Report for 2003MA8B: Copper Removal by Biofilms

- Other Publications:
    - Brussee, K., X. Zhang, and J. Rooney-Varga, 2005 Examination of Cellular Response of Biofilms to Copper Contamination. 3rd Annual Water Resources Conference. Amherst, MA. (Submitted)
    - Brussee, K. and X. Zhang, 2005, Copper Removal by Biofilms, Abstract for the University of Massachusetts Lowell 8th Annual Student Research Symposium.
    - Martinez, K, K. Brussee, and X. Zhang, 2005, Adaptability of Biofilm Exposed to Copper. Abstracts for the University of Massachusetts Lowell 8th Annual Student Research Symposium.
    - Brussee, K. and X. Zhang, 2004, Examination of Cellular Response of Biofilms to Copper Contamination. 2nd Annual Water Resources Conference, Amherst, MA.

Report Follows

Copper Removal by Biofilms

Summary

Title:	Copper Removal by Biofilms
Project Number:	
Start Date:	9/1/2003
End Date:	8/31/2005 (no-cost extension)
Research Category:	Water quality
Focus Category:	Treatment, toxic substances, water quality, wastewater.
Principal Investigators:	Xiaoqi Zhang

The reporting period is March 1, 2004 through February 28, 2005.

Problems and Research Objectives

Heavy metal contamination is of growing concern nationwide because of the numerous health risks to animals and humans. Among the five pollutants of primary concern to MWRA's Toxic Reduction and Control division in Massachusetts, three are heavy metals (i.e. Hg, Cu, and Pb, http://www.mwra.state.ma.us/sewer/html/regs2.htm). Some of the heavy metal contamination comes from agriculture and sewage disposal, although most come from industrial sources, including electroplating plants, mining, nuclear and electronics industries, metal finishing operations, tanneries, and industrial processes utilizing metals as catalysts. Since most of the heavy metal laden effluent will ultimately reach sewerage systems via direct discharge or urban runoff, it is important to remove heavy metals during wastewater treatment processes to reduce the potential harmful effects to ecosystems and public health. In Massachusetts, The Clean Water Act requires that businesses and industries that discharge into the sewerage treatment plants be regulated through an industrial pretreatment program and the discharge limit is set by the local wastewater treatment plant (WWTP). For copper, the state average local limit is 2.187 mg/l (with the maximum being 27.6 mg/l). Such program has greatly reduced the burden of local sewerage treatment plants who usually don't have the capability of handling high concentration industrial pollutants. Wastewater treated by municipal/industrial wastewater treatment plants is usually discharged into local surface water. Although many municipal/industrial wastewater treatment plants can meet the discharge limit set by DEP/EPA, some still have difficulty in meeting the copper discharge limit. Therefore there is an urgent need for an effective treatment technology to remove copper during the wastewater treatment process to meet the ever more stringent discharge limit (6.2 µg/l for copper discharge to Nashua River).

It is hypothesized that microorganisms produce negatively charged extracellular polymeric substances which can sorb positively charged copper. The objective of this research is to evaluate the effectiveness of a biofilm system in treating heavy metal containing wastewater, and determine the cellular response to copper contamination.

Methodology

Two laboratory scale biofilm reactors (Biosurface Technologies, Corp. Jacketed Model 1120LJ) (Figure 1) were used to generate biofilm growth on removable clear polycarbonate slides by seeding the reactor with activated sludge and introducing an influent with a controlled synthetic wastewater and copper concentrations.

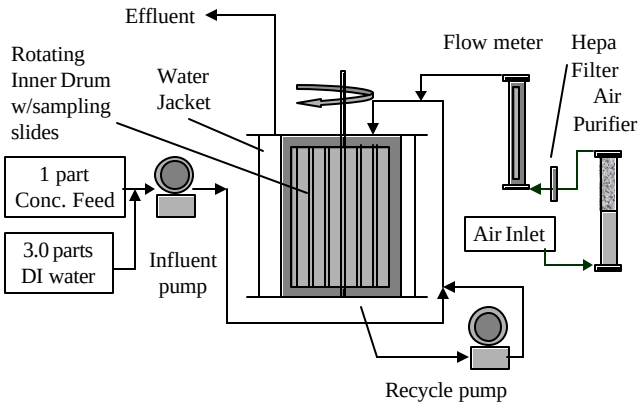

Figure 1. Experimental Flow Chart of Biofilm Reactor.

Influent Design: The synthetic wastewater is introduced to the reactor using one pump (Cole Parmer No. 7553-80) with two pump heads (Cole Parmer, Masterflex No. 77200-60). One pump head utilizes Manostat 1/16th inch tygon silicone tubing to transport the concentrated feed, the other 1/8th inch tubing to transport DI water. The two lines are joined to produce an influent with a ratio of approximately 3.0 mL of DI water to 1 mL of concentrated feed. The influent flow rate to the reactor is being maintained at 8.5 mL/min producing 106 minutes of hydraulic retention time.

Table 1. Desired influent concentrations (Zhang et al., 1999)

Organics	Influent conc. (mg/L)	Inorganics	Influent conc. (mg/L)
beef extract	41.76	NH_4Cl	1.67
yeast extract	45.93	$NaHCO_3$	156.44
peptone	41.76	K_2HPO_4	18.37
glucose	29.48	KH_2PO_4	7.11
		$MgSO_4 \cdot 7H_20$	18.37
		$FeCl_2 \cdot 4H_20$	0.25
		$CaCl_2 \cdot 2H_20$	24.56
		$NH_2 \cdot CO \cdot NH_2$	29.48
		$Na_2HPO_4.7H_2O$	27.56

A fresh twenty liters of synthetic wastewater measured by chemical oxygen demand (COD) of ~150 mg/L is prepared weekly and fed into the reactors. (see Table 1 for the feed composition). The organics (except urea) and approximately eighteen liters of DI water are autoclaved to prevent the feed from fouling (fouling has been shown to reduce the influent COD concentration, pH and ratio of free to total copper). The inorganics, urea, and copper are added once the autoclaved water has cooled and is then placed on a stir plate and hooked up to the influent pump.

Influent/Effluent Parameters: For each new twenty liters of feed, the COD concentration, pH, total copper and free copper concentration (Hach method 8143) of the influent are measured three times a week. The COD concentration, pH, total copper and free copper concentrations are also determined for the effluent on the same schedule as the influent.

Biofilm Sampling: Once the reactor has reached a pseudo-steady state condition indicated by the constant effluent COD concentration (~25 mg/L, within approximately two weeks of forward flow), and the biofilm growth is substantial enough for sampling, biofilm is scraped from one or two of the sampling slides for biofilm analysis and EPS extraction. For each sampling, the surface charge (Morgan et al., 1990), and total and free copper concentrations of the biofilm are determined (Hach method 8143). Total Solids (TS) (APHA, 1998) are determined for the biofilm to represent the total biomass of the biofilm.

EPS Extraction: Biofilm EPS is extracted according to the steaming procedure described in Zhang et al. (1999). EPS is quantified by measuring polysaccharides content (Dubois et al, 1956) and protein content (Bradford, 1976). Figure 2 shows the variety of analyses that are performed on the biofilm samples, and EPS extraction procedure and its measurement.

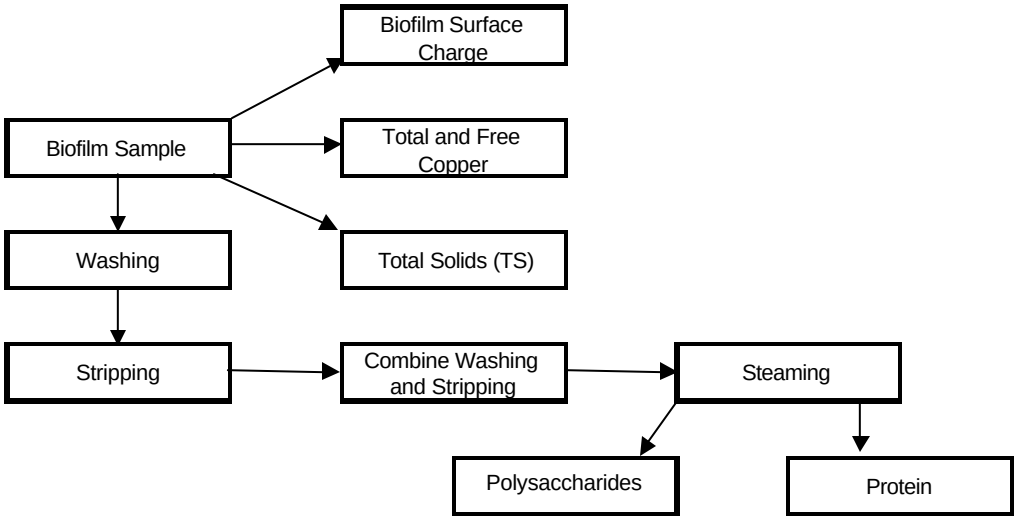

Figure 2. Flowchart of Biofilm and EPS Analyses.

Principal Findings and Significance

During this reporting period, one reactor of 0ppb Cu2+ and one reactor of 250ppb Cu^{2+} were studied.

Reactor Running Conditions: For both reactors, the COD of the synthetic wastewater influent was maintained at 150 mg/L, and the COD in the effluent reached 20-25 mg/L, indicating ~85% organics removal (Figure 3). pH in the influent was maintained at 8.0; pH in the effluent was at 7.2 (Figure 4).

Copper Removal: The total influent copper concentration was maintained at 254 (± 19) ppb. The free copper concentration of the influent was 164 (± 6) ppb and the effluent 110 (± 15) ppb. Although only 33% of the free copper was removed from the influent, either by sorption or forming complexes (Figure 5), the result suggests that a biofilm system could be used as a pretreatment for wastewater contaminated with higher copper concentration. The toxicity of copper mainly comes from free copper, therefore, this biofilm reactor is effective to a certain degree in removing the free copper and reducing copper toxicity.

Cellular Response: Under the reactor conditions of 0ppb copper, EPS-polysaccharide concentrations averaged 11.54mg/g TS, which was only slightly lower than the concentration found in the biofilm exposed to 200ppb copper (13.41mg/g TS). A significant difference was observed between the EPS-protein concentrations of the two biofilms. The EPS-protein concentration for the biofilm exposed to no copper was determined to average approximately 5.99mg/g TS, consistent with the previous finding by Ramasamy and Zhang (2004). However, the biofilm grown under 200ppb copper produced more than double that concentration (14.46mg/g TS). This may be correlated with the significant differences observed in the surface charge of each biofilm. The biofilm grown under 0ppb copper conditions developed an average negative surface charge value of –0.17µequiv/g TS. This value was found to be half the surface charge value observed by the biofilm that was produced under 200ppb copper (-0.32µequiv/g

TS). The results indicate that cells produced more proteins with negative charges as a form of cellular response to copper contamination.

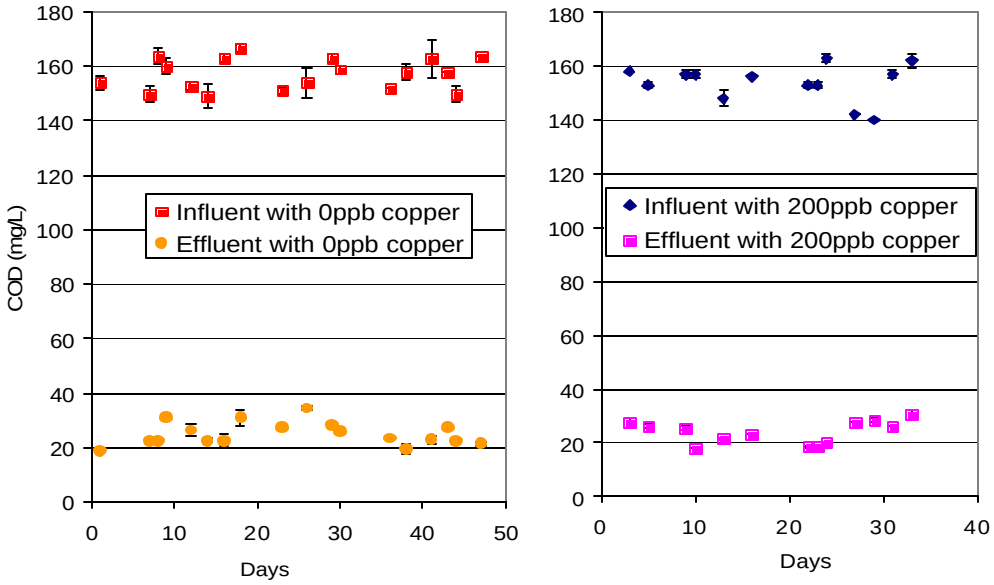

Figure 3. Reactor Running Conditions - COD

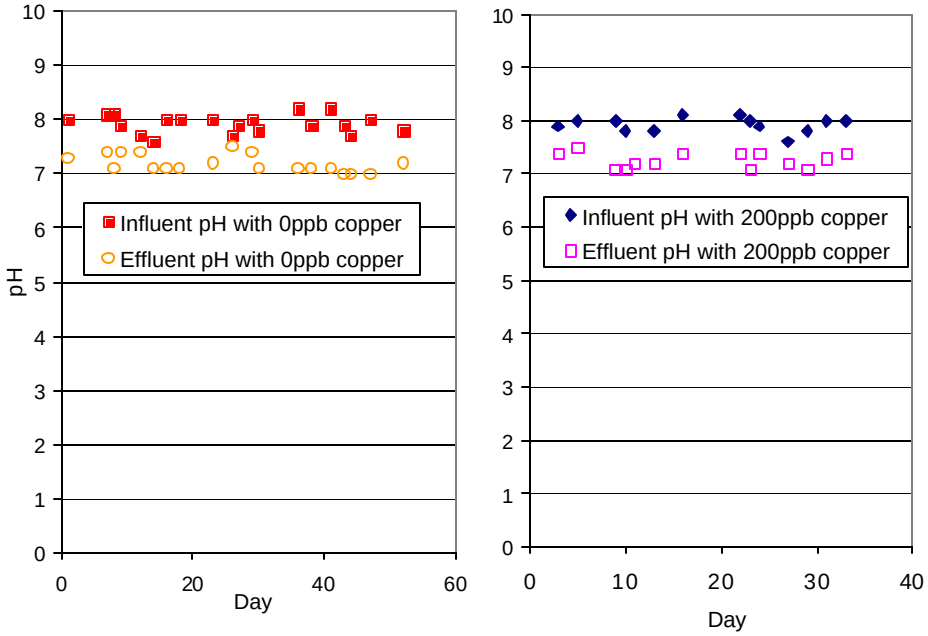

Figure 4. Reactor Running Conditions – pH

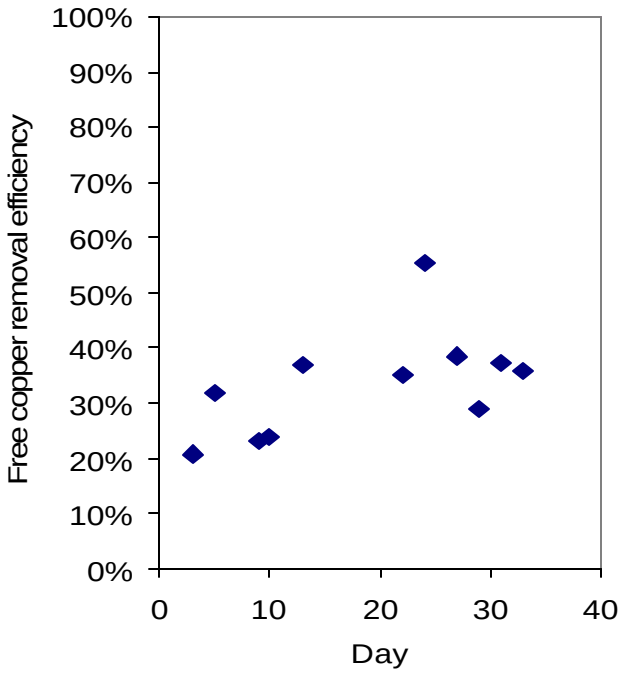

Figure 5. Free Copper Removal Efficiency of 250 ppb Copper Reactor

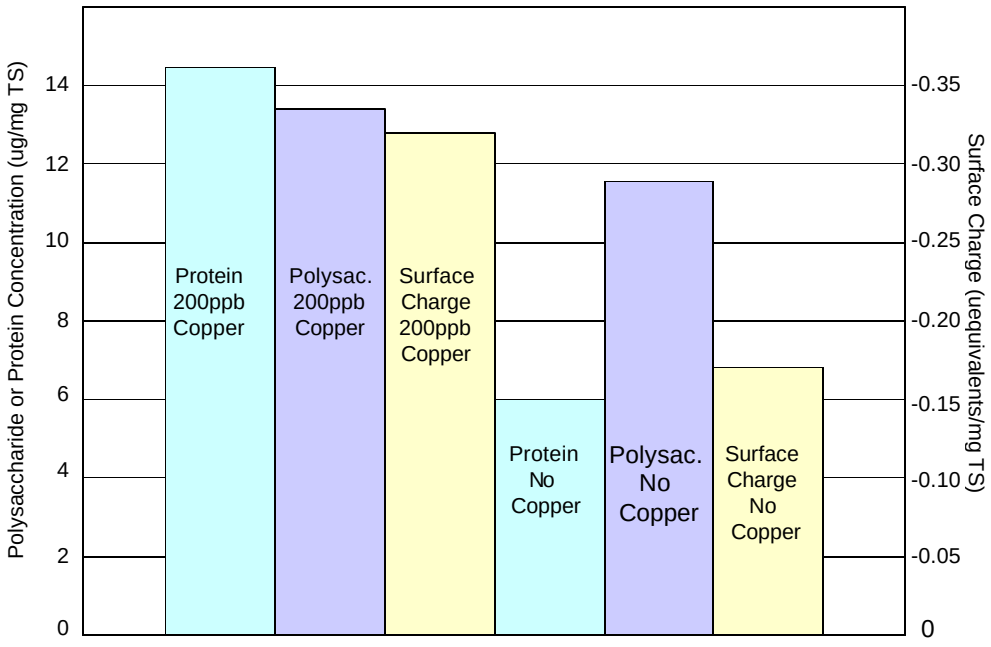

Figure 6. Biofilm Cellular Response to Copper Contamination

Microbial Community: Preliminary molecular analysis of the bacterial communities in both copper-free and copper-exposed biofilms revealed qualitative and semi-quantitative differences in their community compositions. These results indicate that exposure to 250 ppb copper selects for specific microbial populations that are able to tolerate this stress and that may contribute to its remediation.

Work in Progress

Cells may respond differently to different level of copper concentrations. Currently the biofilm exposed to 100ppb and 300ppb Cu^{2+} are being examined, the free copper removal efficiency, and their surface charge, EPS protein and polysaccharide concentrations compared to further determine the cellular response of biofilms to copper contamination.

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Publications

Water Resources Research Institute Annual Report (this report)

Brussee, K., Zhang, X., and Rooney-Varga, J. (2005) Examination of Cellular Response of Biofilms to Copper Contamination. 3rd Annual Water Resources Conference. Amherst, MA. (Submitted)

Brussee, K. and Zhang, X. (2005) Copper Removal by Biofilms. Abstract for the University of Massachusetts Lowell 8th Annual Student Research Symposium.

Martinez, K, Brussee, K. and Zhang, X. (2005) Adaptability of Biofilm Exposed to Copper.

Abstracts for the University of Massachusetts Lowell 8th Annual Student Research Symposium.

Brussee, K. and Zhang, X., (2004) Examination of Cellular Response of Biofilms to Copper Contamination. 2rd Annual Water Resources Conference. Amherst, MA.

Students Supported (number and level)

One Master's student (Kevin Brussee) is being financially supported.
One work study female undergraduate student (Kely Martinez) is conducting research for this project.

Future Funding

The PI is actively seeking funding from NSF to continue research on this topic.