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RE: Evaluation of Zinc Inhibition of Nitrification and BNR
Phases III: Final Report
The Cadmus Group, Inc. Subcontract to VMIRL (069-VMI-1)
EPA Contract 68-C-02-069 – Washington D.C. Water System Direct Implementation Support

Dear Ms. Dufresne:

Please find attached the Final Report for Phase III of this project. The objectives of this document are summarized as follows:

1. Provide a brief summary of Phases I and II – Literature Review and Experimental Proposal.
2. Present an overview of bench-scale studies performed to measure nitrification/BNR inhibition due to zinc at the Arlington County, Virginia Water Pollution Control Plant (WPCP).
3. Provide a detailed methodology for the bench-scale experiments that have been performed.
4. Discuss the results and conclusions from experiments conducted to date.

Based on information available in the technical literature concerning the impact of zinc on nitrifying bacteria, we hypothesized that the increment of 0.3 mg/L total zinc will have no significant impact on nitrification at the Arlington WPCP. The purpose of bench-scale testing is to attempt to refute or reject this hypothesis by several acceptable procedures. Evaluating this hypothesis is a less demanding objective than attempting to answer the question – "what level of influent zinc causes a measurable impact on nitrification and biological nutrient removal (BNR)?" If attempting to simply refute the research hypothesis, it is possible to consider experimentally worst-case inhibition scenarios at the 0.3-0.5 mg/L Zn level - specifically, no removal by primary clarification, no acclimation, 24 hours of chemical exposure (but exposure time issues should be evaluated), addition of a concentrated soluble Zn stock directly to a mixed liquor sample (no prior exposure of the zinc to raw wastewater or PE), and the use of mixed liquor concentrations significantly less than the full-scale process (high Zn/MLSS mass ratio). If inhibition is observed under these "worst-case" conditions, it would then be possible to adjust experimental protocol to consider the factors that would tend to decrease the inhibitory effects of 0.3 mg/L total Zn added to the water supply.

As such, we have been running experiments attempting to make 0.5-1.0 mg/L Zn as inhibitory as possible to nitrifying bacteria:

- Zinc added from completely soluble Zn^{2+} stock solution - not premixed in raw wastewater

- Potential removal in primary clarifier neglected
- Fresh mixed liquor diluted into secondary effluent (which contains ~ 0.5 mg/L PO_4-P -- pretty low)
- High mass ratio of Zn/MLSS in a given experiment (compared to actual operating MLSS of 2000-3000 mg/L).
- Contact for >24 hours up to 5-7 days
- No known prior acclimation of nitrifying bacteria to zinc

To date, we have focused on the following general bench-scale experiments:

- Respirometry
  0.5 to 50 mg/L Zn + target MLSS of ~1000 mg/L
- Nitrate/Nitrite Generation Rate (NOx-N) Measurements – "Snapshot" Experiments
  0.5 to 50 mg/L Zn + target MLSS of ~1000 mg
- High F/M NOx-N generation rate study
  0.5 to 10 mg/L Zn + target MLSS of ~45 mg/L

Summary Results:

- As expected, 20 mg/L allylthiourea (ATU), a selective inhibitor of ammonia oxidizing bacteria (AOB), fully and consistently inhibited nitrification in respirometry, nitrate generation rate, and High F/M nitrate generation rate experiments. Full inhibition was observed for periods up to 5-7 days after initial ATU addition.
- 50 mg/L Zn induced rapid and near-complete inhibition by respirometry.
- 50 mg/L Zn induced rapid and very significant inhibition as measured in a short-term nitrate generation rate experiment.
- 10 mg/L Zn induced rapid and near-complete inhibition in High F/M experiments. This response was repeated during several independent experiments.
- 10 mg/L Zn induced mild to significant inhibition by both respirometry and nitrate generation rate measurements at ~1000 mg/L MLSS, although the degree of inhibition and the contact time required to observed inhibition were somewhat variable and inconsistent. Sometimes the inhibition was immediately observed at the beginning of an experiment. For others, as long as 6-12 hours was required to see significant inhibition. Comparing tap water diluent used with Arlington sludge while at VMI to secondary effluent diluent used with Arlington sludge since starting here in Arlington, there is some indication that secondary effluent offers a protective response - possibly due to PO_4-P or low levels of residual soluble organics that quickly complex the soluble Zn. We may look at this more during the fall semester (after this work).
- Several respirometry experiments conducted as long as 4 to 5 days with a target mixed liquor of 1000 mg/L showed no significant inhibition at 0.5 mg/L Zn.
- Nitrate generation rate experiments conducted as long as 2 days with a target mixed liquor of 1000 mg/L have shown no significant inhibition at 0.5 mg/L Zn.
- Under ammonia-N limiting conditions, respirometry experiments have shown no effect of 0.5 mg/L Zn on the autotrophic half saturation coefficient for ammonia.
- Two valid High F/M experiments were conducted at 0.5 mg/L Zn. In one experiment, there was no significant inhibition over a 4.5 day contact period. In the other, very slight inhibition was observed between 2 and 5 days of contact, but the stressed reactor seemed to acclimate to the added zinc and nitrified at a rate consistent with the negative control reactor from 5 to 7 days after the initial Zn addition.

- Although the High F/M test procedure provides a very sensitive method for detecting nitrification inhibition, the numerical curve fitting procedure used to analyze experimental data to determine the autotrophic maximum specific growth rate may not be appropriate for quantifying the degree of inhibition (see discussion below).
- Based on three High F/M experiments conducted at 1.0 mg/L Zn, there did seem to be some mild inhibition after approximately 24-36 hours of exposure.
- There was no significant inhibition observed at 1.0 mg/L Zn assessed by respirometry at ~1000 mg/L MLSS and over a contact period of 3 days.
- Very slight inhibition, though not statistically significant, was observed at 1.0 mg/L Zn assessed by short-term nitrate generation rate measurement at ~1000 mg/L MLSS and over a contact period of 2 days.
- Experiments were conducted to determine the effect of zinc on nitrite oxidizing bacteria only (nitrite rather than ammonia addition). A single respirometry experiment suggested no inhibition at 0.5 mg/L Zn and only slight inhibition at 10 mg/L. A short-term nitrate generation rate experiment indicated no inhibition at 0.5 mg/L and moderate inhibition at 50 mg/L. As expected for zinc inhibition of nitrification, ammonia oxidizing bacteria are much more sensitive to Zn than nitrite oxidizing bacteria.
- An experiment to determine the effect of zinc on the specific denitrification rate (SDNR) was unsuccessful. It was not possible to repeat the experiment or optimize the procedure due to time limitations.
- It is critical to recognize the research hypothesis when interpreting the inhibition noted above at 1.0 and 10 mg/L Zn. The mild inhibition observed at 1.0 mg/L Zn and the significant but inconsistent inhibition observed at 10 mg/L Zn ***do not*** suggest that 1.0-10 mg/L Zn would cause nitrification inhibition in the full-scale BNR process. Given the experimental design, conclusions regarding the performance of the full-scale system can be made only when no significant inhibition is observed.
- At this point, we have found that 0.5 mg/L Zn induced no significant inhibition under these "ultra-sensitive" conditions. Therefore, it can be concluded that no nitrification inhibition would be expected in the full-scale system if 0.5 mg/L Zn is added to the water supply. It seems that the 1.0 mg/L Zn level is probably right at the point where we start seeing an inhibition response under the experimental conditions we have been using.
- Considering the sensitizing conditions that we used, I speculate that simply premixing 1.0 mg/L Zn with primary effluent diluent would likely eliminate any observed inhibition at this level.

Possible future work to be conducted during the coming academic year:

- Further evaluation of nitrification inhibition at the 1.0 mg/L Zn level by using primary effluent as the diluent and mixing for approximately 2 hours before mixed liquor addition.
- Further experiments to determine the effect of zinc on specific denitrification rate (SDNR) – both by nitrate uptake rate measurements and possible N_2 gas evolution rate using the respirometer.
- Evaluation of nitrification rates using samples of mixed liquor from the Arlington WPCP pilot system before and during zinc stress.
- Evaluation of the effect of soluble ortho-phosphate on zinc inhibition (as well as other heavy metals) of nitrification. This is based on the preliminary observation that secondary effluent diluent offers a protective response as compared tap water - possibly due to either soluble ortho-phosphate or low levels of residual soluble organics that quickly complex the soluble Zn.

- Additional respirometry testing may be conducted to evaluate the effect of zinc on endogenous respiration (tap water only ± zinc) and the effect of zinc on combined and separate heterotrophic and autotrophic respiration (primary effluent ± zinc ± ATU, possibly supplemented with NH_4-N and acetate).

The results of this project could be used to determine whether the zinc concentrations from the proposed zinc ortho-phosphate corrosion control system will exert a toxic impact on BNR at the Arlington WPCP. This assessment could be used by the larger technical review committee evaluating alternatives for corrosion control in water supply in the greater metropolitan Washington D.C. area.

Please contact me at 540-464-7752 if you have questions or comments.

Respectfully,

Charles B. Bott, Ph.D., P.E.
Assistant Professor

cc:
J. Kenneth Klewicki, Ph.D., The Cadmus Group, Inc.
Jack Stoecker, The Cadmus Group, Inc.

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ABBREVIATIONS

AOB	ammonia oxidizing bacteria
Arlington WPCP	Arlington County, Virginia Water Pollution Control Plant
ATU	allylthiourea
AUR	ammonia uptake rate
BNR	biological nutrient or nitrogen removal
BOD	biochemical oxygen demand (typically 5 day)
COD	chemical oxygen demand
DO	dissolved oxygen
F/M	food to microorganism ratio
HFM	high food to microorganism ratio
HRT	aeration basin hydraulic residence time
IC50 or ICxx	concentration producing 50% or xx% inhibition
K_N	Monod half-saturation coefficient
LOT	limit of technology
MLE	modified Ludzack-Ettinger process
MLSS & MLVSS	mixed liquor total and volatile suspended solids
NGR	nitrate or nitrite generation rate
NH_4-N	total ammonia expressed as nitrogen (total ammonia nitrogen)
NOB	nitrite oxidizing bacteria
NO_3-N	nitrate expressed as nitrogen
NO_2-N	nitrite expressed as nitrogen
NUR	nitrate or nitrite uptake rate
OUR	oxygen uptake rate
PE	primary clarifier effluent
RAS	return activated sludge
SDNR	specific denitrification rate
SE	secondary effluent
SOUR	specific oxygen uptake rate
SNR	specific nitrification rate
SRT	solids retention time
TCMP	2-chloro-6-(trichloromethyl)pyridine
TKN	total Kjeldahl nitrogen
TN	total nitrogen
TP	total phosphorus
TSS & VSS	total and volatile suspended solids
USEPA	U.S. Environmental Protection Agency
VPDES	Virginia Pollutant Discharge Elimination System
WAS	waste activated sludge
WPCP	Water Pollution Control Plant
ZnOP	zinc ortho-phosphate

1. BACKGROUND

Portions of the greater metropolitan District of Columbia have water distribution system corrosion problems. This has resulted in elevated levels of heavy metals, in particular lead from the corrosion of some plumbing and service connections. One alternative being considered for corrosion control is the addition of either phosphoric acid (ortho-phosphate) or zinc ortho-phosphate based products at the water treatment plant to inhibit corrosion of plumbing and service connections that contain lead. The benefits of corrosion control in the water supply distribution system are generally understood (reduction in the potential for lead corrosion) and will provide a significant public health benefit. One consequence of the addition of zinc ortho-phosphate (ZnOP) to the water supply is the potential impacts on the operation of the wastewater treatment plants that receive and treat sanitary wastewater from treated water supplies. The objective of this project is to evaluate the impact of zinc ortho-phosphate and specifically zinc on the inhibition of nitrification and biological nutrient removal at the Arlington, Virginia Water Pollution Control Plant (WPCP).

Zinc can have a toxic affect on the biomass responsible for biological treatment of wastewater. In particular, the bacteria responsible for the biological nitrogen removal (BNR) via nitrification and denitrification are particularly sensitive to metals at low concentrations (part per million range). The presence of zinc at low concentrations is known to inhibit BNR and cause upset and interference, typically as a result of nitrification inhibition. The nitrification process involves the biological oxidation of ammonia-N to nitrite-N and subsequently to nitrate-N by two genera of aerobic autotrophic bacteria. This process is accomplished in the aeration basin of the biological wastewater treatment process (e.g. activated sludge) simultaneously with the removal of biodegradable organic compounds (i.e. biochemical or chemical oxygen demand BOD/COD) by heterotrophic bacteria. Nitrifying bacteria have relatively slow specific growth rates in the activated sludge process and are especially sensitive to toxic compounds including both heavy metals and xenobiotic organic chemicals (Barth et al., 1965; Blum and Speece, 1991; Juliastuti et al., 2003a) – much more so than the aerobic heterotrophic bacteria responsible for BOD removal or the heterotrophic/facultative bacteria responsible for denitrification and BOD removal under anoxic conditions (anoxic conversion of nitrate-N to nitrogen gas). Thus, the nitrification process is generally the “weakest link” in terms of chemical inhibition and nitrogen removal, and the point of concern for this project. Since the product of nitrification (nitrate) is the substrate for denitrification, inhibition of nitrifying bacteria affects both nitrification and denitrification, thus limiting the ability for a wastewater treatment facility to meet its ammonia-N and total nitrogen (TN) permit limits. The VPDES discharge limits for the Arlington WPCP for ammonia-N and the Virginia Water Quality Improvement Fund effluent treatment objective for TN are shown in Table 1.

Table 1. Current Effluent Nitrogen and Phosphorus Limits for the Arlington WPCP

Constituent	Units	Monthly Average	Weekly Maximum	Annual Average
Total Phosphorus (TP)	mg/L	0.18	0.27	
Ammonia-N (Apr-Oct)	mg/L	1.0	2.7	
Ammonia-N (Nov-Mar)	mg/L	3.5	4.2	
Total Nitrogen (TN) [a]	mg/L			8.0

a. Annual average treatment objective specified by the Virginia Water Quality Improvement Fund grant.

Previous laboratory and pilot plant studies on the impacts of zinc on nitrification have been documented, and are summarized in Tables 2 and 3 below. The results suggest that the threshold of inhibition to nitrification varies from about 0.1 – 50 mg/L total influent zinc. There is considerable discrepancy in this data however, and there is an indication that the impact of zinc on BNR, and specifically nitrification, is site-specific. The primary objective of this project is to determine whether the maximum expected increase in influent total zinc to the Arlington WPCP based on the anticipated zinc ortho-phosphate dosing scheme, will have an effect on nitrification process performance.

While the secondary maximum contaminant level (MCL) for Zn in drinking water is 5 mg/L, a typical objective of <0.3 mg/L is used in areas where there is concern for the impact of Zn on wastewater treatment and biosolids quality (AWWARF & DVGW-TW, 1996). As a result, the lower Zn:PO_4 ratio products are more widely used today. The ZnOP product under consideration by the Washington DC Water and Sewer Authority (DCWASA) is a 1:10 Zn:PO_4 product. Given the planned initial "passivating" dose of 3 mg/L as PO_4 , a 0.3 mg/L Zn increment in the finished drinking water would result. When the inhibitor dose is reduced to the expected maintenance level of 1 mg/L PO_4 (after 1-6 months), a 0.1 mg/L Zn increment would be expected (The Cadmus Group, 2004).

Current influent total Zn concentrations for the Arlington WPCP are in the range of 0.2 mg/L (The Cadmus Group, Inc., 2004). Based on the information summarized in the previous Phase I/ II Literature Review and Experimental Proposal, it is likely that an increase in the influent total zinc concentration to the Arlington WPCP of 0.3 mg/L will have little or no effect on nitrification, especially after several weeks of acclimation. Considering the published data as a whole, a total influent Zn of 0.5 mg/L seems to be on the low end of the scale for threshold inhibition values. However, it is prudent to verify the site-specific impact on nitrification kinetics to ensure that the process is not more sensitized to other stressors (temperature, ammonia loading, other chemicals, etc.) at the current operating SRT. With acclimation at an influent total zinc concentration of 0.5 mg/L, it is likely that nitrification would not significantly affected, but as indicated above, many site specific parameters affect metal inhibition of nitrification.

This concern is now particularly relevant with detailed evaluations being conducted for complying with "Limit of Technology" (LOT) nutrient discharge criteria in the Chesapeake Bay watershed. As part of the current Chesapeake Bay Program, Virginia wastewater treatment facilities in the watershed are evaluating the requirements and cost for achieving the LOT nutrient removal by 2010. For the Arlington WPCP, the Virginia Department of Environmental Quality has indicated that this would mean achieving an effluent TN of 3.0 mg/L (annual

average) and an effluent TP of 0.18 mg/L (monthly average) at a design flow of 40 MGD. Phosphorus is removed at the Arlington WPCP by chemical precipitation to meet a current TP limit of 0.18 mg/L. It is unlikely that additional phosphorus removal will be required for the Arlington WPCP by the Chesapeake Bay Program based on available information. The Arlington WPCP will soon be converting to multi-point ferric chloride addition for chemical phosphorus precipitation, and will probably not consider enhanced biological phosphorus removal in the future. Thus, it is not anticipated that zinc addition to the potable water supply would have any affect on TP removal at the Arlington WPCP. However, it should be recognized that the additional ortho-phosphate load to the Arlington WPCP will result in increased chemical usage and sludge production (The Cadmus Group, Inc., 2004).

As part of the 2001 Master Plan for the Arlington WPCP, treatment strategies to achieve a TN of 3.0 mg/L were considered, and provisions were made to allow for this in the future. Nevertheless, LOT nitrogen removal would require substantial improvements beyond ongoing work to provide additional nitrification and denitrification capacity. If LOT nitrogen removal is implemented by 2010, it will be critical to maintain consistently low effluent ammonia-N concentrations to ensure that the TN limit of 3.0 mg/L can be achieved on an annual average basis. While it is unclear what level of influent total zinc to the plant might affect nitrification, it would be absolutely critical to ensure that there is no significant impact on the nitrification process if LOT nutrient removal is required.

2. EXPERIMENTAL OVERVIEW

This section reviews the laboratory methods used to evaluate the impact of zinc on nitrification, denitrification and process performance.

2.1 Overview of Methods for Quantifying Biological/BNR Process Inhibition

There are two general types of investigations that can be performed to evaluate the impact of a chemical toxin on nitrification, denitrification, or general process performance. These include:

1. Measurement of Kinetic Parameters: The measurement of kinetic parameters involves the removal of samples of mixed liquor from the biological process, in order to conduct short-term laboratory experiments with appropriate controls to determine the affect of a specific chemical stressor on the "kinetic capability" of the biomass (e.g. respirometry). These experiments can be conducted under aerobic or anoxic conditions, with varying levels of biomass, with a wide range of added substrates, and with or without specific inhibitors of nitrifying bacteria such as allylthiourea (ATU) or 2-chloro-6-(trichloromethyl)pyridine (TCMP) (APHA, 1998; Bennes et al., 2002; Hooper and Terry, 1973). For nitrification assessment, the biomass can be augmented with PE, ammonia-spiked PE, ammonia, or nitrite. If organic substrates are added when conducting nitrification experiments, the testing must be performed with and without nitrification inhibitors (e.g. ATU or TCMP). For denitrification and/or heterotrophic growth, PE, readily biodegradable organic substrates (e.g. acetate), nitrate, or some combination thereof can be used. To determine the effect of exposure time on inhibition, these

experiments can be performed over periods as short as 15 minutes or as long as 24 hours. Representative examples include:

- Measurement of Specific Oxygen Uptake Rate (SOUR) under a wide range of conditions for nitrification and aerobic heterotrophic growth, including endogenous respiration rate (respirometry).
- Measurement of ammonia uptake rate (AUR) or nitrite/nitrate generation rate (NGR) for nitrification. In some cases, the AUR or NGR is used to calculate the specific nitrification rate (SNR).
- Measurement of nitrate uptake rate (NUR) for denitrification – specific denitrification rate (SDNR).

2. Operation of a Bench- or Pilot-Scale System: The operation of a bench or pilot scale system involves the construction and operation of a biological treatment process that simulates as well as possible the full-scale system and uses actual PE feed. The PE feed is spiked with the chemical toxin of interest. The biological treatment system can range in scale/size (bench or pilot) as appropriate to address the type of inhibition being considered, resources available, etc. In most cases, it is best to operate two or more systems in parallel, with at least one control system that receives no chemical stressor. The effect of the chemical stressor is noted simply by the performance of the stressed reactor versus the control in terms of effluent BOD, ammonia-N, NO_3-N/NO_2-N, TN, total suspended solids, effluent concentration of stressor chemical, etc. Due to simplicity and ease of operation in the laboratory, a number of researchers have used bench-scale sequencing batch reactor systems to simulate both MLE and conventional activated sludge processes. If necessary, removal in the primary clarifier can also be simulated/assessed using actual raw wastewater (typically screened and degritted). In this case, the chemical stressor is added to raw wastewater in a small mixed vessel (to simulate the sewage collection system), and then pumped into a tank that simulates primary clarification.

In the case of nitrification, it is possible to use SOUR or AUR/NGR information to determine the effect of the inhibitor on the autotrophic maximum specific growth rate ($\mu_{max,a}$) or the Monod half-saturation coefficient (K_N). This makes it possible to evaluate impacts on the minimum SRT required for nitrification under the exact conditions occurring in the full-scale system. This type of experimentation tends to be a more sensitive measurement of inhibitory effects.

Two important disadvantages of this strategy (Method #1 above) are that it does not allow for acclimation of the biomass to the chemical inhibitor, and it does not provide an indication of metal toxicity as a result of accumulation in the biomass based on the operating SRT. If mixed liquor is removed from the full-scale process and used to address inhibition in short-term "kinetic capability" experiments, this may represent a worst-case scenario in terms of measured inhibition. On the other hand, if metal accumulation in the biomass is an important inhibition mechanism, direct kinetic evaluations could underestimate inhibition. It is typically observed that for heavy metal inhibition of nitrification, maximum inhibition occurs within 12-24 hours after exposure (Hu et al., 2003). However, with continued exposure for a period on the order of 1 to 3 SRTs, acclimation to the chemical contaminant can often occur, and nitrification kinetics approach pre-stress values (Manoharan, 1992; Hu et al., 2004). Since it is normally not

advisable or practical to acclimate a full-scale system to the chemical inhibitor in question, this limitation must be recognized when evaluating the results from these experiments – short-term tests without acclimated biomass (see Table 3).

To best evaluate site-specific nitrification inhibition for a given chemical stressor, the most realistic data are obtained by doing a combination of Methods #1 (kinetic testing) and #2 (bench- or pilot-scale testing) above. In this case, kinetic experiments can be performed using biomass taken from control and stressed bench- or pilot systems (that simulate the full-scale biological process). Both acclimation and metal accumulation in the biomass can be assessed by performing these kinetic measurements over time after exposure to the chemical toxin. Furthermore, data from kinetic measurements can be compared to effluent quality measurements in the stressed versus control systems. Since Arlington personnel have been conducting pilot testing in parallel with the experimental work described below, it would be advantageous to perform similar kinetic evaluation experiments using mixed liquor from this system. For the bench-scale study to measure nitrification/BNR inhibition due to zinc at the Arlington WPCP, laboratory kinetic measurements were performed on stressed and unstressed samples of mixed liquor from the full-scale activated sludge process. The Arlington WPCP was operating one BNR pilot system at the time this investigation was being conducted, but timing of our experiments and performance of the pilot system did not allow us to sample for kinetic measurements. As Arlington will soon be starting a second pilot system run, further experimental work this fall could be completed.

2.2 Kinetic Methods for Assessing Nitrification and Denitrification Inhibition

There are three independent kinetic methods that have been used to evaluate nitrification kinetics, and specifically the effect of chemical toxins on nitrification. These include:

1. Respirometry: Respirometry involves the measurement of oxygen uptake rate (OUR) for a biological treatment culture. A sample of mixed liquor is removed from a full-, pilot-, or bench-scale system, placed in a sealed vessel, possibly amended with substrate or nitrification inhibitor, and the rate of oxygen consumption is monitored over time. To evaluate nitrification kinetics, a sample of mixed liquor is added to a temperature-controlled respirometer vessel with and without (control) a chemical stressor. The mixed liquor is supplemented with PE, ammonia-spiked PE, ammonia, or nitrite. Generally, it is desirable to initiate the experiment with relatively high levels of ammonia to allow for a longer experimental run and to ensure that the maximum nitrification rate is maintained. If this is the case, careful control of pH must be maintained to ensure the pH does not drop below about 6.8-7.0 (alkalinity is typically added in the form of sodium carbonate). If organic substrate is added (e.g. PE), these experiments can be run with and without nitrification inhibitor to distinguish between heterotrophic and autotrophic oxygen uptake. Since endogenous heterotrophic oxygen uptake can occur without organic substrate addition, experiments are often run with and without nitrification inhibitor even when ammonia is the only substrate added to the mixed liquor. It is possible to calculate nitrification kinetic parameters based on specific oxygen uptake rate (SOUR) profiles (note that the term “specific” indicates that the OUR has been normalized to the biomass concentration). The use of respirometry by previous researchers to evaluate zinc

inhibition of nitrification was summarized in the previous Phase I/ II Literature Review and Experimental Proposal. Further information concerning commercial instruments available, respirometer operation, and specific details related to the evaluation of nitrification inhibition are available in the following references: Young and Cowan, 2004; Spanjers et al., 1998; Love and Bott, 2000.

2. Ammonia Uptake Rate – Nitrate/Nitrite Generation Rate: In order to evaluate the nitrification process fully independent of heterotrophic activity, kinetic rates directly related to the consumption or production of reactants and products of the nitrification process itself can be measured, specifically nitrate/nitrite generation rate (NGR) or ammonia uptake rate (AUR). The specific nitrification rate (SNR) can be obtained by normalizing to biomass concentration. The SNR data can then be used to determine the autotrophic kinetic parameters described above. For these experiments, a sample of mixed liquor is added to small temperature controlled reactor vessels (typically less than 1.0-3.0 L). The vessel is mixed and aerated, and the chemical toxin of interest added to the stressed reactor. After the consumption of residual organic substrate associated with the mixed liquor sample itself, ammonia or nitrite is spiked into the reactor, and again care must be taken to ensure that pH and alkalinity remain within acceptable limits – preferably pH 7 to 8 at all times. The nitrate, nitrite, and ammonia concentrations are monitored over time using typical analytical methods (APHA, 1998). It is critical to rapidly separate the mixed liquor from the soluble supernatant as quickly as possible after removing a sample from the reaction vessel. Typically, samples are removed from the reaction vessels at predetermined time intervals and rapidly centrifuged. The supernatant is poured off and immediately filtered through a 0.20 µm membrane filter. The filtrate can then be preserved for subsequent analysis. As discussed in Table 3, these techniques have also been used to evaluate zinc inhibition of nitrification. More detailed procedures can be found in the following references: Kelly et al., 2004; Madoni et al.,1999; Kelly and Love, 2004; Daigger and Sadick, 1997; Panswad and Polprucksa, 1998. In addition, several relatively new experimental protocols that are derivations of these nitrate generation/ammonia uptake rate measurement procedures have been formalized and reported by Melcer et al. (2003) that allow for direct calculation of $\mu_{max,a}$ and b_a (See Chapters 16 and 17 of the Melcer et al. (2003) report).

3. Specific Denitrification Rate (SDNR): In order to evaluate the effect of chemical toxins on denitrification rates, short-term experiments can be conducted in which the rate of nitrate uptake is measured (nitrate uptake rate = NUR). These experiments are performed in a similar manner to that described above for the AUR/NGR nitrification methods. A sample of mixed liquor is added to temperature-controlled reaction vessels with and without a chemical stressor. The sample is spiked with nitrate and a readily degradable organic substrate (e.g. acetate). The reactor contents are mixed but maintained under anoxic conditions by N_2 gas (or N_2/CO_2 blend) sparging. The concentration of nitrate is monitored over time and can be used to calculate the specific denitrification rate (SDNR). These methods were used by Panswad and Polprucksa (1998), Waara (1992), and Petersen et al. (2002).

4. Alkalinity Titration: Titration bioassay systems monitor the rate of alkalinity consumption by ammonia-spiked or wastewater-spiked mixed liquor. Since the nitrification process consumes a significant amount of alkalinity, the rate of alkalinity consumption is proportional to the nitrification rate, and these measurements have been found to be relatively sensitive indicators of inhibition. The system works by measuring the amount of base (e.g. NaOH) needed to maintain constant pH in the reaction vessel. Similarly, titration bioassay systems can be used to monitor denitrification rates by tracking the amount of acid required to maintain constant pH. This technology was reviewed in detail by Love and Bott (2000).

For this investigation, a combination of respirometry and AUR/NGR measurements will be performed to evaluate the impact of zinc on nitrification. Although denitrification inhibition is not expected to be problematic, it is also recommended that a brief investigation be conducted to evaluate of the impact of zinc on SDNR per the NUR method described above.

2.3 Experimental Objectives and Hypothesis

As noted above, the maximum increase in influent total zinc level for the Arlington WPCP is expected to be in the range of 0.3 mg/L, giving a total influent Zn concentration in the range of 0.5 mg/L. The objective of this project was to determine whether the maximum level of Zn that could be contributed by the implementation of a corrosion control program using a 1:10 ZnOP product will cause any negative impacts on the nitrification and BNR process at the Arlington WPCP. Based on the literature review presented above, it seems reasonable to hypothesize that the increment of 0.3 mg/L total zinc will have no significant impact on nitrification at the Arlington WPCP.

> *Hypothesis – Zinc does not cause any significant impact on nitrification or BNR (performance or kinetic parameters) in the range of concentrations anticipated as a result of ZnOP addition to the water supply.*

Based on this hypothesis, the purpose of bench-scale testing was to attempt to refute or reject this hypothesis by several acceptable procedures. Evaluating this hypothesis is a much simpler objective than attempting to answer the question – "what level of influent zinc causes a measurable impact on nitrification and BNR?" If attempting to simply refute the research hypothesis, it was possible to consider experimentally worst-case inhibition scenarios at the 0.3-0.5 mg/L Zn level - specifically, no removal by primary clarification, no acclimation, at least 24 hours of chemical exposure (but exposure time issues were evaluated), and addition of a concentrated soluble Zn stock directly to a mixed liquor sample (no prior exposure of the zinc to raw wastewater or PE). If inhibition were observed under these "worst-case" conditions, it would then be possible to adjust experimental protocol to consider the factors that would tend to decrease the inhibitory effects of 0.3 mg/L total Zn added to the water supply. To be more conservative in the event that other ZnOP formulations are considered, a zinc concentration of something on the order of 1.0 mg/L was also evaluated.

As part of this experimental work, it was necessary to add Zn concentrations much higher than 0.3-0.5 mg/L range, but this was done in an attempt to validate the experimental method being used (e.g. high F/M NGR measurement) and its sensitivity to detect nitrification inhibition relative to the target Zn concentration range. The experimental protocol would become much more complicated and intensive if it were necessary to determine the maximum level of zinc that could be added to the water supply without resulting in inhibition of nitrification and BNR. As a result, it is critical to recognize the research hypothesis when interpreting inhibition. The observation of inhibition by the protocol outlined above ***does not*** suggest that the dosed Zn concentration would cause nitrification inhibition in the full-scale BNR process. Given the experimental design, conclusions regarding the performance of the full-scale system can be made only when no significant inhibition is observed in kinetic bench-scale testing (i.e. if no inhibition is observed in bench-scale kinetic testing, no inhibition in the full-scale process would be expected at that Zn concentration).

While this sounds like a relatively simple task, experiments involving kinetic measurements in actual biological treatment cultures require considerable trial and error to produce reliable and repeatable data. These experiments are time consuming and require very careful planning and

execution. Often, the data are somewhat difficult to interpret, and subsequent experiments must be designed to address this. It should be recognized that these experiments are much different than analyzing a sample of mixed liquor for a series of chemical constituents. It is very difficult to predict *a priori* what procedures or protocols will produce the best experimental data. Many site-specific factors are involved including SRT, wastewater characteristics, process configuration, behavior of the chemical toxin in the mixed liquor, background alkalinity, pH, and temperature.

The experimental overview is presented below in terms of personnel, timing, and logistics, followed by a short discussion of the three broad experimental protocols that were used for this investigation.

2.4 Overview of Experimental Methods

Three broad experimental protocols were used for this work using mixed liquor samples from the full-scale biological treatment system. These include:

- Respirometry – A new temperature-controlled, 4-cell (parallel reactor vessels) continuous respirometer with computer data acquisition was purchased for this project. The system has the capability to be operated with continuous OUR measurements for periods up to 24-48 hours. The respirometer was purchased by the Arlington WPCP, installed in the VMI Environmental Engineering Laboratory, used for Research Assistant (RA) training purposes, and was transported to the Arlington WPCP lab for experimental work. Respirometry experiments generally have been performed per the protocols of the equipment manufacturer and that described by Young and Cowan (2004) and Spanjers et al. (1998) to evaluate nitrification inhibition.
- AUR/NGR – Experiments were conducted using mixed/aerated and temperature-controlled reactor vessels per the "High F/M batch test" method described by Melcer et al. (2003) with some modification. Direct measurements of AUR and NGR were also conducted at MLSS concentrations typical of the full-scale process ("Snapshot Experiments"). Substrate was added in the form of either ammonia or nitrite (i.e. NH_4Cl or $NaNO_2$), and pH and alkalinity were controlled. The concentrations of ammonia (total ammonia nitrogen – NH_4-N), NO_3-N and NO_2-N were monitored over time.
- SDNR – A single experiment was conducted using mixed/N_2 sparged and temperature-controlled reactor vessels at MLSS concentrations typical of the full-scale process. Substrate was added in the form of a readily degradable organic compounds (i.e. acetate) and nitrate (i.e. $NaNO_3$). The concentration of NO_3-N was monitored over time.

Two research assistants (RAs) from the Department of Civil and Environmental Engineering at the Virginia Military Institute conducted the experimental work over the months of June and July, 2005 in the Arlington WPCP laboratory under the direct supervision and guidance of Charles Bott. While working at the Arlington WPCP, the RAs also worked under the direct supervision of plant personnel, including the Laboratory Manager, the Pretreatment Program Coordinator, and the Operations Specialist. The RAs and Charles Bott conducted the standard laboratory safety training (and other safety training) as prescribed by the Arlington Laboratory Manager and/or other supervisors. Charles Bott was onsite at the Arlington WPCP to work with

the RAs in the laboratory, review data, and meet with Arlington personnel one to two days per week over the duration of the experiments.

The RAs were trained over the course of the spring semester (February – May, 2005) in the Environmental Engineering Lab at VMI. Actual experiments, similar to those that were subsequently performed at the Arlington WPCP, were conducted in the Environmental Engineering Lab using fresh mixed liquor samples from the Lexington wastewater treatment plant (nitrifying oxidation ditch that achieves some denitrification) and from the Arlington WPCP, when available. One of the RAs focused on the operation of the respirometer and the other on the AUR/NGR protocols. Experiments were conducted using a wide range of zinc concentrations (0.5-50 mg/L), but focusing on the 0.3-0.5 mg/L range as described above. While working in the VMI Environmental Engineering Laboratory, the RAs prepared and analyzed samples for MLSS, MLVSS, NO_3-N, NO_2-N, and NH_4-N using equipment and instruments available.

3. METHODOLOGY FOR BENCH-SCALE TESTING

The following represents a rough chronological procedure for evaluating nitrification inhibition using both respirometry and NGR/AUR measurements and for evaluating denitrification inhibition using NUR measurements. It is important to recognize that these experiments were repeated several times to refine test variables before conducting the first useful experiment or producing the first informative data that actually provides an indication of the impact of zinc on nitrification. Training experiments with Zn stress in the 0.5-50 mg/L range were conducted in the VMI Environmental Engineering Laboratory to begin this effort of refining test variables. After refining test procedures, experiments were repeated multiple times over a period of several days or weeks to provide statistical validity. In addition, a wide range of positive and negative controls were run to validate the methods used. The basic methodology for the bench-scale experiments included:

1. Collect grab sample of return activated sludge (RAS) in a 5 gallon carboy from the sampling valve in the RAS/WAS gallery. If needed, collect a sample of PE or secondary effluent (SE) in a 5 gallon carboy by directly pumping the sampler from the existing plant composite sampler pump. Transport these samples to the laboratory.

2. While setting up experiments, aerate the RAS sample in the laboratory for approximately 2 hours to consume residual BOD and ammonia. This was done on a daily basis when experiments were performed.

3. Respirometry and AUR/NGR experiments were conducted in parallel using the same sample of mixed liquor.

The following compounds were dissolved in distilled/deionized water to make concentrated stock solutions: zinc sulfate, sodium nitrate, sodium nitrite, ammonium chloride, sodium carbonate, sodium bicarbonate, sulfuric acid, ATU, TCMP, sodium azide, and sodium acetate. RAS was typically diluted with SE. Depending on the experiment, one or more stock solutions

were added at target initial concentrations in the experiment vessel (already containing RAS + SE).

3.1 Respirometry –Challenge AER-200 Series Continuous Respirometer

The continuous respirometer was operated with 4 parallel 500 mL reaction vessels at constant temperature. To each reaction vessel RAS and primary or secondary effluent were added to simulate the full-scale process or to achieve an appropriate oxygen uptake rate (OUR) range for measurement by the respirometer.

- The respirometer system was configured and operated per the manufacturer's instructions. Data acquisition by the respirometer was initiated, and stock solutions of various chemicals were added either initially or after some reaction period had elapsed. Data acquisition was continued for 1-72 hours depending on the data produced and the goal of that specific experiment.

- Testing was conducted by adding excess ammonia-N to the reaction vessels ± zinc and ± nitrification inhibitor (ATU). For these experiments, four ammonia-spiked reaction vessels were run in parallel: zinc only, nitrification inhibitor only, zinc + nitrification inhibitor, and no zinc/no nitrification inhibitor. In some cases, two zinc concentrations were investigated and the zinc + nitrification inhibitor vessel was not run.

- For these experiments, it was critical to maintain careful control of pH in the target range of 7.0-7.8. In most scenarios, the dilution water could be simply supplemented with a phosphate-based buffer to control pH. However, since high levels of phosphate would be expected to precipitate the added Zn, it was not possible to use phosphate-based buffers for pH control, because the phosphate would likely alleviate the inhibition potential of Zn on nitrification. Rather, sodium bicarbonate or sodium carbonate was supplemented as needed to control the pH in the target range (see procedure below).

To assess the impact of zinc on NOB rather than AOB, nitrite-N was spiked to the mixed liquor rather than ammonia-N. Alkalinity in the form of sodium bicarbonate or sodium carbonate was added as needed to ensure that the pH remained between 7 and 8 throughout the test period. Sufficient ammonia/nitrite must also be added (or additional supplemented during the experiment) to ensure that the measured nitrification rate was not limited by ammonia/nitrite. However, to avoid free ammonia inhibition of nitrification (typically nitrite oxidation), the reactor ammonia-N concentration added should not exceed roughly 75-150 mg/L NH_4-N. This was determined by tracking the total oxygen consumed in each test vessel and/or measuring the residual ammonia-N remaining in the reaction vessel at the end of an experiment.

- The MLSS, MLVSS, NH_4-N, NO_3-N, and NO_2-N concentrations were measured at the end of formal experimental runs, but not for preliminary experiments unless needed as information for optimizing SOUR measurements. Total and soluble zinc were also measured for formal experimental runs, and a 0.45 µm membrane filters were used to be consistent with typical sampling protocol for heavy metals.

3.1.1 Materials

- Fresh activated sludge cultures were removed from the RAS line at the Arlington WPCP
- Arlington WPCP secondary effluent sampled fresh from the composite sampler pump.
- Challenge AER-200 bench-scale respirometer system with 4x500 mL reaction vessels, a magnetic stirring base for mixing contents of reaction vessels at rates to provide sufficient oxygen transfer, and water bath for maintaining constant temperature.
- Sample storage vials suitable for subsequent chemical analysis.
- Reagent or analytical grade chemicals for stock solutions:
    - Ammonium chloride, NH_4Cl (Stock Solution 25 g/L NH_4-N)
    - Allylthiourea, ATU (Stock Solution 10 g/L ATU)
    - Zinc sulfate, $ZnSO_4$ (Stock Solution 153.8 g/L $ZnSO_4 \cdot 7H_2O$)
    - Alkalinity, $NaHCO_3$ or Na_2CO_3 (added neat/dry or from stock solution 75 g/L as $CaCO_3$)
    - Potassium hydroxide, KOH (Stock Solution 30% w/w KOH)
    - Sulfuric Acid, H_2SO_4 (Stock Solution 10 N H_2SO_4)
    - Sodium nitrite, $NaNO_2$ (Stock Solution 25 g/L NO_2-N)

3.1.2 Respirometry Method in General

3.1.2.1 Test Vessel Setup

1. All test bottles, caps, Teflon inserts were cleaned using conventional laboratory cleaners. Each of the above were subsequently rinsed thoroughly.
2. A Teflon–coated magnetic stirring bar was inserted in each bottle.
3. The required volume of secondary effluent (tap water for some preliminary experiments) was added to each of the 4 respirometer vessels. (Note: respirometer bottles/test vessels were always operated at a final volume of 500 mL.)
4. A bicarbonate solution made from a 75 g/L stock solution was added to each test vessel to achieve 250 mg/L alkalinity as $CaCO_3$. The amount initially added was based on the alkalinity of the secondary effluent.
5. Each of the 4 bottles was placed in a water bath to achieve the desired temperature of 20^oC.
6. Approximately 7 mL of 30% w/w potassium hydroxide solution was added to the carbon dioxide absorption tubes.
7. The activated sludge dosage was added to each of the 4 bottles and they were placed on the stirring base of the respirometer to mix at approximately 900-1000 rpm's.
8. The pre-determined doses of ammonia (or nitrite), zinc, and ATU were added to the respective bottles in a timely fashion.
9. The carbon dioxide absorption tubes and their respective inserts were inserted into each bottle and the bottles were capped tightly.
10. The needles attached to the respective flow-measuring cells were inserted into the septa of the test vessels. For most dependable operation, the needle was inserted in the edge of the septum at an angle that did not cause strain on the septum.
11. The headspace gas was withdrawn from each vessel by inserting a 10-mL syringe and a 20-gage needle. 4-5 bubbles were allowed to pass through the respective cells.

3.1.2.2 Respirometer Startup Procedure

1. Each of the respirometer components was turned on, with the exception of the stirring base, which was started after the test vessels had been placed on the stirring base.
2. The constant temperature circulator was adjusted to run the experiment at the desired temperature of 20^{o}C.
3. The flow valve on the oxygen supply cylinder pressure regulator was adjusted to deliver 2 to 3 psi pressure. The oxygen flow was adjusted using the outlet needle valve and pressure regulator to provide about 2 bubbles per second through the oxygen bypass bottle.
4. The oxygen bypass line was clamped to purge the cells and the oxygen lines with pure O_2.
5. The computer software was then setup and initialized.
6. Constituents were added to the reaction vessels, and the experiment was started.

3.1.2.3 Respirometer Operation

1. The respirometer was left to run on approximate 12 hour intervals after which the instrument was stopped to determine the ammonia or nitrite consumed by subtracting the oxygen uptake in the fully inhibited positive control vessel from the nitrifying negative control vessel.
2. The ammonia consumed was calculated via the stoichiometric factor of 4.25 g O_2 consumed per g NH_4-N oxidized to NO_3-N; the nitrite consumed was calculated by the factor of 1.0 g O_2 consumed per g NO_2-N oxidized to NO_3-N.
3. Ammonia or nitrite was then added at a concentration sufficient enough to bring levels back to the original 50 mg/L.
4. Alkalinity was also added on approximate 12 hour intervals from a 75 g/L bicarbonate solution at a concentration sufficient to raise the pH between the desired levels of 7.0-8.0.

3.1.3 Analytical Methods

At the conclusion of each successful respirometry experiment, the reactor vessels were sampled for NH_4-N, NO_3-N, NO_2-N, MLSS, MLVSS, ortho-phosphate-P, total Zn, and soluble Zn. For NH_4-N, NO_3-N, NO_2-N analysis, approximately 55 mL samples were removed from the bottles using a 60 mL syringe and filtered through a 0.20 μm membrane filter. Approximately 40 mL was filtered into a 60 mL HDPE sample bottle for nitrogen analysis by Arlington WPCP personnel, and the remaining sample was filtered into 40 mL U.S. Environmental Protection Agency (EPA) bottle for analysis using Hach colorimetric methods for NO_2-N and NO_2-N+NO_3-N. Arlington WPCP personnel performed sample analysis for these constituents using an in-house flow-injection analyzer. Sample dilutions were prepared by the VMI RAs prior to submitting samples and dilutions were performed by the Arlington WPCP personnel. Samples were removed for total Zn analysis, pre-filtered using 0.45 μm membrane filters for soluble Zn analysis, and preserved using nitric acid prior to being shipped to a contract laboratory for analysis. Samples for MLSS and MLVSS were removed and submitted to Arlington WPCP

personnel for analysis by *Standard Methods*. In addition, the temperature and pH were measured for each bottle after the conclusion of the respirometry run to determine if the experiment was running at the desired pH and temperature.

3.1.4 Evaluation of Kinetic Parameters

Nitrification kinetic parameters were calculated based on the specific oxygen uptake rate (SOUR). The specific oxygen uptake rate was measured under a wide range of conditions for nitrification and aerobic heterotrophic growth, including endogenous respiration. It is important to recognize that the term "specific" indicates that the OUR has been normalized to the biomass concentration (MLVSS). The SOUR, which is represented in units of mg O_2/g MLVSS/hour, was used to determine the effect of zinc on the maximum SNR and on K_N. The following general calculation procedure were performed:

1. Respirometer data was evaluated to determine average SOURs for each of the four reaction vessels.
2. The ATU-stressed SOUR was subtracted from the control SOUR ($NH4\text{-}_N$ only) to determine the SOUR due to nitrification. The same procedure was used to determine the SOUR due to nitrification and impacted by Zn.
3. The SOUR due to nitrification was divided by the common factor 4.25 g O_2 used per g NH_4-N nitrified to NO_3-N. As long as the NH_4-N concentration in the respirometer vessel remained above approximately 10 mg/L (well above the K_N value), this provided an estimate of the maximum SNR in units of mg NH_4-N oxidized per mg MLVSS per hour – which is related to the maximum specific autotrophic growth rate, $\mu_{max,a}$, through the autotrophic yield (which can be assumed constant). The impact of Zn on the maximum SNR can be assessed in this manner.

For experiments where the NH_4-N concentration was permitted to decline to levels less than the 15-20 mg/L range, a general indication of the effect of Zn on K_N can be assessed by comparing the SOUR curves due to nitrification (mathematical interpretation was not performed). The SOUR curve due to uninhibited nitrification would be expected to look like a straight line, then curving to zero-slope as the NH_4-N concentration approaches K_N and below.

3.2 Nitrate Generation Measurements

3.2.1 Overview

These experiments were conducted in four aerated and mixed 4 L reactor vessels operated in parallel in a constant temperature room. Aeration was provided by aquarium-style air pumps and a small diffuser stone, and mixing was provided with a paddle driven by an electric motor at 100 RPM. Over the course of each experiment, NH_4-N, NO_3-N, and NO_2-N were measured to calculate the NGR.

Two different experimental protocols were considered for these experiments (Table 2). The two protocols that were used to evaluate zinc inhibition of nitrification includes:

1. Full-Scale Simulation - NGR (similar to SNR protocol used for respirometry)
To each reactor vessel, RAS + tap water/secondary effluent were added to simulate the full-scale process or to achieve an appropriate NGR for measurement over a target exposure period (2-72 hours). Testing was conducted by adding excess ammonia-N to the reaction vessels ± zinc and ± nitrification inhibitor (ATU). Nitrification inhibitor was used as a positive control for nitrification inhibition. For these experiments, a control, zinc-stressed reactors at two different concentrations, and an ATU-stressed reactor at 20 mg/L were operated. Alkalinity in the form of sodium bicarbonate was added as needed to ensure that the pH remained between 7.2 and 7.8 throughout the test period (established during initial experiments). Sufficient ammonia was added and intermittently supplemented during the experiment, to ensure that the measured nitrification rate was not limited by ammonia. However, to avoid ammonia inhibition of NOB, the reactor ammonia-N concentration added did not exceed roughly 50 mg/L NH_4-N (as determined in preliminary experiments). It was also confirmed via use of a DO probe/monitoring system that the reactor vessel DO was consistently maintained above 3-4 mg/L. The MLSS and MLVSS concentrations were measured at the end of formal experimental runs, but not for preliminary experiments. Total and soluble zinc was measured for formal experimental runs, and a 0.45 µm filter was used to be consistent with the sampling protocol described below.

2. High F/M Batch Test (per report by Melcer et al., 2003)
This protocol was similar to that described above for the NGR experiments except that the test was conducted over a period of several (4-7) days and a very small amount of nitrifying mixed liquor was used (in the range of 30-35 mg/L MLVSS). Due to the low initial concentration of nitrifying biomass, rapid growth of autotrophs over the experimental period results in an exponential curve describing ammonia-N consumption and nitrate-N generation. If the concentration of NH_4-N concentration remains significantly above the K_N value (typically 0.5-2 mg/L NH_4-N) and if the DO is maintained consistently above 3-4 mg/L, the data generated by this procedure provide a direct estimate of the quantity $\mu_{max,a}$ minus b_a ($\mu_{max,a}$-b_a). Alkalinity and pH were considered as discussed above, and preliminary experiments were used to develop techniques for proper control. The MLSS and MLVSS concentrations were measured at the end of formal experimental runs, but not for preliminary experiments. Total and

soluble zinc were measured for formal experimental runs, and 0.45 µm filters were used to be consistent with the sampling protocol described below.

Table 2. Summary of NGR and HFM Experimental Protocols

Protocol	Duration	Target MLSS/MLVSS	Inhibitor used	Zn Source	Nitrogen Source	Target pH	pH Control
NGR	2-72 hours	1000 mg/L MLSS	ATU	$ZnSO_4$-$7H_2O$	NH_4Cl or $(NH_4)_2SO_4$	7.2-7.8	NaHCO3 or Na2CO3
HFM	4-7 days	35 mg/L MLVSS	ATU	$ZnSO_4$-$7H_2O$		7.2-7.8	NaHCO3 and Na2CO3 + sulfuric acid as needed

Protocol	Zinc Dose Range	Sampling Frequency	Sample Filtration	Data Analysis
NGR	0.5 – 50 mg/L Zn	Every 2-6 hours	0.20 µm for N species; 0.45 µm for Zn	Simple Linear Regression
HFM	0.5-10 mg/L Zn	2-3 times per day	0.20 µm for N species; 0.45 µm for Zn	Sigma Plot non-linear regression or manual non-linear least squares regression using Excel Solver

3.2.2 Full-Scale Simulation Method - NGR

3.2.2.1 Initial experiment considerations

As established previously, zinc inhibition is a function of MLSS, which was verified in preliminary experiments. Therefore concentrations of MLSS concentrations less than 3000 mg/L (typical aeration basin concentration), were considered for all experiments in attempt to make zinc as inhibitory as possible.

3.2.2.2 Apparatus Set-up

All experiments used four 3.5L glass beakers as reactors. An aquarium style pump provided aeration through vinyl and glass tubing and diffused through an air stone. Each reactor had a 3" paddle stirrer attached to a 100 rpm, 115V and 0.3 amp electric motor. There was some concern for Zn sorption to the glass reactors, and this was addressed by total and soluble Zn analyses at the end of critical experiments (see Section 5.5 below).

3.2.2.3 Reactor Overview

The four reactors used for each trial consisted of a positive control, a negative control and two concentrations of zinc. All four reactors were started at the same concentration of MLSS (typically a target of 1000 mg/L), initial concentration of NH4-N (typically 50 mg/L –N), and alkalinity added with sodium bicarbonate. The positive control reactor received 20 mg/L ATU, which is a known and complete inhibitor of AOB. Two reactors were stressed with zinc, with one typically at the target concentration of 0.5 mg/L.

3.2.2.4 Experiment start-up and additions

The amount of diluent (secondary effluent) added was based on the amount of mixed liquor added to achieve 1000 mg/L, and to dilute to a volume of 3.5 L. Ammonia was added to the reactors using a stock solution of $(NH_4)_2SO_4$ or NH_4Cl. Sodium bicarbonate was added as necessary into all four reactors. Alkalinity was consumed throughout the duration of each test,

and an appropriate amount was added based on pH monitoring. The next addition was of ATU to the positive control reactor only. Finally, zinc and ATU were added from individual stock solutions.

3.2.2.5 pH Monitoring and Control

As discussed previously, pH control issues were one of the most challenging aspects of these experiments. Careful pH monitoring was essential to maintain the desired pH of 7.2-7.8 throughout the test. Preliminary experiments demonstrated that uninhibited reactors consumed much more alkalinity and need intermittent dosages of sodium bicarbonate through the testing period. Similarly, inhibited reactors maintained pH and typically required no additional alkalinity. If the pH increased to a level above the desired level of 7.8 due to excessive CO_2 stripping, then concentrated sulfuric acid (H_2SO_4) was added to decrease the pH.

3.2.2.6 Dissolved Oxygen Monitoring and Control

To maintain uninhibited nitrification and to avoid simultaneous denitrification (which would significantly affect NGR measurements), reactors must remain under aerobic conditions at DO concentrations above 2-4 mg/L. The DO concentration was monitored using a DO probe during preliminary experiments at VMI. It became apparent that the technique used for aeration was more than adequate, and that future experiments would not require DO monitoring. To avoid excessive CO_2 stripping, the aeration rate was controlled by adjusting the air pumps and by partially clamping off the tubing from the air pump.

3.2.2.7 Sampling and Analysis

Test duration determined the frequency and quantity of sampling. When test duration was 24 hours or less, samples were taken approximately every 3 hours. When test duration was greater than 24 hours, sampling occured 3-5 times a day. During each sample, the temperature of the reactors was measured and recorded. Initial samples were always taken within the first 15 minutes of the experimental period. Samples were removed from the Reactors using a 30 mL syringe. Approximately 5-10 mL (or larger depending on analysis technique) were removed from each reactor with a 30 or 60 mL syringe (once again dependent on sample size) at the designated sampling time, then filtered through a 0.20 μm filter into a vial. Filtered samples were analyzed for NH_4-N, NO_3-N, and NO_2-N. Arlington WPCP personnel performed sample analysis for these constituents using an in-house flow-injection analyzer. Sample dilutions were indicated by the VMI RAs prior to submitting samples and dilutions were performed by the Arlington WPCP personnel. Analysis of these constituents was also performed for NO_2-N and NO_2-N+NO_3-N by Hach colorimetric methods by the VMI RAs. At the end of formal experiments, samples were removed for total Zn analysis, pre-filtered using 0.45 μm membrane filters for soluble Zn analysis, and preserved using nitric acid prior to be shipped to a contract laboratory for analysis. Samples were also removed for soluble ortho-phosphate-P, MLSS and MLVSS with analysis by *Standard Methods* and conducted by Arlington WPCP personnel.

3.2.2.8 Data Interpretation

Data were typically plotted from these experiments as NO_x-N versus time, where NO_x-N represents the sum of NO_3-N + NO_2-N. Linear regressions were performed to calculate respective NGRs, which were then divided by the MLVSS concentration, providing a specific NGR in units of mg NO_x-N/g MLVSS hr.

3.2.3 High F/M Method (per Melcer et al., 2003)

The High F/M experiments were conducted identically to the NGR tests described above with the exception of the following (Table 2):

- Initial concentration of MLVSS in the range of 35 mg/L
- Test duration ranges from 4-7 days.
- Sampling volume was considered and accounted for during subsequent additions of alkalinity and ammonia.
- Since excessive aeration can magnify the problem of evaporation, aeration rates were controlled to a very low level, yet still capable of maintaining sufficient DO throughout the experiment.
- Analysis was performed using the nonlinear regression package in Sigma Plot 9.01 (Systat Software, Inc.) to solve for an estimate of the maximum specific growth rate ($\mu_{aut} = \mu_{max,a}$).
- All data was modeled to fit the equation fully developed in Mercer et al. (2003):

$$S_{NO,t} = S_{NO,0} + \frac{\mu_{aut} * X_{AUT,0}}{Y_{aut} * (\mu_{aut} - b_{aut})} * [e^{(\mu_{aut} - b_{aut})t} - 1] \qquad (1)$$

  where, $S_{NO,t}$ is the NO_x-N concentration at time t, $S_{NO,0}$ is the NO_x-N concentration at time zero, Y_{aut} is the nitrifier yield coefficient, b_{aut} is the nitrifier decay rate, $X_{aut,0}$ is the initial nitrifier concentration, and μ_{aut} is the maximum autotrophic specific growth rate.

Based on well accepted values, Y_{aut} (mg/mg) and b_{aut} (d^{-1}) were both assumed to be 0.15 (Melcer, et al., 2003). Using these established values, nonlinear regression analysis was performed for a plot of NO_x-N versus time, and estimates of $X_{aut,0}$, $S_{NO,0}$ and of particular interest μ_{aut} were computed. The resulting μ_{aut} is indicative of nitrifier health, which can be compared to well-accepted established values and computed values of zinc-stressed mixed liquor.

3.3 Denitrification Inhibition - NUR (SDNR) Measurements

This experiment was conducted exactly like the Full-Scale Simulation NGR/AUR investigations, except for the following:

- As noted above, the reactor vessels were mixed using a stirring paddle. However, to maintain anoxic conditions in the reactors, nitrogen gas (supplemented with ~315ppmv CO_2) was sparged from a compressed gas cylinder/regulator and using small diffuser stones.
- The reactors were spiked with sodium nitrate (~50 mg/L NO_3-N) and sodium acetate (~500 mg/L COD).
- Sodium azide was used as a positive control for denitrification inhibition at a concentration of 20 mg/L.
- Consumption of NO_3-N was monitored over time. Samples were removed from the reactor vessel and processed per the above procedures.
- Since denitrification produces alkalinity, it was necessary to monitor pH to maintain a target range of 7-8. When necessary, sulfuric acid was added to maintain this range.

4. RESPIROMETRY RESULTS AND CONCLUSIONS

In the early stages of this experimental work, zinc concentrations significantly higher than the proposed 0.3-0.5 mg/L dosage were used to validate the experimental method used. Thus, higher doses of zinc were used as a control and measured the ability of the respirometry method's sensitivity to detect nitrification inhibition. In each of the experiments conducted at the Arlington WPCP, it was assumed that the vessel dosed with only 50 mg/L NH_4-N represented uninhibited nitrification. From NGR experiments, it was confirmed that the vessel dosed with 50 mg/L NH_4-N + 20 mg/L ATU exhibited no significant nitrification even over a contact period up to 5 days, and that the only oxygen uptake in this vessel was due to endogenous decay associated with heterotrophic organisms. It is important to recognize that the difference in the measured SOUR between the vessel dosed with only 50 mg/L NH_4-N and the vessel dosed with 50 mg/L NH_4-N + 20 mg/L ATU represents the oxygen consumed by nitrification. In several instances, especially with higher zinc concentrations, the oxygen uptake associated with the vessel dosed with 50 mg/L NH_4-N + 20 mg/L ATU + zinc was lower than the vessel dosed with 50 mg/L NH_4-N + 20 mg/L ATU. This suggests that zinc combined with 20 mg/L ATU caused slight inhibition of heterotrophic respiration even under endogenous decay conditions (no substrate added).

4.1 Experiment Conducted with 50 mg/L Zinc

A series of experiments were conducted with extremely high levels of zinc in order to determine the sensitivity of the respirometer for detecting zinc-induced nitrification inhibition. The first preliminary experiment conducted in the VMI Environmental Engineering Laboratory was run with a zinc concentration of 50 mg/L and other dosages as shown in Table 3.

Table 3. Dosages for Preliminary Respirometry Experiment #1.

	Bottle 1	Bottle 2	Bottle 3	Bottle 4	Order of Addition
Legend ID	N50	N50ATU20	N50Zn50	N50ATU20Zn50	N/A
Target MLSS	1000 mg/L	1000 mg/L	1000 mg/L	1000 mg/L	2
NH_4-N	50 mg/L	50 mg/L	50 mg/L	50 mg/L	3
Zn^{2+}	0 mg/L	0 mg/L	50 mg/L	50 mg/L	5
ATU	0 mg/L	20 mg/L	0 mg/L	20 mg/L	4
Secondary Effluent	Balance	Balance	Balance	Balance	1

As indicated in Table 3, no alkalinity was initially added to maintain consistent pH between 7.0-8.0. However, 100 mg/L alkalinity as $CaCO_3$ was added to vessels 1 and 3 after roughly 22 hours based on pH declines in a similarly dosed experiment where the nitrate generation rate was measured and pH was closely monitored. It appears, however, that the low pH values are somewhat irrelevant because nitrification inhibition occurs in the bottle dosed with 50 mg/L zinc from the onset of the experiment as shown in Figure 1. It is important to recognize that as

indicated by the graph of cumulative oxygen uptake versus time, there were several complications relating to pH and alkalinity issues. The negative control vessel dosed with only 50 mg/L NH_4-N was likely inhibited by low pH conditions roughly 12 hours into the experiment. When 100 mg/L alkalinity was added 22 hours into the experiment, the oxygen uptake rate increased to a level consistent with conditions prior to pH limitations. The pH limitations observed after 12 hours of exposure represents important information for future experiments but did not impact the assessment of these data. This is because, as previously noted, zinc dosed at 50 mg/L began to inhibit nitrification from the beginning of the experiment.

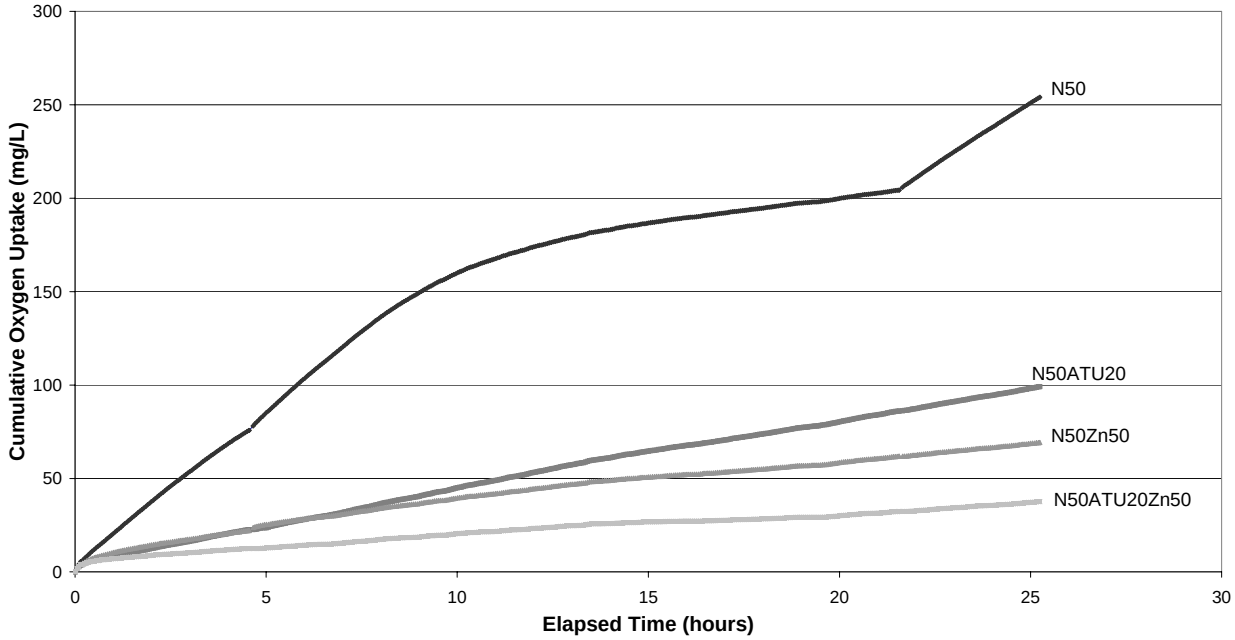


Figure 1. Cumulative Oxygen Uptake versus Time for Zinc at 50 mg/L

4.2 Experiments Conducted with 10 mg/L Zinc

Multiple experiments were run with a zinc concentration of 10 mg/L at the Arlington WPCP. Zinc at 10 mg/L induced rather dramatic inhibition in each of the experiments conducted at that concentration. Slight nitrification inhibition typically began after 2-5 hours of contact. More significant inhibition was observed to be somewhat variable and inconsistent and typically occurred between 6-12 hours into the experiment. Table 4 illustrates a representative experiment conducted with 10 mg/L zinc and diluted with secondary effluent. The secondary effluent initially contained 93.6 mg/L alkalinity as $CaCO_3$. Alkalinity was then added in the form of sodium carbonate to accomplish a total initial alkalinity concentration of roughly 250 mg/L.

Table 4. Dosages for Zinc at 10 mg/L

	Bottle 1	Bottle 2	Bottle 3	Bottle 4	Order of Addition
Legend ID	N50	N50ATU20	N50Zn50	N50ATU20Zn10	N/A

Target MLSS	1000 mg/L	1000 mg/L	1000 mg/L	1000 mg/L	3
NH_4-N	50 mg/L	50 mg/L	50 mg/L	50 mg/L	4
Zn^{2+}	0 mg/L	0 mg/L	10 mg/L	10 mg/L	6
ATU	0 mg/L	20 mg/L	0 mg/L	20 mg/L	5
Alk as $CaCO_3$	150 mg/L	150 mg/L	150 mg/L	150 mg/L	2
Secondary Effluent	Balance	Balance	Balance	Balance	1

Two experiments, one diluted with secondary effluent (Figure 2A) and the other diluted with tap water (Figure 2B) both contained zinc at 10 mg/L and were run under similar conditions to compare the diluents used. These experiments, along with additional experiments at the VMI Laboratory conducted with Arlington sludge and tap water suggest that secondary effluent may provide some protection as compared to tap water. This is possibly due to PO_4-P or low levels of residual soluble organics that quickly precipitate/complex the soluble zinc. Due to time constraints, further investigation of secondary effluent versus tap water will most likely be investigated in the fall of 2005.

Figures 2A and 2B show the specific oxygen uptake rate (SOUR) versus time for zinc at a concentration of 10 mg/L diluted with secondary effluent and tap water, respectively. Again after about 12 hours of contact, it appears that the low alkalinity/low pH possibly combined with limiting NH_4-N concentrations began to inhibit nitrification as indicated by the declining SOUR of the negative control vessel. The maximum specific nitrification rate of the control vessel (SNR_{cont}) diluted with secondary effluent was 2.712 mg NH_4-N/g MLVSS hr during roughly the 5-12 hour period when there were no pH/ammonia/alkalinity problems. During the same time period, the maximum specific nitrification rate of the vessel impacted by zinc (SNR_{zinc}) and diluted with secondary effluent was 2.178 mg NH_4-N/g MLVSS/hr, a statistically significant reduction suggesting the inihibition potential of zinc at 10 mg/L. The SNR_{cont} for the tap water experiment was 2.237 mg NH_4-N/g MLVSS/hr and the SNR_{zinc} was 2.017 mg NH_4-N/g MLVSS hr, which is also a statistically significant difference indicating the presence of nitrification inhibition caused by 10 mg/L zinc.

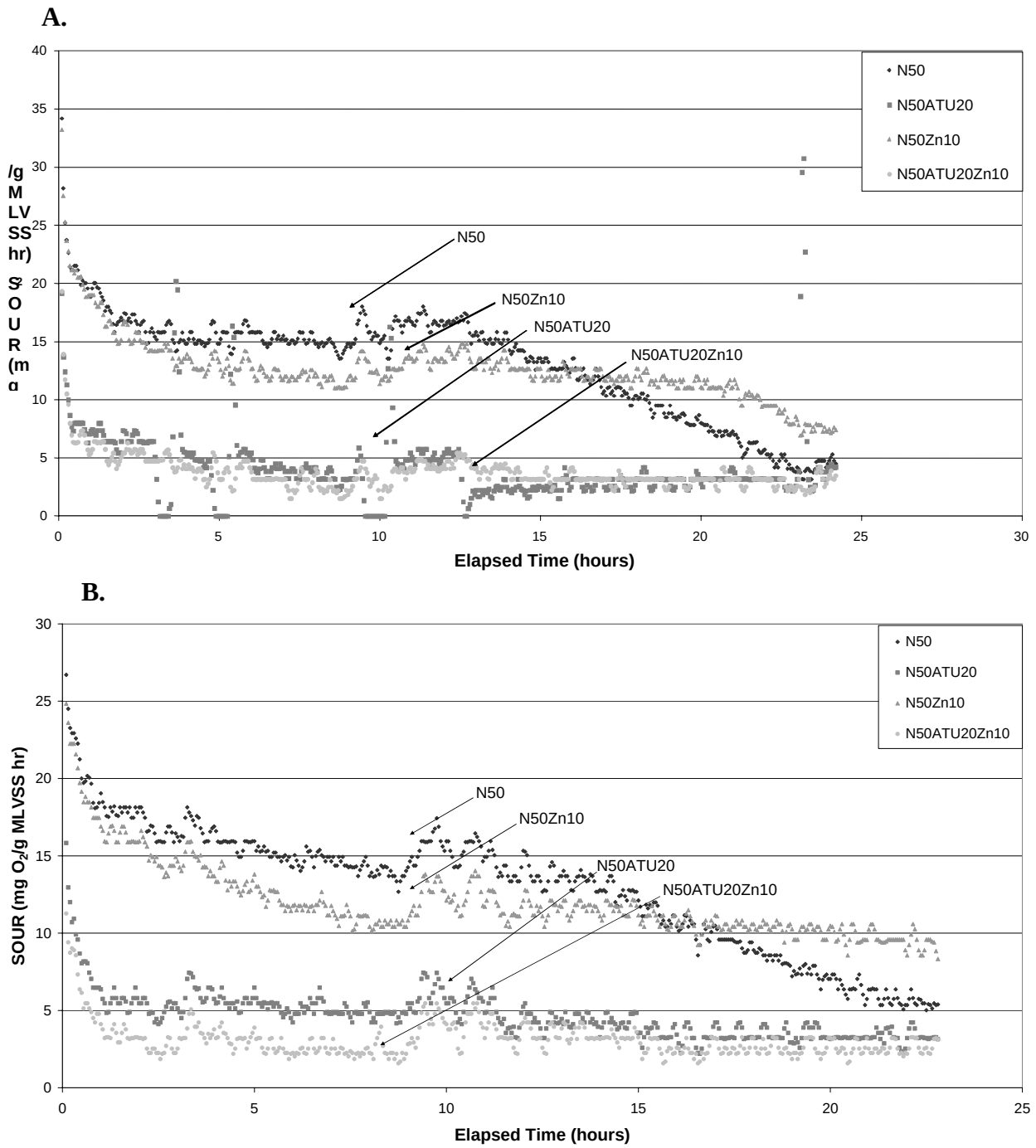


Figure 2. Specific Oxygen Uptake Rate versus Time for Zinc at 10 mg/L diluted with Secondary Effluent (A) or Tap Water (B)

4.3 Experiments Conducted with 0.5 mg/L Zinc

A series of experiments were conducted with a zinc concentration of 0.5 mg/L. The length of these experiments varied from approximately 10-72 hours. Two representative experiments running for 70 and 90 hours are discussed herein. Other short-term experiments conducted with 0.5 mg/L zinc showed similar trends, but were terminated after a shorter contact due to pH/alkalinity/ammonia problems.

The experiments conducted with 0.5 mg/L zinc were diluted with secondary effluent and setup per Table 5. The target MLSS concentration was 1000 mg/L as per previous experiments. Alkalinity was initially added to each vessel at a concentration of 150 mg/L as $CaCO_3$ to maintain the desired pH and so that the initial total alkalinity was approximately 250 mg/L as $CaCO_3$ including the alkalinity from the secondary effluent. It should be noted that the alkalinity concentrations listed in the table represent only the initial alkalinity added to each of the vessels. In many instances additional alkalinity was added throughout the experiment to maintain the desired pH.

As expected, 0.5 mg/L zinc did not appear to cause significant nitrification inhibition during any of the short-term experiments; however, several problems were encountered resulting from pH/ammonia/alkalinity/mixing problems. Since zinc at a concentration of 0.5 mg/L did not appear to inhibit nitrification over short-term contact periods, additional experiments were conducted with the same concentration over longer periods of time. Additionally, several measures were taken to ensure that nitrification inhibition due to pH/ammonia/alkalinity/mixing limitations did not occur. For instance, a battery backup and surge protector was purchased to prevent future power failures resulting in insufficient mixing. Furthermore, the experiments were monitored at intervals no longer than 12 hours. Ammonia was added to vessels 1 and 3 at a concentration sufficient enough to bring levels back to the initial 50 mg/L NH_4-N. Ammonia was added based on the oxygen required for the complete oxidation of ammonia based on the 4.25 mg O_2/mg N stoichiometric factor. It was assumed that ammonia was being completely oxidized to nitrate and this assumption was valid because there was negligible nitrite measured at the end of all of the experiments. Unlike ammonia, alkalinity was not added to bring concentrations back to the initial 250 mg/L concentration, but rather was added until the pH of vessels 1 and 3 fell into the desired range of 7.0-8.0.Thus, before pH/ammonia/alkalinity issues could inhibit nitrification, ammonia and alkalinity were added to ensure continuous uninhibited nitrification.

Table 5. Dosages for Zinc at 0.5 mg/L

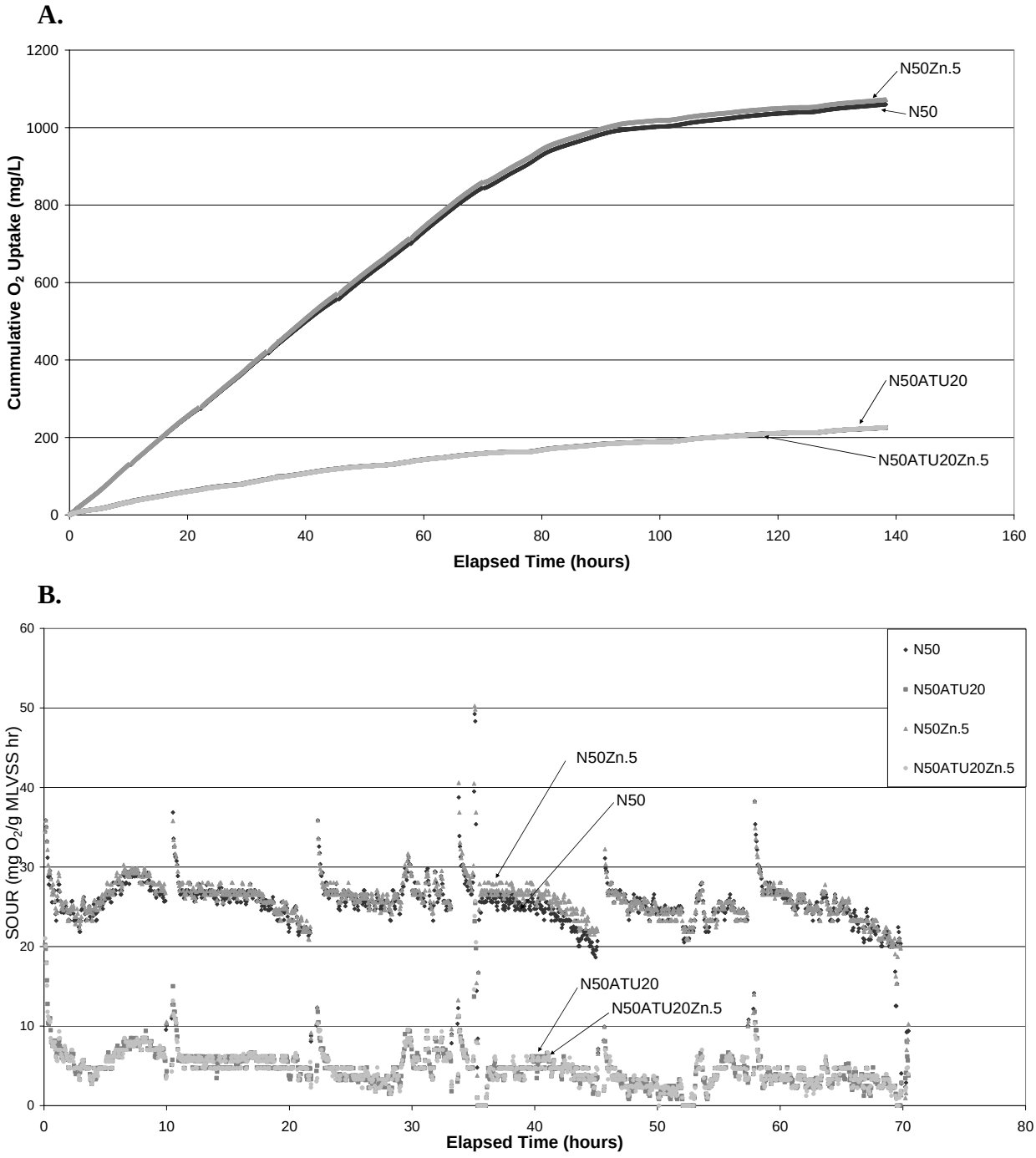
	Bottle 1	Bottle 2	Bottle 3	Bottle 4	Order of Addition
Legend ID	N50	N50ATU20	N50Zn.5	N50ATU20Zn.5	N/A
Target MLSS	1000 mg/L	1000 mg/L	1000 mg/L	1000 mg/L	3
NH_4-N	50 mg/L	50 mg/L	50 mg/L	50 mg/L	4
Zn^{2+}	0 mg/L	0 mg/L	0.5 mg/L	0.5 mg/L	6
ATU	0 mg/L	20 mg/L	0 mg/L	20 mg/L	5
Alk as $CaCO_3$	150 mg/L	150 mg/L	150 mg/L	150 mg/L	2
Secondary Effluent	Balance	Balance	Balance	Balance	1

An experiment conducted with 0.5 mg/L zinc and set up per Table 5 was monitored and run for 70 hours. The final dose of ammonia was added roughly 60 hours into the experiment and the final dose of alkalinity was added at 70 hours. The experiment was then left to run until ammonia became limiting to evaluate the effect of zinc on the autotrophic half-saturation coefficient for ammonia-N. In Figure 3A, the slope of the cumulative oxygen uptake versus time curves for the vessel dosed with 50 mg/L NH_4-N and the vessel dosed with 50 mg/L NH_4-N + 0.5 mg/L zinc were quite similar. Since, the curves remain similar even after ammonia became limiting, it is likely that zinc at a concentration of 0.5 mg/L also does not have an impact on the autotrophic half-saturation coefficient.

Over the course of 70 hours, the experiment was stopped 6 times to measure pH, add alkalinity, and add ammonia. On approximate 12 hour intervals, ammonia was added at an average concentration of 26.5 mg/L and alkalinity was added at an average concentration of 230 mg/L to maintain continuous uninhibited nitrification. As previously mentioned, it should again be noted that each time the experiment was stopped, ammonia was added to reach a concentration of 50 mg/L; however, alkalinity was added until the pH of vessels 1 and 3 fell into the desired range of 7.0-8.0.

As expected, zinc at a concentration of 0.5 mg/L did not inhibit nitrification over the 70 hour time period during which the experiment was run. The specific oxygen uptake rate (SOUR) versus time is shown in Figure 3B. The SOUR was generally consistent during the 70 hour time period during which the experiment was conducted. It is important to recognize that variability of the data that occurred roughly at 12 hours intervals represents the times when the experiment was stopped, the pH was measured, and ammonia and alkalinity were added. At roughly 70 hours into the experiment, the specific oxygen uptake rate sharply fell which represents nitrification inhibition due to insufficient ammonia concentrations. The specific oxygen uptake rates for the negative control vessel dosed with only 50 mg/L NH_4-N and the vessel dosed with 50 mg/L NH_4-N + 0.5 mg/L were generally identical which indicates that no inhibition was caused by zinc at a concentration of 0.5 mg/L. It appears that the specific oxygen uptake rate of the vessel dosed with zinc was slightly above that of the negative control vessel; however, the magnitude was not significant enough to conclude that small dosages of zinc would enhance the

nitrification process. The $SNR_{cont.}$ was 4.787 mg NH4-N/g MLVSS/hr and the SNR_{zinc} was 4.912 mg NH4-N/g MLVSS/hr.



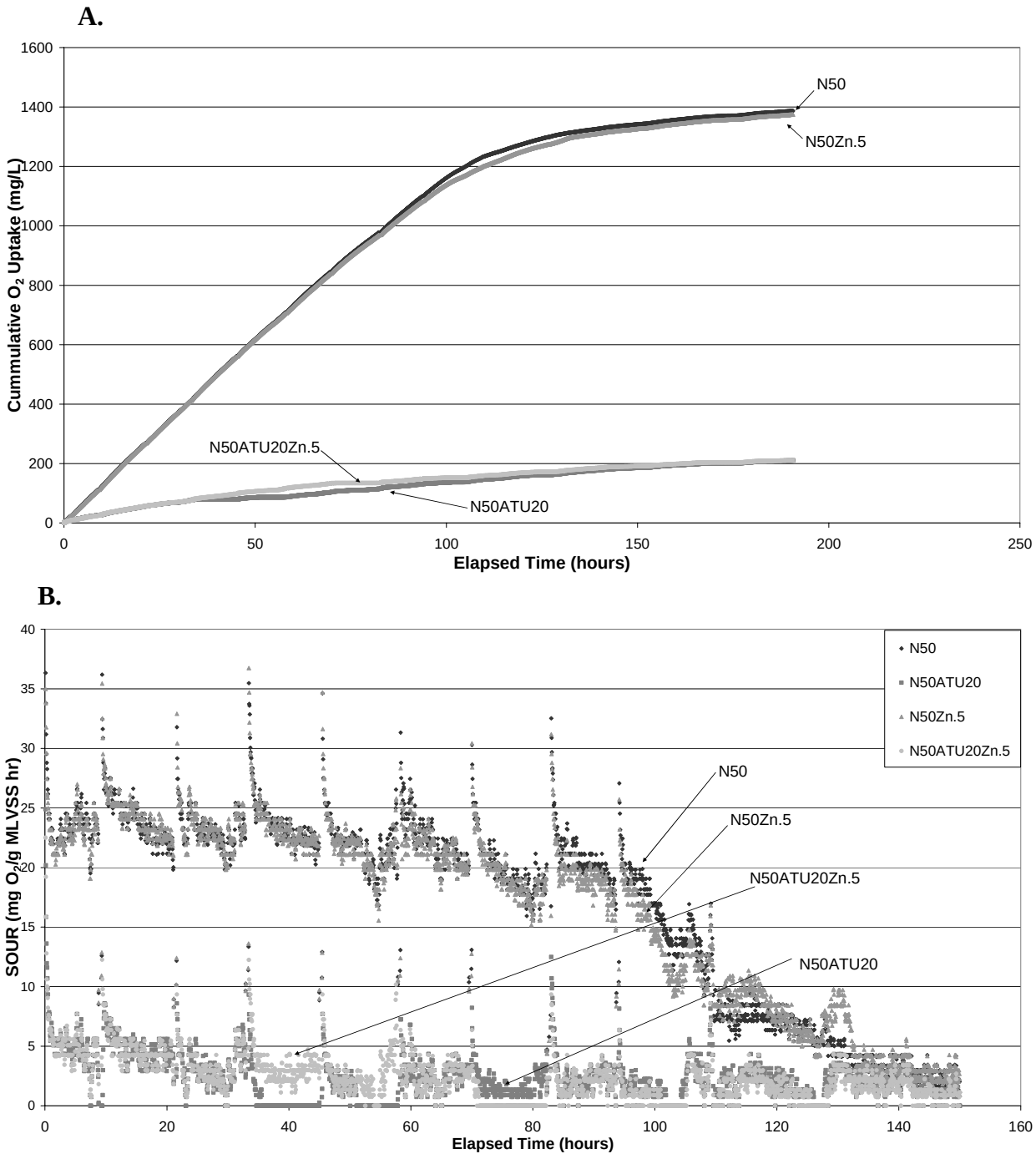
Figures 3A & 3B. Cumulative Oxygen Uptake (A) and SOUR(B) versus Time for Zinc at 0.5 mg/L (Replicate Experiment #1)

A second experiment conducted with 0.5 mg/L zinc and set up per Table 5 was monitored and run for 90 hours to determine if nitrification inhibition would occur between 70 and 90 hours

(Figure 4). The final dose of ammonia was added roughly 82 hours into the experiment and the final dose of alkalinity was added at 94 hours. As with the previous experiment conducted with 0.5 mg/L zinc, the experiment was left to run until ammonia became limiting to evaluate the effect of zinc on the autotrophic half-saturation coefficient for ammonia-N. Since the cumulative oxygen uptake of the vessel dosed with 50 mg/L NH_4-N remained consistent with the vessel dosed with 50 mg/L NH_4-N + 0.5 mg/L zinc even after ammonia became limiting, it again appeared that zinc at 0.5 mg/L had no significant impact on the autotrophic half-saturation coefficient for ammonia-N.

Over the course of 94 hours, the experiment was stopped 8 times at roughly 12 hour intervals and ammonia and alkalinity were added at average concentrations of 28.97 mg/L and 231.25 mg/L respectively. The cumulative oxygen uptake versus time plot is shown in Figure 4A. As with previous experiments conducted with 0.5 mg/L zinc, the oxygen uptakes associated with the negative control vessel dosed with only 50 mg/L NH_4-N and the vessel dosed with 50 mg/L NH_4-N + 0.5 mg/L zinc were quite similar. Additionally, the SOUR plots for both of these vessels were roughly identical as shown in Figure 4B. As previously mentioned, the drastic spikes illustrated on the SOUR plot occur when the experiment was stopped, the pH was monitored, and ammonia and alkalinity were added.

As illustrated by both the cumulative oxygen uptake (4A) and the SOUR (4B) versus time, it is reasonable to conclude that a zinc concentration of 0.5 mg/L does not cause nitrification inhibition. The SNR_{cont} and the SNR_{zinc} were 4.70 mg NH4-N/g MLVSS/hr and 4.65 mg NH4-N/g MLVSS/hr respectively, a statistically insignificant difference.



Figures 4A & 4B. Cumulative Oxygen Uptake (A) and SOUR (B) versus Time for Zinc at 0.5 mg/L (Replicate Experiment #2)

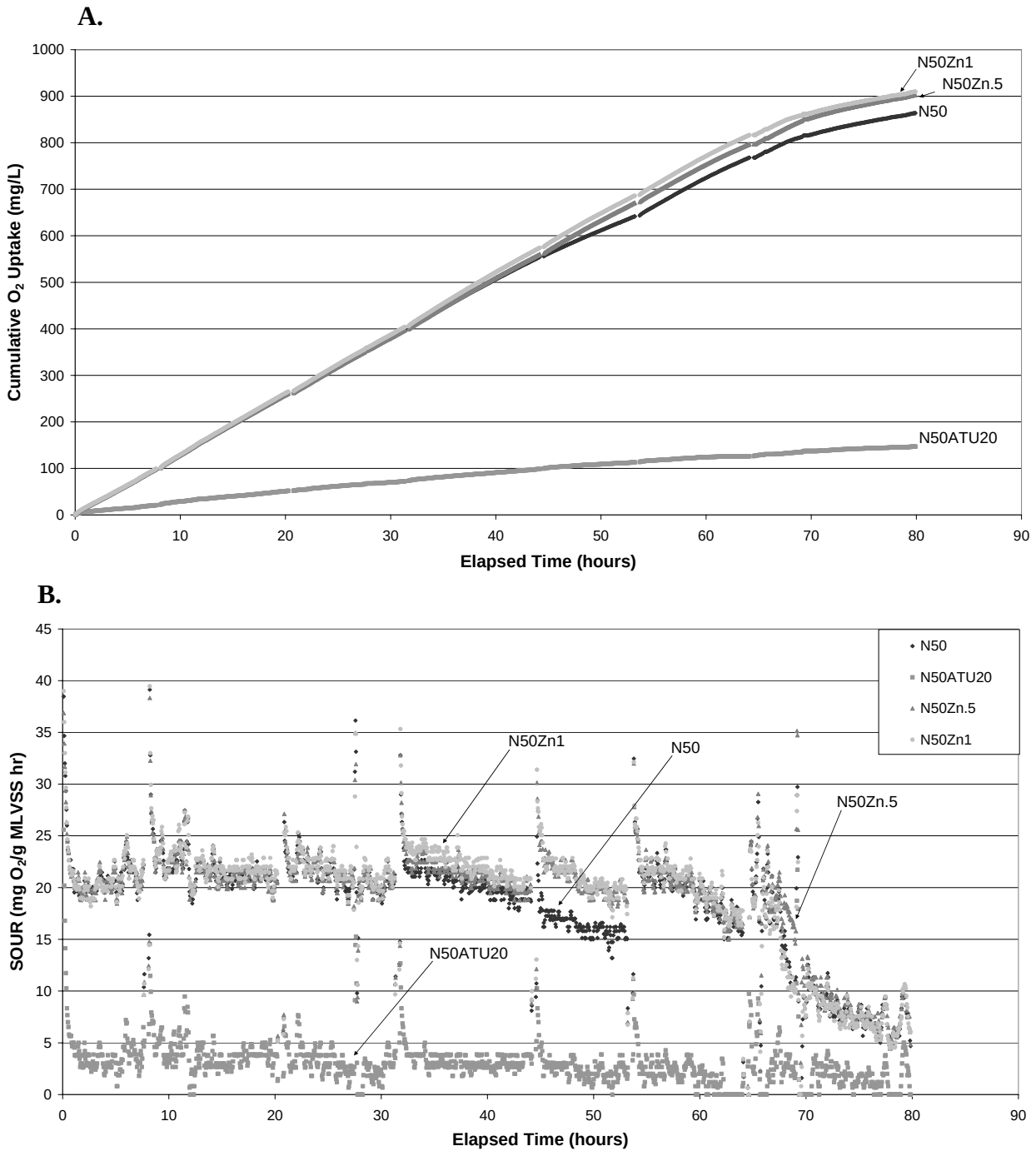
4.4 Experiments Comparing 1.0 and 0.5 mg/L Zinc

An experiment was conducted to compare zinc concentrations of 0.5 and 1.0 mg/L. As with previous experiments, 4 respirometer vessels were run in parallel: vessel 1 was dosed with only 50 mg/L NH_4-N (negative control), vessel 2 was dosed with 50 mg/L NH_4-N + 20 mg/L ATU (positive control), vessel 3 was dosed with 50 mg/L NH_4-N + 0.5 mg/L zinc, and vessel 4 was dosed with 50 mg/L NH_4-N + 1.0 mg/L zinc. It is again important to recognize that the negative control illustrated continuous uninhibited nitrification and the positive control was fully-inhibited.

Table 6. Dosages for the Experiment Comparing Zinc at 0.5 and 1.0 mg/L

	Bottle 1	Bottle 2	Bottle 3	Bottle 4	Order of Addition
Legend ID	N50	N50ATU20	N50Zn.5	N50Zn1	N/A
Target MLSS	1000 mg/L	1000 mg/L	1000 mg/L	1000 mg/L	3
NH_4-N	50 mg/L	50 mg/L	50 mg/L	50 mg/L	4
Zn^{2+}	0 mg/L	0 mg/L	0.5 mg/L	1.0 mg/L	6
ATU	0 mg/L	20 mg/L	0 mg/L	0 mg/L	5
Alk as $CaCO_3$	150 mg/L	150 mg/L	150 mg/L	150 mg/L	2
Secondary Effluent	Balance	Balance	Balance	Balance	1

The pH was closely monitored and alkalinity and ammonia were added to bring concentrations back to the original levels; this was done on approximate 12 hour intervals. It should once again be noted that the spikes and fluctuations in Figure 5 were not caused by nitrification inhibition due to zinc, but rather were generated with variations in pH and ammonia levels. Thus, spikes represent instances when the experiment was stopped and ammonia and alkalinity were added. The graphical fluctuations are relatively insignificant in determining the potential for zinc inhibition of nitrification. There was no statistically significant difference between the specific oxygen uptake rates or cumulative oxygen uptake of either of the zinc dosed vessels and the negative control vessel (Figure 5B). This suggests that zinc at both 1.0 and 0.5 mg/L had no significant impact on nitrification.



Figures 5A & 5B. Cumulative Oxygen Uptake (A) and SOUR (B) versus Time for the Experiment Comparing Zinc at 0.5 and 1.0 mg/L Concentrations

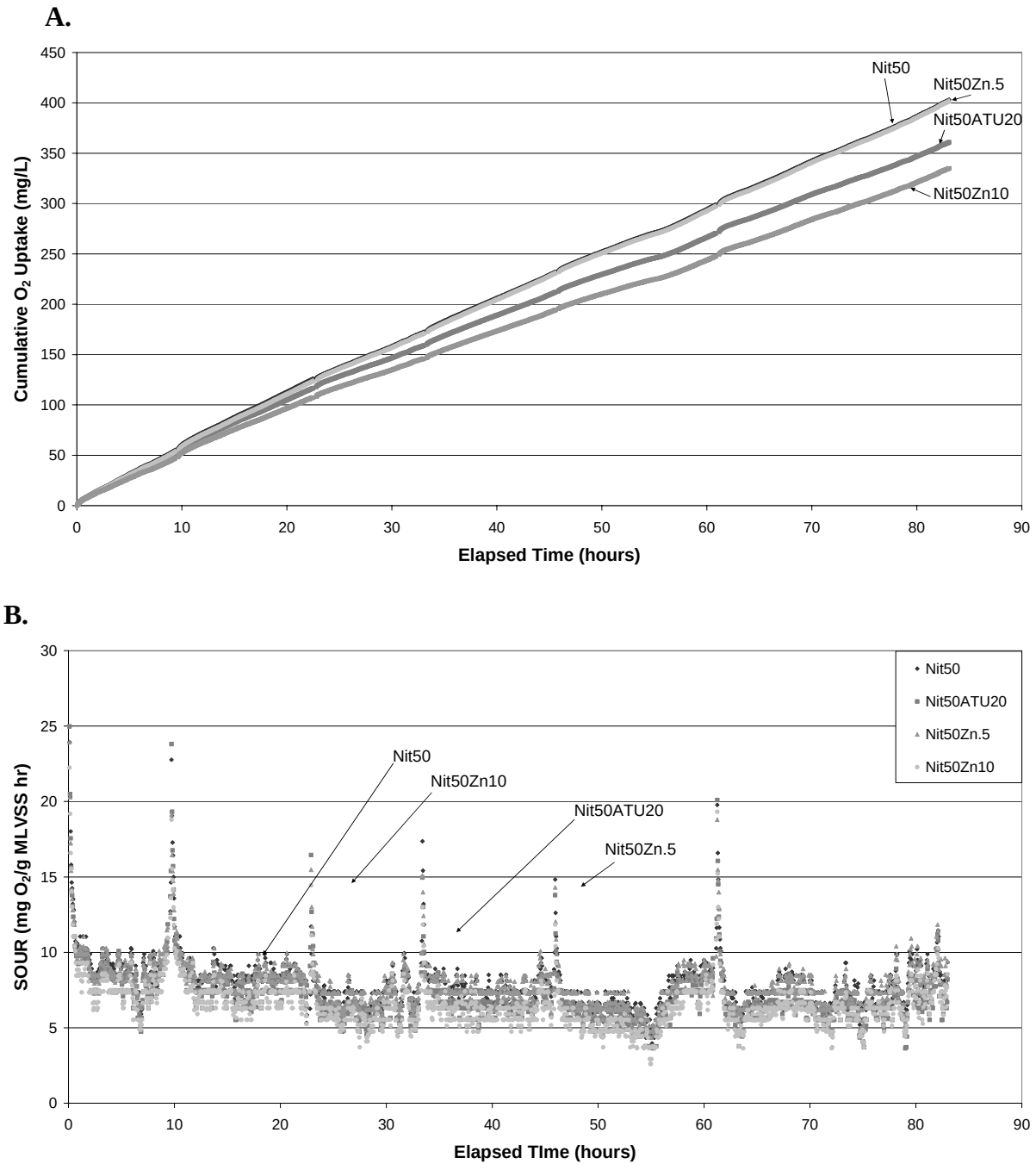
4.5 Zinc Inhibition of Nitrite Oxidizing Bacteria

An experiment was conducted to determine the effect of zinc on the second step of the nitrification process or more specifically the oxidation of nitrite to nitrate. Thus, nitrogen was added from a 25 g/L NO_2-N stock solution instead of as ammonia. Since the majority of the alkalinity consumption noted above is due to the oxidation of ammonia to nitrite, in this case, the target initial alkalinity was only 200 mg/L as $CaCO_3$, and no subsequent alkalinity addition during the experiment was required with pH levels consistently between 7.0 and 7.4. As with previous experiments, the respirometer was stopped on approximate 12 hour intervals and nitrite was added at a concentration sufficient to obtain the original 50 mg/L level. The respirometer vessels were setup in a similar manner to previous experiments. Although a positive control was run at 20 mg/L ATU, it is commonly accepted that ATU only inhibits the oxidation of ammonia to nitrite (AOB only) – not NOB.

Table 7. Experiment Considering Nitrite Oxidizing Bacteria

	Bottle 1	Bottle 2	Bottle 3	Bottle 4	Order of Addition
Legend ID	Nit50	Nit50ATU20	Nit50Zn.5	Nit50Zn10	N/A
Target MLSS	1000 mg/L	1000 mg/L	1000 mg/L	1000 mg/L	3
NO_2-N	50 mg/L	50 mg/L	50 mg/L	50 mg/L	4
Zn^{2+}	0 mg/L	0 mg/L	0.5 mg/L	10 mg/L	6
ATU	0 mg/L	20 mg/L	0 mg/L	0 mg/L	5
Alk as $CaCO_3$	100 mg/L	100 mg/L	100 mg/L	100 mg/L	2
Secondary Effluent	Balance	Balance	Balance	Balance	1

There was no significant impact of 0.5 mg/L zinc on nitrite oxidizing bacteria as shown by the cumulative oxygen uptake and the specific oxygen uptake rate versus time graphs in Figures 6A and 6B, respectively. As expected, the bottle dosed with 20 mg/L ATU did not show any significant reduction in the nitrite oxidation rate, as ATU is typically associated with inhibiting ammonia oxidizing bacteria and not nitrite oxidizing bacteria. The vessel dosed with 10 mg/L Zn only showed slight inhibition of NOB. Thus, there was no credible basis for a positive control in this experiment and a specific nitrification rate could not be determined. This is not critical given that oxygen uptake rate for the vessel dosed with 0.5 mg/L Zn was consistent with the negative control. Thus, it can be concluded that zinc at a concentration of 0.5 mg/L has no significant impact on nitrite oxidizing bacteria in the Arlington mixed liquor.



Figures 6A & 6B. Cumulative Oxygen Uptake (A) and SOUR (B) versus Time for the Experiment Assessing the Effect of Zn on NOB

4.6 Summary of Respirometry Experiments

Table 8. Summary of Significant Respirometry Experiments

Respirometry experiments								
Experimental conditions						Degree of inhibition		
Zn (mg/L)	NH_4-N (mg/L)	NO_2-N (mg/L)	Target MLSS (mg/L)	Alk (mg/L as $CaCO_3$)	Make-up water (SE or tap)	Impact of Zn on cumulative O_2 uptake (vs. ctrl)	SNR (control)[a]	SNR (Zn)[a]
0.5 (1)	50	--	1000	150	SE	None	4.80	4.91
0.5 (2)	50	--	1000	150	SE	None	4.70	4.65
0.5 (3)	50	--	1000	150	SE	None	4.08	4.11
0.5	--	50	1000	150	SE	None	--	--
1	50	--	1000	150	SE	None	4.08	4.20
10	50	--	1000	150	TAP	Slight	2.24	2.02
10	50	--	1000	150	SE	Slight	2.71	2.18
50	50	--	1000	150	SE	Severe	--	--

a. SNR units are mg NH_4-N/g MLVSS-hr.

5. NITRATE GENERATION RATE EXPERIMENT RESULTS AND CONCLUSIONS

5.1 NGR- Preliminary experiment using Lexington Mixed Liquor and Lexington Tap Water

A preliminary experiment was conducted to evaluate methods and to develop a repeatable and effective procedure. A sample of mixed liquor was removed from the Lexington, VA wastewater treatment plant on April 3, 2005 and aerated overnight. Three reactors were set-up such that, reactor A was the negative control, reactor B was dosed with 0.5 mg/L of zinc and reactor C was dosed with 50 mg/L of zinc (Table9). It should be noted that there was no positive control reactor in this experiment. Alkalinity was initially added using Na_2CO_3 (sodium carbonate) at 172 mg/L as $CaCO_3$, and the desired pH range was between 6.8 and 8.0. NH_4-N was dosed at 100 mg/L at the beginning of the experiment using an ammonium chloride stock solution. MLSS and MLVSS were analyzed in reactor A and all samples were analyzed for nitrite and nitrate by ion chromatography.

Table 9. Reactor Setup of Preliminary NGR Experiment performed on April 4, 2005

	A	B	C
NH_4-N	100 mg/L	100 mg/L	100 mg/L
Na_2CO_3 as $CaCO_3$	172 mg/L	172 mg/L	172 mg/L
$ZnSO_4$	n/a	0.5 mg/L Zn	50 mg/L Zn
Mixed Liquor	1.0 L	1.0 L	1.0 L
Tap Water	2.5 L	2.5 L	2.5 L

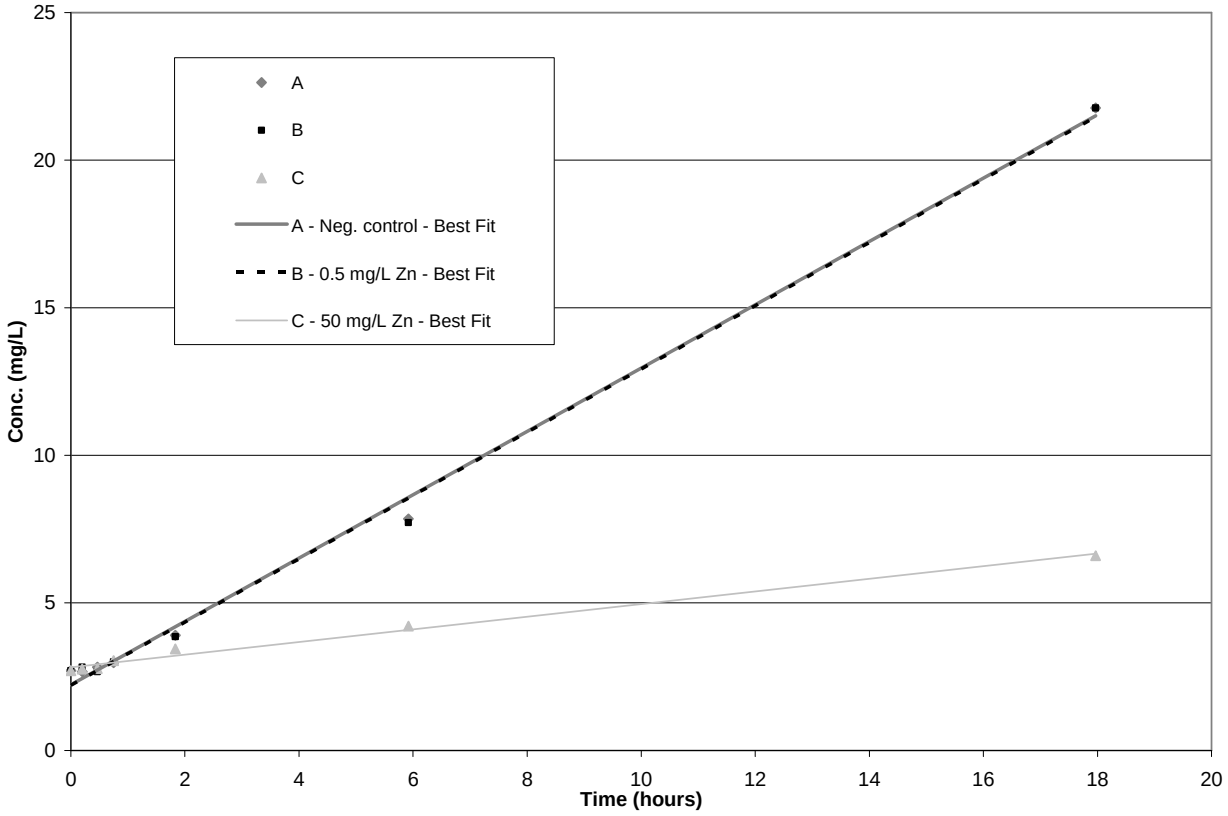


Figure 7. Nitrate Concentration as Nitrogen (mg/L) versus Time (hours) (Note: nitrite concentrations were not included due to complication from interference with chloride).

Table 10. Specific Nitrification Rates of Reactors A, B and C

	NGR
Reactor	mg NO_3-N/g MLVSS/hr
A	1.16
B	1.16
C	0.23

The reactors were run for a period of nearly 18 hours. After the experiment had been running for 4 hours, the pH was measured to be 8.35, 8.36 and 8.32 in reactors A, B and C, respectively. The cause for the high pH was likely a combination of over dosing of alkalinity and CO_2 stripping. The NGR for the negative control and the 0.5 mg/L Zn stressed system were both 1.16 g NO_3-N /g MLVSS/hr, and the 50 mg/L Zn stressed reactor NGR was 0. 23 g NO_3-N/g MLVSS/hr. This suggests that 50 mg/L Zn has an extreme inhibitory effect on nitrification, but that 0.5 mg/L Zn does not significantly affect nitrification rates.

5.2 NGR using Arlington Mixed Liquor and Lexington Tap Water

A preliminary NGR experiment was conducted using Arlington mixed liquor and Lexington Tap Water. The initial dosage of NH_4-N was 50 mg/L in all four reactors, and zinc was tested at 50 mg/L. Alkalinity was added intermittently based on reactor pH, and a positive control reactor was dosed with 20 mg/L ATU (Table 11).

Table 11. Reactor Setup of NGR experiment performed May 19, 2005

	A	B	C	D
NH_4-N	50 mg/L	50 mg/L	50 mg/L	50 mg/L
Na_2CO_3 as $CaCO_3$	50 mg/L	50 mg/L	50 mg/L	50 mg/L
ATU		20 mg/L		20 mg/L
$ZnSO_4$	n/a	n/a	50 mg/L as Zn	50 mg/L as Zn
MLSS	0.5 L	0.5 L	0.5 L	0.5 L
Tap Water	3.0 L	3.0 L	3.0 L	3.0 L

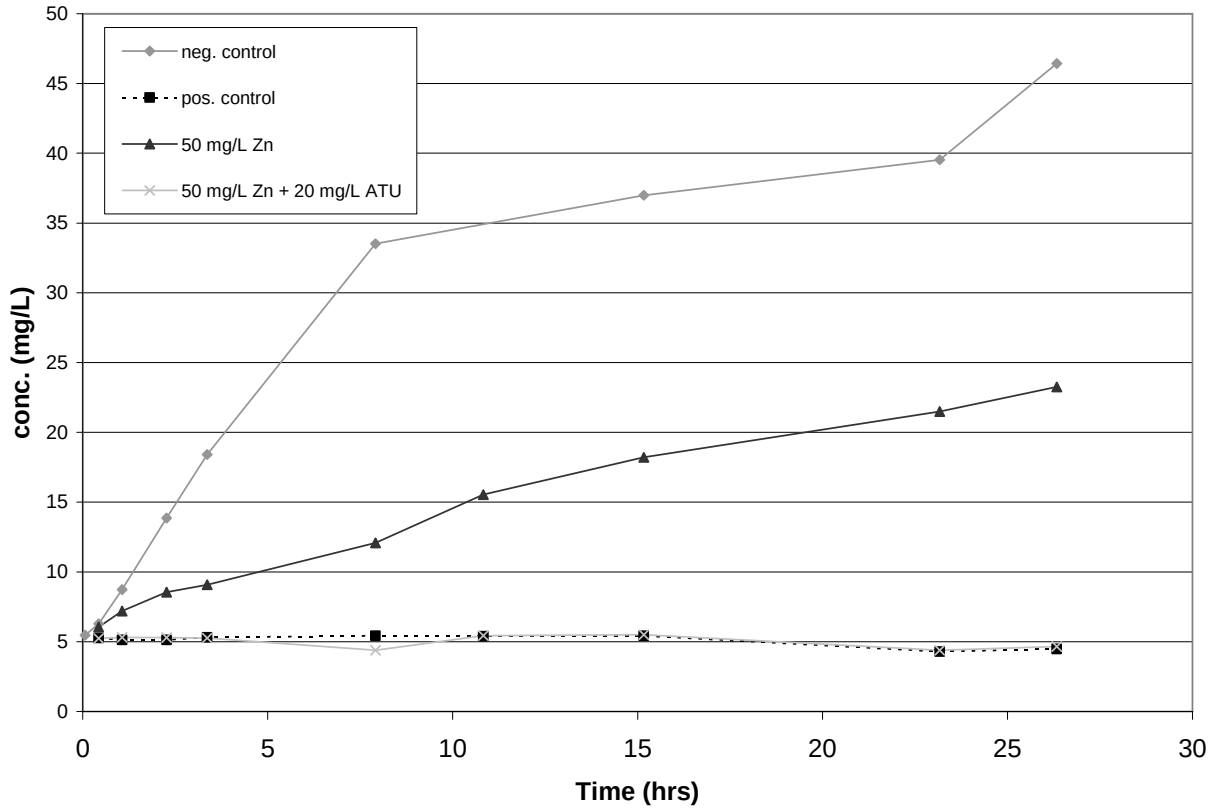


Figure 8. Nitrate as nitrogen concentration (mg/L) versus Time (hours), (note that nitrite has not been included due to chloride interference during analysis.

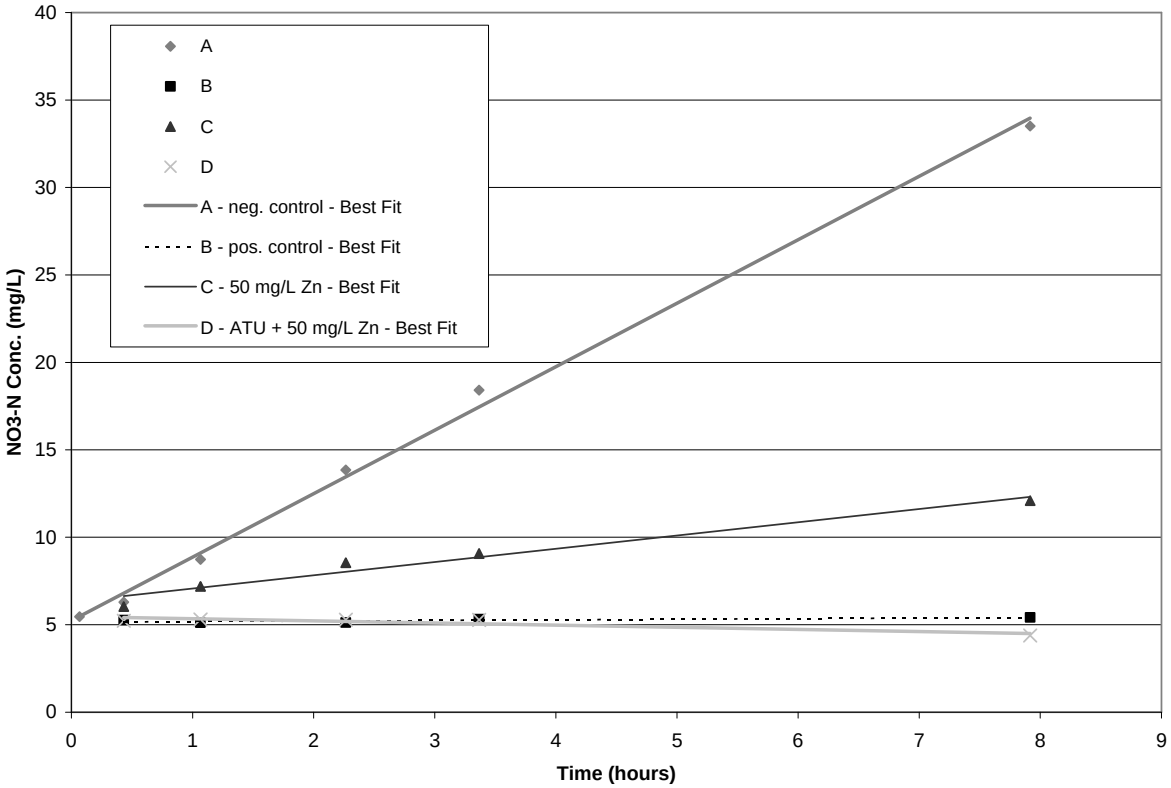


Figure 9. Nitrate Concentration versus Time for first 8 hours, prior to insufficient Ammonia inhibition, with linear regression shown.

Table 12. Specific Nitrate Generation Rates for Reactors A, B, C and D

	NGR
Reactor	mg NO_3-N/ g MLVSS hr
A	3.9
B	0.04
C	0.8
D	-0.1

Figures 8 and 9 illustrate that inhibition of nitrification occurred within the first hour of contact at 50 mg/L Zn. Also, ATU completely inhibited nitrate generation at 20 mg/L, and there was no further inhibition when 20 mg/L of ATU and 50 mg/L Zn were added together. Thus, 20 mg/L of ATU was adequate for use as a positive control through the rest of the experiments.

Due to improper alkalinity addition, the control reactor was likely inhibited by low pH conditions after 8 hours. As a result, only the first 8 hours of the experiment were considered for the computation of NGRs. The data in Table 12 suggest that 50 mg/L of Zn decreased the NGR by approximately five-fold.

5.3 NGR using Arlington Mixed Liquor and Lexington Tap Water

In an attempt to overcome pH problems, a buffer solution using tap water, sulfuric acid and sodium bicarbonate was created and utilized in this experiment. Also, the aeration rate was reduced to minimize carbon dioxide stripping. This experiment was run with a target MLSS of 1000 mg/L, and NH_4-N was added as necessary to maintain nitrification. Although mixed liquor from the Arlington WPCP was used, the sample was old and had been aerating in the laboratory for over a week.

Table 13. Reactor Setup for NGR conducted on May 30, 2005

	A	B	C	D
NH_4-N	50 mg/L	50 mg/L	50 mg/L	50 mg/L
Na_2CO_3 as $CaCO_3$	50 mg/L	50 mg/L	50 mg/L	50 mg/L
ATU	n/a	20 mg/L	n/a	n/a
$ZnSO_4$	n/a	n/a	0.5 mg/L as Zn	10 mg/L as Zn
Mixed liquor	270 mL	270 mL	270 mL	270 mL
Tap Water	3223 mL	3223 mL	3223 mL	3223 mL
Conc. H_2SO_4	1.0 mL	1.0 mL	1.0 mL	1.0 mL
$NaHCO_3$	5.415 g	5.415 g	5.415 g	5.415 g

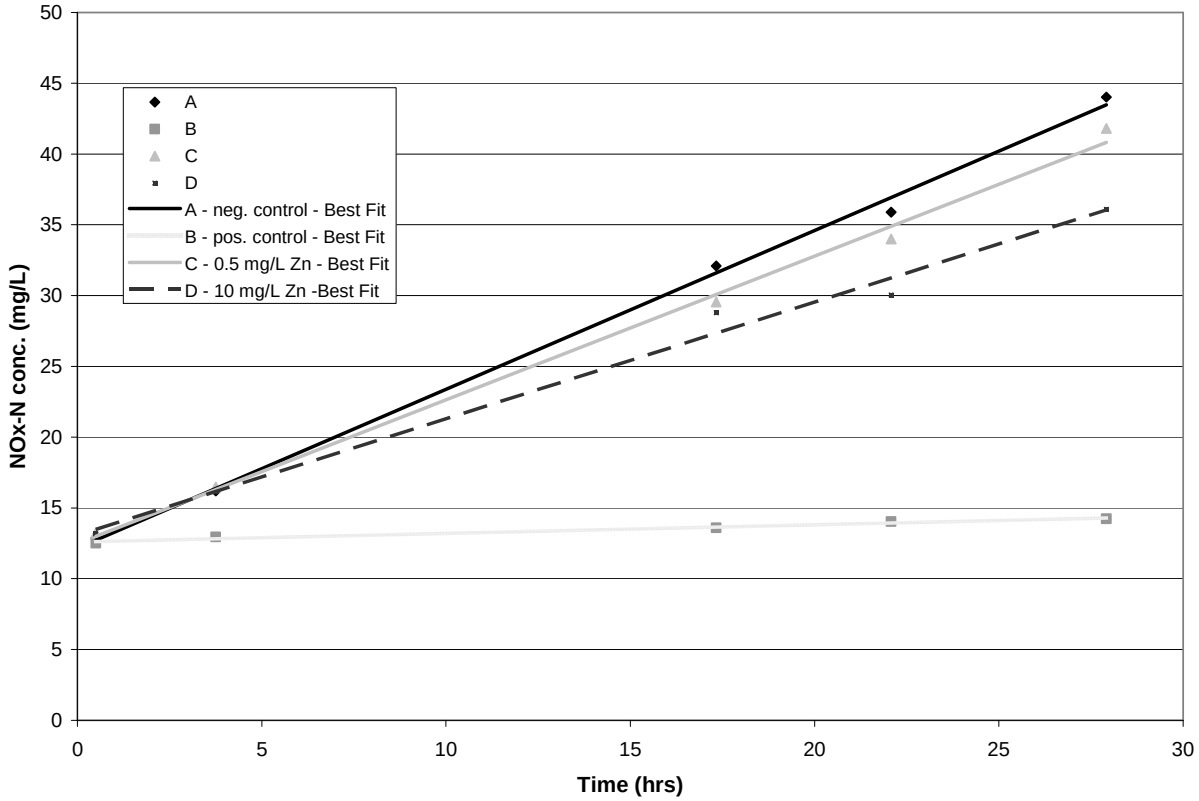


Figure 10. Concentration of NOx-N (mg/L) verses Time (hours) with linear regression.

Table 14. Specific NOx-N Generation Rates for all Reactors

	NGR
Reactor	mg NO_x-N/g MLVSS/hr
A	2.3
B	0.1
C	2.1
D	1.7

During the course of the experiment, the pH was monitored to examine the effectiveness of the buffer solution. From the start of the experiment, the pH was too high (>8.0) and sulfuric acid was added in several doses. After sulfuric acid was added, the pH was within the desired range only for a short period (~1-2 hours). With CO_2 stripping, the pH gradually increased to above 8.0 after several additions of sulfuric acid. As expected, the elevated pH resulted in nitrite accumulation as a result of free ammonia inhibition of NOB. Although the NGR in the control and 0.5 mg/L Zn-stressed reactors were similar, pH control issues make the interpretation of these data suspect.

5.4 NGR using Arlington Mixed Liquor and Arlington Secondary Effluent

A subsequent NGR experiment was conducted from June 22, 2005 until June 24, 2005 per similar protocol as above, but dosing alkalinity on an as needed basis. The target MLSS concentration was 1000 mg/L however, after lab analysis the MLSS concentration was determined to be 565 mg/L. Secondary effluent, the diluent, was aerated for several hours to remove carbon dioxide, which elevated the pH as it was removed. After aeration, sulfuric acid and sodium bicarbonate were added to adjust the pH to 7.6, while maintaining the necessary alkalinity. Hach colorimetric methods were used to analyze the nitrite and nitrate concentrations. Ammonia-N was added as needed to maintain reactor concentrations above 15-20 mg/L and below 50 mg/L.

Table 15. Reactor Setup

	A	B	C	D
NH_4-N	50 mg/L	50 mg/L	50 mg/L	50 mg/L
$NaHCO_3$ as $CaCO_3$	as needed	as needed	As needed	as needed
ATU	n/a	20 mg/L	n/a	n/a
$ZnSO_4$	n/a	n/a	0.5 mg/L as Zn	10 mg/L as Zn
Mixed Liquor	390 mL	390 mL	390 mL	390 mL
Secondary Effluent	3.11 L	3.11 L	3.11 L	3.11 L

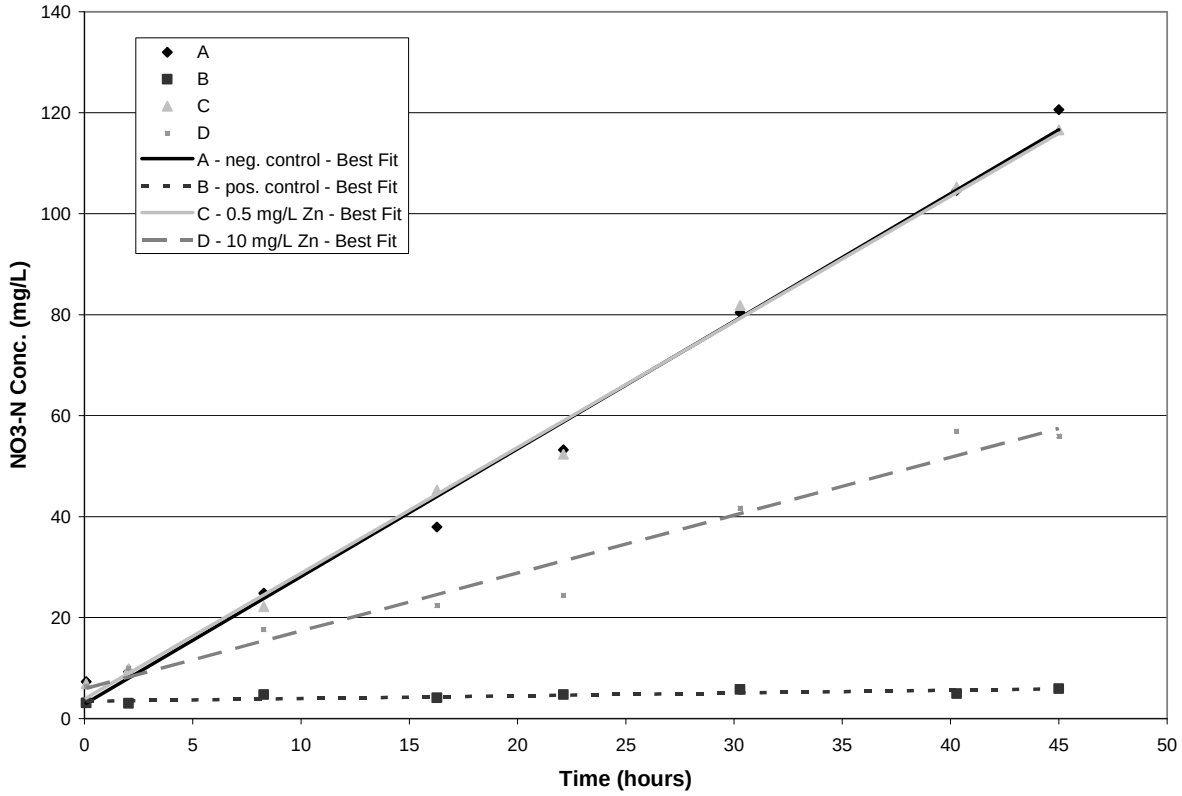


Figure 11. Nitrate concentration (mg/L) plotted versus Time (hours) with the line of Best Fit from linear regression shown.

Table 16. Specific nitrate generation rates of all reactors.

	NGR
Reactor	mg NO_3-N/ g MLVSS hr
A	5.7
B	0.10
C	5.6
D	2.6

As shown in Table 14, the specific NGR for the control reactor and 0.5 mg/L Zn-stressed reactor was 5.7 and 5.6 mg NO_3-N/g MLVSS/hr, respectively. While a zinc dose of 10 mg/L reduced the NGR by more than 50%, there seems to be no significant inhibition of nitrification at an MLSS concentration of 565 mg/L and a Zn concentration of 0.5 mg/L. Since no inhibition was detected at a conservatively low MLSS, nitrification inhibition in the full-scale plant would be unlikely.

5.5 NGR – Arlington Mixed Liquor and Arlington Secondary Effluent

A NGR experiment was conducted to evaluate the effects of Zinc at a concentration of 0.5 mg/L and 1.0 mg/L simultaneously (Table 17). The experiment was run for a period of just over 48 hours.

Table 17. Reactor Set-up

	A	B	C	D
NH_4-N	50 mg/L	50 mg/L	50 mg/L	50 mg/L
$NaHCO_3$ as $CaCO_3$	as needed	as needed	As needed	as needed
ATU	n/a	20 mg/L	n/a	n/a
$ZnSO_4$	n/a	n/a	0.5 mg/L as Zn	1.0 mg/L as Zn
Mixed Liquor	390 mL	390 mL	390 mL	390 mL
Secondary Effluent	3.11 L	3.11 L	3.11 L	3.11 L

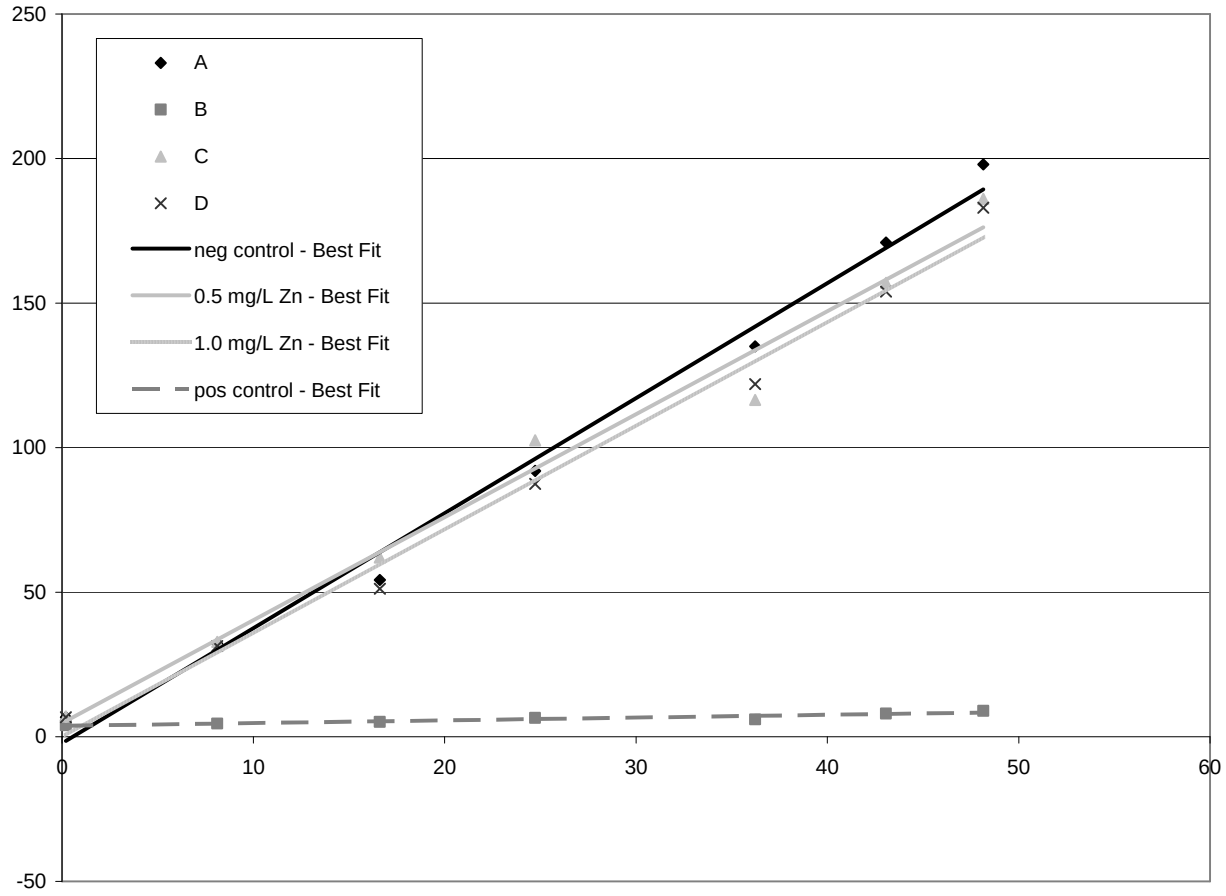


Figure 12. Plot of NOx-N concentration (mg/L) versus Time (hours) with linear regression shown for entire experiment.

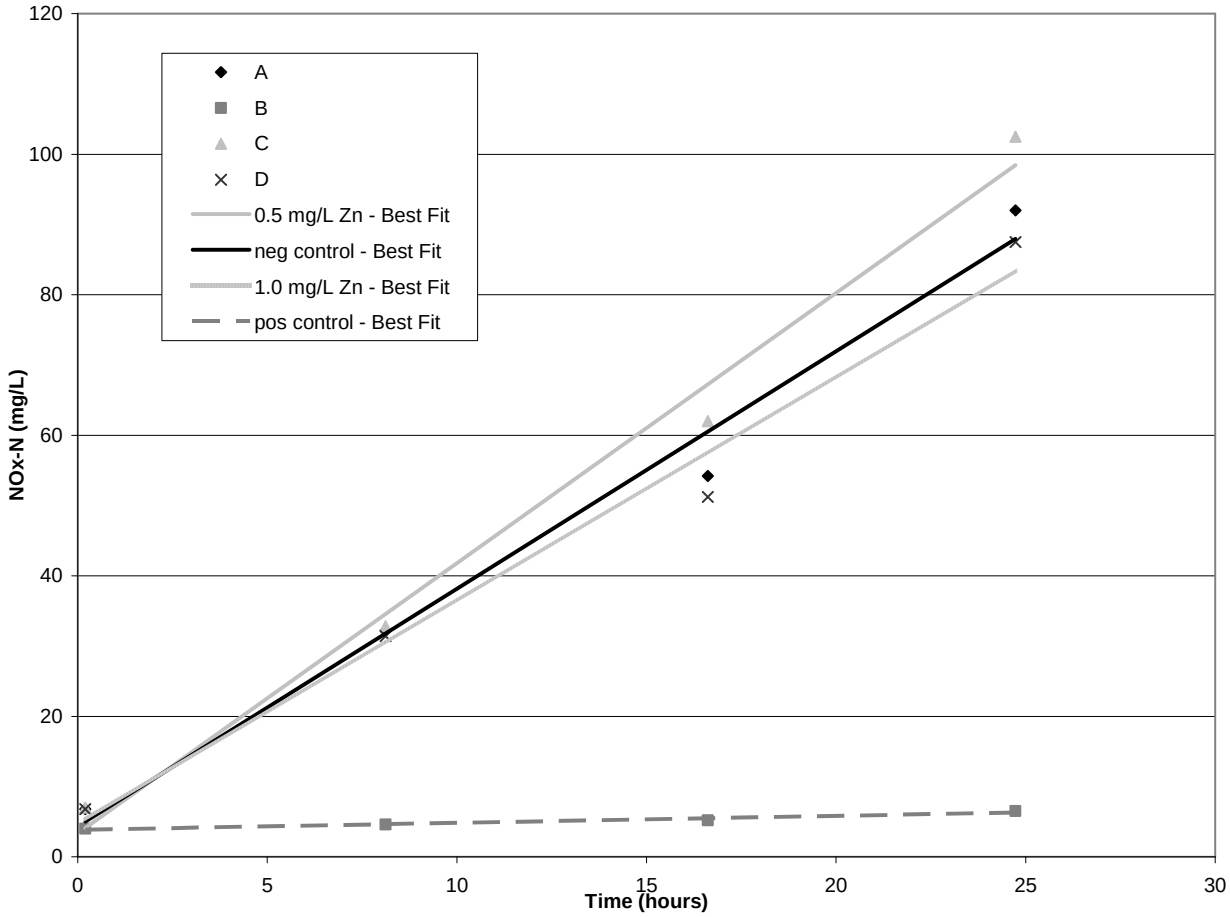


Figure 13. Plot of NOx-N concentration (mg/L) versus Time (hours) with linear regression shown of first 24 hours.

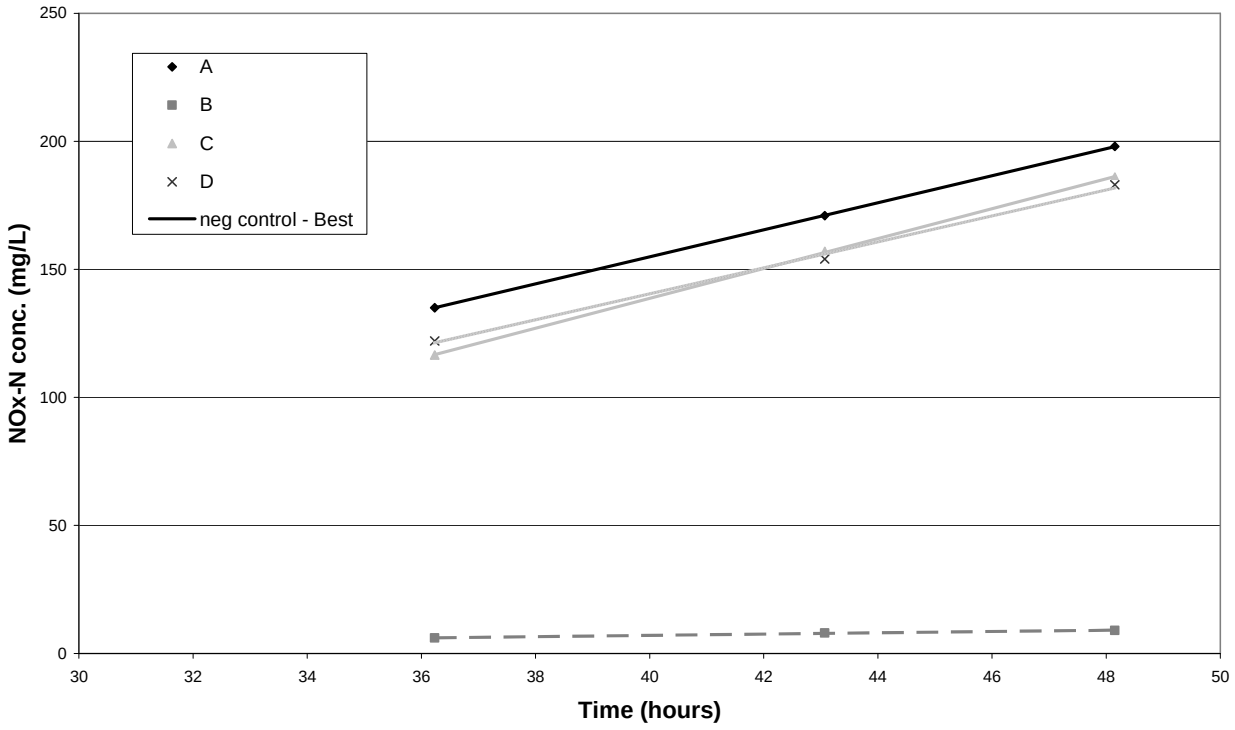


Figure 14. Plot of NOx-N concentrations (mg/L) versus Time (hours) after initial 24 hours, with linear regression shown.

Table 18. NGR summary for each reactor, overall, first 24 hours and after 24 hours.

Full Experiment	NGR	First 24 hours	NGR	After 24 hours	NGR
A	4.0	A	3.4	A	5.3
B	0.095	B	0.099	B	0.25
C	3.6	C	3.9	C	5.8
D	3.6	D	3.2	D	5.1

*NGR in mg NOx-N/L/hour

Although the plots shown in Figure 12 suggest very mild inhibition at 0.5 mg/L Zn and slight inhibition at 1.0 mg/L Zn, the analytical data exhibited some variability for this experiment, and there is no statistically significant difference (p=0.05) between the NGRs noted in Table 18 for the full time series (Reactors A, C, and D). Furthermore, if the plot is split into two time periods – one less than 24 hours (Figure 13) and one from 36 to 48 hours – the data show no inhibition at 0.5 mg/L Zn and mild but statistically insignificant inhibition at 1.0 mg/L.

For this experiment, total and soluble zinc data are provided in Table 19 to demonstrate that loss of zinc from the system due to zinc sorption onto the sidewalls of the glass reactors was not problematic. It is apparent that the zinc concentration in the negative control reactor was in range of the established average influent Zn level of 0.2 mg/L for the Arlington WPCP. The total Zn measured in the Zn-stressed reactors is consistent with what was added (plus

background), suggesting that losses due to sorption were minimal. Soluble/dissolved Zn measurements suggest significant partitioning of Zn to a particulate phase (i.e. mixed liquor solids), as expected.

Table 19. Total and Soluble Zinc concentrations for NGR experiment

Reactor	Total Zinc (mg/L)	Soluble Zinc (mg/L)
A - Neg. Control (no Zinc added)	0.230	0.190
C - 0.5 mg/L Zinc	0.750	0.200
D - 1.0 mg/L Zinc	1.100	0.210

5.6 Summary of NGR Experiments

Table 20. NGR Experiment Summary[b]

NGR Experiments								
Experimental Conditions						Degree of inhibition		
Zn (mg/L)	NH_4-N (mg/L)	NO_2-N (mg/L)	Target MLSS (mg/L)	Alk (mg/L as $CaCO_3$)	Make-up water (SE or tap)	Impact of Zn on NGR (vs. control)	NGR (control)[a]	NGR (Zn)[a]
0.5	50	--	1000	50	Tap	none	2.3	2.1
0.5	50	--	1000	as needed	SE	none	5.7	5.6
0.5	50	--	1000	as needed	SE	none	NA	NA
1.0	50	--	1000	as needed	SE	mild	NA	NA
10	50	--	1000	50	Tap	mild	2.3	1.7
10	50	--	1000	as needed	SE	significant	5.7	2.6
50	50	--	1000	50	Tap	severe	3.9	0.8

a. NGR units are mg NO_3-N/g MLVSS-hr.

b. Results shown for experiment in Table 18 are presented qualitatively in Table 20.

5.6 Nitrite Uptake Rate – Inhibition of NOB

NGR experiments suggested that 0.5 mg/L Zinc has no significant inhibitory effect on nitrification – specifically ammonia oxidation and AOB. A single nitrite uptake rate experiment was conducted to determine the impact of zinc on NOB. The target mixed liquor concentration was 1000 mg/L. The primary differences between nitrite uptake and NGR experiments are that no alkalinity was required and nitrite was added initially instead of ammonia.

Table 21. Reactor Set-up

	A	B	C	D
NO_2-N	50 mg/L	50 mg/L	50 mg/L	50 mg/L
$NaHCO_3$ as $CaCO_3$	none	none	none	None
ATU	n/a	20 mg/L	n/a	n/a
$ZnSO_4$	n/a	N/a	0.5 mg/L as Zn	50 mg/L as Zn
Mixed Liquor	390 mL	390 mL	390 mL	390 mL
Secondary Effluent	3.11 L	3.11 L	3.11 L	3.11 L

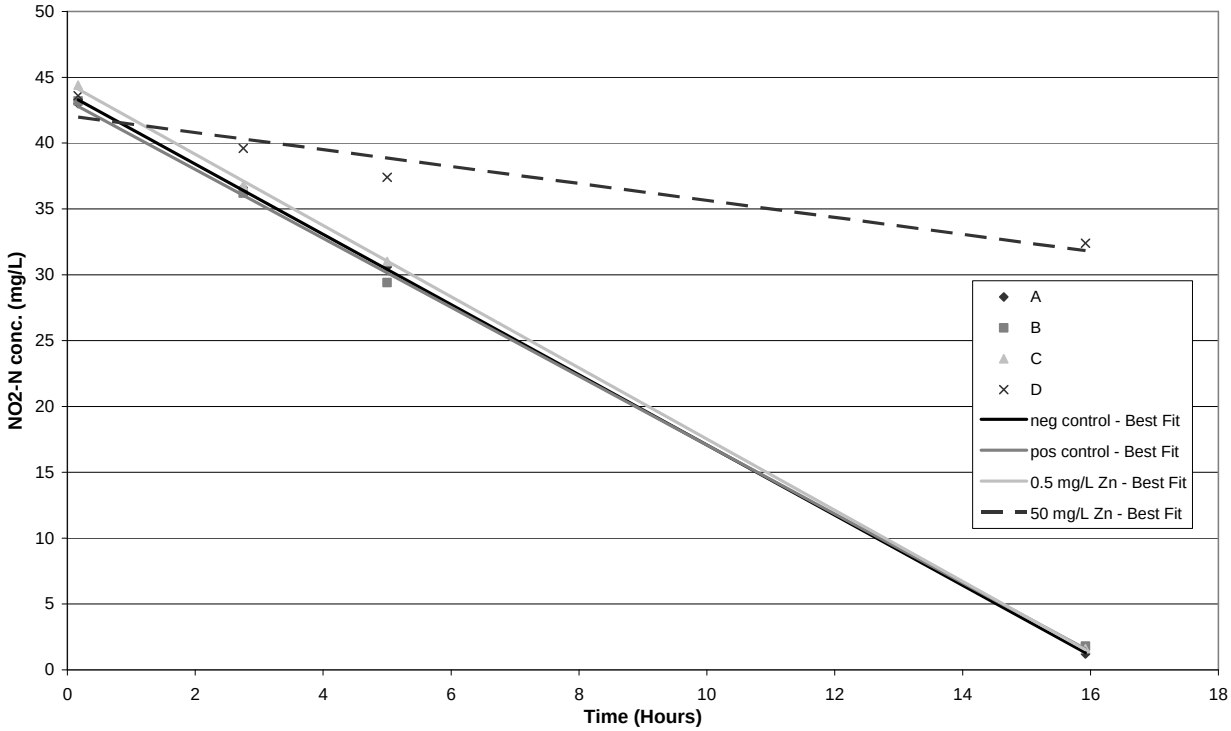


Figure 15. Plot of Nitrite concentration (mg/L) versus Time (hours) with linear regression shown.

Table 22. Nitrite Uptake Rates, independent of MLVSS concentrations

	NUR
Reactor	mg NO_2-N/g MLVSS hr
A	4.2
B	4.0
C	4.2
D	0.9

As expected,0.5 mg/L Zn had no inhibitory effect on the oxidation of nitrite to nitrate, and 50 mg/L Zn was found to decrease the nitrite uptake rate to one-fourth of the negative control. Interestingly, ATU had no inhibitory effects on the oxidation of nitrite to nitrate, and this verifies that ATU is a selective AOB inhibitor.

6. HIGH F/M EXPERIMENT RESULTS AND CONCLUSIONS

6.1 High F/M Experiment using Arlington Mixed Liquor and Arlington Tap Water

A preliminary High F/M (HFM) experiment was conducted with Arlington, VA tap water and mixed liquor sampled from Arlington WPCP on May 22, 2005. After sampling, a small portion of the mixed liquor was analyzed for TSS and VSS while the remaining sample was aerated to remove BOD and residual NH_4. The TSS and VSS measurements were used to determine the required amount of mixed liquor to achieve 35 mg/L of VSS at the beginning of the experiment.

Alkalinity was added intermittently using sodium bicarbonate to maintain a target pH between 6.8 and 7.2. The reactors were set up per Table 24. Sampling for N species occurred at the onset of the experiment, and roughly three times per day for the duration of the experiment.

Table 24. Preliminary High F/M Batch Test (HFM)

	A	B	C	D
NH_4-N	50 mg/L	50 mg/L	50 mg/L	50 mg/L
$NaHCO_3$ as $CaCO_3$	50 mg/L	50 mg/L	50 mg/L	50 mg/L
ATU	n/a	20 mg/L	n/a	n/a
$ZnSO_4$	n/a	n/a	10 mg/L as Zn	1 mg/L as Zn
Mixed Liquor	15.4 mL	15.4 mL	15.4 mL	15.4 mL
Arlington Tap Water	3.478 L	3.478 L	3.478 L	3.478 L

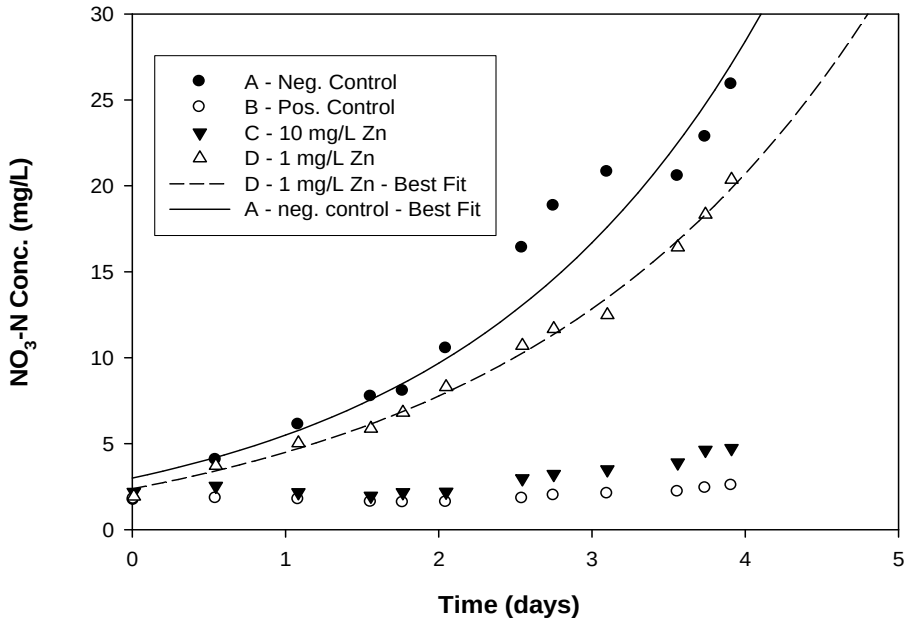


Figure 16. Concentration of NO_3-N (mg/L) versus Time (days) for

Table 25. Calculated Maximum Specific Growth Rates.

	Sigma Plot	Excel
Reactor	$\mu_{max,a}$ (d^{-1})	$\mu_{max,a}$ (d^{-1})
A	0.67	0.66
D	0.59	0.60

Table 26. Calculated Initial Concentration of Nitrifying Bacteria

Reactor	$X_{aut,o}$ (mg/L)
A	0.43
D	0.43

It is likely that excessive aeration in this experiment resulted in significant evaporation near the end of the experiment, CO_2 stripping, and pH fluctuations. This probably caused the significant scatter in the negative control after 2 days (Figure 16). Following this experiment, much better control of aeration rates was instituted. Fitting the NO_3-N data in Figure 16 to the Equation 1 above, an estimate for the maximum specific autotrophic growth rate was determined using both the Sigma Plot nonlinear regression package and manual fitting procedure using Microsoft Excel's Solver optimization algorithm per the method of Melcer et al. (2003). In this case, both approaches provided similar results. The initial concentration of nitrifying bacteria is reported in Table 26 for comparison to latter experiments discussed below.

As shown in Figure 16, the ATU control completely inhibited nitrification, and 10 mg/L Zn resulted in near complete inhibition with only minor NO_3-N generation over 4 days, suggesting the significant sensitivity of this method to detect nitrification inhibition at such a low MLSS concentration. As a result of the scatter in the negative control reactor, the calculated $\mu_{max,a}$ of 0.67 day^{-1} was determined to have a standard error of 0.19 day^{-1}, which suggests that the apparent inhibition at 1.0 mg/L Zn is not statistically significant. The calculated $\mu_{max,a}$ for the 1.0 mg/L Zn-stressed reactor was found to be 0.59 day^{-1} with a standard error of 0.05 day^{-1}. Regardless of the mathematical interpretation of the data, it is clear that mild inhibition was caused by 1.0 mg/L of Zn, and it was deemed critical to determine whether this response was repeatable.

6.2 HFM using Arlington Mixed Liquor and Arlington Secondary Effluent

The first experiment at the Arlington WPCP was started on June 6, 2005 . All experiments conducted at the Arlington WPCP used a combination of Hach colorimetric methods and flow injection analyzer (Lachet) for nitrogen species. In all cases, NO_2-N was analyzed immediately after sampling by Hach method 371N (Hach Odyssey model DR/2500 spectrophotometer). Samples were preserved using H_2SO_4 for Arlington WPCP analysis of NH_4-N and NO_3-N + NO_2-N using the flow injection analyzer. Duplicates were also analyzed for NO_3-N + NO_2-N immediately after sampling by Hach method 355N.

For this experiment, the aeration rate was carefully controlled and secondary effluent was used as the diluent. Arlington WPCP MLSS and MLVSS process control for the return activated sludge stream were used to calculate an appropriate dilution to provide ~35 mg/L MLVSS in the

reactors. Zinc was added at 1 and 10 mg/L, and the experiment was run for approximately 3.5 days.

Table 27. Reactor Overview of HFM Experiment

	A	B	C	D
NH_4-N	50 mg/L	50 mg/L	50 mg/L	50 mg/L
$NaHCO_3$ as $CaCO_3$	As needed	As needed	As needed	As needed
ATU	n/a	20 mg/L	n/a	n/a
$ZnSO_4$	n/a	n/a	1 mg/L as Zn	10 mg/L as Zn
Mixed Liquor	17.68 mL	17.68 mL	17.68 mL	17.68 mL
Secondary Effluent	3.482 L	3.482 L	3.482 L	3.482 L

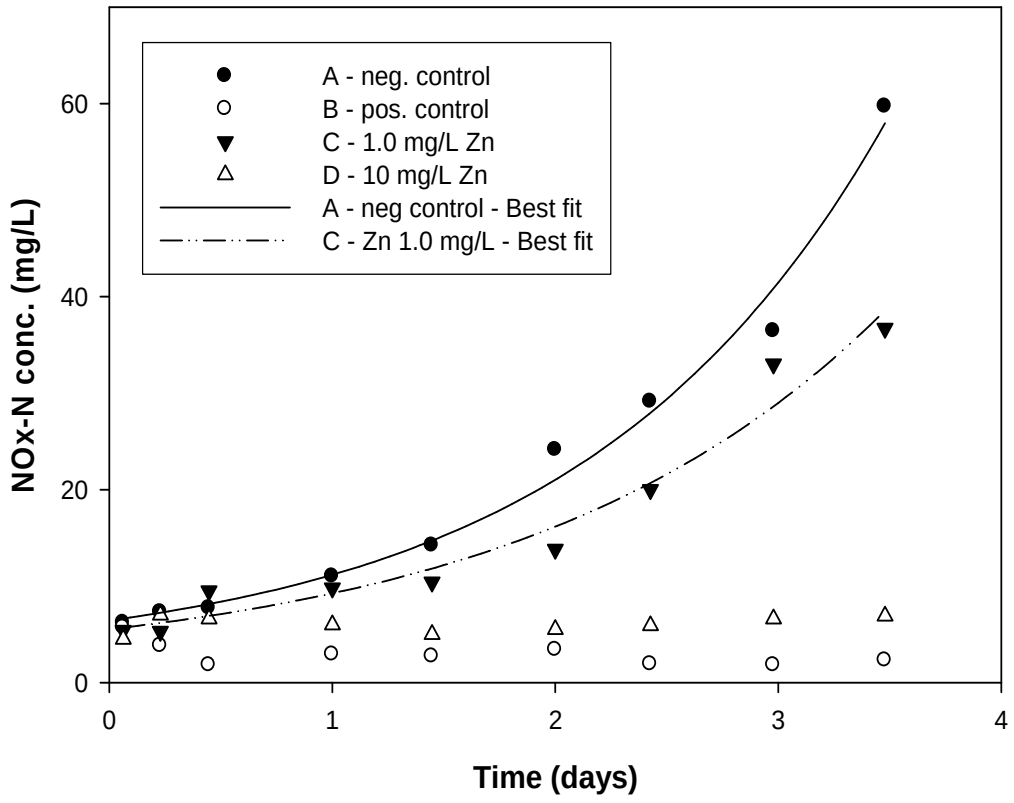


Figure 17. Concentration of NOx-N (mg/L) plotted versus Time (days) with the Best Fit of the negative control and 1.0 mg/L Zinc

Table 28. Values of the maximum specific growth rate, from regression performed on Excel and Sigma Plot solver programs

	$\mu_{max,a}$ (d^{-1})	$\mu_{max,a}$ (d^{-1})
Reactor	Sigma Plot	Excel
A	0.88	0.88
C	0.77	0.77

Table 29. Computed concentrations of Initial Nitrifying Bacteria

Reactor	X_{aut} (mg/L)
A	0.55
C	0.53

Due to previous problems with NO_2-N analysis by ion chromatography in the VMI lab (chloride interference), samples analyzed in Arlington allowed NO_x-N (NO_3-N + NO_2-N) to be plotted versus time (Figure 17). Although there was again significant scatter in the data, it is clear that 10 mg/L Zn caused near complete inhibition and 1.0 mg/L caused a slight reduction in the nitrification rate. However, on evaluation of the standard errors of the $\mu_{max,a}$ estimates for the negative control and the 1.0 mg/L Zn-stressed reactors (0.12 and 0.18 day^{-1}, respectively), the inhibition response caused by 1.0 mg/L Zn was not statistically significant.

Using the HFM method as well as other techniques, Melcer et al. (2003) showed that the autotrophic maximum specific growth rate for a number of municipal wastewater treatment facilities in North America ranged from 0.9 to 0.95 d^{-1} – a very narrow range. Although the negative control from the previous HFM experiment exhibited values below this range (Table 25), this was likely due to the accumulation of NO_2-N and the failure to account for this in the estimation of $\mu_{max,a}$. As shown in Table 28, the negative control was consistent with what would be expected for fully uninhibited nitrification.

6.3 HFM using Arlington Mixed Liquor and Arlington Secondary Effluent

In this experiment, 0.5 and 10 mg/L Zn were tested. Ammonia-N concentrations were kept in the range of approximately 20-50 mg/L through intermittent additions based on Hach NO_3-N + NO_2-N measurements. The alkalinity of the secondary effluent diluent was determined through titration as 95 mg/L as $CaCO_3$, and thus no initial alkalinity addition was required. However, careful pH monitoring was used to determine appropriate points for intermittent alkalinity addition.

Table 30. Reactor Set-up of HFM test

	A	B	C	D
NH_4-N	50 mg/L	50 mg/L	50 mg/L	50 mg/L
$NaHCO_3$ as $CaCO_3$	As needed	As needed	As needed	As needed
ATU	n/a	20 mg/L	n/a	n/a
$ZnSO_4$	n/a	n/a	0.5 mg/L as Zn	10 mg/L as Zn
Mixed Liquor	17.7 mL	17.7 mL	17.7 mL	17.7 mL
Secondary Effluent	3.482 L	3.482 L	3.482 L	3.482 L

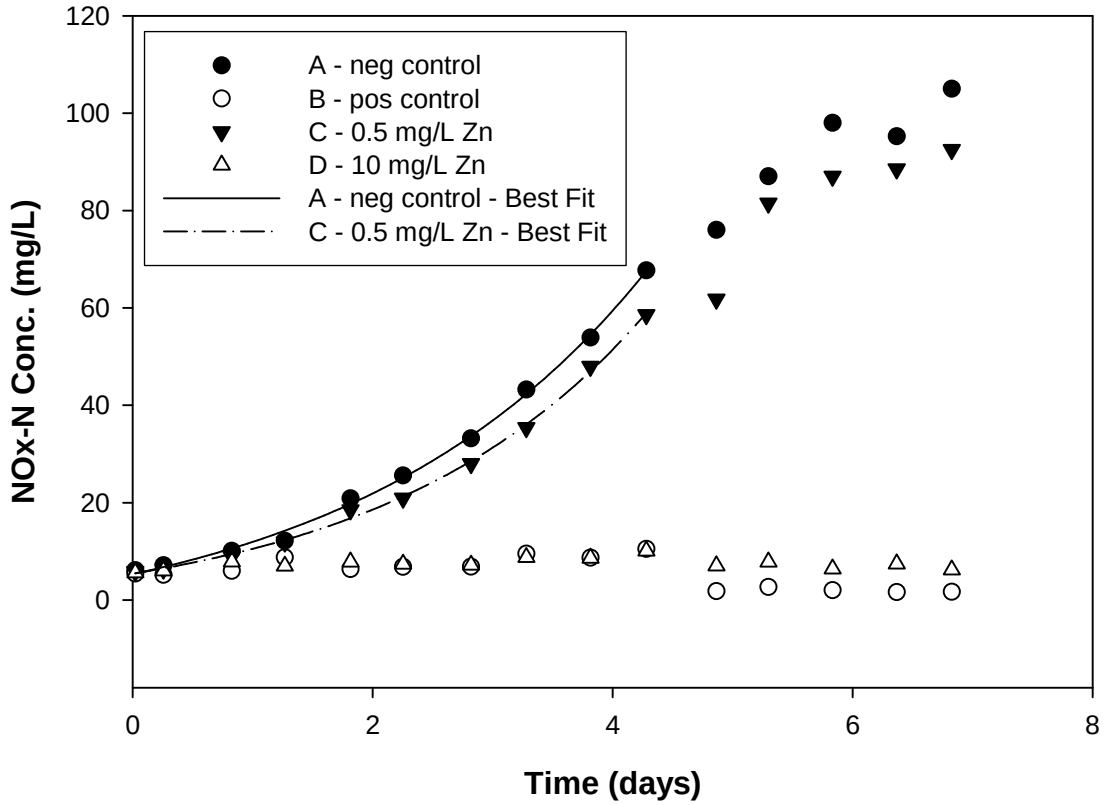


Figure 18. Concentration of NOx-N (mg/L) plotted versus Time (days) with Best Fit Curves for Reactors A and C.

Table 31. Specific Growth Rate and Initial Nitrifier Concentration of Reactor A and C.

	Sigma Plot
Reactor	$\mu_{max,a}$ (d^{-1})
A	0.56
C	0.61

	Sigma Plot
Reactor	$X_{aut,o}$ (mg/L)
A	1.4
C	1.0

The duration of this experiment was eight days (Figure 18), however, after day four the NO_x-N concentration no longer increased exponentially. The numerical fitting of the data was performed for only the shown range above. During this period, it is clear that 10 mg/L Zn induced near complete inhibition of nitrification, while 0.5 mg/L Zn induced very slight inhibition during the first 4 days of the experiment, but was consistent with the control after only four days of contact.

The numerical curve fitting procedure produced unexpected results as shown in Table 31, whereby the 0.5 mg/L Zn-stressed reactor was found to have a higher $\mu_{max,a}$ than the negative control, although again this difference was not statistically significant. It was found that the curve fitting procedure was very sensitive to the fitted initial nitrifier concentration, $X_{aut,o}$, and attempts to force these values to be more consistent (which they should be) only exacerbated the discrepancy shown above. These results suggest that while the data produced from a HFM experiment can be used as an indicator of nitrification inhibition and the procedure itself is very sensitive for detecting nitrification inhibition (with such a low test MLSS concentration), the curve fitting procedure to get $\mu_{max,a}$ may not be an appropriate method for evaluating the data. This is certainly the case when it is necessary to measure very slight reductions in nitrification rates. It seems that very subtle changes in the shape of the HFM curves produce unexpected parameter fits.

6.4 HFM-Arlington Mixed Liquor and Arlington Secondary Effluent

A HFM test was conducted with dosages of 0.5 and 1.0 mg/L Zn. The methodology was as per previous discussion, and the reactor setup is shown below in Table 32.

Table 32. Reactor Setup for HFM Experiment

	A	B	C	D
NH_4-N	50 mg/L	50 mg/L	50 mg/L	50 mg/L
$NaHCO_3$ as $CaCO_3$	As needed	As needed	As needed	As needed
ATU	n/a	20 mg/L	n/a	n/a
$ZnSO_4$	n/a	n/a	0.5 mg/L as Zn	1.0 mg/L as Zn
Mixed Liquor	17.7 mL	17.7 mL	17.7 mL	17.7 mL
Secondary Effluent	3.482 L	3.482 L	3.482 L	3.482 L

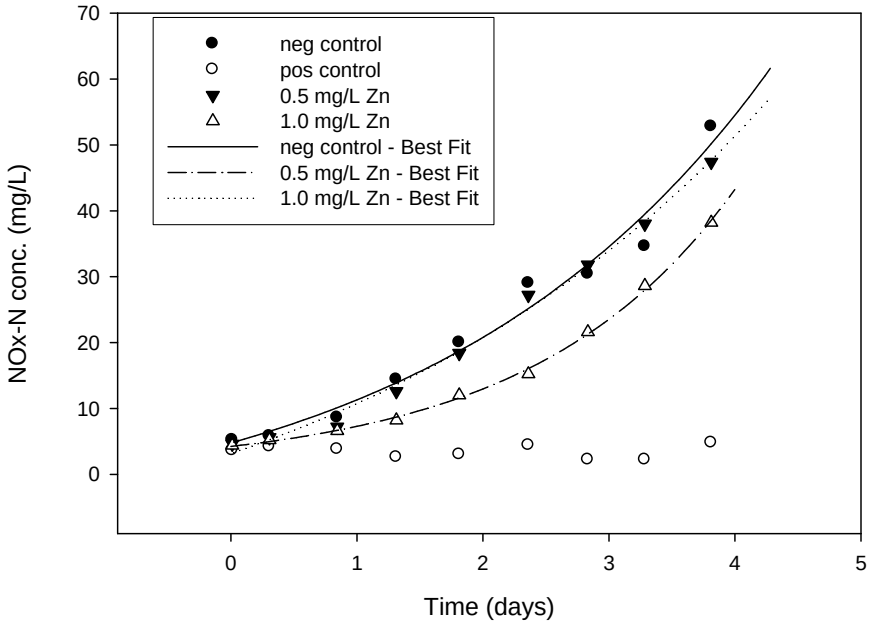


Figure 19. Nitrate and Nitrite concentration (mg/L) versus Time (days) with Best Fit curves shown for Reactors A, C and D.

Table 33. Maximum specific autotrophic growth rates, of each Reactor

	$\mu_{max,a}$
Reactor	d^{-1}
A	0.52
B	0
C	0.43
D	0.77

In this case, the 0.5 mg/L Zn induced no significant nitrification inhibition, while 1.0 mg/L Zn cause a slight decrease in the nitrification rate. The fitted $\mu_{max,a}$ estimates shown in Table 33 clearly do not represent the apparent trends in Figure 19.

6.5 Summary of HFM Experiments

Table 34. HFM Experiment Summary

HFM Experiments								
Experimental Conditions						Degree of inhibition		
Zn (mg/L)	NH_4-N (mg/L)	NO_2-N (mg/L)	Target MLSS (mg/L)	Alk (mg/L as $CaCO_3$)	Make-up water (SE or tap)	Impact of Zn on $\mu_{max,a}$ (vs. control)	$\mu_{max,a}$ (control)	$\mu_{max,a}$ (Zn)
0.5	50		35	as needed	SE	none	0.56	0.61
0.5	50		35	as needed	SE	none[a]	0.52	0.43
1	50		35	50	Tap	mild	0.67	0.59
1	50		35	as needed	SE	mild	0.88	0.77
1	50		35	as needed	SE	mild[a]	0.52	0.77
10	50		35	50	Tap	severe	0.67	--
10	50		35	as needed	SE	severe	0.88	--
10	50		35	as needed	SE	severe	0.56	--

a. Impact based on Figure 19, not fitted valued of $\mu_{max,a.}$

7. SPECIFIC DENITRIFICATION RATE EXPERIMENT - SDNR

A single SDNR experiment was attempted late in July, 2005 but the resulting nitrate uptake rate (NUR) data were inconsistent and suggested that very little nitrate uptake/denitrification occurred even in the negative control. In fact, nitrate levels may have actually increased somewhat during the experiment indicating that nitrification was occurring and that low levels of oxygen must have transferred. Due to time constraints it was not possible to repeat/optimize this experiment. In addition, the N_2/CO_2 blend was mistakenly sparged into the reactors at a very high flow rate and all of the gas was consumed over a 24 hour period. The presence of low levels of dissolved oxygen in the reactors was likely caused by either oxygen contamination of the compressed gas cylinder or entrainment at the surface of the reactor due to excessive sparging and mixing.

As indicated in the previous Phase I/ II Literature Review, it is quite likely that 0.5 mg/L Zn would have no significant effect on heterotrophic denitrification. This may be studied further during the fall. In addition to nitrate uptake rate measurements, it was determined that it might also be possible to assess denitrification continuously using the Challenge respirometer, by measuring N_2 gas production rate. Others have had significant difficulty maintaining anoxic conditions in open bench-scale reactors, and the closed respirometer bottles offer an opportunity to minimize oxygen input.

8. REFERENCES

See the previous Phase I/ II Literature Review and Experimental Proposal