentrations below 25

ppm Vaporooter product (in these tests, 12.5 ppm and below), there appears to be very little impact on ammonia consumption. It should be noted that the threshold concentration of 25 ppm does not eliminate nitrification, but is an approximate product level at which an observed effect is more distinct (see Figures 1-5). This threshold is also based on a worst-case scenario of the lowest hourly flow at the smallest HRSD facility combined with the largest daily amount of Vaporooter likely to be applied. In practice, it would be unlikely that the entire amount of Vaporooter product would impact any treatment plant in a single hourly event, due to the specific characteristics of the Vaporooter “foam” delivery system which helps retain much of the active ingredient at the application site (Justin Fearn, Douglas Products, personal communication). Additionally, there was not any detectable cyanide produced from the metam sodium active ingredient in these tests, either in the controls or “X” concentration, and thus this was not considered a significant problem.

Table 1. Data from the Nitrification Inhibition tests for HRSD using raw influent and mixed liquor from the Traverse City Wastewater Treatment Plant, Traverse City, Michigan. Values shown for each concentration are ammonia concentration (mg/L). Rate is expressed mg NH₃ consumed/L/Hr. Start and End ammonia concentrations are mean of three replicates.

Ammonia (mg/L)

Test 1	Control	0.125X	0.25X	0.5X	X	2X	Test Duration
Start	20.69	22.19	24.10	26.13	25.04	21.75	3.5 hrs
End	10.26	13.90	15.98	20.91	20.39	18.39	
Rate	2.98	2.37	2.32	1.49	1.33	0.96	
Test 2	Control	0.125X	0.25X	0.5X	X	2X	Test Duration
Start	19.39	21.02	22.25	25.16	26.37	28.87	5.0 hrs
End	10.62	17.72	18.63	22.46	24.86	26.94	
Rate	1.75	0.66	0.72	0.54	0.30	0.39	
Test 3	Control	0.125X	0.25X	0.5X	X	2X	Test Duration
Start	22.26	22.50	22.34	22.90	22.89	23.21	2.25 hrs
End	11.53	12.62	12.89	14.37	15.98	15.91	
Rate	4.77	4.39	4.20	3.79	3.07	3.24	
Test 4	Control	0.125X	0.25X	0.5X	X	2X	Test Duration
Start	22.30	23.00	24.44	22.01	24.16	24.21	3.0 hrs
End	11.32	11.81	15.41	14.83	14.00	18.24	
Rate	3.66	3.73	3.01	2.39	3.40	1.99	
Test 5	Control	0.125X	0.25X	0.5X	X	2X	Test Duration
Start	21.97	21.67	22.50	23.52	23.98	24.23	3.5 hrs
End	11.02	11.57	13.02	18.56	19.33	20.34	
Rate	3.13	2.89	2.71	1.42	1.33	1.11	

Table 2. Ammonia data (mg/L) from Vaporooter II test to determine if the test product produces a background ammonia reading using the Orion Model 9512 Ammonia electrode. Test was run as a companion, set up normally using raw water influent and mixed liquor compared to only deionized water. The average measured ammonia background concentration using the ammonia probe calibration blank from each of the five test runs (Table 1) is shown for comparison.

Ammonia (mg/L)

	Control	0.125X	0.25X	0.5X	X	2X
Normal Test Set-Up	21.61	21.70	22.19	22.96	23.38	23.55
DI Water Only	0.17	0.21	0.23	0.19	0.18	0.22
Test Blank Average (5 Tests)		0.22				

Table 3. Comparison of ammonia concentrations as measured by the Orion Model 9512 Ammonia electrode and by EPA Method 350.2. Concentrations expressed in mg/L. Start and End indicate when sample was taken during the test run.

Ammonia (mg/L)

Test Concentration	Orion 9512 Probe	EPA Method 350.2	% Difference
Control (Start)	21.6	22.6	4.4
Control (End)	13.1	14.3	8.4
0.125 X (End)	14.4	15.2	5.3
0.25X (End)	14.9	16.2	8.0
0.5X (End)	15.6	15.9	1.9
X (End)	17.0	18.5	8.1
2X (End)	17.8	18.3	2.7

Table 4. Measured concentrations of cyanide (CN) in controls and concentration X at start and end of each test.*

Test Concentration	Test 1	Test 2	Test 3
Control (Start)	ND**	ND	ND
Control (End)	ND	ND	ND
X (Start)	ND	ND	ND
X (End)	ND	ND	ND

* Cyanide analysis not done on Test 4 and 5 due to Non Detects on Tests 1 through 3.

**ND = Not Detected. Limit of Detection = 0.005 mg/L

Figure 1. Ammonia consumption rate (mg NH₃/L/Hr) for Test #1. Concentration “X” is based on the maximum Vaporooter II product usage and lowest hourly flow rate at any HRSD treatment plant.

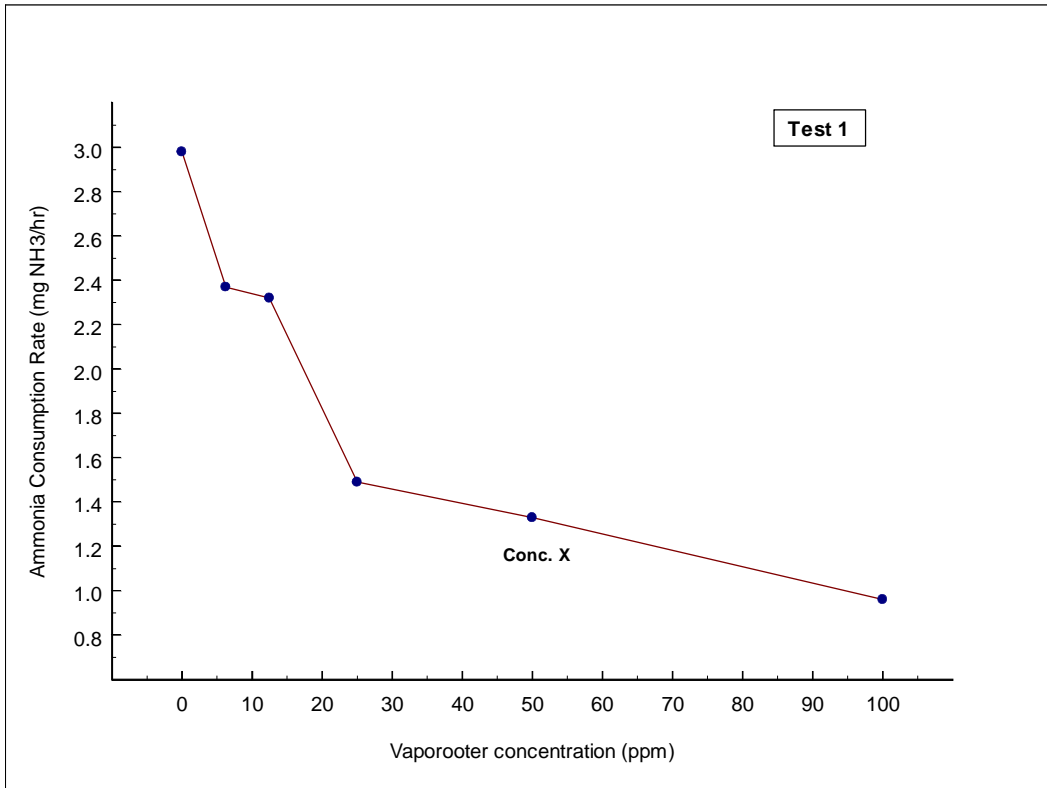


Figure 2. Ammonia consumption rate (mg NH₃/L/Hr) for Test #2. Concentration “X” is based on the maximum Vaporooter II product usage and lowest hourly flow rate at any HRSD treatment plant.

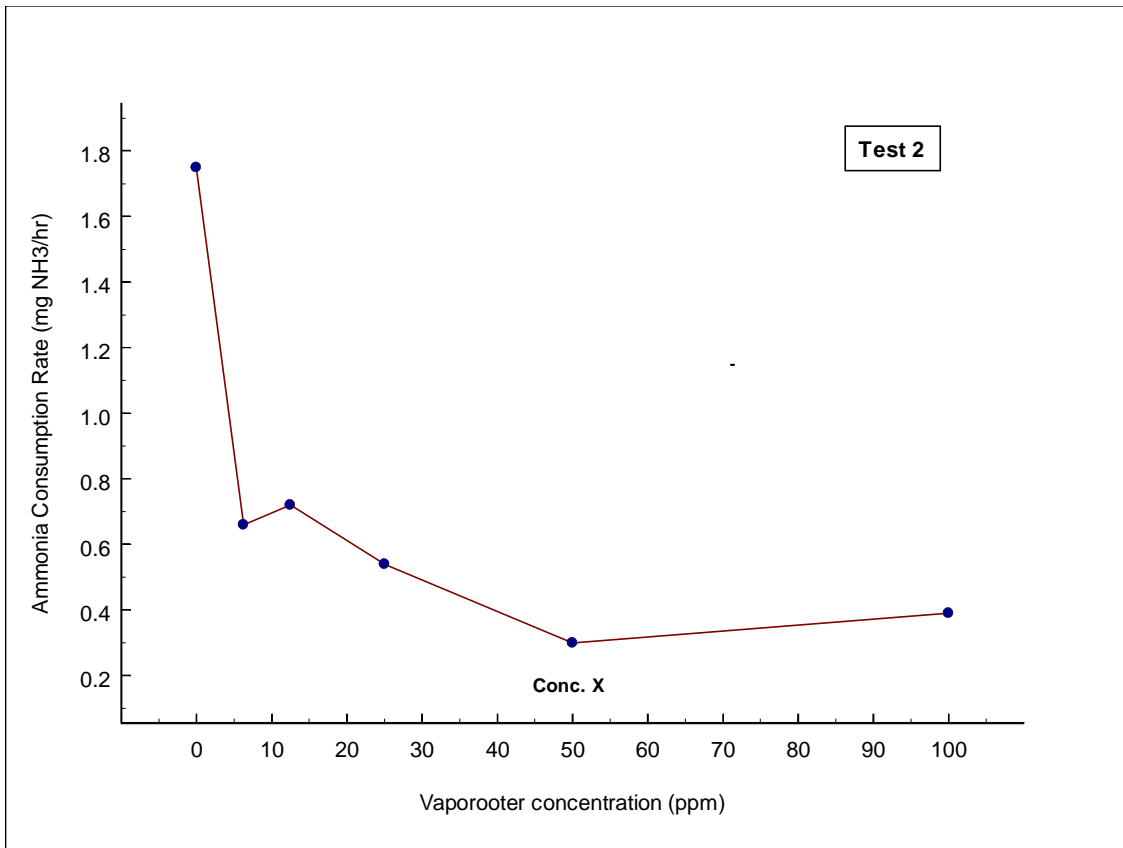


Figure 3. Ammonia consumption rate (mg NH₃/L/Hr) for Test #3. Concentration “X” is based on the maximum Vaporooter II product usage and lowest hourly flow rate at any HRSD treatment plant.

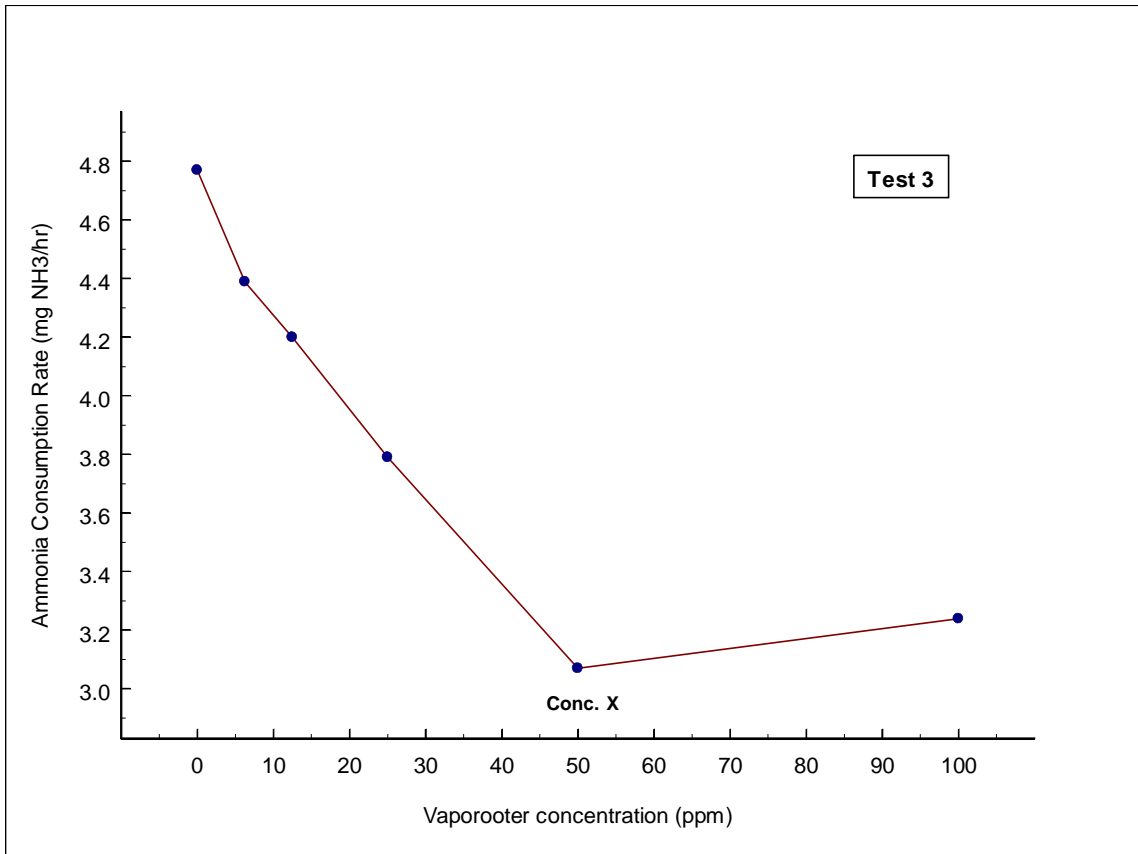


Figure 4. Ammonia consumption rate (mg NH₃/L/Hr) for Test #4. Concentration “X” is based on the maximum Vaporooter II product usage and lowest hourly flow rate at any HRSD treatment plant.

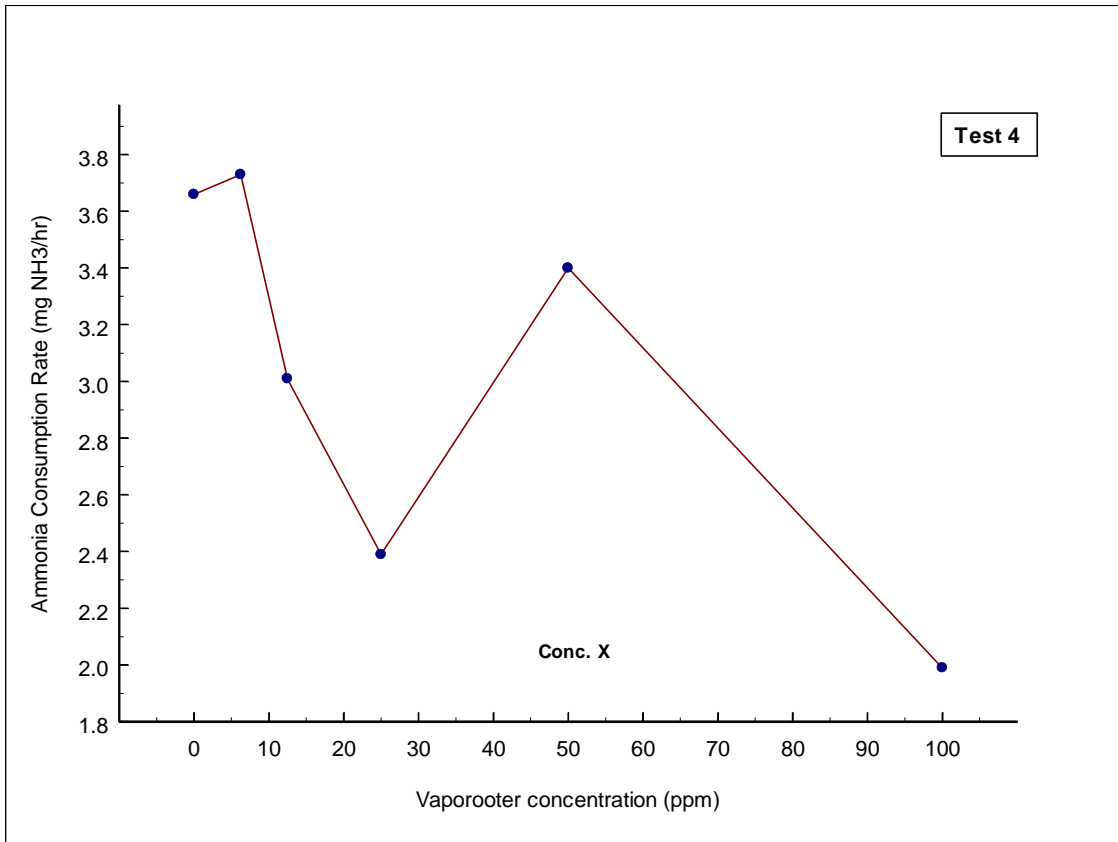
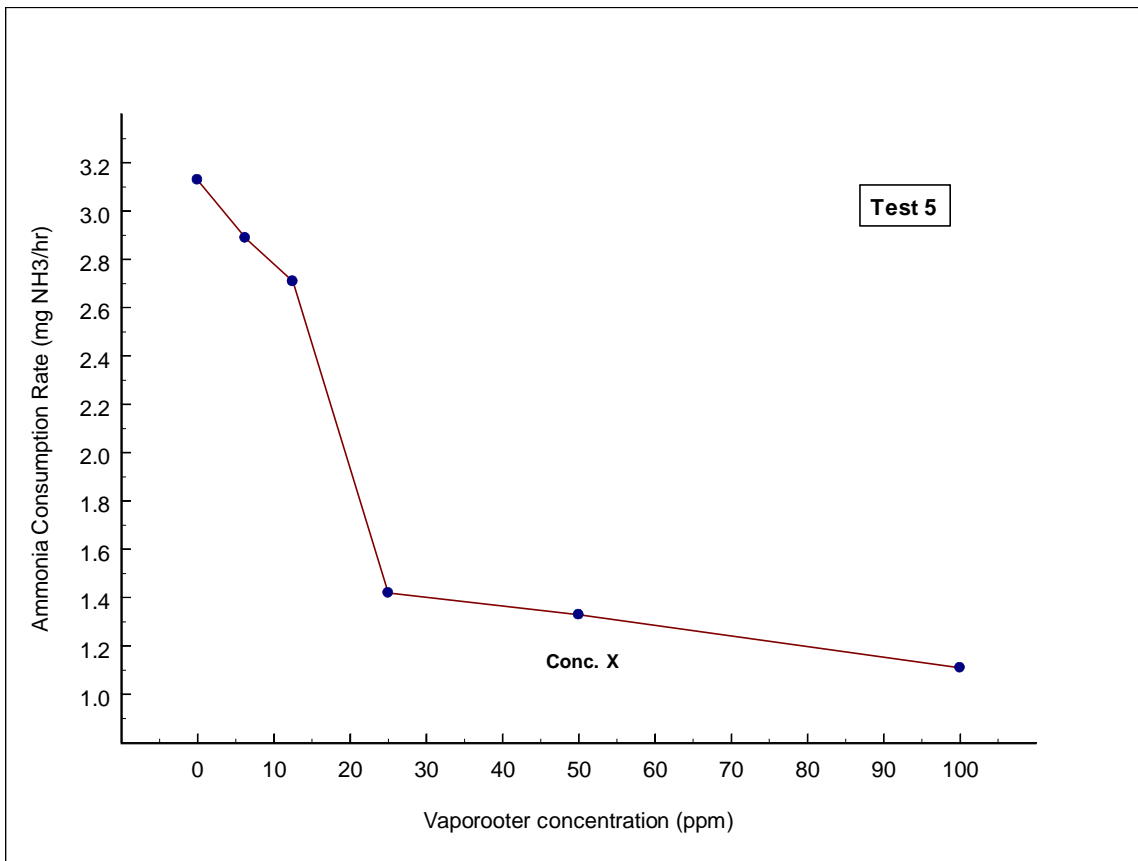


Figure 5. Ammonia consumption rate (mg NH₃/L/Hr) for Test #5. Concentration “X” is based on the maximum Vaporooter II product usage and lowest hourly flow rate at any HRSD treatment plant.



APPENDIX A

NITRIFICATION INHIBITION STUDY

This test can be used to determine the potential of industrial waste discharges and/or internal POTW process streams to inhibit nitrification. Two control waters have been referenced; each is specific to the study objectives. Control #1 represents the raw influent of a facility which is unaffected by its constituents and is different than that of the plant in question, which is presumably already affected. Control #1 is necessary if toxicity is suspected in the service area of a facility (due to toxicity in the raw influent of the plant in question). Control #2 represents the raw influent of the facility in question if that influent is known to be non-toxic. Control #2 is necessary if toxicity is suspected of an internal process stream within the plant. Control #1 should be selected carefully from a facility with a service area similar to that of the plant experiencing problems or projected to experience problems. The influent from an affected plant can not be used to study industrial waste discharges because that waste already affects the influent of that plant. If the problem has not been determined to be service area or plant-specific, the first step of the study should be to compare Control #1 to Control #2 in terms of ammonia conversion rates. Significant differences in the rates will determine if the problem is within the plant or service area. It is a good idea to run both controls at all times to determine baseline responses for each. These baselines can then be used as a reference point for changes which may be implemented either in the service area or in the plant, or both.

The critical steps in this test which influence variability and interpretation of results are the assigning of representative aliquots of solids and sample to each test chamber and the recording of time at initial and final ammonia measurements. The utility of this test is maximized when intra-treatment variability is minimized. Questions can be addressed to Jim Pletl, Environmental Scientist, Hampton Roads Sanitation District, 757-460-4246.

1. Collect 2.5- 5 liters of mixed liquor and allow to settle. The mixed liquor tested should be obtained from a facility which currently does not suffer from inhibition of nitrification. TSS of mixed liquor should be 2- 3000 mg/l and TVSS should be 65- 75%. Final settling volume of the 2.5- 5 liter collection should be approximately 1.0 liter. This will be enough to test 4 samples. Allow at least 10 minutes for settling. Prepare ammonia probe and meter for operation. Fresh, untested mixed liquor should be used each day.
2. Bring control water(s) to room temperature, depending of study objectives.

3. Decant off liquid from mixed liquor sample. Aerate solids ASAP after settling, bringing DO to 2.0- 4.0 mg/l.
4. Prepare a 1000 ppm NH₃ standard with 3.147 g anhydrous NH₄Cl in a one liter volumetric flask. Use DI water for dilution.
5. Bring samples to room temperature.
6. Measure ammonia in all samples, including controls. You will need to calibrate ammonia probe at 10 mg/l and 50 mg/l, therefore prepare standards for these concentrations from the 1000 mg/l standard prepared above. Prepare the ammonia probe standards as follows using DI water every Monday:

<u>Standard</u>	<u>ml 1000 mg/l std/liter</u>
10 mg/l	10 ml
50 mg/l	50 ml

7. Label 3 500- ml beakers for each control and sample. Label each of the three beakers for each treatment as "a", "b", and "c". Three replicates per treatment are required, at a minimum, to address intra- treatment variability.
8. Pour exactly 50 mls of solids into each beaker, being sure that the solids are well mixed while aliquots are drawn, measuring with a graduated cylinder. Pour 100 mls of well- mixed solids into a TSS sample bottle and submit for analysis of TSS and TVSS. Date sample and label appropriately. (Note: Use larger proportionate volumes of solids and sample to get more reproducible results, if available. Volumes of 100- 200 mls of solids and 450- 900 mls of sample are preferable, but reproducing the ratio of sample to solids existing full- scale is very important.)
9. Normalize ammonia concentrations in all samples and controls as follows. This example only shows one industrial waste sample, but the approach can be used on any number of industrial waste and/or process stream samples (control water used is study objective and sample specific). This approach will insure that the ammonia levels between controls and treatments will be similar. Significantly different ammonia levels between treatments may result in different ammonia uptake rates by mixed liquor independent of treatment(s) being tested, which will confound the study results. This example only assumes that 1-liter of each control and samples are being prepared, the ammonia adjustments will vary for the controls if more control water is required.

<u>Treatment</u>	<u>B</u>	<u>C</u> <u>ml 1000 ppm NH3</u>	<u>ml DI</u>
ind.wst.	ind. wst. NH3	highest B - ind.wst. B	highest C - ind. wst.C
Cntrl #2	Cntrl #2 NH3	highest B - Cntrl #2 B	highest C - Cntrl #2C
Cntrl #1	Cntrl #1 NH3	highest B - Cntrl #1 B	highest C - Cntrl #1 C

For example, if ind. wst. sample = 30 mg/l total NH3, Cntrl #2 = 25 mg/l total NH3 and Cntrl #1 = 20 mg/l total

<u>Treatment</u>	<u>B</u>	<u>NH3: C</u> <u>ml 1000 ppm NH3</u>	<u>ml DI</u>
ind. wst.	30	30-30 = 0	10 - 0 = 10
Cntrl #2	25	30-25 = 5	10 - 5 = 5
Cntrl #1	20	30-20 = 10	10 - 10 = 0

The final ammonia concentration in all samples for this example should be $30 \text{ mg/l NH}_3 / (1.0 + .015 \text{ l}) = 29.6 \text{ mg/l NH}_3$. Remember that the calculations are based on the highest "B" and "C" achieved, regardless of sample identification. Each sample should be adjusted to 25-30 mg/l total ammonia if initial concentrations of all samples are relatively low. This will facilitate measurable differences in controls and treatments, which is required to calculate conversion rates and to determine differences between controls and treatments. However it is also important that the concentrations be similar between all samples, even if all concentrations are raised to some arbitrary level.

10. Normalization of pH between controls may be necessary if they differ significantly (more than 0.5 pH units). pH influences ammonia conversion rates and will confound data interpretation if significantly different between treatments and controls. The pH of samples should be adjusted only after preparing dilutions.
11. Prepare industrial waste dilutions by combining these samples with the Control #1 water as a percentage equal to that occurring full-scale at the plant in question. Prepare process stream dilutions by combining with the Control #2 water as a percentage equal to that occurring full-scale at the plant in question. For example, if an industrial waste makes up 5% of a facilities raw influent, then add 50 mls of the wastewater from that industry to 950 mls of raw, non-toxic influent (Control #1). Measure the ammonia concentration of each control and prepared treatment at this time.
12. Pour exactly 225 ml of each treatment (when using 50 mls of solids) into the appropriately labeled beakers and aerate gently immediately to bring DO between 2.0-4.0 mg/l in each beaker. Addition of treatments to the solids should occur immediately after the treatments are prepared in #11 above. Record the time at which each treatment is added to its respective test beaker. Monitor DO for the entire test to keep within the specified

range in all beakers. The use of stir plates and bars should be considered if aeration is not sufficient to keep each beaker's contents evenly mixed throughout the test.

13. Measure ammonia after 2 hours in one beaker of Control #1 and note the time. The test can be terminated if 50% or more of the original ammonia has been consumed since the test began for that beaker. Test termination consists of taking final ammonia measurements for all remaining beakers and noting the time of each measurement. The time to be recorded is when the sample is fixed for analysis. The test should continue if 50% of the ammonia has not been consumed. Extrapolate how much longer the test should last based on the rate of uptake thus far measured. If the initial ammonia is 25 mg/l and the final is 15 mg/l for a two-hour period, then the amount of time left in the test is approximately:

$$[(.5)(2 \text{ hrs})(25 \text{ mg/l})]/(25 \text{ mg/l} - 15 \text{ mg/l}) - 2 \text{ hrs} = 0.5 \text{ hrs} = 30 \text{ additional minutes}$$

Please note that this is an example. If the time that has passed since the initial ammonia measurement for the Control #1 replicate in question is different than 2 hours, insert the actual time into the parts of the equation which are in bold type. Wait the additional time projected and then measure the ammonia concentration and time for all test beakers. Adjustment of data for the first control beaker may be necessary since the solids:sample ratio is now higher due to removal of sample for the initial ammonia analysis used to terminate the test.

14. Calculate the mg of NH₃ consumed per hour for each beaker. Compare average uptake rate for each treatment against Control #1 for each day tested. Normalize data for TVSS (mg NH₃ consumed/hr/mg TVSS) for comparisons between days.

MEMORANDUM

TO: Industrial Waste Manager

FROM: Environmental Scientist

DATE: June 17, 2005

SUBJECT: Nitrification Inhibition and CN testing of metam sodium

The following represents an outline for a study plan to investigate the potential for metam- sodium based products to inhibit nitrification and liberate CN:

1. Follow the SOP entitled "Nitrification Inhibition Study" to conduct each experiment.
2. Use HRSD's VIP or Nansemond plant mixed liquor as a source of nitrifying biomass; check with HRSD as to which one when the study is to begin. If other sources of biomass are to be used, they must be approved for use by HRSD prior to beginning the study.
3. Calculate the target concentration of product to test by dividing the largest volume of product to be used in any single project, per day and in gallons, by 300,000 gallons (the lowest hourly flow expected at any HRSD plant at any time of day). This will provide the worse case concentration occurring at any plant at any time. For the purposes of this study, this will be referred to as concentration X.
4. Each day's study will test the product at 0.5X, X and 2X to establish a dose response curve. This study, therefore, will only confirm potential for toxicity at these concentrations and less. The use of higher concentrations must be verified through further study.
5. Those funding the study may opt to use product in the test that has been pre-exposed to conditions similar to that in the field to address any product degradation/transformation issues that may influence study conclusions. This is acceptable, but the process for preparing the product in this fashion must be outlined and approved by HRSD prior to beginning testing.
6. The control for this study should be, if possible, Nansemond Plant raw influent. This influent will be used to dilute the product to the desired concentrations prior to testing and without the product as a benchmark for comparison. Only one control needs to be tested (in triplicate) in contrast to that described in the SOP. If another control is selected it must be approved by HRSD prior to beginning the study.
7. The product, in whatever form tested using the nitrification inhibition

SOP, will be tested for cyanide at concentration X using a 40 CFR Part 136 approved procedure. The Nansemond plant raw influent should also be sampled for cyanide prior to exposure to the product or the inhibition tests. At the completion of the inhibition tests each day, samples are also to be taken from the concentration X replicates and the control for cyanide analysis.

8. Calculate the ammonia conversion rate for each concentration of product and the control, each day, by dividing the difference between the initial and final ammonia readings for each replicate by the duration of the test for each replicate. Report these conversion rates for each replicate and treatment, for each day of study.
9. The inhibition test must be conducted a minimum of 5 different calendar days using mixed liquor and raw influent collected from the plants on the same day as each respective test. This is necessary to address variability in nitrifying biomass and influent quality. Mixed liquor and raw influent cannot be used unless they are collected the same day they are used and tested.
10. Questions can be addressed by Jim Pletl of HRSD, 757- 460- 4246, jpletl@hrsd.com.

This study may raise issues that require further study; HRSD must reserve the right to request additional information if necessary.