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Assessing wastewater metal toxicity with bacterial bioluminescence in a bench-scale wastewater treatment system

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Abstract

The effectiveness of a previously developed toxicity monitoring method for activated sludge wastewater treatment employing a bioluminescent bacterium (Shk1) was evaluated in batch experiments and a bench-scale activated sludge system exposed to heavy metals (Cu, Zn, Ni, and Cd). Influent wastewater (primary clarifier supernatant) and activated sludge from a municipal wastewater treatment plant were used in both batch experiments and in the bench-scale wastewater treatment system. Shk1 bioluminescence was most sensitive to Cd and Zn, followed by Cu, and then Ni in order of decreasing sensitivity. In contrast, activated sludge specific oxygen uptake rate was most sensitive to Cu, followed by Cd and Zn, and finally Ni. The same pattern of sensitivity was observed in batch and bench-scale evaluations.

Batch experiments examining the effect of metal adsorption were performed. The adsorption of metals to activated sludge and reduction in bioavailability due to chelation by soluble organics or by precipitation in wastewater was found to be an important effect in mediating differences in toxicity response between bioluminescence and respirometry. Batch adsorption experiments indicated that the activated sludge adsorption capacity was highest for Cu, followed by Cd, Ni, and then Zn. A simple mathematical model for the soluble metal concentration in the aeration basin and clarifier was developed utilizing metal distribution coefficients determined from the batch adsorption experiments. Model predictions compared well with results from the bench-scale activated sludge experiments.

Keywords: Bioluminescence; Bioreporter; *Lux*; Activated sludge; Wastewater; Toxicity; Respirometry; Metals

1. Introduction

Heavy metals are commonly found in municipal sewage and industrial effluents. Removal of heavy metals at low concentrations by the activated sludge process is particularly effective, producing effluents with minimal metal contamination. A portion of the heavy metals present in raw sewage is removed during primary sedimentation by precipitation or adsorption onto particulate matter [1,2]. Soluble and suspended forms of the metals pass into the biological stage of sewage treatment and may be removed by adsorption to the activated sludge biomass. However, a loss in activated sludge viability, changes in sludge community structure [3], loss of floc structure [3,4], and/or decreases in treatment efficiency [1,3,5] may occur at high concentrations of heavy metals. Because the conventional activated sludge process is unsuitable for removing heavy metals in high concentration, it is desirable to develop toxicity assessment methods that can detect these pollutants.

The first stage of metal adsorption is rapid uptake between 3 and 10 min, in which a large quantity of metal ions is adsorbed by the cell flocs. The second stage is a slow phase, which may extend over many hours [6,7]. Mechanisms proposed for metal removal in activated sludge include physical trapping of precipitated metals in the sludge floc matrix, binding of soluble metal to extracellular polymers [8], accumulation of soluble metal by the cell, and volatilization of metal to the

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Nomenclature

Subscripts

E	aeration basin effluent
F	feed
O	clarifier overflow
R	recycle
C	liquid phase metal concentration (mg metal) L^{-1}
E	flow rate of the aeration basin effluent ($L\,h^{-1}$)
F	flow rate of the feed stream ($L\,h^{-1}$)
K_P	sorption partitioning coefficient (mg metal adsorbed) $\times$ L (mg suspended solids)$^{-1}$ (mg metal in liquid phase)$^{-1}$
O	flow rate of the clarifier overflow effluent ($L\,h^{-1}$)
R	flow rate of recycle from clarifier to aeration basin ($L\,h^{-1}$)
S	mass metal adsorbed per mass of suspended solids (mg metal) (mg suspended solids)$^{-1}$
t	time (h)
X	activated sludge suspended solids concentration (mg suspended solids) L^{-1}

atmosphere. Adsorption on extracellular polymers produced by activated sludge species, adsorption to cell walls, and accumulation within the cytoplasm of activated sludge [3,2,9] have been shown to play important roles in metal removal. Heavy metals present in a precipitated form may be removed either by independent settling or by physical trapping in the sludge floc matrix. Metal present in ionic form may be removed from solution by adsorption to sites on sludge floc surfaces [3,9].

Toxicity assays used to screen for the presence and effects of toxicants in wastewater include respirometry, measurement of the inhibition of growth and viability of bacterial cells [10], chemical analyses, microscopic analyses [11,12], and bioluminescent analyses.

An ideal assessment method is simple, sensitive, easy to use, online, inexpensive, and relevant to activated sludge microorganisms [13]. Chemical analysis for toxicants requires knowledge of he toxicant compound, is time consuming, and is expensive [14]. Microscopic analysis can document toxic shock by examining the change in activated sludge microorganisms that have already been exposed to the toxicant. The community structure of protozoan species is an effective biological indicator of functional conditions of wastewater treatment plants [15–17]. However, this assay is not an ideal assessment method because it is time consuming and cannot be used for online measurement. Respirometric inhibition methods are the most commonly used toxicity assessment method in biological wastewater treatment plants [18,19]. The oxygen consumption rate of activated sludge decreases when the wastewater contains toxicants. This method examines the metabolic rate of the entire sludge community, and can be used for online measurement, but is also affected by changes in organic matter loading.

Bioluminescent methods use bioluminescent bacteria to detect toxicity in wastewater. Bioluminescence is light produced by a series of enzymatic reactions within the cell. These enzymes are products of the *lux* genes. The biochemical, physiological and genetic basis for bioluminescence have been reviewed by Hastings et al. [20] and Meighen [21]. Bioluminescent bacteria toxicity tests have the advantage of being sensitive, simple, rapid, and inexpensive. The Microtox system (AZUR Environmental, Carlsbad, CA) is a well-known toxicity testing method that uses a naturally luminescent marine bacterium to produce bioluminescence, and has been used in effluent toxicity testing. Other bioluminescent reporters have recently been developed to assess toxicity in wastewater treatment systems [13], detect nitrification inhibitors [22], and determine metal in soils [23,24].

Shk1 is a constitutive bioluminescent reporter constructed by inserting *lux* genes into an activated sludge bacterium [13]. Shk1 was identified using 16S rDNA sequencing and comparison to known sequences, and was determined to be *Pseudomonas fluorescens*. This strain may be more relevant to activated sludge microorganisms than marine bioluminescent microorganisms because it was isolated from an activated sludge process. The Shk1 bioluminescent toxicity assay is simple, rapid, easy to use, sensitive to toxicants, and has the capability for online toxicity monitoring [25]. Using multidimensional scaling, Ren and Frymier [26] found that an Shk1-based bioluminescent assay delivered comparable toxicity information compared to the Polytox® (utilizes a consortium of activated sludge isolates) assay, an activated sludge respiration inhibition assay, and a *Tetrahymena* assay.

The purpose of this research was to investigate the toxic effect of heavy metals (Cu, Zn, Ni, and Cd) on activated sludge oxygen uptake rate (OUR), and to correlate these effects to decreases in bioluminescence from Shk1. In batch experiments, activated sludge and Shk1 were exposed to heavy metals. Bioluminescent and respirometric methods were used to evaluate the toxic effect of these four hazardous metals. In addition, a bench-scale activated sludge system was used to evaluate the effect of the same toxicants. Metal adsorption kinetics on activated sludge were investigated to develop an adsorption model of heavy metals onto activated sludge biomass.

2. Materials and methods

Shk1 bioluminescence, specific oxygen uptake rate (SOUR), and metal concentration were the parameters measured in the batch and bench-scale activated sludge experiments.

2.1. Bioluminescent assays

Bioluminescent assays were used to investigate the light response of Shk1 to toxicants. A standard assay was performed as described by Lajoie et al. [25]. The assay consisted of combining a sample from the bench-scale system or activated sludge obtained from Syracuse Metropolitan Wastewater Treatment Facility (METRO) with Shk1 cells. In all assays, bioluminescence was measured from triplicate samples using a luminometer (MiniLumat LB9506; USA Wallac, Inc., Gaithersburg, MD). Shk1 growth medium (100 mL of 8 g L^{-1} nutrient broth and 10 mg L^{-1} tetracycline) was inoculated with 1 mL of frozen (−80°C) Shk1 cells. These reporter cells were incubated at room temperature on a shaker for about 24 h to achieve an optical density (OD_{600}) between 0.9 and 1 measured with a spectrophotometer (Spectronic Genesys 5; Spectronic Instruments, Rochester, NY) at 600 nm wavelength. This test culture was refrigerated before use and was useable for approximately 1 month. For the assay, 10 μL of the Shk1 test culture was added to 1 mL of nutrient broth (NB) and incubated at room temperature for 20 min for activation before use in the toxicity test. Each sample test tube contained 0.1 mL activated Shk1 cells and 0.9 mL test sample (e.g., activated sludge, influent wastewater, clarifier supernatant). The Shk1 cells were added last and the light was measured following 7 min of exposure. The Shk1 bioluminescent assay, as well as the Microtox assay, can be time dependent; therefore, a constant exposure time of 7 min was utilized for all assays. Results were calculated as the bioluminescence in the test samples divided by the bioluminescence in the control samples (samples with no added toxicant). EC_{50} was calculated from the experimental graphs of bioluminescence versus metal concentration, and is the toxicant concentration that causes a 50% reduction in bioluminescence.

2.2. Respirometry assays

Respirometry was used to measure the oxygen consumption rate by the activated sludge microorganisms. The oxygen consumption rate was measured from a 3 mL sample in a small stirred well equipped with an oxygen probe and no vapor headspace (YSI Model 5300 Biological Oxygen Monitor; Yellow Springs Instrument CO., Inc., Yellow Springs, OH). The sludge microorganisms consumed the oxygen in the chamber, and the dissolved oxygen concentration decreased. The slope of the dissolved oxygen concentration versus time plot was divided by mixed liquor suspended solids concentration (MLSS) to obtain the SOUR expressed as mg O_2 min^{-1} (mg activated sludge)$^{-1}$. The effective concentration (EC_{50}) was calculated, which is the toxicant concentration that causes a 50% reduction in OUR by the activated sludge microorganisms.

2.3. Metal concentration

The soluble metal concentration was measured using atomic adsorption (AA) spectroscopy (model 2380, Perkin-Elmer, Pittsford, NY). Samples (1 mL) were centrifuged to remove activated sludge or other suspended materials in the samples. Supernatant ($\frac{1}{2}$ mL) was transferred to a microcentrifuge tube and acidified with one drop of nitric acid (HNO_3). These samples were stored in a freezer at −20°C until measurement, and diluted as required for the AA assay. A standard curve for each metal was used to quantify metal concentrations.

2.4. Batch bioluminescent toxicity experiments

The effects of the test media (wastewater, activated sludge, and nutrient broth) on bioluminescence were examined in batch experiments. Influent wastewater (primary clarifier supernatant) and activated sludge were obtained from the METRO plant. Dilutions of each metal were made in distilled water or influent wastewater to produce concentrations ranging from 1000 to 0 mg L^{-1} of each metal. Samples of the metal dilutions (100 μL) were added to 800 μL of the test (sample) medium in each test tube. The dilutions in distilled water were added to the tubes containing nutrient broth and the dilutions in influent wastewater were added to the tubes containing either influent wastewater or activated sludge. Triplicate samples were assayed using the standard bioluminescence assay.

2.5. Batch respirometry toxicity experiments

Samples were taken from the METRO facility during the same time period as the samples for batch bioluminescence experiments. Metal dilutions were made in influent wastewater. In all, 300 μL of each metal dilution was added to 2.7 mL of activated sludge, and the standard respriometric assay was performed. Due to high variability typically observed in respiration measurements, samples from three different days were taken. SOUR was determined for each activated sludge sample at each metal concentration and compiled on the same graph. An exponential curve was fitted to the data points to determine the EC_{50}.

2.6. Shk1 bioluminescence versus oxygen uptake rate

Both the bioluminescence assay and the respirometry assay were performed with the Shk1 bioreporter to determine if the different mechanistic measures of toxicity (bioluminescence versus OUR) resulted in differences in sensitivity to metals. Shk1 cells were grown in nutrient broth with 10 mg L^{-1} tetracycline at room temperature to an OD_{600} of 1.0, and then refrigerated at 4°C according to the standard protocol. These cells were then centrifuged and resuspended in phosphate buffered saline (PBS). At the start of each OUR measurement, a 300 μL sample of nutrient broth (8 g L^{-1}) was added to 2.4 mL of room temperature PBS suspended Shk1 cells. This culture was incubated for 5 min at room temperature before the addition of 300 μL of each metal stock solution. To determine the effect of a metal on the OUR at the time of each measurement, the OUR of a control sample with no added metal was determined. Results are expressed as the OUR of the metal amended sample divided by the OUR of the control sample (normalized OUR).

The effects of the same metal concentrations on bioluminescence were determined using the same PBS suspended Shk1 cells. In these experiments, 100 μL of nutrient broth was added to 0.8 mL cells, and the culture was incubated for 5 min. In all, 100 μL of toxicant in distilled water was then added, the culture was vortexed, and then incubated for 2 min at room temperature. Next 10 μL of this culture was added to 1 mL of PBS, and bioluminescence measured immediately thereafter. These results were normalized using corresponding cultures with no added toxicant.

2.7. Batch metal adsorption experiments

Batch adsorption experiments were conducted examining the adsorption of metal to activated sludge. The general adsorption experiment procedure was to add activated sludge, supernatant, or distilled water to triplicate 50 mL glass or plastic centrifuge tubes. Metal stock solutions ($ZnCl_2$, $CuSO_4 \cdot 5H_2O$, $3[CdSO_4] \cdot 8H_2O$, and $NiSO_4 \cdot 6H_2O$) in distilled water were added to achieve the experimental concentration, and the tubes were placed on a tube rotator for mixing. Samples were taken at 0.5, 1, 2, 4, 8, and 24 h. In all, 1 mL of sample was removed and centrifuged for 5 min at 3600*g*, and 0.5 mL of supernatant was then transferred to a 1.5 mL microcentrifuge tube. Each tube was acidified to pH 2 with one drop of nitric acid solution (HNO_3) and then stored at –20°C before analysis. AA spectroscopy was used to analyze the metal concentration in the supernatant of the samples.

Cu and Zn experiments were performed three times within 3 months, while Ni experiments were performed twice, and Cd once in the same time period. Triplicate adsorption experiments were performed at each concentration (10, 30, and 50 mg L^{-1}) examined. Experiments employed activated sludge, whereas controls consisted of activated sludge supernatant (activated sludge was centrifuged for 4 min at 4600 rpm to remove biomass) or distilled water with concentrations of 30 and 50 mg metal L^{-1}. Based on the decline in soluble metal concentration, the sorption partitioning coefficient (K_P) [27], as defined in Eq. (1), was determined by calculating the slope of $[S]$ versus $[C]$.

$$[S] = K_P [C]. \quad (1)$$

This analysis simplifies the approach of Parker et al. [27] by considering adsorbed and precipitated metal fractions as a single adsorbed metal fraction.

2.8. Bench-scale experiments

A bench-scale activated sludge wastewater treatment system was previously constructed [28] in duplicate to provide a control and experimental system. The control system was operated under standard conditions, whereas the experimental system was subject to toxicants in the influent wastewater stream. Each system consisted of an influent feed tank (8 L working volume, plastic carboy), an aeration tank (2 L working volume), and a clarifier (2 L working volume glass cylinder). The activated sludge was maintained in suspension via a stir plate and magnetic stirrer and aeration. Oxygen concentration in the aeration basins was controlled with an oxygen controller (Model PHDG-71; Omega Engineering, Inc., Stamford, CT) and aquarium air pump. Influent wastewater was added and sludge was recycled from the clarifier to the aeration basin using timer controlled peristaltic pumps. The effluent from the clarifier was collected in plastic carboys.

The influent wastewater and activated sludge were obtained directly from the top of a primary clarifier and the aeration basin, respectively, from the METRO municipal wastewater treatment plant. The hydraulic residence time and the oxygen set-point concentration in the bench-scale system were adjusted to the corresponding values in the full-scale plant (2.1 h and 1.5 mg L^{-1}, respectively). Activated sludge was not wasted over the course of the 4 h experiments. Four bench-scale experiments were performed with the toxicants Cu, Zn, Cd, and Ni, at influent metal concentrations of 100, 100, 100, and 200 mg L^{-1}, respectively. During the 4 h test the metal concentration in the aeration basin would increase to the influent value, effectively examining the range from no metal to the influent concentration. Barth et al. [1] found that 4 h toxic shock loads of 75 mg L^{-1} for Cu, 50–200 mg L^{-1} for Ni, and 160 mg L^{-1} for Zn, resulted in increases in effluent chemical oxygen demand.

2.9. Model of soluble metal concentration in the aeration basin and clarifier

Assuming (1) equilibrium of metal adsorption by sludge is achieved rapidly and can be described by the sorption partitioning coefficient, (2) no biomass in the feed stream or clarifier effluent, (3) the activated sludge volume is small compared to the liquid volume, (4) there is negligible biomass growth in the 4 h experiments, and (5) the total metal concentration $= C(1 + K_P X)$, a mass balance on metal in the aeration basin and the clarifier was performed resulting in Eqs. (2) and (3). These equations were solved to predict the soluble metal concentration in the aeration basin and clarifier to validate the use of the sorption partitioning coefficient to describe metal adsorption:

$$V_R(1 + K_{SD}X_E)\frac{dC_E}{dt} = FC_F - EC_E - S_E X_E E + RC_R + S_R X_R R, \quad (2)$$

$$V_C(1 + K_{SD}X_R)\frac{dC_R}{dt} = EC_E + S_E X_E E - OC_O - RC_R - S_R X_R R. \quad (3)$$

3. Results

3.1. Batch bioluminescent toxicity experiments

The bioluminescence EC_{50} for each metal based on batch exposure to metals is indicated in Table 1. The results indicate that cadmium and zinc have similar toxicities to Shk1, and these two metals are more toxic than copper or nickel. Nickel is the least toxic of the four metals.

Generally, each of the metals causes the greatest decrease in bioluminescence in wastewater, followed by nutrient broth, and activated sludge. It is important to note that comparisons of toxicity in nutrient broth and activated sludge or influent wastewater are not directly comparable as the metal dilutions for the nutrient broth experiments were made in distilled water (where the metal is soluble at least up to 1000 mg L^{-1}), whereas the metal dilutions for the wastewater and sludge experiments were made in influent wastewater (metals much less soluble and added as a slurry).

3.2. Batch respirometry toxicity experiments

The respirometric EC_{50} values were determined using an exponential decay to fit the experimental decreases in activated sludge SOUR in response to metals, and are indicated in Table 1. Activated sludge is much more sensitive to Cu than to the other metals. The sensitivities to Zn and Cd are similar, whereas the toxicity of Ni is much less.

3.3. Shk1 bioluminescence versus oxygen uptake rate

This experiment was conducted to determine if the differences in sensitivities between the bioluminescence assay (with Shk1) and the respirometric assay (with activated sludge) were due to the difference in the manner in which metals affect SOUR and bioluminescence. The results of these experiments for Zn, Cu, and Cd impacts on both OUR and bioluminescence are presented in Fig. 1. For Zn and Cu, the OUR and bioluminescence curves are in close agreement. For Cd, there is some discrepancy at lower Cd concentrations, but at the higher concentrations the results are similar.

3.4. Batch metal adsorption experiments

Batch metal (Cu, Zn, Cd, and Ni) experiments were conducted to investigate the equilibrium capacity and kinetics of adsorption of metal to activated sludge. All

Table 1
Experimentally determined bioluminescence and SOUR EC_{50} and the sorption partitioning coefficient values

Toxicity assay	Sample	Zinc (mg L^{-1})	Copper (mg L^{-1})	Nickel (mg L^{-1})	Cadmium (mg L^{-1})
Bioluminescence	Wastewater	2.4	14	>100	6.0
	Sludge	19	63	>100	15
	Nutrient broth	9.5	79	>100	6.0
SOUR	Sludge	41	15	76	39
K_P (L mg^{-1}) This study	Sludge	0.00030 ±0.000058[a]	0.00350 ±0.0011[a]	0.0001 0.0002[b]	0.00170
K_P (L mg^{-1}) [27]	Sludge	0.00627	0.0139	0.00394	>0.0199

[a] Standard error of the mean.
[b] Duplicates.

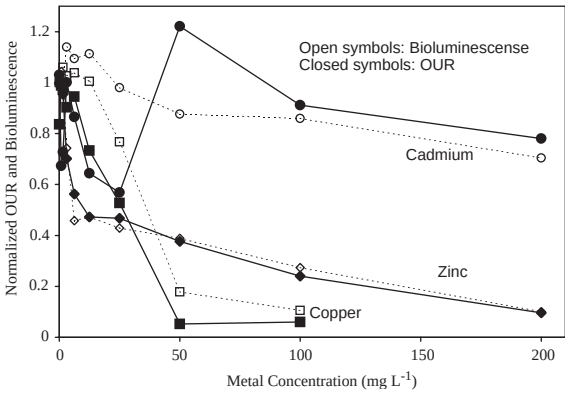

Fig. 1. OUR and bioluminescence of Shk1 upon exposure to Cd, Zn, and Cu.

of the metals (Cu, Zn, Cd, and Ni) were adsorbed rapidly by activated sludge in less than 8 min. There were no differences observed between glass and plastic tubes. None of the soluble metals concentrations declined with time in distilled water. The soluble Cu concentration declined in the supernatant controls to about 50–70% of the initial value, while Zn and Ni soluble metal concentration did not decline significantly in activated sludge supernatant. Cd was not measured in supernatant controls.

Metal adsorption kinetics and equilibrium by activated sludge differ with different metals. In these experiments, Cu was adsorbed the fastest, followed by Cd and then Zn. The activated sludge capacity to adsorb Cu was the highest, followed by Cd, Zn, and then Ni. The slope of $[S]$ versus $[C]$ is equal to the sorption partitioning coefficient, K_P. The sorption partitioning coefficient for each metal was determined using three different initial metal concentrations, and is shown in Table 1. The adsorbed concentrations of the metals were calculated as the initial concentration minus the soluble equilibrium concentration.

3.5. Bench-scale experiments

Transport of each of the metals through the bench-scale wastewater treatment system was detected by decreases in bioluminescence. The results from the Cd experiment are indicated in Fig. 2. The rates of decline in activated sludge SOURs and Shk1 bioluminescence in activated sludge samples were compared for all metals following addition to the influent wastewater. SOURs decreased faster than Shk1 bioluminescence for Cu and Ni, but decreased more slowly than bioluminescence for Zn and Cd, as indicated in Fig. 3. This was the same result observed in batch experiments using the Shk1 standard toxicity protocol and activated sludge respirometry experiments.

3.6. Model of soluble metal concentration in the aeration basin and clarifier

Using the experimentally set or measured values for each experiment that are listed in Table 2, Eqs. (2) and (3) were solved for aeration basin and clarifier soluble metal concentrations and compared to measured concentrations. In the case of Cu and Cd, the measured influent soluble metal concentrations were considerably

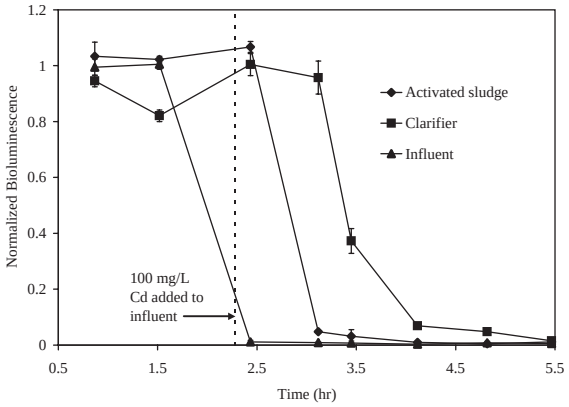

Fig. 2. Bioluminescent response of Shk1 in influent, aeration, and clarifier samples upon exposure to cadmium.

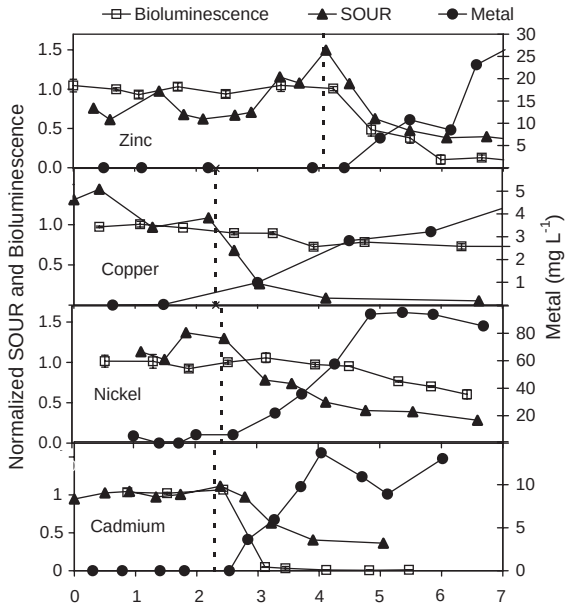

Fig. 3. Bioluminescence, SOUR, and soluble metal from aeration basin samples upon exposure to metal: (A) Cu, (B) Cd, (C) Ni, and (D) Zn. Vertical dashed lines indicate the time of addition of the metal to the influent basin.

Table 2
Operating parameters in the bench-scale toxic shock experiments

Parameter	Value	Determined by
F	$0.576\,L\,h^{-1}$	Experimentally set and measured
R	$0.370\,L\,h^{-1}$	Experimentally set and measured
E	$0.946\,L\,h^{-1}$	$E = F + R$
O	$0.576\,L\,h^{-1}$	$O = E - R$
C_f (Cu)	$46\,mg\,L^{-1}$	Experimentally measured
C_f (Zn, Ni)	$100\,mg\,L^{-1}$	Experimentally measured
C_f (Cd)	$162^{-0.27(t)}$	Empirically fit to experimentally measured data
X_E	1242 (Cu), 2038 (Zn), 1594 (Cd), 1465 (Ni)	Experimentally measured according to standard methods for the examination of water and wastewater [32]
X_R	3174 (Cu), 5210 (Zn), 4076 (Cd), 3745 (Ni)	$X_R = (E/R)X_E$

different than the nominal influent concentrations due to chelation and precipitation with components in the influent wastewater. The measured soluble Cu influent concentration, and an empirical fit to the measured soluble Cd concentration, which declined with time, were used in the models. Order of magnitude agreement between the predicted and the experimentally measured soluble metal concentrations in the aeration basin and clarifier was observed, as indicated in Fig. 4.

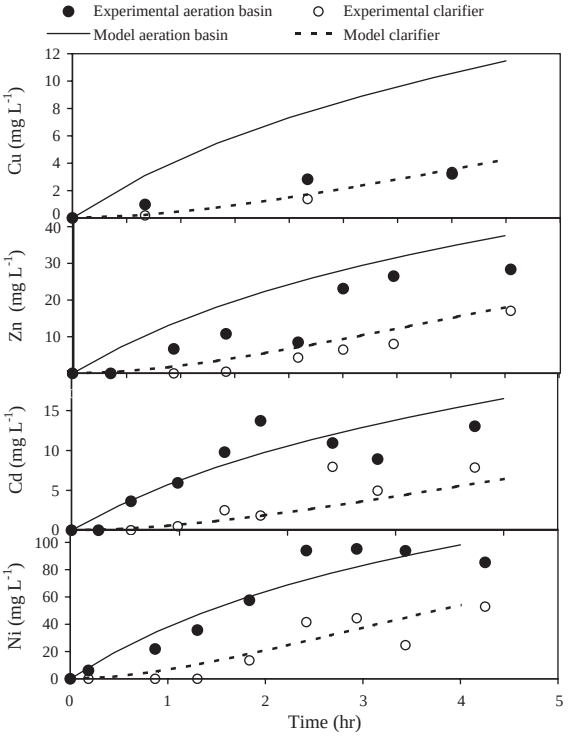

Fig. 4. Model prediction and experimental aeration basin and clarifier soluble metal concentrations.

4. Discussion

The Shk1 bioluminescent assay was able to detect the heavy metals Zn, Cu, Ni, and Cd in background matrices encountered in wastewater systems: influent wastewater, aeration basin samples with activated sludge, and clarifier overflow samples. Variations in concentrations of the metals could be detected using bioluminescence, and the propagation of metal toxicant through the bench-scale wastewater treatment plant was observed using the bioluminescence assay (Fig. 2). Therefore, batch experiments may be able to predict assay behavior in continuous wastewater treatment systems. However, the bioluminescent assay predicted a different relative toxicity for each metal tested than the standard respirometric assay. In this study, the metals were utilized individually, further investigation with multiple metal toxicants is warranted. A slight stimulatory effect at very low metal concentrations was observed in batch tests, this may mask low level toxicity in a multiple metal system.

The respirometric assay was performed with the bioluminescent organism to ascertain if the differences in relative metal toxicities were due to differences in the mechanistic response (bioluminescence versus OUR). The OUR and bioluminescence of Shk1 are directly related (Fig. 1), and give the same relative order of sensitivity for the three metals tested. Therefore, the difference between the assays is not due to a difference in the mechanistic response.

Previous studies have also used respirometry and bioluminescence to assess relative toxicities of metals. Ren and Frymier [29] found identical relative toxicities measured with a 5 min Shk1-based bioluminescent assay, with bioluminescence slightly more sensitive to Cd than Zn, followed by Cu and then Ni. *Rhizobium*-based bioluminescent reporters to assess soil metals were less sensitive to Cu than to Zn [24], Cd, or Ni [23], due to reduced soluble Cu concentrations as a result of adsorption, chelation, and precipitation. The relative toxicities assessed with bioluminescence for these studies agreed with the relative toxicities determined in this study. However, variations using respirometry are observed in previous studies [8,30,31], presumably due to differences in methods and source sludges.

Adsorption of metal to activated sludge and reduction in solubility due to precipitate or complex-forming components in wastewater appeared to be important mechanisms mediating toxicity in the wastewater treatment system, and was likely responsible for the differences between results from the two assays for toxicity (bioluminescence and respirometry). Metal adsorption was quantified by defining a sorption partitioning coefficient. Using this single parameter, determined from batch experiments, the soluble metal concentration in the aeration basin and clarifier was predicted qualitatively with the correct magnitude, verifying that the sorption partitioning coefficient can be used to indicate the capacity for the sludge to adsorb metal. The relative order of adsorption capacity determined in this study was Cu, Cd, Zn, Ni (greatest to least). Cheng et al. [7] and Battistoni et al. [8] found similar uptake patterns with adsorption of Cu > Cd > Ni and Cd > Ni, respectively.

In this study, the relative order of activated sludge adsorption capacity (K_P) is similar to the order of sensitivity of respirometry to metals, which are both different than the bioluminescent assay results as seen in the following equations:

$$\text{Bioluminescence}: \ \text{Cd} \approx \text{Zn} > \text{Cu} > \text{Ni}, \tag{4}$$

$$\text{Respirometry}: \ \text{Cu} > \text{Cd} \approx \text{Zn} > \text{Ni}, \tag{5}$$

$$\text{Capacity for adsorption}: \ \text{Cu} \geqslant \text{Cd} > \text{Zn} > \text{Ni}. \tag{6}$$

These results indicate that the bioluminescent assay assesses the soluble metal concentration, while the respirometric assay assesses metal impact through the adsorbed fraction. This is why adsorption capacity, as indicated by the sorption partitioning coefficient, has a significant impact on metal sensitively in the respirometric assay. In this assay, significant adsorption of Cu to the sludge reduces the toxicity to the bioluminescent organisms.

5. Conclusions

The Shk1 bioluminescent assay was able to detect increasing concentrations of metals in common matrices encountered in wastewater treatment systems, and track the transport of metal through a bench-scale wastewater treatment system. The relative order of sensitivity to Cu, Zn, Ni, and Cd was different than the standard respirometric toxicity assay due to differences in adsorption capacities for each metal.

Activated sludge microorganisms typically have a residence time in wastewater treatment systems on the order of days. If metals are present, the organisms are exposed to an accumulated impact of metals over time. The bioluminescent assay consists of combining a sample from the wastewater treatment system and the Shk1 bacteria, and measuring bioluminescence after minutes of exposure to the wastewater. The bioluminescence system in the grab-sample-type assays may be more valuable for effluent monitoring, but for protecting activated sludge microorganisms an assay should be developed that simulates the accumulated impact of toxicants through adsorption.

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References

[1] Barth EF, Ettinger MB, Salotto BV, McDermott GN. Summary report on the effects of heavy metals on the biological treatment processes. J Water Pollut Control Fed 1965;37(1):86–96.
[2] Oliver BG, Cosgrove EG. The efficiency of heavy metal removal by a conventional activated sludge treatment plant. Water Res 1974;8:869–74.
[3] Brown MJ, Lester JN. Metal removal in activated sludge: the role of bacterial extracellular polymers. Water Res 1979;13:817–37.
[4] Neufeld RD. Heavy metals-induced deflocculation of activated sludge. J Water Pollut Control Fed 1976;48:1940–7.
[5] Beyenal NY, Ozbelge (Baser) TA, Ozbelge HO. Combined effects of Cu^{2+} and Zn^{2+} on activated sludge process. Water Res 1997;31(4):699–704.
[6] Chen KY, Young CS, Rohatgi N. Trace metals in wastewater effluents. J Water Pollut Control Fed 1974;46:2663–75.
[7] Cheng MH, Patterson JW, Minear RA. Heavy metals uptake by activated sludge. J Water Pollut Control Fed 1975;47:362–76.
[8] Battistoni P, Fava G, Ruello ML. Heavy metal shock load in activated sludge uptake and toxic effects. Water Res 1993;27(5):821–7.
[9] Brown MJ, Lester JN. Role of bacterial extracellular polymers in metal uptake in pure bacterial culture and activated sludge—I. Effect of metal concentration. Water Res 1982;16(11):1539–48.
[10] Alsop GM, Waggy GT, Conway RA. Bacterial growth inhibition test. J Water Pollut Control Fed 1980;52(10):2452–6.
[11] Curds CR, Cockburn A. Protozoa in biological sewage-treatment process II. Protozoa as indicators in the activated-sludge process. Water Res 1970;4:237–49.
[12] Madoni P, Davoli D, Gorbi G, Vescovi L. Toxic effect of heavy metals on the activated sludge protozoan community. Water Res 1996;30:135–41.

[13] Kelly CJ, Lajoie CA, Layton AC, Sayler GS. Bioluminescent reporter bacterium for toxicity monitoring in biological wastewater treatment systems. Water Environ Res 1999;71:31–5.
[14] Paton GI, Palmer G, Kindness A, Campbell C, Glover LA, Killham K. Use of luminescence-marked bacteria to assess copper bioavailability in malt whisky distillery effluent. Chemosphere 1995;31:3217–24.
[15] Madoni P, Davoli D, Chierici M. Comparative analysis of the activated sludge microfauna in several sewage treatment works. Water Res 1993;27:1485–91.
[16] Madoni P. A sludge biotic index (SBI) for the evaluation of the biological performance of activated sludge plants based on the microfauna analysis. Water Res 1994;28: 67–75.
[17] Salvado H, Gracia MP. Determination of organic load rate of activated sludge plants based on protozoa analysis. Water Res 1993;27:891–5.
[18] Kong Z, Vanrolleghem P, Willems P, Verstraete W. Simultaneous determination of inhibition kinetics of carbon oxidation and nitrification with a respirometer. Water Res 1996;30:825–36.
[19] Strotmann UJ, Eglsaer H. The toxicity of substituted phenols in the nitrification inhibition test and luminescent bacteria test. Ecotoxicol Environ Saf 1995;30:269–73.
[20] Hastings JW, Potrikus CJ, Gupta SC, Kurfhrst M, Makemson JC. Biochemistry and physiology of bioluminescent bacteria. Adv Microbiol Physiol 1985;26: 235–91.
[21] Meighen EA. Molecular biology of bacteria bioluminescence. Microbiol Rev 1991;55:123–42.
[22] Iizumi T, Mizumoto M, Nakamura K. A bioluminescence assay using nitrosomonas europaea for rapid and sensitive detection of nitrification inhibitors. Applied and Environmental Microbiology 1998;64(10):3656–62.
[23] Paton GI, Palmer G, Burton M, Rattray EAS, McGrath SP, Glover LA, Killham K. Development of an acute and chronic ecotoxicity assay using *lux*-marked *Rhizobium leguminosarum* biovar *trifolii*. Lett Appl Microbiol 1997;24:296–300.
[24] Chaudri AM, Lawlor K, Preston S, Paton GI, Killham K, McGrath SP. Response of a *Rhizobium*-based luminescence biosensor to Zn and Cu in soil solutions from sewage treated soils. Soil Biol Biochem 2000;32:383–8.
[25] Lajoie CA, Lin S-C, Nguyen H, Kelly CJ. A toxicity testing protocol using a bioluminescent reporter bacterium from activated sludge. J Microbiol Methods 2002;50(3):273–82.
[26] Ren S, Frymier PD. Use of multidimensional scaling in the selection of wastewater toxicity test battery components. Water Res 2003;37:1655–61.
[27] Parker WJ, Monteith HD, Bell JP, Melcer H, Berthouex PM. Comprehensive fate model for metals in municipal wastewater treatment. J Environ Eng 1994;120:1266–83.
[28] Lajoie C, Lin S-C, Kelly C. Comparison of bacterial bioluminescence with activated sludge oxygen uptake rates during zinc toxic shock loads in a wastewater treatment system. J Environ Eng 2003;129(9):879–83.
[29] Ren S, Frymier PD. Kinetics of the toxicity of metals to luminescent bacteria. Adv Environ Res 2003;37:1655–61.
[30] Cabrero A, Fernandez S, Mirada F, Garcia J. Effects of copper and zinc on the activated sludge growth kinetics. Water Res 1998;32:1355–62.
[31] Madoni P, Davoli D, Guglielmi L. Response of SOUR and AUR to heavy metal contamination in activated sludge. Water Res 1999;33:2459–564.
[32] American Public Health Association. In: Clesceri LS, Greenberg AE, Eaton AD, editors. Standard Methods for the examination of water and wastewater, 20th ed. Washington: American Public Health Association; 1998.