

Project Title

Development of Predictive Tools to Infer Inhibition of Biological Nitrogen Removal at POTWs via Long Term Bench Scale and Full Scale Monitoring

Principal Investigators

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Summary of proposed activities**CHEMICAL ANALYTICAL MEASURES OF NITRIFICATION INHIBITORY CHARACTER**

We will develop rapid screening tools to measure wastewater composition and correlate bulk composition measures to nitrification inhibition. Initially, we will focus on metals (cadmium, zinc, nickel and copper, which have been implicated in nitrification inhibition, moderate to high toxicity to nitrifying microorganisms. Prior to the start of the monitoring study, we will optimize and validate the bulk chemical characterization tool in a synthetic matrix, such as the cultivation medium for nitrifying bacteria. The bulk metal content of a wastewater matrix will be determined by titrating against excess sulfide (in an anoxic environment) and measuring remaining sulfide concentrations and expressed in terms of a lumped metal-sulfide solubility product of the constituent metal-sulfide solubility product.

MONITORING OF CONTINUOUSLY OPERATED BENCH-SCALE BNR REACTORS

To verify proposed measurement techniques, two bench scale bioreactors initiated during our current Long Island Research Foundation funded study, will be operated in parallel at the Stamford WPCA. The bioreactors have an operating volume of 40L, and a flow rate of 10 gallons per day. The reactors consist of one anoxic basin followed by three aerated basins, followed by an internal clarifier and will be constructed at the Technical Services Center, University of Connecticut. These reactors will be operated under identical HRT, SRT, aeration and mixing regimes as the full-scale treatment WPCA (*i.e.* as a Modified LÜdzack Ettinger configuration). Once established, baseline nitrification and denitrification performance and kinetics in each of these reactors -operated in tandem- will be measured using the chemical specific and respirometric assays, respectively described above.

We will validate application of the batch respirometric assay to measuring nitrification kinetics in a full-scale BNR reactor treating actual domestic and industrial wastewater, across a

wide range of seasonal, wastewater composition and biocatalyst activity dynamics, during a prolonged monitoring campaign.

RESPIROMETRIC ASSAY TO MEASURE NITRIFICATION KINETICS

The kinetics of nitrification and nitrification inhibition in continuously operated bench-scale biological nitrogen removal (BNR) bioreactors will be measured using an extant respirometric assay developed in our laboratories. In the proposed study, we will validate application of the batch respirometric assay to measuring nitrification inhibition in continuously operated BNR reactors treating actual domestic wastewater, across a wide range of seasonal, wastewater composition and biocatalyst activity dynamics, during a prolonged monitoring campaign.

Summary of concluded project activities

CHEMICAL ANALYTICAL MEASURES OF NITRIFICATION INHIBITORY CHARACTER

The metal content of a wastewater matrix was determined by titrating against sulfide (in an anoxic environment) and measuring remaining sulfide concentrations. The bulk measure of heavy metal content will be expressed in terms of a the total metal concentration and a lumped metal-sulfide solubility product of the constituent metal-sulfide solubility product (Table I). Sulfide precipitation assays were carried out in deionized water at pH 7.0, achieved using 10mM MOPS. The design matrix below was used for conducting ΔS and free-metal ion concentration measurements. ΔS measurements will be performed on metal solutions containing total metal concentrations in the range 10^{-7} – 10^{-3} M (prepared by serial dilution). Metal mixtures were be prepared with individual total metal concentrations at 10^{-7} M, 10^{-6} M, 10^{-5} M, 10^{-4} M and 10^{-3} M. ΔS measurements are performed on metal solutions containing total metal concentrations in the range 10^{-5} , 10^{-4} and 10^{-3} M.

Table I : Experimental design for determination of metal-sulfide complexation capacity, columns denote different metal constituents tested

Ni	+	-	-	-	+	-	-	+	-	+	+	-	+	+	+	-
Cu	-	+	-	-	+	+	-	-	+	-	+	+	-	+	+	-
Zn	-	-	+	-	-	+	+	+	-	-	+	+	+	-	+	-
Cd	-	-	-	+	-	-	+	-	+	+	-	+	+	+	+	-

Metal concentrations were determined based on measurements of their sulfide complexation capacity according to protocol outlined in the appendix. Following the sulfide complexation tests, the resulting sulfide precipitation profiles were subject to linear regression

analysis. Two measures of total metal concentration were calculated based on the region of the sulfide evolution profile during which the added sulfide concentration exceeds the analytical total metal concentration in solution.

1. The first measure was based on the difference between the added and measured sulfide concentrations. Ideally, this difference should reflect the equivalents of the metal ions and should be constant with increasing added sulfide concentrations.

2. The second measure was the x-intercept of the best-fit line thorough the measured sulfide concentrations higher than zero. Ideally, the x-intercept should reflect the equivalents of the metal ions and should be constant with increasing added sulfide concentrations.

Results

Since all the metals tested are divalent, a 1:1 molar stoichiometry between total metal concentration was theoretically expected. Among the individual metals tested, the metal concentration determined for Ni(II) using the sulfide complexation assay was the highest (Table II). For all the tests conducted, the measure based on the x-intercept was more accurate and precise compared to the measure based on the average difference between the added and the measured residual sulfide concentrations. In general, there was a positive bias in the sulfide complexation based method for measuring lumped metal concentrations (Table II).

Table II : Two measures of lumped metal concentration normalized with respect to the known initially added metal concentration, $M_{T,added}$ for individual metals and metal mixtures

	$\Delta S^2/M_{T,added}$	x-intercept/ $M_{T,added}$	n
Cd(II)	1.24 ± 1.45	0.69 ± 0.41	4
Cu(II)	1.17 ± 0.07	1.37 ± 0.56	6
Ni(II)	1.97 ± 1.5	2.48 ± 1.04	6
Zn(II)	1.33 ± 1.44	1.28 ± 0.88	3
Cu(II)-Cd(II)	1.21	Not calculated	1
Cu(II)-Zn(II)	1.88	Not calculated	1
Cu(II)-Ni(II)	1.53	1.66	1
Cd(II)-Zn(II)	1.63	1.81	1
Cd(II)-Ni(II)	1.3	1.46	1
Ni(II)-Zn(II)	0.71	0.8	1

Cu(II)-Cd(II)-Ni(II)-Zn(II)	1.26	1.27	1
Cu(II)-Cd(II)-Ni(II)	1.27	0.83	1
Cu(II)-Cd(II)-Zn(I)	1.09	1.09	1
Cu(II)-Ni(I)-Zn(II)	1.27	1.41	1
Ni(II)-Zn(II)-Cd(II)	1.22	1.5	1
Overall	1.67 ± 1.05	1.43 ± 0.65	30

Concluding remarks

The method developed in this study presents a simple alternative to comprehensive ion-coupled plasma mass spectrophotometry or voltammetry based metals measurement. However, a positive bias was observed in the results from the method when applied to solutions containing test heavy metals. In general, the lumped metal concentration based on the x-intercept of the sulfide complexation profile was more accurate and precise than that based on the average difference between the added and the residual sulfide concentration. Based on these results, further verification of this method is required before routine application at POTWs.

MONITORING OF CONTINUOUSLY OPERATED BENCH-SCALE BNR REACTORS

Fabrication of the bench-scale reactors to be installed at the Stamford WPCA was performed by the University of Connecticut Technical Services Center and completed in March 2001. Bench-scale operation at Stamford commenced during July 2001. The reactors consisted of four in-line baffled stages followed by a gravity secondary clarifier. The first and third stages of the bench-scale reactors were anoxic and the second and fourth stages were aerobic. The system included full-recycle from the secondary clarifier to the head of the treatment train. The two biological BNR reactors were housed in a temporary project laboratory located near the primary clarifiers at the Stamford treatment plant. The reactors were designed to simulate a biological nitrogen removal treatment plant (Figures 1 and 2). After installation at the Stamford WPCA, the reactors were seeded with biomass from the full-scale reactors at the same facility.

The reactors were continuously fed from a reservoir of primary effluent that was pumped from the primary clarifier tanks at the Stamford plant. Oxygen was applied with fine stone aeration and mixing. Peristaltic pumps were used to feed each bench-scale system and to provide recycle flow (4 pumps all together). A syringe pump located at the head of the treatment train (1st anoxic stage) was used to feed inhibitory compounds at a specific dose to one of the bench systems, while the other system acted as a control.

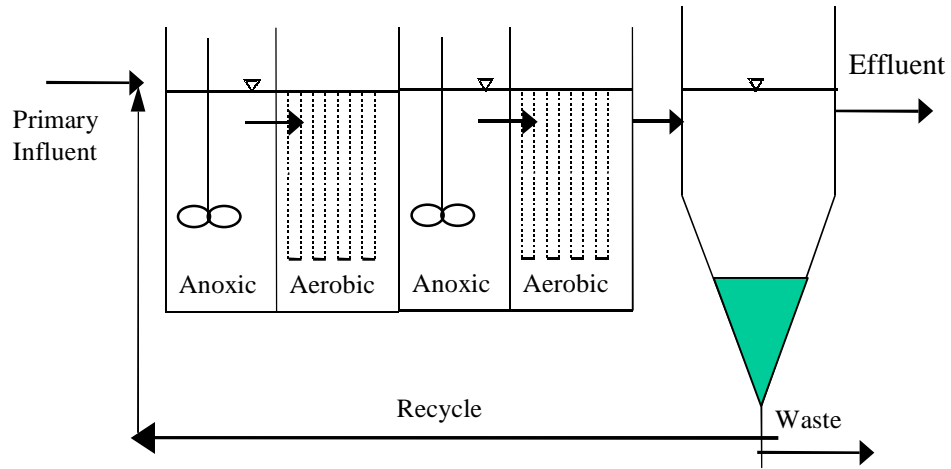
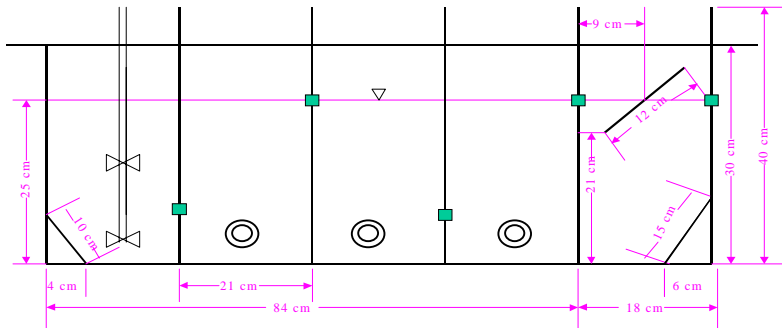
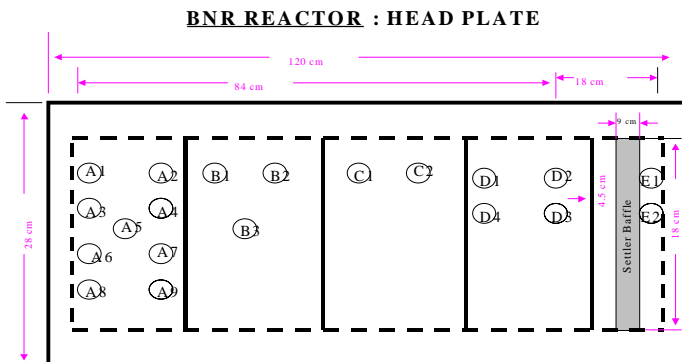
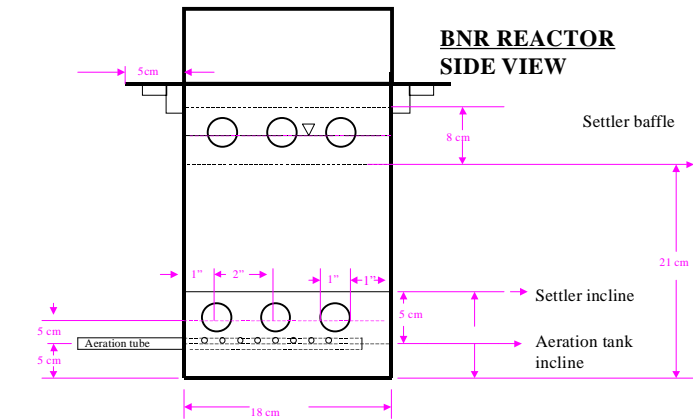


Figure 1: Schematic of Bench scale Nitrification-Denitrification Reactors at the Stamford WPCA.

BNR REACTOR
FRONT VIEW





All hole diameters : 0.5" except A1 (DO), A4(pH), D2(pH)
 Diameters of A1, A4 and D2 per existing design and fittings

Figure 2: Design Specifications of Bench scale Nitrifying Reactor Stamford WPCA

Start-up and Sampling

The Stamford WPCF bench-scale reactors were officially started in July 2001. The Stamford WPCF staff spent many hours designing and modifying the process piping, pumping and airflow systems. The staff relied on trouble-shooting assistance and equipment from Manhattan College, with the Stamford staff performing the majority of the analysis and bench-scale operation. Figures 3a - 3c includes photographs of the continuous flow bench-scale system and the enclosure that was built to house the reactors.

Running bench-scale reactors on actual plant primary effluent proved to be very problematic, but by August 2001, the reactors had reached steady state and the WPCF staff was able to begin monitoring. Samples were taken from each bench scale reactor at various locations

along the treatment train. Reactor samples were analyzed for dissolved nitrogen species and suspended solids concentrations (Table 3). The pH and dissolved oxygen concentration measurements in the reactor were automated. Due to the long piping length needed to bring the primary effluent to the bench-system and the need for a reservoir holding tank to feed the bench system, the raw water had lost some of its BOD and ammonia by the time it entered the reactor systems. However, there were still adequate amounts of BOD and ammonia to maintain a continuous flow system.

Table 3 : Sampling Specifications, Bench-scale Reactors, Stamford WPCA

PARAMETER	TYPE
pH (Bench scale Reactor)	Continuous
Chemical Oxygen Demand	Grab
NH ₃ -N	Grab
NO ₂ ⁻ -N	Grab
NO ₃ ⁻ -N	Grab
TKN	Grab
MLSS	Grab
MLVSS	Grab
Toxicity (Extant Respirometry)	Grab
Dissolved oxygen (Extant Respirometry)	Grab
Dissolved oxygen (Bench scale Reactor)	Continuous



Figure3a - Reactor building constructed to maintain reasonable operating temperatures.

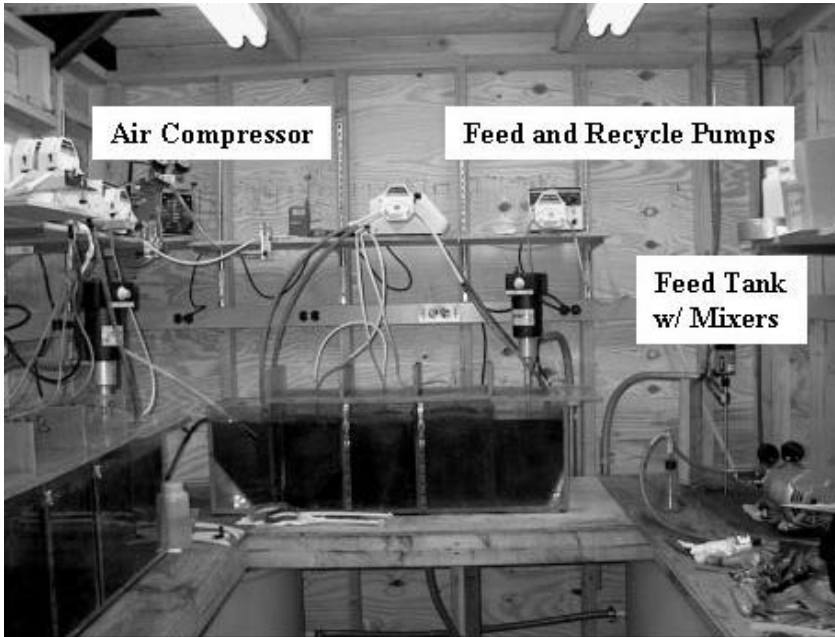


Figure 3b - Both reactor system (Test and Control) with pumps, feed tank and aeration system noted.

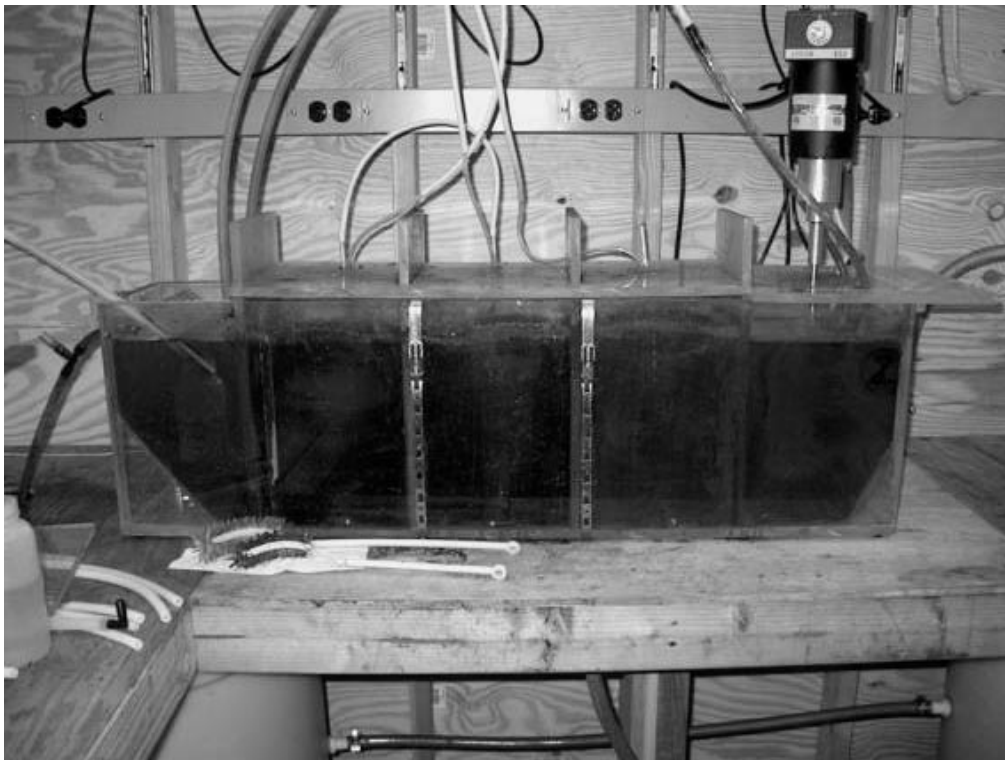


Figure 3c - Close-up of bench scale reactor system in operation.

RESPIROMETRIC ASSAY TO MEASURE NITRIFICATION KINETICS

Respirometric tests were carried out to screen various possible nitrification inhibitors at Stamford WPCA. To prepare for inhibition testing, special attention was paid to get the two bench-scale reactors working as effectively as possible and to ensure that pseudo-steady state operation had been reached. Sodium lauryl sulfate (SLS) was chosen as the inhibitor to test since it is commonly found in municipal wastewaters in significant concentrations. A dose of 10 mg/l of SLS was applied to the one of the bench systems via a syringe pump, while the second reactor acted as the control. A dose of 10 mg/l of SLS resulted in a 42% reduction in specific nitrification activity when tested in the batch studies performed during the Field Assay testing using Stamford BNR sludge. The SLS was fed to the system for a period of 6 hydraulic residence times (3 days) prior to measuring the nitrification inhibition. Nitrification inhibition was measured using two methods; the first was based on ammonia removal performance ; the second method used the respirometric assay to directly measure the biological nitrification kinetics. Results from the test reactor were compared to those of the control to determine percent reduction in ammonia removal. The ammonia measured reduction was compared to the respirometric results.

Prior to SLS addition both the test reactor and the control reactor were operating very similarly, with both system removing 98% of the influent ammonia. After three days of SLS addition, ammonia analysis showed the control reactor was still removing 98% of the influent ammonia (20.7 mg/l to 0.4 mg/l), while the test reactor removed only 78.8% (20.35 mg/l to 4.35 mg/l) of the influent ammonia. The SLS reduced total ammonia removal by 20% . Figure 4 shows a set of respirograms for the test and control reactor after 3 days of SLS addition. The Field Assay results indicate that a 10 mg/l dose of SLS in the continuous flow systems resulted in a 19% reduction in specific NOUR. These results compared very well with the ammonia removal reductions, and seem to indicate that the Field Assay results are comparable to direct ammonia measurements. These results also indicate that the inhibitory effects of SLS are not as extreme in continuous flow systems as they are in batch systems. There are many possible explanations for the reduced inhibition in the continuous flow systems, some of these possible explanations include: a) dilution of SLS due to recycle flow; b) reserve and recycle of active nitrifiers; and c) potential degradation of SLS by other active organisms in the BNR sludge resulting in a lower applied dose.

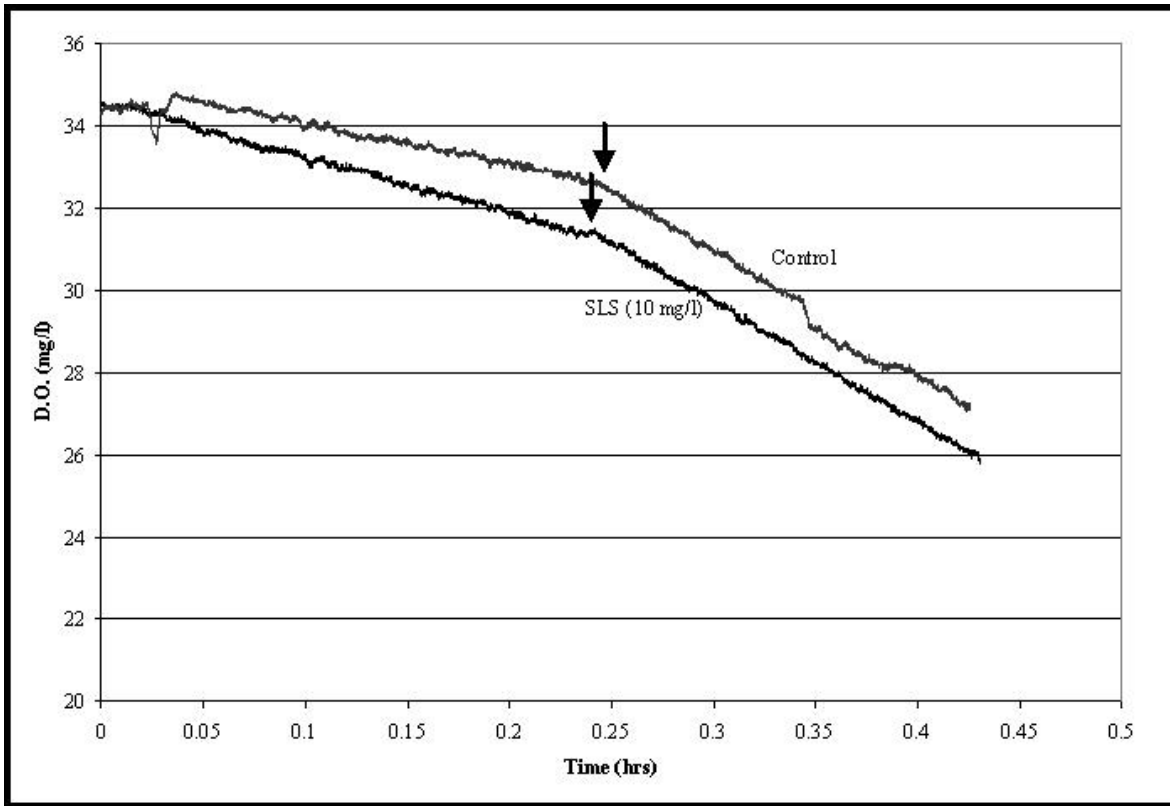


Figure 4: Results of SLS inhibition on continuous flow BNR bench reactors as measured with the Field Respirometric Assay. A 10 mg/l dose of SLS resulted in a 20% reduction in specific NOUR.

Project Personnel Supported

During the course of this study, the following personnel were supported by the awarded project funding.

- ◇ Kartik Chandran Ph.D., Post Doctoral Fellow, Environmental Engineering Program, University of Connecticut, currently with Metcalf and Eddy, New York.
- ◇ Monika Siwek, Undergraduate student, Microbiology Program, University of Connecticut, graduated Fall 2001.
- ◇ Wojciech Krach, Undergraduate student, Environmental Engineering Program, University of Connecticut, graduated Spring 2003.