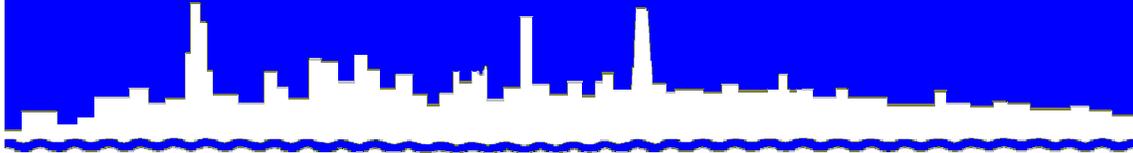


*Protecting Our Water Environment*



***Metropolitan Water Reclamation District of Greater Chicago***

***RESEARCH AND DEVELOPMENT  
DEPARTMENT***

***REPORT NO. 07-61***

***SYNERGISTIC INHIBITORY EFFECTS OF HEAVY METAL MIXTURE  
ON ACTIVATED SLUDGE NITRIFICATION***

***SEPTEMBER 2007***

**Metropolitan Water Reclamation District of Greater Chicago**

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ACTIVATED SLUDGE NITRIFICATION**

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## **DISCLAIMER**

Mention of proprietary equipment and chemicals in this report does not constitute endorsement by the Metropolitan Water Reclamation District of Greater Chicago.

## BACKGROUND

Nitrification is an extremely important process in wastewater treatment because ammonia nitrogen ( $\text{NH}_4^+\text{-N}$ ) effluent limits are regulated and expected to become more stringent. Nitrification proceeds as described below,



This reaction is a combination of two intermediate reactions driven by the autotrophic genera *Nitrosomonas* and *Nitrobacter*. In this series of biochemical reactions,  $\text{NH}_4^+\text{-N}$  is consumed and nitrate ( $\text{NO}_3^-$ ) is produced. Alkalinity is consumed and pH decreases with the production of hydrogen ions ( $\text{H}^+$ ). Nitrification is a very sensitive process to external factors such as toxic compounds, pH, temperature, heavy metals, priority pollutants, and many other pollutants.

The Calumet Water Reclamation Plant (WRP) experienced inhibited nitrification and produced elevated  $\text{NH}_4^+\text{-N}$  levels in the plant effluent during the period of March 30–April 2, 2005. However, no violation of the NPDES permit occurred. The plant restored full nitrification gradually by April 6, 2005.

Unfortunately, the Research and Development Department (R&D) could not identify the nitrification-inhibiting substance(s) responsible for the interference based on our intensive analytical and investigative efforts. Analytes such as cyanide, phenol, influent ammonia, heavy metals, and other priority pollutants were not found at high enough levels to cause such an upset. However, slightly elevated levels of some heavy metals, particularly cadmium (Cd), hexavalent chromium ( $\text{Cr}^{+6}$ ), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn) were detected during the plant upset. Maximum concentrations of these heavy metals over the period in question are summarized in [Table 1](#). The concentrations of these individual heavy metals were not high enough to upset the nitrification process. However, this left a possibility that a mixture of heavy metals may have exerted synergistically inhibitive effect on the nitrifying biomass in the activated sludge.

As a first step in this evaluation, a detailed literature survey was conducted to examine the synergism of heavy metals on the nitrification process. The findings of literature survey are presented in [Appendix AI](#). Briefly, the individual metals studied were shown to significantly decrease nitrification and microbial growth. The first step in nitrification, oxidation of ammonium to nitrite, seems to be the limiting step. Less effect is observed with the second step, nitrite oxidation to nitrate. Varying objectives, methods and analysis in the studies reviewed produced mixed results regarding trends in metal toxicity.

A literature review did not reveal a single study that addressed synergism of heavy metals on the nitrification process. The R&D did find certain studies with a range of experiments conducted with metal combinations investigating nitrification inhibition with varying objectives and mixed results. The lack of understanding regarding synergistic metal inhibition triggered the present study.

TABLE 1: METAL CONCENTRATIONS OBSERVED IN RAW SEWAGE  
COLLECTED FROM SOUTH DISCRETE SAMPLER AT THE CALUMET WRP ON  
MARCH 30, 2005, AT 6 A.M.

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Metal	Concentration (mg/L)
Zn	1.2777
Cr	0.0814
Cd	0.0024
Ni	0.1306
Pb	0.1612
Cu	0.3422

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## **OBJECTIVE**

The objective of this study was to investigate the effect of individual heavy metals of concern and a combination of heavy metals (“metal cocktails” [MCs]) on nitrification at a concentration range found at the Calumet WRP during the March/April 2005 upset. To study potential impacts of single metals and MCs on microbial response, laboratory experiments using Stickney WRP Battery B mixed liquor (ML) were conducted. The Stickney Battery B ML was preferred for these experiments due to its accessibility and consistency in the samples. Samples were immediately available; the R&D laboratories are located at the Stickney WRP.

## METHODS AND MATERIALS

### Sample Collection

Approximately 7.5 liters of fresh Stickney WRP Battery B ML were collected on ten separate occasions from August 22 through September 15, 2006.

Stickney ML was collected for the following analyses in place of Calumet ML. The 2005 influent metal concentrations for each WRP are summarized in [Table 2](#). The average metal concentrations were calculated from measurements above the analysis detection limit.

Comparatively, the individual total and soluble metal concentrations between the two WRPs are within an order of magnitude of each other. Conventional wisdom suggests the soluble fraction is more harmful to cellular processes than the insoluble metal species due to the ability to diffuse across bacterial cell walls. Based on the average values above, it can be assumed that the activated sludge at both WRPs experience the same heavy metal concentrations. Currently, no information is available with respect to acclimation of the microbial populations to elevated metal concentrations at either plant.

### Solids Analysis

Total solids (*TS*), total dissolved solids (*TDS*), and total volatile solids (*TVS*) analyses were conducted on ML samples using the respective standard methods (Eaton et al., 1995). Mixed liquor volatile suspended solids (*MLVSS*) concentrations were determined using the following equation,

$$MLVSS = \frac{TVS}{TS}(TS - TDS) \quad (2)$$

To minimize analysis, %TVS was used rather than performing volatile suspended solids analysis.

### Nitrification Analysis

**Mixed Liquor Sample Preparation.** Ten treatments, consisting of a control, six with individual metals, and three with different combinations of metals were prepared by adding metals to the collected ML sample as summarized in [Table 3](#). Each of the six individual metals was added at a concentration of 10 ppm. The Calumet MC treatment (Treatment 8) was prepared to replicate the heavy metal concentrations observed during the Calumet WRP upset as shown in [Table 1](#). This is the most dilute treatment of the MC treatments (total metal concentration of 2.0 ppm) while MC5 treatment is the strongest treatment with a total metal concentration of 30 ppm.

TABLE 2: AVERAGE 2005 INFLUENT TOTAL METAL CONCENTRATIONS

WRP	Flow MGD	Total Metals						Soluble Metals					
		Cd mg/L	Cr mg/L	Cu mg/L	Pb mg/L	Ni mg/L	Zn mg/L	Cd Sol mg/L	Cr Sol mg/L	Cu Sol mg/L	Pb Sol mg/L	Ni Sol mg/L	Zn Sol mg/L
Calumet	224	0.0037	0.0066	0.0421	0.0103	0.0104	0.3634	ND	0.00138	0.00522	ND	0.00683	0.10000
Stickney	653	0.0016	0.0564	0.1360	0.0506	0.0247	0.3729	0.00049	0.00301	0.00596	0.00636	0.00635	0.01635
Southwest	320	0.00225	0.07318	0.19928	0.08191	0.03223	0.55373	0.00055	0.00125	0.00800	0.00600	0.00533	0.01215
West	333	0.0010	0.0403	0.0752	0.0205	0.0174	0.1992	0.00043	0.00470	0.00400	0.00670	0.00733	0.02038

ND = Not determined.

TABLE 3: MIXED LIQUOR TREATMENTS EXAMINED FOR NITRIFICATION TESTS

Treatment Number	Treatment Name	Metal Salts Used	Specification
1	Control	None	ML (control)
2	Zn	ZnSO <sub>4</sub> ·7H <sub>2</sub> O	ML + 10 ppm Zn
3	Cr	K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	ML + 10 ppm Cr
4	Cd	Cd(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O	ML + 10 ppm Cd
5	Ni	NiSO <sub>4</sub> ·6H <sub>2</sub> O	ML + 10 ppm Ni
6	Pb	Pb(NO <sub>3</sub> ) <sub>2</sub>	ML + 10 ppm Pb
7	Cu	CuSO <sub>4</sub> ·5H <sub>2</sub> O	ML + 10 ppm Cu
8	Cal MC	All the above	ML + Calumet cocktail concentrations as shown in Table 1
9	MC1.67	All the above	ML + 1.67 ppm of each metal of concern
10	MC5	All the above	ML + 5 ppm of each metal of concern

The MC1.67 treatment had a combined metal concentration of 10 ppm. These treatments were investigated on ten separate days with freshly collected ML.

Three separate 2500-mL aliquots of fresh ML were added to 3.78-liter plastic containers. These three ML samples were dosed with metal salt solutions described in [Table 3](#) in order to achieve the concentrations selected for each treatment. The salt solutions were prepared by adding the necessary stoichiometric mass of individual metal salts to one liter of deionized (DI) water in order to obtain a 1000 ppm concentration of each metal. The individual metal concentrations in the prepared salt solution were verified by argon inductively coupled plasma analysis; all metal concentrations were within 6.1% of the intended concentration of 1000 ppm. Serial dilutions were prepared and added in appropriate quantities to achieve 10 ppm concentration in ML samples. The background metal concentration of the ML before treatment was not determined. However, the average metal concentrations for Stickney influent during the course of the study (August-September 2006) were 0.004 mg/L Cd, 0.077 mg/L Cr, 0.165 mg/L Cu, 0.079 mg/L Pb, 0.027 mg/L Ni, and 0.520 mg/L Zn. Except for cadmium, the individual metal concentrations are at least 50% lower than the concentrations observed during the Calumet WRP upset. It should be noted that the metal treatments in [Table 3](#) do not account for the residual metal concentrations in the sampled ML. However, for this study the ML metal concentrations are assumed to be on the order of the measured influent concentrations. Therefore, the treatment concentrations could be slightly higher than suggested in [Table 3](#). For example, Treatment 2 could have as high as 10.520 ppm Zn. For simplification, the treatment is still considered to be 10 ppm Zn.

The salt addition in the solutions contributed less than 0.25% of the total volume of the treated ML and therefore did not dilute the ML. For each treatment, three samples were analyzed with three different contact times: at or very close to the time the metal solutions were added, and 2 hours and 6 hours after the metal solutions were added.

Up to six hours of contact time is expected to show an effect on microbial activity. The 2- and 6-hour metal contact spans were chosen to mimic the time for the heavy metal(s) to completely mix into the ML in the aeration tank and exhibit impact on nitrification.

**Nitrification Tests.** Each of the three treated ML samples ( $T = 0, 2, \text{ and } 6$  hrs) were tested for nitrification rates. Approximately 2000 mL of sample were added to a 4000-mL beaker. One end of tygon tubing was attached to an air spigot while the open end was centered at the bottom of the vessel. Air was bubbled at a constant pressure for two minutes to provide aeration and adequate mixing of the ML. The airflow was then stopped, and three samples were withdrawn, 80 mL, 80 mL, and 100 mL for pH,  $\text{NH}_3$ , and  $\text{NO}_3^-$  analysis, respectively. The tygon tubing was immediately placed back in the vessel, and the treated ML was aerated for another 22 minutes, and again the three aliquots were taken for pH,  $\text{NH}_3$ , and  $\text{NO}_3^-$  analysis.

*pH.* A magnetic stirring rod was added to an 80-mL sample. This system was placed on a stir plate to ensure constant mixing. The pH of the sample was determined with a pH ion selective electrode (Orion combined pH).

*Ammonia Nitrogen.* An 80-mL sample was allowed to sit for 3 minutes, thereby letting most solids to settle to the bottom of the beaker. A 10-mL volumetric pipette was used to draw off 10 mL of the supernatant liquid of the sample, which was added to a 100-mL volumetric flask. The remainder of the flask was brought to 100 mL volume with DI water. The volumetric flask was then shaken.

A 25-mL graduated cylinder was filled to volume with the diluted sample solution (10%). Likewise, a second cylinder was treated as a blank and filled with DI water. Three drops of mineral stabilizer, three drops of polyvinyl alcohol, and 1.0 mL of Nessler reagent was added to each cylinder. The contents of each cylinder were then mixed. One minute of reaction time was allowed for each cylinder, and the solutions were poured into separate 10-mL sample cells.

A Hach DR 2800 portable spectrophotometer was used for ammonia analysis (380 N). The blank cell was placed in the spectrophotometer cell holder and zeroed, i.e., set at 0 mg NH<sub>3</sub>/L prior to analysis of the prepared sample.

*Nitrate.* A magnetic stirring rod was added to the 100-mL sample. This system was placed on a stir plate to ensure constant mixing. Two milliliters of an ionic strength adjustment buffer (2 M ammonium sulfate) was added, and an Orion Ion Plus Nitrate electrode was used to measure the voltage. A calibration curve with a range of 0–2.5 mg NO<sub>3</sub>/L was used in order to convert the voltage measured to the nitrate concentration of the sample in mg NO<sub>3</sub>/L.

*Nitrification Rate.* With ammonia and nitrate concentrations at the beginning and end of sample aeration, i.e.  $t = 2$  min and  $t = 24$  min ( $\Delta t = 22$  min), the nitrification rate ( $NR$ ) can be determined using the following equations,

$$NR_{NH_3} = \frac{([NH_3]_{t_{final}} - [NH_3]_{t_{initial}})}{(t_{final} - t_{initial})} \frac{1440 \text{ min}}{\text{day}} \frac{1}{MLVSS} \quad \text{and}; \quad (3)$$

$$NR_{NO_3} = \frac{([NO_3]_{t_{final}} - [NO_3]_{t_{initial}})}{(t_{final} - t_{initial})} \frac{1440 \text{ min}}{\text{day}} \frac{1}{MLVSS}$$

Thus, R&D determined the  $NR$ s considering ammonia depletion ( $NR_{NH_3}$ ) and, conversely, considering nitrate production concentrations ( $NR_{NO_3}$ ). However,  $NR$ s using NH<sub>4</sub><sup>+</sup>-N depletion were used as the primary data in our analysis, and the  $NR$ s obtained from nitrate increase were viewed as corroborative information.

## Oxygen Uptake Rate Analysis

Each of the treated ML samples ( $T = 0, 2$  and  $6$  hrs) was then subjected to oxygen uptake rate (OUR) tests to determine microbial respiration. The OUR is directly related to the microbes' ability to consume carbon and nitrogen food sources.

A well mixed, aerated, and treated 300-mL sample was added to a biochemical oxygen demand (BOD) bottle. A YSI dissolved oxygen (DO) meter was inserted into the BOD bottle, and the attached oscillating baffle was activated to ensure rapid, constant mixing. Both time and DO values were recorded until the DO concentration dropped to ~2.5 mg DO/L. Additionally, the temperature was recorded during the experiment. For the DO versus time measurements, the OUR was calculated according to the following relation:

$$OUR = \frac{([DO]_n - [DO]_{n+1})}{t_{n+1} - t_n} \quad (4)$$

Regression analysis of individual DO versus time data was used to calculate a single OUR value. Because the OUR tests were performed under different temperatures, the Arrhenius temperature relationship was used to normalize the average OUR to test temperature as follows (Brey, 1958),

$$OUR_{T_2} = OUR_{T_1} \theta^{(T_2 - T_1)} \quad (5)$$

Here,  $T_1$  represents the actual temperature and  $T_2 = 20^\circ\text{C}$ . The value of  $\theta$ , a dimensionless temperature coefficient, is 1.015. Finally, the OUR was normalized using the *MLVSS* concentration to give the specific oxygen uptake rate ( $SOUR_{20}$ ),

$$SOUR_{20} = \frac{OUR_{20}}{MLVSS} \quad (6)$$

## RESULTS AND DISCUSSION

All raw data collected and used in this study are shown in Appendix AII.

### Solids Analysis

*TS*, *TDS*, *TVS*, and *MLVSS* analyses were performed on each ML sample. These values are summarized in Table 4. The *MLVSS* concentration ranged from 1,318 to 2,553 mg/L. The percent *TVS* ranged from 59% to 72%.

### Nitrification Test Analysis

**Nitrification Rate.** The initial and final ammonia nitrogen and nitrate concentrations observed for each treatment are summarized in Table 5. These concentration values were used to calculate nitrification rates as presented in Table 6. The greater magnitude for either calculated rate value (Equation 3) does not reflect necessarily higher nitrification. Nitrification rates should ideally agree with each other because  $\text{NO}_3^-$  is generated as  $\text{NH}_3$  is depleted during microbial nitrification. However, the lack of nitrate production as  $\text{NH}_3$  is depleted may be an indication of inhibition of the second stage of nitrification; nitrite concentrations were not measured. However, from the literature review, the first stage of nitrification is considered the rate-limiting step. Madoni et al. (1999) found significant inhibition in terms of  $NR_{\text{NH}_3}$  due to short-term (1 hour) and long-term (24 hours) exposure to Cu, Zn, Pb, Cd, and Cr. Hu et al. (2004) verified that the first stage nitrification is much more vulnerable compared to the second stage. They observed that as little as one hour of exposure to higher concentrations of individual metals, Cu, Zn, Ni, and Cd, reduced  $\text{OUR}_{\text{NH}_4}$  from 28.5% to 87.5% but did not reduce as much  $\text{OUR}_{\text{NO}_2}$ . Based on the results summarized in Table 6, metal addition decreases the *NRs*, both with respect to nitrate and ammonia observations. Among the individual metal treatments, Cr (10 ppm) has the greatest inhibitory effect on nitrification, and MC5 (30 ppm) has the greatest inhibitory effect among the MC treatments. No clear trend is observed with respect to metal contact time and the nitrification rates. Longer time windows of contact time may need to be used in order to see a significant difference in nitrification inhibition.

Bar graphs presenting the average  $NR_{\text{NO}_3}$  and  $NR_{\text{NH}_3}$  rates vs. treatment are shown in Figures 1a and 1b, respectively. Table 7 summarizes the percent difference in nitrification rates of each treatment compared to the Control. Again, Cr and MC5 had the lowest nitrification rates.

The Control treatment was expected to have no inhibition of *NRs*. As shown in Figures 1a and 1b, this hypothesis was verified. Almost all other treatments were observed to have  $NR_{\text{NO}_3}$  and  $NR_{\text{NH}_3}$  rates lower than the Control rates. The one exception was found with the Cal MC  $NR_{\text{NO}_3}$  that was 4.6% higher than the Control rate; however, for the purposes of this study, this increase may be considered negligible because the percent difference in  $NR_{\text{NO}_3}$  from Control ranged from -89.3 to 4.6%. On the contrary, the Cal MC  $NR_{\text{NH}_3}$  rate was 68.1% lower than the

TABLE 4: SOLIDS ANALYSIS SUMMARY

Treatment	Sample Date	TS (mg/L)	TDS (mg/L)	TVS (mg/L)	TVS (%)	MLVSS (mg/L)
Control A	8/22/2006	3129.7	457.5	2034.2	65.0	1736.8
Zn 10 ppm	8/23/2006	2676.2	555.0	1740.6	65.0	1379.6
Cr 10 ppm	8/24/2006	3223.8	430.0	2314.2	71.8	2005.5
Cd 10 ppm	8/25/2006	2500.6	407.5	1691.9	67.7	1416.2
Ni 10 ppm	8/26/2006	2886.8	522.5	2078.9	72.0	1702.7
Pb 10 ppm	9/15/2006	4358.2	432.5	2834.6	65.0	2553.3
Cu 10 ppm	9/11/2006	3986.4	395.0	2417.2	60.6	2177.7
Calumet Metal Cocktail	9/1/2006	2676.6	345.0	1766.3	66.0	1538.7
Mixed Metals (1.6 ppm)	9/21/2006	4417.1	407.5	2607.6	59.0	2367.1
Mixed Metals (5 ppm)	8/31/2006	2534.6	392.5	1559.4	61.5	1317.9

TABLE 5: AVERAGE INITIAL AND FINAL AMMONIA AND NITRATE CONCENTRATIONS FOR EACH TREATMENT

Treatment	Average NH <sub>3</sub> Initial (mg/L)	Average NH <sub>3</sub> Final (mg/L)	Average NO <sub>3</sub> Initial (mg/L)	Average NO <sub>3</sub> Final (mg/L)
Control	9.9	6.4	1.0	2.5
Zn	9.1	6.6	0.2	0.9
Cr	12.8	12.3	2.6	2.7
Cd	8.8	7.6	2.1	2.8
Ni	8.7	7.8	1.4	2.3
Pb	7.3	6.0	1.8	3.0
Cu	5.7	4.3	3.7	4.5
Cal MC	6.6	5.6	0.3	1.6
MC1.67	9.1	9.0	2.3	2.8
MC5	4.1	2.6	4.5	6.3

TABLE 6: NITRIFICATION RATES FOR EACH TREATMENT AND METAL CONTACT TIME

Treatment	Time (hrs)	$NR_{NO_3}$ (mg/L NO <sub>3</sub> )/ (mg/L MLVSS/day)	Average $NR_{NO_3}$	$NR_{NH_3}$ (mg/L NH <sub>3</sub> )/ (mg/L MLVSS/day)	Average $NR_{NH_3}$
Control	0	0.049	0.055	-0.157	-0.129
	2	0.046		-0.064	
	6	0.072		-0.166	
Zn	0	0.011	0.036	-0.033	-0.119
	2	0.030		-0.176	
	6	0.066		-0.147	
Cr	0	0.001	0.006	0.003	-0.014
	2	0.019		-0.020	
	6	-0.002		-0.026	
Cd	0	0.027	0.033	-0.074	-0.055
	2	0.042		-0.051	
	6	0.030		-0.042	
Ni	0	0.054	0.037	0.004	-0.035
	2	0.024		-0.092	
	6	0.034		-0.015	
Pb	0	0.047	0.048	-0.046	-0.050
	2	0.046		-0.038	
	6	0.050		-0.067	
Cu	0	0.038	0.024	-0.039	-0.041
	2	-0.009		-0.033	
	6	0.044		-0.051	
Cal MC	0	0.024	0.058	-0.021	-0.041
	2	0.014		-0.030	
	6	0.137		-0.072	
MC1.67	0	0.045	0.048	-0.035	-0.040
	2	0.053		-0.053	
	6	0.047		-0.033	

TABLE 6 (Continued): NITRIFICATION RATES FOR EACH TREATMENT  
AND METAL CONTACT TIME

Treatment	Time (hrs)	$NR_{NO_3}$ (mg/L NO <sub>3</sub> )/ (mg/L MLVSS/day)	Average $NR_{NO_3}$	$NR_{NH_3}$ (mg/L NH <sub>3</sub> )/ (mg/L MLVSS/day)	Average $NR_{NH_3}$
MC5	0	0.024	0.024	-0.015	-0.005
	2	0.022		0.000	
	6	0.024		0.000	

FIGURE 1: AVERAGE NITRIFICATION RATES FOR EACH TREATMENT AS A FUNCTION OF (A) NITRATE GENERATION AND (B) AMMONIA DEPLETION

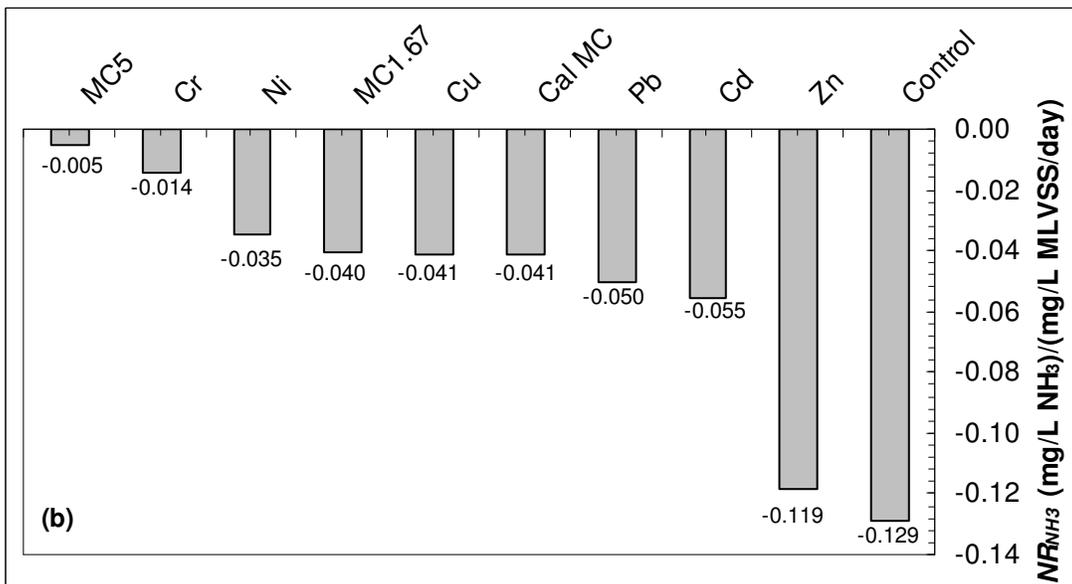
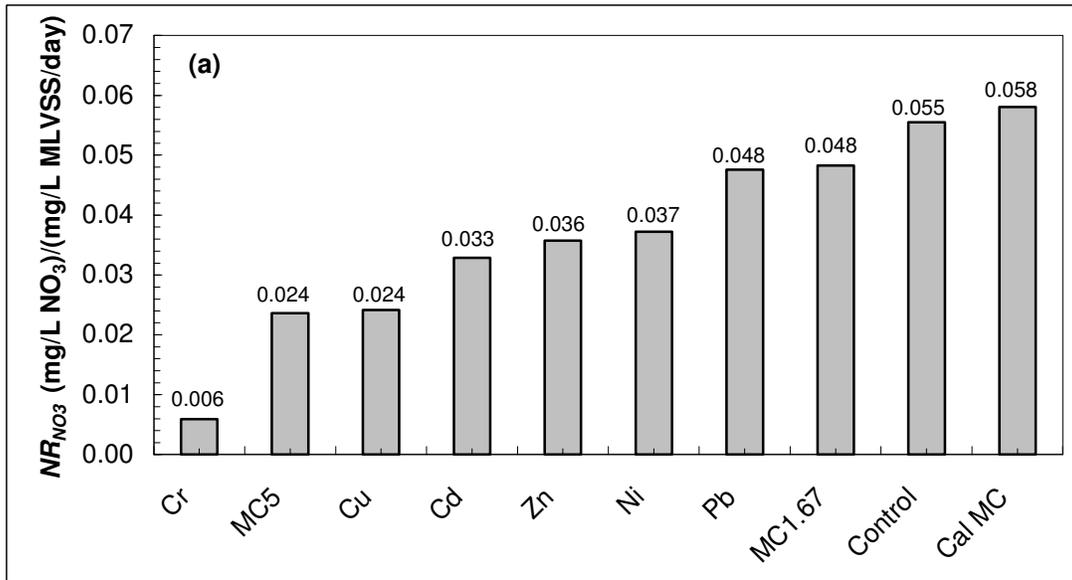


TABLE 7: PERCENT DIFFERENCE IN  $NR_{NO_3}$  AND  $NR_{NH_3}$  FOR EACH TREATMENT WITH RESPECT TO THE CONTROL

Treatment	% Difference in $NR_{NO_3}$ from Control	% Difference in $NR_{NH_3}$ from Control
MC5	-57.5	-96.1
Cr	-89.3	-89.0
Ni	-32.9	-73.2
MC1.67	-13.0	-68.8
Cu	-56.5	-68.1
Cal MC	4.6	-68.1
Pb	-14.2	-60.9
Cd	-40.7	-57.0
Zn	-35.6	-8.0
Control	0.0	0.0

Control rate. In our analysis of the results, R&D considered  $NR_{NH_3}$  rates as primary information and  $NR_{NO_3}$  as corroborative information though, due to first stage ( $NH_3 \rightarrow NO_2^-$ ) inhibition, the  $NR_{NO_3}$  may not equal  $NR_{NH_3}$ . In general, the Cr and MC5 treatments exhibited the greatest inhibitory effects, which had  $NR_{NH_3}$  rates 89% and 96%, respectively, lower than the Control rate.

Based on the nitrification results of the individual metal treatments, the order of metal inhibition is as follows: Cr > Ni > Cu > Pb > Cd > Zn. Based on the literature review ([Appendix AI](#)), Juliastuti et al. (2003) found Cu as a stronger inhibitor than Zn. Hu et al. (2004) found a similar inhibitory trend for short-term (1 hour) and long-term (24-hour) exposure as the following: Cd > Zn > Ni > Cu. Madoni et al. (1999) observed inhibition as follows: Cd > Cu > Zn > Pb > Cr. Hu et al. (2004) and Madoni et al. (1999) both concluded that long-term metal exposure caused higher inhibition for each individual metal as the exposure time increased. Thus, the cited research observed different orders of inhibitory effects of heavy metals. Therefore making comparisons with our study results was difficult. Additionally, as the Stickney ML historically has Zn concentrations of 4.40 mg/L, it could be hypothesized that the activated sludge has become acclimated to higher concentrations of Zn. Therefore, dosing the ML with an additional 10 mg/L of Zn may not have the same nitrification inhibitory effect as other metals.

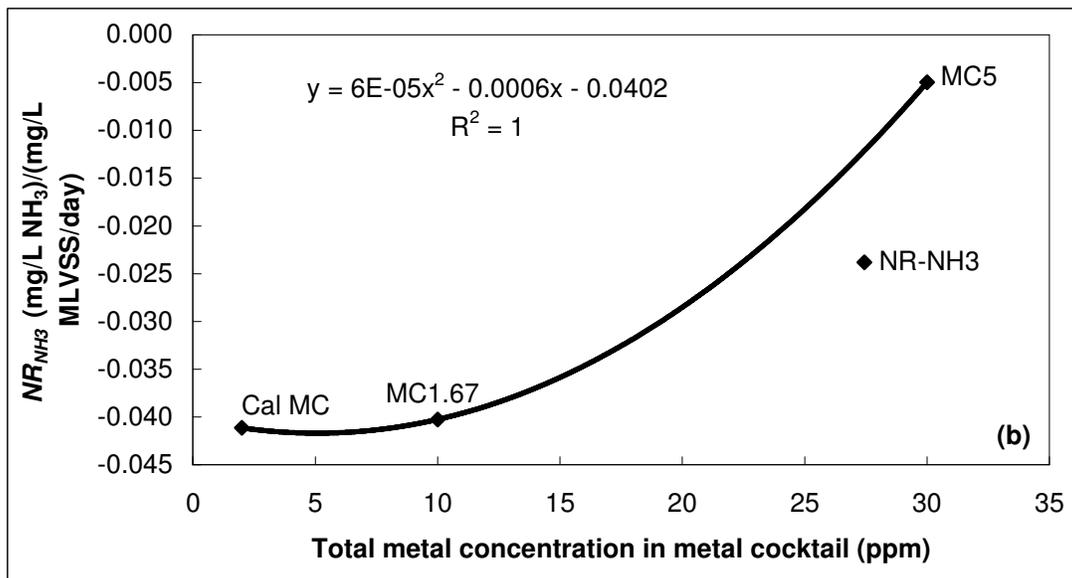
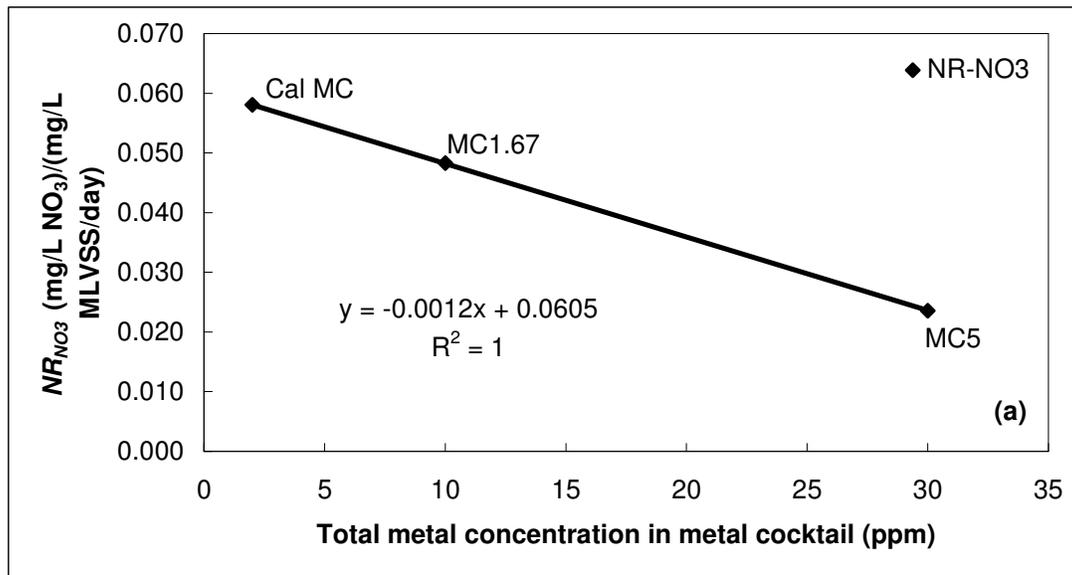
The order of inhibition for the MC treatments is: MC5 > MC1.67 > Cal MC. This trend is reasonable since inhibition should increase with increasing heavy metal concentrations. Based on the protease enzymatic activity, Gonzalez et al. (2001) concluded that a combined exposure of Cu and Zn concentration of 40 mg/L each showed neither synergistic nor antagonistic effect. Cabrero et al. (1997) showed that a combination of metals had a greater inhibitory effect on microbial growth than individual metals. Shuttleworth and Unz (1991) found a synergistic inhibitory effect of metal mixtures, Cu-Ni and Cu-Zn.

In this study, chromium and nickel are the most individual inhibitory metals, and MC5 is the most inhibitory MC. Lower nitrification rates of the MC5 tests may be due to the presence of the two inhibitory metals, Cr and Ni, and the total metal concentration of 30 ppm. The nitrification rate of MC5 is approximately 74.6% and 69.4% lower than the Cal MC and MC1.67 rates, respectively. [Figures 2a](#) and [2b](#) present total metal concentrations of the MCs vs. their respective  $NR_{NO_3}$  and  $NR_{NH_3}$  rates. [Figure 2a](#) shows a linear trend, while [Figure 2b](#) shows an exponential trend. The linear trend may reflect an additive effect, whereas the exponential trend may reflect a synergistic effect. It appears that an additive effect may be occurring considering  $NR_{NO_3}$ , but a synergistic effect is observed considering  $NR_{NH_3}$  rates. It is not clear, however, that what R&D interpret as synergy of heavy metals is indeed occurring or apparent synergy is attributed due to increase in total metal concentrations. Based on the test results, it is very difficult to distinguish the impact on nitrification caused due to synergism of metals and increase in total metal concentrations.

## pH Analysis

As shown in [Equation 1](#), theoretically pH should decrease during nitrification. Invariably, every nitrification test showed an average increase in pH of 0.18 units; a maximum increase of 0.47 was observed during one of the Cr nitrification tests. Alkalinity in ML was not measured

FIGURE 2: PLOT OF TOTAL METAL CONCENTRATIONS OF EACH METAL COCKTAIL TREATMENT VS. (A)  $NR_{NO_3}$  AND (B)  $NR_{NH_3}$



but because of bubbling air in ML sample, alkalinity may have increased due to removal of carbon dioxide (CO<sub>2</sub>) from the system resulting in minor increase in pH. This increase in pH from CO<sub>2</sub> stripping evidently overshadowed the pH decrease from nitrification. Average change in pH for each treatment is summarized in [Table 8](#).

### Oxygen Uptake Rate Analysis

In addition to the nitrification tests, OUR tests were performed as corroborative tests. The OURs were normalized to *MLVSS* due to fluctuations in solids concentration during our testing period. The results of the OUR tests are summarized in [Table 9](#). Unlike the nitrification rate results, [Table 9](#) shows that there is a clear trend with respect to oxygen uptake and metal contact time with the ML. OURs decreased with increasing metal contact time. The only deviation from this trend was observed with the MC1.67 (10 ppm) treatment.

The average values for all three contact times from [Table 9](#) are summarized in [Table 10](#). [Table 11](#) summarizes the percent difference in OURs and SOURs of each treatment compared to the Control OUR and Control SOUR. Bar graphs reflecting results from [Table 11](#) for the average OUR and SOUR data vs. treatment are shown in [Figures 3a](#) and [3b](#). The higher OUR or SOUR relates to higher nitrification rates, as oxygen is used as the electron acceptor in the oxidation of ammonia to nitrate. The Control treatment was expected to have the highest OURs and SOURs as no heavy metal was added to inhibit nitrification. [Table 12](#) summarizes the percent difference in  $NR_{NH_3}$ ,  $NR_{NO_3}$ , and SOURs of each treatment with respect to the  $NR_{NH_3}$  and SOUR of the Control.

From [Figures 3a-3b](#) and [Table 11](#), two treatments were observed to have higher SOURs than the Control: Zn (+10.7%), and Cal MC (+11.9%). However, changes under 15% are considered relatively insignificant for this study because of the very nature of the test limitations. The OUR tests are not very accurate in reproducing results because of many variables such as type and health of microbes. Also, as noted before, the Stickney ML has higher Zn background concentrations. This somewhat corroborates the nitrification results as Zn was found to be the least inhibitory individual metal, and Cal MC was found to be the least inhibitory MC. However, these results still contradict the hypothesis that any metal addition will stem nitrification and therefore oxygen uptake. Much like the nitrification analysis, the Cr and MC5 treatments were observed to have the lowest SOURs and were 55.3% and 41.2% lower than the Control SOUR, respectively.

Thus, based on OUR/SOUR results, Cr (10 ppm) has the greatest inhibitory effect on oxygen uptake among the individual metal treatments and MC5 (30 ppm) has the greatest inhibitory effect among the MC treatments.

Based on the SOUR results of the individual metals, the order of metal inhibition is as follows: Cr > Ni > Cu > Cd > Pb > Zn. This is the same trend observed with the nitrification analysis with the exception of the Cd and Pb placement. Based on nitrification rates, Cd was found to be less inhibitory than Pb by 4%. The order of inhibition for the MC treatments is: MC5 > MC1.67 > Cal MC. This is the same trend observed with the nitrification analysis.

TABLE 8: AVERAGE CHANGE IN pH FOR EACH TREATMENT

Treatment	$\Delta$ pH
Control	0.13
Zn	0.20
Cr	0.26
Cd	0.20
Ni	0.16
Pb	0.17
Cu	0.21
Cal MC	0.29
MC1.67	0.11
MC5	0.09

TABLE 9: OUR AND SOURs FOR EACH TREATMENT AND METAL CONTACT TIME

Treatment	Time	OUR (mg/L-h)	OUR <sub>i</sub> - $\Delta$ OUR <sub>cntrl</sub>	SOUR (mg/L-h)/ (mg/L MLVSS) mg/mg/hr	$\Delta$ SOUR <sub>i</sub> - $\Delta$ SOUR <sub>cntrl</sub>
Control	0	32.1	0.0	0.018	0.000
	2	29.6	0.0	0.017	0.000
	6	27.6	0.0	0.016	0.000
Zn	0	29.4	-2.7	0.021	0.003
	2	25.1	-4.5	0.018	0.001
	6	24.1	-3.5	0.017	0.002
Cr	0	24.3	-7.7	0.012	-0.006
	2	12.3	-17.4	0.006	-0.011
	6	9.5	-18.2	0.005	-0.011
Cd	0	25.9	-6.2	0.018	0.000
	2	17.3	-12.3	0.012	-0.005
	6	18.1	-9.5	0.013	-0.003
Ni	0	26.8	-5.3	0.016	-0.003
	2	19.5	-10.1	0.011	-0.006
	6	18.6	-9.0	0.011	-0.005
Pb	0	40.0	7.9	0.016	-0.003
	2	40.2	10.6	0.016	-0.001
	6	39.6	12.0	0.016	0.000
Cu	0	35.1	3.0	0.016	-0.002
	2	26.3	-3.3	0.012	-0.005
	6	22.6	-5.0	0.010	-0.006
Cal MC	0	29.8	-2.3	0.019	0.001
	2	29.4	-0.2	0.019	0.002
	6	29.3	1.7	0.019	0.003

TABLE 9 (Continued): OUR AND SOURs FOR EACH TREATMENT AND METAL CONTACT TIME

Treatment	Time	OUR (mg/L-h)	OUR <sub>i</sub> - ΔOUR <sub>ctrl</sub>	SOUR (mg/L-h)/ (mg/L MLVSS) mg/mg/hr	ΔSOUR <sub>i</sub> - ΔSOUR <sub>ctrl</sub>
MC1.67	0	37.3	5.2	0.016	-0.003
	2	34.6	5.0	0.015	-0.002
	6	39.0	11.3	0.016	0.001
MC5	0	22.8	-9.3	0.017	-0.001
	2	9.7	-19.9	0.007	-0.010
	6	7.4	-20.2	0.006	-0.010

TABLE 10: AVERAGE OUR AND SOUR<sub>s</sub> FOR EACH TREATMENT

Treatment	OUR (mg/L-h)	$\Delta\text{OUR}_i - \Delta\text{OUR}_{\text{ctrl}}$	SOUR (mg/L-h)/(mg/L MLVSS)	$\Delta\text{SOUR}_i - \Delta\text{SOUR}_{\text{ctrl}}$
Control	29.8	0.0	0.017	0.000
Zn	26.2	-3.6	0.019	0.002
Cr	15.4	-14.4	0.008	-0.009
Cd	20.4	-9.3	0.014	-0.003
Ni	21.6	-8.1	0.013	-0.004
Pb	39.9	10.1	0.016	-0.002
Cu	28.0	-1.8	0.013	-0.004
Cal MC	29.5	-0.3	0.019	0.002
MC1.67	37.0	7.2	0.016	-0.002
MC5	13.3	-16.5	0.010	-0.007

TABLE 11: PERCENT DIFFERENCE IN OXYGEN UPTAKE AND SPECIFIC OXYGEN UPTAKE RATES FOR EACH TREATMENT WITH RESPECT TO THE CONTROL

Treatment	% Difference in OUR from Control	% Difference in SOUR from Control
Cr	-48.4	-55.3
MC5	-55.4	-41.2
Ni	-27.4	-25.9
Cu	-6.0	-25.0
Cd	-31.3	-15.8
MC1.67	24.1	-5.9
Pb	34.1	-5.9
Control	0.0	0.0
Zn	-12.1	10.7
Cal MC	-0.9	11.8

FIGURE 3: AVERAGE (A) OURS AND (B) SOURS FOR EACH TREATMENT

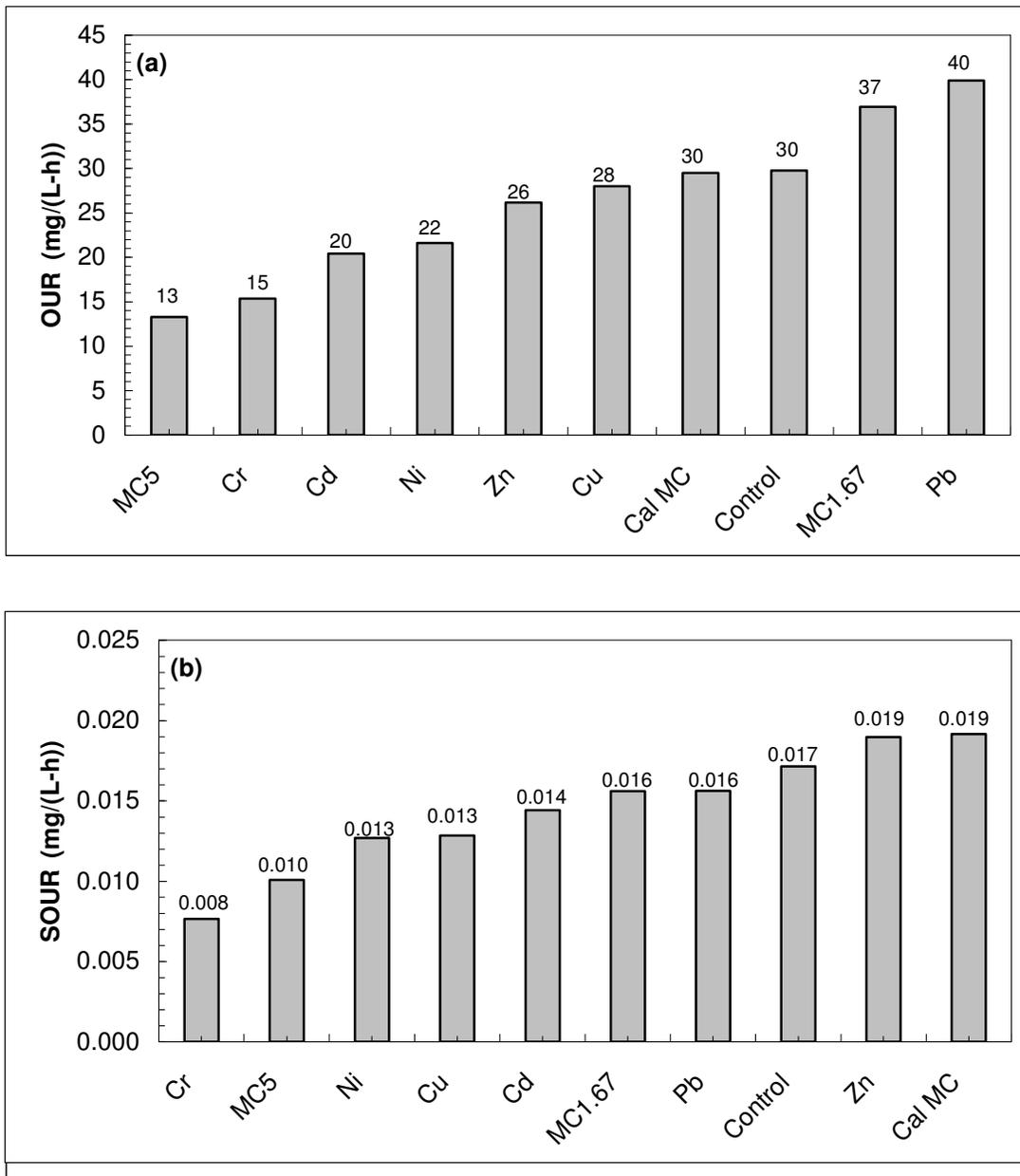


TABLE 12: PERCENT DIFFERENCE IN  $NR_{NH_3}$ ,  $NR_{NO_3}$ , AND SOURs FOR EACH TREATMENT WITH RESPECT TO THE CONTROL

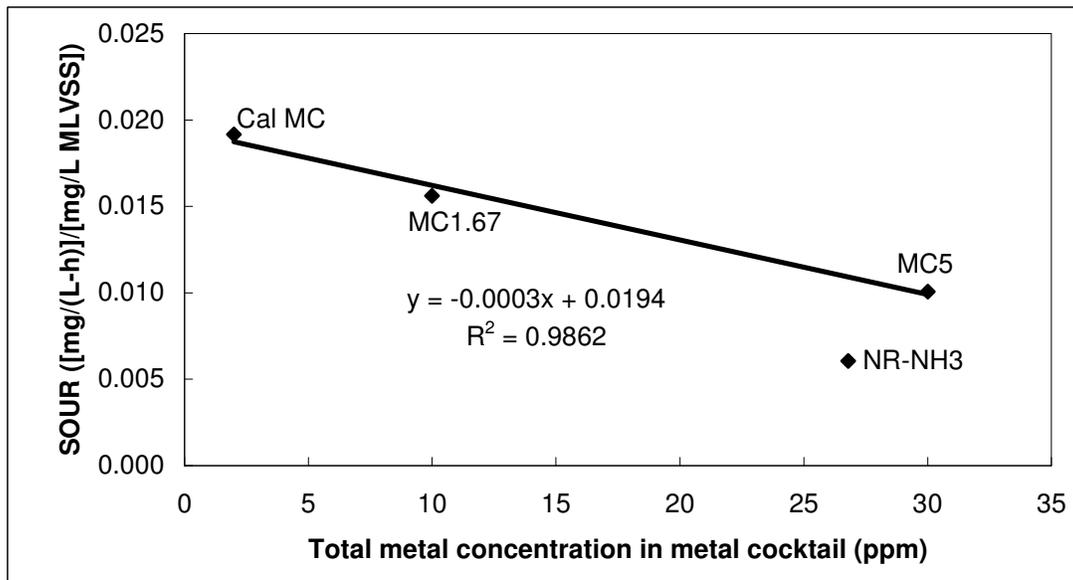
Treatment	% Difference in $NR_{NH_3}$ from Control	% Difference in $NR_{NO_3}$ from Control	% Difference in SOUR from Control
MC5	-96.1	-57.5	-41.2
Cr	-89.0	-89.3	-55.3
Ni	-73.2	-32.9	-25.9
MC1.67	-68.8	-13.0	-8.9
Cu	-68.1	-56.5	-25.0
Cal MC	-68.1	4.6	11.9
Pb	-60.9	-14.2	-8.8
Cd	-57.0	-40.7	-15.8
Zn	-8.0	-35.6	10.7
Control	0.0	0.0	0.0

Thus, nitrification rates determined in this study corroborate OURs/SOURs. However, in order to qualify our findings SOUR results within 15% of the control value are not considered significantly different from the control. Percent difference in SOUR from Control varied from 11.8 to -55.3 %. Again, no clear trend in metal toxicity was observed that was consistent with the literature studies cited in Appendix AI. For example, Hu et al. (2004) showed that normalizing the  $OUR_{NH_4}$  results for the shorter term experiments, the order of inhibition was:  $Cd > Zn > Ni > Cu$ . While from the initial SOUR results of our study using the Stickney WRP ML, the order of inhibition was:  $Cr > Ni > Cu > Cd > Pb > Zn$ . The trends from the studies cited in Appendix AI were not parallel with our study. The differences may be due to the length of respiratory assay period, microbial characteristics of the nitrifying biomass, or experimental protocol.

Based on this study, the treatments can be grouped according to their inhibitory effect on SOUR: 1) Cr and MC5 had a severe effect; 2) Ni, Cu, and Cd had a medium effect; and 3) Pb, Zn, Cal MC, and MC1.67 had a negligible effect.

Additionally, the MC5 SOUR is 47.4% and 35.5% lower than the Cal MC and MC1.67 rates, respectively. Figure 4 shows the total metal concentrations of the MCs vs. their respective SOURs. The linear trend line fit to the data reflects more of an additive effect. Much like nitrification analysis, while an additive effect may be occurring, there is no clear synergistic effect caused by the MCs reflected in the oxygen uptake.

FIGURE 4: PLOT OF TOTAL METAL CONCENTRATIONS OF EACH METAL COCKTAIL TREATMENT VS. SPECIFIC OXYGEN UPTAKE RATES



## CONCLUSIONS

Based on the laboratory study results, the following conclusions are drawn:

1. As anticipated, the Control treatment achieved the highest  $NR_{NH_3}$  rates, the highest  $NR_{NO_3}$  rates with the exception of the Zn treatment, and the highest SOURs with the exception of the Zn and Cal MC treatments.
2. Chromium was observed to have the greatest inhibitory effect on nitrification and oxygen uptake among the individual metals. The MC5 treatment, 30 mg/L total metal concentration, was observed to have the greatest inhibitory effect on nitrification and oxygen uptake among the MCs.
3. Of the individual metals, Zn was observed to have the least inhibitory effect on nitrification or oxygen uptake. Of the MCs, the Cal MC treatment was observed to have the least effect on oxygen uptake and nitrification.
4. Based on the nitrification and oxygen uptake test results, the order of inhibitory effects for individual metals is: Cr > Ni > Cu > Pb > Cd > Zn. The order of inhibition for the MC treatments is: MC5 > MC1.67 > Cal MC.
5. Preliminary nitrification test results ( $NR_{NH_3}$ ) suggest synergistic nitrification inhibition with increasing metal concentrations in the MC treatments. However, it was difficult to corroborate this synergy of MCs from the SOUR results.

Nonetheless, an additive effect was observed with  $NR_{NO_3}$  rates among the MCs, and potentially a synergistic effect was observed with  $NR_{NH_3}$  rates among the MCs. More of an additive effect was observed with SOURs among the MCs.

## SUMMARY AND RECOMMENDATIONS

Results presented herein showed nitrification inhibition for some individual metal additions and MC additions at concentrations above that detected during the Calumet WRP upset that occurred during March 30 through April 2, 2005. Researchers did not observe nitrification inhibition at the concentrations recorded during the upset.

One possible explanation for these results is that the Calumet WRP ML might have reached a mixture of heavy metals concentrations at higher than recorded levels initially during the upset. "After the fact" samples collected and analyzed from the aeration tanks and south discrete sampler perhaps did not capture the peak concentrations.

In light of this possibility, R&D recommends that further tests should be performed to fully assess the effect of heavy metals on ML nitrification and oxygen uptake. Finer increases of MC concentrations and their effect on ML nitrification and OURs may provide a clearer picture on whether combined metals inhibit nitrification processes additively or synergistically. Additionally, different combinations of the heavy metals may need to be examined with respect to ML nitrification, e.g. does Cr + Cd have a higher inhibitory effect than Pb + Zn? Statistically designed experiments would provide more definitive answers to the questions regarding synergistic inhibition of mixtures of metals during the activated sludge process.

Also, the future experiments should focus on longer metal contact time and long-term effects on oxygen uptake capabilities of microorganisms. Documenting the time taken to restore nitrification should also be a part of the experimental plan. More importantly, the future study should attempt to distinguish the effects on nitrification due to increase in metal concentrations and combination of metals. This will help confirm whether metal mixtures result in synergistic inhibition of nitrification or act independently to inhibit process performance. As soluble metal forms are considered the most inhibitory species of the examined metals, future studies will be examined in light of the soluble species as well as total metals. Additionally, care will be taken to analyze the residual metal concentrations in the activated sludge biomass in order to better quantify the measured dose received by the biomass.

## BIBLIOGRAPHY

1. Eaton, A.D., L.S. Clesceri, and A.E. Greenberg. 1995. *Standard Methods for the Examination of Water and Wastewater*. 19th ed. Washington, DC: American Public Health Association.
2. Brey, W.S. 1958. *Principles of Physical Chemistry*. New York: Appleton-Century-Crofts.
3. Madoni, P., D. Cavoli, and L. Guglielmi. "Response of SOUR and AUR to Heavy Metal Contamination in Activated Sludge," *Water Resources*, Vol. 22, No. 3, pp. 349-353, 1988.
4. Hu, A., K. Chandran, D. Grasso, and B. Smets. "Comparison of Nitrification Inhibition by Metals in Batch and Continuous Flow Reactors," *Water Research*, Vol. 38, pp. 3949-3959, 2004.
5. Cabrero, A., S. Fernandez, F. Mirada, and J. Garcia. "Effects of Copper and Zinc on the Activated Sludge Bacteria Growth Kinetics," *Water Resources*, Vol. 32, No. 5, pp. 1355-1362. 1998.

APPENDIX AI

LITERATURE SURVEY ON SYNERGISTIC INHIBITORY EFFECTS OF HEAVY METAL  
MIXTURE ON ACTIVATED SLUDGE NITRIFICATION

# LITERATURE SURVEY ON SYNERGISTIC INHIBITORY EFFECTS OF HEAVY METAL MIXTURE ON ACTIVATED SLUDGE NITRIFICATION

## General Objective

A literature review was performed to investigate synergistic effects of a mixture of heavy metals on the activated sludge nitrification process.

## Literature Review

**Heavy Metal Nitrification Inhibition Background.** Nitrification is an extremely important process in wastewater treatment because ammonia nitrogen ( $\text{NH}_4^+\text{-N}$ ) in effluent is regulated by the NPDES permit limits. Conventional aerobic nitrification involves the two-step conversion of ammonia to nitrate ( $\text{NO}_3^-\text{-N}$ ) by two different genera of bacteria: the ammonia-oxidizing bacteria (AOB) and the nitrite ( $\text{NO}_2^-\text{-N}$ )-oxidizing bacteria (NOB). AOB first convert ammonia to hydroxylamine using an enzyme called ammonia monooxygenase. Hydroxylamine is then converted to nitrite by another enzyme, hydroxylamine oxidoreductase. NOB then use nitrite as an electron donor and oxidize it to nitrate using the nitrite oxidoreductase enzyme. These reactions are intimately coupled with the electron transport chain in each organism. Nitrite serves as the energy source for these autotrophic bacteria (Kelly et al., 2004).

It is well established that nitrification is highly susceptible to upsets in wastewater treatment. Full-scale and laboratory-scale studies have shown that industrial pollutants, specifically heavy metals, are frequently the source of such upsets or inhibitory events. This literature review emphasizes the following heavy metals of interest; cadmium (Cd), copper (Cu), chromium (Cr), nickel (Ni), lead (Pb), and zinc (Zn). Furthermore, restoration of nitrification after an inhibitory event can take time, leaving the treatment system vulnerable to permit violations (Kelly et al., 2004).

In general, it is believed that the first step in the microbial response to heavy metals is the uptake of free metal cations via a nonspecific metal inorganic transport system. Once inside the cell, heavy metals can interact with thiol groups and destroy protein structure and related functions, i.e., the enzymes responsible in the nitrification process (Hu et al., 2002).

Several factors that influence the degree of inhibition by heavy metals are pH, metal concentration, metal speciation, temperature, mixed liquor suspended solids concentration, sludge age, solubility of metal, and the concentration of other cations and molecules present.

**Single Metal Nitrification Inhibitory Experimental Analysis.** Many studies have been performed to test the extent of single metal inhibition on nitrification and microbial growth in activated sludge and soil.

Juliastuti et al. (2003) investigated the effects of different heavy metal inhibitors on the activated sludge process by measuring the net maximum specific growth rate of an autotrophic biomass by measuring oxygen uptake rate (OUR) measurements. Experiments were performed with mixed liquor concentrations of approximately 2000 mg/L. The ranges of Zn and Cu in the mixed liquor solution were 0.3-1.2 mg/L and 0.1-0.5 mg/L, respectively (Juliastuti et al., 2003).

The OUR was continuously monitored throughout the 24-hour experiments. The percent inhibition using OUR data was measured as follows:

$$\% \text{ Inhibition} = \frac{(OUR_{control} - OUR_{sample})}{OUR_{control}} \times 100 \quad (1)$$

The concentration for each metal that produced 50% inhibition ( $IC_{50}$ ) was determined. The free Cu cation ( $Cu^{+2}$ ) was found to be a stronger inhibitor than the free Zn cation ( $Zn^{+2}$ ) with  $IC_{50}$  values of 0.08 mg/L for  $Cu^{+2}$  and 0.35 mg/L for  $Zn^{+2}$ . Inhibition with  $Cu^{+2}$  was apparent at concentrations above 0.05 mg/L, while  $Zn^{+2}$  inhibition was apparent at 0.3 mg/L. Inhibition was complete at 1.2 mg/L for both metals (Juliastuti et al., 2003).

Hu et al. (2004) determined the nitrification inhibition under short- and long-term exposure of nitrifying biomass to Cu, Zn, Ni, and Cd using respiratory assays. After cultivating a nitrifying biomass, the short-term effects of Cu, Zn, Ni, and Cd were investigated individually in batch assays for one hour. The metal concentrations were tested up to 121 mg/L.

In the short-term batch assays, the ammonium oxidation rate (AOR) decreased significantly as the metal dose increased. The ammonia oxidation rate is the change in DO as ammonia is being converted to nitrite, and the nitrite oxidation rate (NOR) is the change in DO as the nitrite is being converted to nitrate. The following results were observed:

- 120.7 mg/L Cu reduced the AOR by 28.5%.
- 112.4 mg/L Cd reduced the AOR by 87.5%.
- 62.1 mg/L Zn reduced the AOR by 56%.
- 102.7 mg/L Ni reduced the AOR by 56%.

In contrast, the specific NOR was affected less. The maximum reduction in NOR was observed for Ni at 16.6%.

For the long-term experiment, an instantaneous shock load of an individual metal was applied for a 24-hour duration into a continuous flow bioreactor, and the DO was measured continuously. Reactor effluent was periodically collected for  $NH_4^+$ -N,  $NO_3^-$ -N,  $NO_2^-$ -N, and metal speciation measurements. The metal concentrations were tested at individual doses of 46.4 mg/L Cu, 179.9 mg/L Cd, 65.4 mg/L Zn, and 101.0 mg/L Ni (Hu et al., 2004). Similar results were observed with the long-term experiments relative to the short-term experiments. However,

a higher degree of ammonium oxidation inhibition occurred with each metal due to the increase in exposure time (Hu et al., 2004).

Madoni et al. (1999) tested the inhibitory effect of free metal ions on nitrifiers using ammonium uptake rates (AUR) and OUR. Activated sludge samples were spiked with elevated and varying concentrations of  $\text{Cu}^{+2}$  (0-0.92 mg/L),  $\text{Zn}^{+2}$  (0-2.10 mg/L),  $\text{Pb}^{+2}$  (0-16.9 mg/L),  $\text{Cd}^{+2}$  (0-3.71 mg/L), and  $\text{Cr}^{+6}$  (0.5-284 mg/L). The MLSS and MLVSS of activated sludge varied from 2300 to 4800 mg/L and 1800 to 4200 mg/L, respectively. All metal tests produced significant AUR and OUR inhibition. Short exposure (one hour) showed less inhibition than longer exposure times (24 hours). The metals in order of inhibition were as follows:  $\text{Cd} > \text{Cu} > \text{Zn} > \text{Pb} > \text{Cr}$ . The  $\text{IC}_{50}$  values based on one-hour OUR measurements for Cd, Cu, Zn, Pb, and Cr are summarized in [Table AI-1](#) (Madoni et al., 1999). The  $\text{IC}_{50}$  values for these selected metals from other sources are also given in [Table AI-1](#) (Madoni et al., 1999; Anderson et al., 1988; Vismara, 1982; Kong et al., 1993; Cenci and Morozzi, 1979; Dutka and Kwan, 1984).

**Mixed Metals Nitrification Inhibitory Experimental Analysis.** The aforementioned studies verify inhibitory effects by single metals on nitrification in activated sludge. A few studies investigating inhibition to a combination of heavy metals are summarized herein.

Garcia-Gonzalez et al. (2001) examined the combined effects of  $\text{Cu}^{+2}$  and  $\text{Zn}^{+2}$  at different pH levels on the enzymatic activity of activated sludge. Enzymatic assays based on either dehydrogenase or hydrolase activities have been used to study heavy metal inhibition on activated sludge. Individually, at 40 mg/L for each metal, 35% and 21% inhibition occurred for Cu and Zn, respectively. Based on the protease activity, a combination showed neither a synergistic nor antagonistic activity (Garcia-Gonzalez et al., 2001); supporting data was not provided.

Rusk et al. (2004) examined soil biological nitrification adaptability over time to elevated concentrations of Zn and Pb. In this study soil samples were spiked with a solution containing different concentrations of Zn and Pb salts giving three soil Zn treatments and two soil Pb treatments. These soil treatments were incubated for 21 months. The lowest metal concentration treatments were considered “unexposed” and the highest concentration treatments were considered “exposed.” The microbial communities in the exposed treatments were considered to be acclimated to the high metal conditions. Unexposed soils were spiked with different heavy metal concentrations as summarized in [Table AI-2](#). These unexposed samples had initial concentrations of 20-39 mg/kg of their respective metals. All test soils were supplemented with ammonium sulfate and allowed to cure for 28 days. From the aforementioned experiments, the  $\text{IC}_{50}$  value for Zn and Pb were determined through nitrate and nitrite concentrations at the onset and conclusion of the 28-day test period. The  $\text{IC}_{50}$  values for the unexposed soils from the five experiments are summarized in [Table AI-2](#). Antagonistic effect from the mixture of metals was observed in all cases instead of anticipated synergistic effects on nitrification inhibition (Rusk et al., 2004).

Cabrero et al. (1997) determined the response of microbial growth from a medium shock dosed to a combination of Cu and Zn. Suppressed microbial growth can be an indicator of nitrification inhibition. Cu and Zn were added to a batch-growth system at concentrations of 0/0,

TABLE AI-1: SUMMARY OF EXPERIMENTAL RESULTS OF METAL CONCENTRATIONS THAT REDUCE AMMONIA UPTAKE OR OXYGEN UPTAKE BY 50% (IC<sub>50</sub>) IN MIXED LIQUOR FROM VARIOUS WASTEWATER TREATMENT PLANTS (MODONI ET AL., 1999)

Cadmium (mg/L)	Chromium (mg/L)	Copper (mg/L)	Lead (mg/L)	Zinc (mg/L)	Nickel (mg/L)	References
0.83	180.00	0.07	13.75	0.60	—	Modoni et al. (1999)*
—	—	0.10	—	0.50	—	Juliastuti et al. (2002)*
—	—	~0.25	—	~0.29	—	Vismara(1982)*
—	—	0.50	—	0.70	—	Kong et al. (1993)*
14	—	6.00	23	25	38	Anderson et al. (1988)**
27	—	—	—	—	—	Kelly et al. (2004)**
19	—	40	—	16	42	Cenci and Morozzi (1979)**
—	—	29	350	1.20	4.30	Dutka and Kwan (1984)**

\*Metal concentrations measured in mixed liquor solution.

\*\*Dose concentrations.

TABLE AI-2: METAL CONCENTRATIONS THAT REDUCE NITRIFICATION IN UNEXPOSED SOIL MICROBIAL POPULATIONS BY 50% (IC<sub>50</sub>) (RUSK ET AL., 2004)

Metal Treated Soil	Metal Spike	IC <sub>50</sub> (mg/kg), Metal
Zn	Zn	210, Zn
Pb	Pb	1,960, Zn
Zn	Zn and Cd	350, Pb
Pb	Zn	230, Zn
Zn	Pb	2,590, Pb

5/5, 5/10, and 10/5 mg/L of Cu and Zn, respectively. The MLSS were monitored periodically for three days. The MLSS was used as an indicator of the microbial growth response (Cabrero et al., 1998).

The maximum biomass and maximum growth rates for the different metal combinations are summarized in Table AI-3. It was observed that the combination of metals had a greater inhibitory effect on microbial growth than individual metals. The Cu/Zn concentration of 10/5 mg/L is the most inhibitory with the maximum biomass concentration and growth rate approximately 50% that of the reference values. While the combined metal effect was not synergistic, it may be considered somewhat additive (Cabrero et al., 1998).

Shuttleworth and Unz (1991) examined the effects of Cu, Ni, and Zn on the growth characteristics of strains of filamentous bacteria obtained from bulking activated sludge. In their experiment, inocula were prepared from actively growing biomass resuspended in a fresh lactate-thiosulfate-HEPES medium. Copper, nickel, and zinc chloride solutions of different concentrations were added to the medium, and growth - no growth studies were conducted. Ni-Cu and Zn-Cu solutions were examined for lag time of bacterial growth initiation (Shuttleworth and Unz, 1991).

The investigators found that although Zn functioned as an antagonist to the inhibitory effect of Ni, combinations of Cu-Ni and Cu-Zn appeared to act synergistically to suppress growth. For individual metal doses, growth occurred in one day with 0.65 mg/L Zn and after 5 days with 0.59 mg/L Ni. However, no growth occurred when using a combination of 0.59 mg/L of Ni and 0.32 mg/L of Cu. Additionally, no growth occurred when using a combination of 0.65 mg/L of Zn and 0.32 mg/L of Cu (Shuttleworth and Unz, 1991).

Lin et al. (2003) examined the joint effect of Cu and Zn on nonacclimated activated sludge microorganisms. Cu and Zn were added to the test reactors at concentrations ranging from 10 to 100 mg/L both individually and combined. The specific growth and substrate removal rates were calculated over a growth period of 300 minutes. Table AI-4 summarizes the microbial growth and substrate removal rates for the individual and combined metal tests (Lin et al., 2003). Based on the results of the combined metal treatments, an additive effect was observed compared to the specific growth rate, i.e., the growth rate notably decreased with increasing Cu or Zn concentrations (Lin et al., 2003).

Blais et al. (1993) examined acidophilic and less-acidophilic thiobacillus growth on 21 different sludges contaminated with individual heavy metals and combinations of heavy metals. Thiobacilli are sulfur-oxidizing bacteria which can decrease the pH of the system if sulfur is present to effectively leach heavy metals from sludge. The acidophilic bacteria were added to each sludge sample along with elemental sulfur and incubated for 12 to 22 days in order to acclimate the bacteria to the subsequent acidic conditions; the less-acidophilic bacteria were not acclimated in this manner (Blais et al., 1993).

A summary of the results with the corresponding sludge and metal type are provided in Table AI-5. The sludge type is characterized by the metals exceeding recommended levels. In general, the combined metal sludges have less microbial growth than the single metals which

TABLE AI-3: MEAN VALUE PARAMETERS OF LOGISTIC MODEL FIT TO DATA ON THE BACTERIA CELL GROWTH (CABRERO ET AL., 1997)

Metal	Metal Concentration (mg/L)	Maximum Biomass Concentration (g/L)	Maximum Growth Rate (g/L-h)
None	0	0.916	0.040
Cu	5	0.759	0.037
Cu	10	0.394	0.022
Zn	5	0.852	0.041
Zn	10	0.723	0.035
Cu/Zn	5/5	0.689	0.034
Cu/Zn	5/10	0.507	0.026
Cu/Zn	10/5	0.445	0.021

TABLE AI-4: INDIVIDUAL AND COMBINED EFFECT OF Zn AND Cu ON THE SPECIFIC GROWTH RATE AND PERCENT COD REMOVAL (LIN ET AL., 2003)

Metal	Metal Concentration (mg/L)	Specific Growth Rate (h <sup>-1</sup> )	% COD Removal
None	0	0.210	83.0
Cu	10	0.160	—
Cu	20	0.145	—
Zn	10	0.245	—
Zn	20	0.220	—
Zn	80	0.190	—
Cu/Zn	10/20	0.180	77.0
Cu/Zn	20/20	0.170	72.5
Cu/Zn	20/80	0.160	72.0
Cu/Zn	20/100	0.145	71.5

TABLE AI-5: TEMPORAL VARIATION OF THIOBACILLUS POPULATIONS  
IN SEWAGE SLUDGES (BLAIS ET AL., 1993)

Sludge	Metal	Less-Acidophilic Concentrations (cfu/mL) x 10 <sup>4</sup>	Acidophilic Concentrations (cfu/mL) x 10 <sup>4</sup>
C*	Cu	2.80	1.48
C2*	Cu/Ni	1.22	1.50
C3*	Cu/Ni	1.05	—
C4*	Cu/Ni	1.05	—
D**	Cu	19.2	4.40
D3**	Cu/Ni	4.73	3.80
S***	Cu	1.15	—
S2***	Pb	5.24	—

\*C, C2, C3, C4 = Secondary activated sludge.

\*\*D, D3 = Aerobically activated sludge.

\*\*\*S, S2 = Anaerobically activated sludge.

may be due to at least an additive inhibitory effect of both metals. The biggest discrepancy to this trend is observed with respect to the S and S2 sludge tests where the less-acidophilic thiobacilli grew much better on the Cu and Pb sludge compared to the Cu sludge alone (Blais et al., 1993).

## SUMMARY

Based on the literature review, individual metals studies showed significant decrease in nitrification and microbial growth. The first step in nitrification, oxidation of ammonium to nitrite, seems to be the most vulnerable and limiting step. Less effect is observed with respect to the second step, nitrite oxidation to nitrate.

Studies investigating the combined effects of metals on nitrification and microbial growth were limited and proved inconclusive. Gonzalez et al. (2001) found neither antagonistic nor synergistic effects with combined Cu and Ni on enzyme activity in activated sludge tests. Rusk et al. (2004) found antagonistic effects on biological nitrification with respect to combined heavy metal contaminations in soil. Cabrero et al. (1997) showed an additive effect of Cu and Zn on microbial growth in activated sludge. In contrast, Shuttleworth and Unz (1991) found the synergistic suppressive effects on growth characteristics of filamentous bacteria due to Cu, Ni, and Zn. Garcia et al. (1990) indicated that composting sludges with higher metal concentrations of Zn, Pb, Cr, Cd, Ni and Cu led to lower nitrification rates. However, nitrification inhibition cannot be directly attributed to a synergistic effect or an increase in single metal concentration.

This literature search did not reveal a strict nitrification study conducted with a mixture of heavy metals in mixed liquor. The studies cited do not provide sufficient guidance to support our objective because most of the studies were conducted with different objectives and wide range of experimental strategies to evaluate combined metal nitrification inhibition.

## REFERENCES

1. Kelly, R.T., D.S. Henriques, and N.G. Love. "Chemical Inhibition of Nitrification in Activated Sludge," *Biotechnology and Bioengineering*, Vol. 85, No. 6, pp. 683-694, 2004.
2. Hu Z., K. Chandran, D. Grasso, and B.F. Smets. "Effect of Nickel and Cadmium Speciation on Nitrification Inhibition." *Environmental Science and Technology*, Vol. 36, No. 14, pp. 3074-3078, 2002.
3. Juliastuti, S.R., J. Baeyens, C. Creemers, D. Bixio, and E. Lodewyckx. "The Inhibitory Effects of Heavy Metals and Organic Compounds on the Net Maximum Specific Growth Rate of the Autotrophic Biomass in Activated Sludge," *Journal of Hazardous Materials*, Vol. B100, pp. 271-283, 2003.
4. Hu, A., K. Chandran, D. Grasso, and B. Smets. "Comparison of Nitrification Inhibition by Metals in Batch and Continuous Flow Reactors," *Water Research*, Vol. 38, pp. 3949-3959, 2004.
5. Madoni, P., D. Cavoli, and L. Guglielmi. "Response of SOUR and AUR to Heavy Metal Contamination in Activated Sludge," *Water Resources*, Vol. 33, No. 10, pp. 2459-2464, 1999.
6. Anderson, K., B. Koopman, and G. Bitton. "Evaluation of Int-Dehydrogenase Assay for Heavy Metal Inhibition of Activated Sludge," *Water Resources*, Vol. 22, No. 3, pp. 349-353, 1988.
7. Vismara, R. "Depurazione biologica," *Ingegneria Ambientale*, Vol. 11, No. 8, pp. 634-643, 1982.
8. Kong, Z., P.A. Vanrolleghem, and W. Verstraete. "An Activated Sludge-Based Biosensor for Rapid IC<sub>50</sub>) Estimation and On-line Monitoring," *Biosens. Bioelectron*, Vol. 8, pp 49-58, 1993.
9. Cenci, G. and G. Morozzi. "The Validity of the TTC-Test for Dehydrogenase Activity of Activated Sludges in the Presence of Chemical Inhibitors," *Zentbl. Bakt. Hyg. I. Abt. Orig. B.*, Vol. 169, pp. 320-330, 1979.
10. Dutka, B.J. and K.K. Kwan. "Studies on a Synthetic Activated Sludge Toxicity Screening Procedure with Comparison to Three Microbial Toxicity Tests," In *Toxicity Screening Procedures Using Bacterial Systems*, Marcel Dekker, New York, 1984.
11. Garcia-Gonzalez, J., F. Mirada, and F. Lopez. "Evaluation of a Protease Assay Based on a Natural Protein for Heavy Metals Inhibition of Activated Sludge," *Journal of Environmental Science and Health*, Vol. A36, No. 7, pp. 1349-1360, 2001.

12. Rusk, J.A., R.E. Hamon, D.P. Stevens, and M.J. McLaughlin. "Adaptation of Soil Biological Nitrification to Heavy Metals," *Environmental Science and Technology*, Vol. 38, No. 11, pp. 3092-3097, 2004.
13. Cabrero, A., S. Fernandez, F. Mirada, and J. Garcia. "Effects of Copper and Zinc on the Activated Sludge Bacteria Growth Kinetics," *Water Resources*, Vol. 32, No. 5, pp. 1355-1362, 1998.
14. Shuttleworth, K.L. and R.F. Unz. "Influence of Metals and Metal Speciation on the Growth Filamentous Bacteria," *Water Resources*, Vol. 25, No. 10, pp. 1177-1186, 1991.
15. Lin, Y., X. Yang, and Y. Liu. "Kinetic Responses of Activated Sludge Microorganisms to Individual and Joint Copper and Zinc," *Journal of Environmental Science and Health*, Vol. A38, No. 2, pp. 353-360, 2003.
16. Blais, J.F., R.D. Tyagi, and J.C. Auclair. "Bioremediation of Metals from Sewage Sludge: Microorganisms and Growth Kinetics," *Water Resources*, Vol. 27, No. 1, pp. 101-110, 1993.
17. Fait, G., K. Broos, S. Zrna, E. Lombi, and R. Hamon. "Tolerance of Nitrifying Bacteria to Copper and Nickel," *Environmental Toxicology and Chemistry*, Vol. 25, No. 8, pp. 2000-2005, 2006.
18. Garcia, C., J.L. Moreno, T. Hernandez, and F. Costa. "Composting of Heavy-Metal Contaminated Sewage Sludge," *Biological Wastes*, Vol. 30, No. 4, pp. 291-301, 1990.

APPENDIX AII  
EXPERIMENTAL DATA

TABLE AII-1: SOLIDS ANALYSES DATA OF MIXED LIQUOR FOR ALL TREATMENTS

Treatment	TS (mg/L)	TDS (mg/L)	TSS (mg/L)	MLVSS (mg/L)																																																												
Control	3,138	445	2,672	1,737																																																												
	3,121	470			Zn	2,724	585	2,121	1,380	2,628	525	Cr	4,083	525	2,794	2,006	2,365	335	Cd	2,591	400	2,093	1,416	2,411	415	Ni	2,887	505	2,364	1,703		540	Cu	5,415	455	3,591	2,178	2,558	335	Pb	4,329	440	3,926	2,553	4,388	425	Cal MC	2,697	350	2,332	1,539	2,656	340	MC1.67	4,497	385	4,010	2,367	4,337	430	MC5	2,491	420	2,142
Zn	2,724	585	2,121	1,380																																																												
	2,628	525			Cr	4,083	525	2,794	2,006	2,365	335	Cd	2,591	400	2,093	1,416	2,411	415	Ni	2,887	505	2,364	1,703		540	Cu	5,415	455	3,591	2,178	2,558	335	Pb	4,329	440	3,926	2,553	4,388	425	Cal MC	2,697	350	2,332	1,539	2,656	340	MC1.67	4,497	385	4,010	2,367	4,337	430	MC5	2,491	420	2,142	1,318	2,579	365				
Cr	4,083	525	2,794	2,006																																																												
	2,365	335			Cd	2,591	400	2,093	1,416	2,411	415	Ni	2,887	505	2,364	1,703		540	Cu	5,415	455	3,591	2,178	2,558	335	Pb	4,329	440	3,926	2,553	4,388	425	Cal MC	2,697	350	2,332	1,539	2,656	340	MC1.67	4,497	385	4,010	2,367	4,337	430	MC5	2,491	420	2,142	1,318	2,579	365											
Cd	2,591	400	2,093	1,416																																																												
	2,411	415			Ni	2,887	505	2,364	1,703		540	Cu	5,415	455	3,591	2,178	2,558	335	Pb	4,329	440	3,926	2,553	4,388	425	Cal MC	2,697	350	2,332	1,539	2,656	340	MC1.67	4,497	385	4,010	2,367	4,337	430	MC5	2,491	420	2,142	1,318	2,579	365																		
Ni	2,887	505	2,364	1,703																																																												
		540			Cu	5,415	455	3,591	2,178	2,558	335	Pb	4,329	440	3,926	2,553	4,388	425	Cal MC	2,697	350	2,332	1,539	2,656	340	MC1.67	4,497	385	4,010	2,367	4,337	430	MC5	2,491	420	2,142	1,318	2,579	365																									
Cu	5,415	455	3,591	2,178																																																												
	2,558	335			Pb	4,329	440	3,926	2,553	4,388	425	Cal MC	2,697	350	2,332	1,539	2,656	340	MC1.67	4,497	385	4,010	2,367	4,337	430	MC5	2,491	420	2,142	1,318	2,579	365																																
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MC5	2,491	420	2,142	1,318																																																												
	2,579	365																																																														

TABLE AII-2: INITIAL AND FINAL pH, NO<sub>3</sub>, NH<sub>3</sub> DATA FROM  
NITRIFICATION TESTS FOR ALL TREATMENTS

Sample	Date	Time (hr)	Initial			Final		
			pH	NO <sub>3</sub> (ppm)	NH <sub>3</sub> (ppm)	pH	NO <sub>3</sub> (ppm)	NH <sub>3</sub> (ppm)
Control	8/22/2006	0	7.18	1.42	12.00	7.35	2.73	7.84
		2	7.23	0.89	8.70	7.22	2.10	7.00
		6	7.06	0.66	8.90	7.29	2.56	4.50
Zn	8/23/2006	0	7.18	0.09	8.80	7.39	0.33	8.10
		2	7.08	0.20	8.70	7.25	0.84	5.00
		6	7.18	0.18	9.80	7.41	1.56	6.70
Cr	8/24/2006	0	7.07	2.81	12.50	7.22	2.83	12.60
		2	7.21	2.69	12.60	7.37	3.28	12.00
		6	7.16	2.19	13.20	7.63	2.13	12.40
Cd	8/25/2006	0	7.12	2.27	8.50	7.18	2.85	6.90
		2	7.05	2.16	8.60	7.39	3.08	7.50
		6	7.09	1.80	9.30	7.30	2.45	8.40
Ni	8/26/2006	0	7.07	1.46	7.70	7.30	2.87	7.80
		2	7.24	1.44	10.10	7.30	2.06	7.70
		6	7.14	1.22	8.30	7.34	2.10	7.90
Pb	9/15/2006	0	7.05	3.79	4.20	7.20	5.62	2.40
		2	7.14	3.10	4.50	7.28	4.91	3.00
		6	7.02	2.46	6.00	7.36	4.39	3.40
Cu	9/11/2006	0	6.98	0.44	4.70	7.08	1.69	3.40
		2	7.05	0.50	4.70	7.07	0.18	3.60
		6	6.89	10.10	7.60	7.05	11.57	5.90
Cal MC	9/1/2006	0	7.12	0.21	6.20	7.12	0.77	5.70
		2	7.27	0.35	6.60	7.37	0.68	5.90
		6	7.05	0.20	6.90	7.28	3.41	5.20
MC1.67	9/21/2006	0	7.13	5.16	3.10	7.21	6.97	1.70
		2	7.01	4.24	4.40	7.19	6.15	2.50
		6	7.04	3.99	4.70	7.22	5.69	3.50

TABLE AII-2 (Continued): INITIAL AND FINAL pH, NO<sub>3</sub>, NH<sub>3</sub> DATA FROM  
NITRIFICATION TESTS FOR ALL TREATMENTS

Sample	Date	Time (hr)	Initial			Final		
			pH	NO <sub>3</sub> (ppm)	NH <sub>3</sub> (ppm)	pH	NO <sub>3</sub> (ppm)	NH <sub>3</sub> (ppm)
MC5	8/31/2006	0	6.90	1.99	9.00	7.14	2.48	8.70
		2	6.98	2.02	9.00	7.32	2.46	9.00
		6	6.93	3.04	9.40	ND	3.53	9.40

TABLE AII-3: OXYGEN UPTAKE DATA AT t = 0, 2, AND 6 HOURS FOR ALL TREATMENTS

Control, 8/22/2006					
t = 0hr, temp = 23.26°C		t = 2h, temp = 23.26°C		t = 6h, temp = 22.75°C	
Time (s)	DO (mg/L)	Time (s)	DO (mg/L)	Time (s)	DO (mg/L)
0	6.83	0	7.03	0	6.880
8	6.71	12	6.89	7	6.140
22	6.56	21	6.79	13	5.930
32	6.46	29	6.68	18	5.860
45	6.30	39	6.59	25	5.800
69	6.00	51	6.43	37	5.700
90	5.75	70	6.25	46	5.600
112	5.50	91	6.00	57	5.500
134	5.25	116	5.75	67	5.400
155	5.00	140	5.50	86	5.250
179	4.75	164	5.25	112	5.000
199	4.50	188	5.00	138	4.750
222	4.25	213	4.75	167	4.500
253	3.90	236	4.50	206	4.110
266	3.75	258	4.25	219	4.000
318	3.19	283	4.00	245	3.750
336	3.00	308	3.75	270	3.500
360	2.75	332	3.50	298	3.250
383	2.50	357	3.25	326	3.000
407	2.25	382	3.00	353	2.750
429	2.00	434	2.5	381	2.500

TABLE AII-3 (Continued): OXYGEN UPTAKE DATA AT t = 0, 2, AND 6 HOURS FOR ALL TREATMENTS

Zinc, 8/23/2006					
t = 0hr, temp = 23.37°C		t = 2h, temp = 23.62°C		t = 6h, temp = 24.02°C	
Time (s)	DO (mg/L)	Time (s)	DO (mg/L)	Time (s)	DO (mg/L)
0	7.89	0	6.67	0	7.05
8	7.64	8	6.53	9	6.94
13	7.55	14	6.48	15	6.88
19	7.42	28	6.38	22	6.81
26	7.35	37	6.29	29	6.74
23	7.24	53	6.16	39	6.63
44	7.13	64	6.04	47	6.54
50	7.04	78	5.90	63	6.40
64	6.91	95	5.75	79	6.25
75	6.77	127	5.50	105	6.00
91	6.60	156	5.25	135	5.75
111	6.40	182	5.00	161	5.50
126	6.25	210	4.75	190	5.25
147	6.00	236	4.50	220	5.00
171	5.75	264	4.25	252	4.67
194	5.50	289	4.00	276	4.50
220	5.25	317	3.75	303	4.25
241	5.00	347	3.50	331	4.00
267	4.75	375	3.25	362	3.75
290	4.50	403	3.00	389	3.50
317	4.25	433	2.75	418	3.25
341	4.00	461	2.50	448	3.00
367	3.75	488	2.25	475	2.75
392	3.50	519	2.00	504	2.50
415	3.25			535	2.25
441	3.00			563	2.00
466	2.75				
494	2.50				
521	2.25				

TABLE AII-3 (Continued): OXYGEN UPTAKE DATA AT t = 0, 2, AND 6 HOURS FOR ALL TREATMENTS

Chromium, 8/24/2006					
t = 0hr, temp = 23.26°C		T = 2h, temp = 23.11°C		t = 6h, temp = 22.91°C	
Time (s)	DO (mg/L)	Time (s)	DO (mg/L)	Time (s)	DO (mg/L)
0	7.29	0	6.21	0	7.10
8	6.96	7	6.16	6	7.02
13	6.89	12	6.10	13	6.98
23	6.79	19	6.07	27	6.92
30	6.72	29	6.02	39	6.87
42	6.59	41	5.95	55	6.82
56	6.49	59	5.83	75	6.75
69	6.39	76	5.80	112	6.60
82	6.25	92	5.70	146	6.50
106	6.00	113	5.60	217	6.25
136	5.75	135	5.50	287	6.00
165	5.50	183	5.25	366	5.75
192	5.25	241	5.00	448	5.50
220	5.00	299	4.75	534	5.21
251	4.75	360	4.50	599	5.00
281	4.50	420	4.25	686	4.75
338	4.00	487	4.00	773	4.50
370	3.75	547	3.75	853	4.25
399	3.50	614	3.50	939	4.00
431	3.25	681	3.25		
463	3.00	750	3.00		
493	2.75				
526	2.50				
558	2.25				
590	2.00				

TABLE AII-3 (Continued): OXYGEN UPTAKE DATA AT t = 0, 2, AND 6 HOURS FOR ALL TREATMENTS

Cadmium, 8/25/2006					
t = 0hr, temp = 22.91°C		T = 2h, temp = 23.27°C		t = 6h, temp = 23.81°C	
Time (s)	DO (mg/L)	Time (s)	DO (mg/L)	Time (s)	DO (mg/L)
0	7.42	0	7.87	0	7.70
8	7.20	5	7.71	5	7.54
12	7.12	10	7.62	9	7.48
18	7.09	19	7.54	15	7.40
24	7.01	26	7.46	24	7.34
32	6.95	42	7.38	32	7.28
39	6.88	52	7.30	45	7.17
49	6.80	69	7.20	57	7.10
58	6.70	84	7.10	69	7.00
68	6.60	116	6.92	105	6.75
80	6.50	138	6.75	149	6.50
107	6.25	175	6.50	183	6.25
133	6.00	219	6.25	219	6.00
160	5.75	260	6.00	258	5.75
187	5.50	301	5.75	292	5.50
213	5.25	342	5.50	330	5.25
239	5.00	387	5.25	371	5.00
268	4.75	424	5.00	411	4.75
296	4.50	466	4.75	451	4.50
322	4.25	512	4.50	494	4.25
352	4.00	552	4.25	532	4.00
383	3.75	596	4.00	569	3.75
413	3.50	638	3.75	606	3.50
443	3.25	682	3.50	645	3.25
473	3.00	727	3.25	685	3.00
506	2.75				
538	2.50				
570	2.25				
605	2.00				

TABLE AII-3 (Continued): OXYGEN UPTAKE DATA AT t = 0, 2, AND 6 HOURS FOR ALL TREATMENTS

Nickel, 8/26/2006					
t = 0hr, temp = 23.46°C		t = 2h, temp = 23.41°C		t = 6h, temp = 22.96°C	
Time (s)	DO (mg/L)	Time (s)	DO (mg/L)	Time (s)	DO (mg/L)
0	7.78	0	7.51	0	7.31
5	7.52	6	7.43	19	7.27
10	7.42	10	7.38	26	7.22
15	7.36	17	7.33	39	7.17
20	7.26	23	7.27	47	7.12
26	7.17	36	7.20	57	7.06
32	7.13	50	7.10	67	7.00
37	7.07	69	7.00	84	6.90
43	6.99	106	6.75	103	6.80
49	6.94	143	6.50	118	6.70
56	6.86	179	6.25	132	6.60
62	6.80	217	6.00	149	6.50
70	6.70	253	5.75	189	6.25
82	6.60	287	5.50	229	6.00
95	6.43	326	5.25	267	5.75
117	6.25	357	5.00	306	5.50
137	6.00	392	4.75	345	5.25
164	5.75	429	4.50	383	5.00
188	5.50	467	4.25	422	4.75
217	5.25	503	4.00	462	4.50
241	5.00	541	3.75	499	4.25
269	4.75	577	3.50	540	4.00
298	4.50	614	3.25	577	3.75
324	4.25	651	3.00	619	3.50
352	4.00			659	3.25
382	3.75			700	3.00
413	3.50				
443	3.25				
488	3.00				

TABLE AII-3 (Continued): OXYGEN UPTAKE DATA AT t = 0, 2, AND 6 HOURS FOR ALL TREATMENTS

Lead, 9/15/2006					
t = 0hr, temp = 22.02°C		t = 2h, temp = 22.37°C		t = 6h, temp = 22.91°C	
Time (s)	DO (mg/L)	Time (s)	DO (mg/L)	Time (s)	DO (mg/L)
0	8.10	0	8.29	0	8.20
4	7.91	5	8.11	5	8.01
9	7.82	10	8.02	10	7.92
14	7.75	15	7.94	17	7.80
19	7.70	19	7.88	24	7.70
26	7.60	27	7.80	32	7.60
35	7.50	33	7.70	41	7.50
45	7.40	41	7.60	61	7.25
54	7.30	50	7.50	77	7.00
62	7.20	70	7.25	97	6.75
79	7.00	89	7.00	116	6.50
101	6.75	108	6.75	135	6.25
121	6.50	128	6.50	152	6.00
137	6.25	147	6.25	171	5.75
159	6.00	166	6.00	191	5.50
176	5.75	183	5.75	208	5.25
195	5.50	202	5.50	227	5.00
214	5.25	222	5.25	245	4.75
235	5.00	241	5.00	262	4.50
253	4.75	259	4.75	282	4.25
272	4.50	278	4.50	301	4.00
293	4.25	299	4.25	319	3.75
311	4.00	317	4.00	338	3.50
330	3.75	336	3.75	357	3.25
351	3.50	354	3.50	375	3.00
369	3.25	372	3.25	392	2.75
388	3.00	391	3.00	412	2.50
409	2.75	410	2.75		
427	2.50	428	2.50		

TABLE AII-3 (Continued): OXYGEN UPTAKE DATA AT t = 0, 2, AND 6 HOURS FOR ALL TREATMENTS

Copper, 9/12/2006					
t = 0hr, temp = 21.75°C		t = 2h, temp = 21.97°C		t = 6h, temp = 22.35°C	
Time (s)	DO (mg/L)	Time (s)	DO (mg/L)	Time (s)	DO (mg/L)
0	8.28	0	8.41	0	8.37
5	8.23	5	8.36	8	8.30
10	8.17	10	8.31	20	8.20
15	8.12	15	8.25	35	8.10
21	8.07	27	8.15	46	8.00
27	8.00	31	8.10	61	7.90
36	7.90	45	8.00	87	7.70
44	7.80	58	7.90	113	7.50
63	7.60	70	7.80	148	7.25
73	7.50	104	7.50	182	7.00
95	7.25	132	7.25	215	6.75
121	7.00	164	7.00	249	6.50
141	6.75	195	6.75	281	6.25
167	6.50	224	6.50	317	6.00
189	6.25	254	6.25	355	5.75
213	6.00	285	6.00	386	5.50
245	5.60	314	5.75	429	5.17
254	5.50	343	5.50	451	5.00
277	5.25	374	5.25	505	4.60
299	5.00	405	5.00	518	4.50
322	4.75	434	4.75	553	4.25
345	4.50	462	4.50	587	4.00
367	4.25	492	4.25	613	3.80
391	4.00	524	4.00	675	3.35
412	3.75	552	3.75	722	3.00
435	3.50	580	3.50	773	2.65
457	3.25	615	3.21	816	2.33
482	3.00	642	3.00		
507	2.75	673	2.75		

TABLE AII-3 (Continued): OXYGEN UPTAKE DATA AT t = 0, 2, AND 6 HOURS FOR ALL TREATMENTS

Cal MC, 9/1/2006					
t = 0hr, temp = 22.6°C		t = 2h, temp = 22.77°C		t = 6h, temp = 23.12°C	
Time (s)	DO (mg/L)	Time (s)	DO (mg/L)	Time (s)	DO (mg/L)
0	8.21	0	7.91	0	0.00
5	8.05	4	7.84	4	7.91
10	7.98	8	7.76	8	7.79
15	7.93	12	7.70	13	7.70
20	7.88	17	7.64	17	7.66
28	7.80	22	7.60	23	7.60
41	7.70	33	7.50	34	7.50
60	7.50	46	7.40	41	7.40
87	7.25	63	7.25	53	7.30
110	7.00	87	7.00	87	7.00
139	6.75	111	6.75	111	6.75
161	6.50	138	6.50	137	6.34
185	6.25	164	6.25	162	6.25
210	6.00	188	6.00	186	6.00
234	5.75	212	5.75	210	5.75
260	5.50	236	5.50	234	5.46
285	5.25	260	5.25	258	5.23
311	5.00	286	5.00	282	5.00
336	4.75	311	4.75	308	4.75
361	4.50	338	4.50	331	4.50
384	4.25	363	4.25	354	4.25
412	4.00	388	4.00	380	4.00
437	3.75	411	3.75	405	3.75
463	3.50	437	3.50	427	3.50
489	3.25	463	3.25	454	3.25
523	2.95	488	3.00	479	3.00
544	2.71	516	2.75	503	2.71

TABLE AII-3 (Continued): OXYGEN UPTAKE DATA AT t = 0, 2, AND 6 HOURS FOR ALL TREATMENTS

MC1.67, 9/15/2006					
t = 0hr, temp = 22.72°C		t = 2h, temp = 22.72°C		t = 6h, temp = 23.36°C	
Time (s)	DO (mg/L)	Time (s)	DO (mg/L)	Time (s)	DO (mg/L)
0	7.86	0	7.97	0	7.75
4	7.72	5	7.89	6	7.58
9	7.66	10	7.85	12	7.52
18	7.60	15	7.82	16	7.47
23	7.66	26	7.70	23	7.40
31	7.47	36	7.60	33	7.30
38	7.38	45	7.50	40	7.20
43	7.33	67	7.25	49	7.10
54	7.20	89	7.00	57	7.00
62	7.10	110	6.75	74	6.75
71	7.00	130	6.50	93	6.50
91	6.75	150	6.25	115	6.25
110	6.50	174	6.00	134	6.00
131	6.25	192	5.75	153	5.75
151	6.00	213	5.50	170	5.50
170	5.75	234	5.25	190	5.25
190	5.50	253	5.00	207	5.00
209	5.25	276	4.75	225	4.75
230	5.00	296	4.50	244	4.50
256	4.66	319	4.25	260	4.25
269	4.50	340	4.00	276	4.00
289	4.25	362	3.75	292	3.75
308	4.00	385	3.50	310	3.50
329	3.75	411	3.25	330	3.25
348	3.50	433	3.00	346	3.00
369	3.25	457	2.75	364	2.75
390	3.00	483	2.50	383	2.50
410	2.75				
431	2.50				

TABLE AII-3 (Continued): OXYGEN UPTAKE DATA AT t = 0, 2, AND 6 HOURS FOR ALL TREATMENTS

<u>t = 0hr, temp = 22.63°C</u>		<u>MC5, 8/31/2006</u> <u>t = 2h, temp = 22.77°C</u>		<u>t = 6h, temp = 23.12°C</u>	
<u>Time (s)</u>	<u>DO (mg/L)</u>	<u>Time (s)</u>	<u>DO (mg/L)</u>	<u>Time (s)</u>	<u>DO (mg/L)</u>
0	7.86	0	8.22	0	8.22
6	7.76	5	7.87	5	7.98
10	7.64	10	7.75	12	7.89
15	7.60	15	7.71	17	7.85
21	7.54	20	7.67	29	7.80
27	7.50	27	7.63	50	7.70
36	7.40	37	7.60	70	7.60
45	7.40	57	7.50	105	7.50
56	7.20	83	7.40	192	7.22
68	7.10	112	7.25	256	7.00
76	7.00	177	7.00	336	6.75
106	6.75	240	6.75	457	6.34
135	6.50	302	6.50	489	6.25
169	6.19	306	6.25	567	6.00
191	6.00	441	6.00	657	5.75
221	5.75	507	5.75	763	5.46
253	5.50	581	5.50	855	5.23
284	5.25	652	5.25	943	5.00
317	5.00	746	4.94	1045	4.75
349	4.75	810	4.75	1154	4.50
384	4.50	889	4.50	1265	4.25
418	4.25	982	4.23	1375	4.00
457	4.00	1060	4.00	1500	3.75
492	3.75	1152	3.75	1613	3.50
534	3.50	1250	3.48	1743	3.25
572	3.25	1343	3.23	1874	3.00
616	3.00	1436	3.00	2030	2.71
661	2.75	1535	2.75	2143	2.50
707	2.50	1639	2.50		