Report for 2003MA8B: Copper Removal by Biofilms

- Conference Proceedings:
  - Brussee, K. and X. Zhang, Copper Removal by Biofilms, 2003, in First Annual Water Resources Research Center Conference: Water Resources in the Northeast: Science and Policy. http://www.umass.edu/tei/wrrc/presentations/Zhang.pdf
- Other Publications:
  - Brussee, K. (2004) Copper removal by biofilms. Abstracts for the University of Massachusetts Lowell 7th Annual Student Research Symposium, #10.

Report Follows

Copper Removal by Biofilms

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/03sewer/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 and the effects of substrate concentration on such cellular response and copper removal.

Methodology

A laboratory scale biofilm reactor (Biosurface Technologies, Corp. Jacketed Model 1120LJ) (Figure 1) is being used to generate biofilm growth on twenty removable clear polycarbonate slides by seeding the reactor with activated sludge and introducing an influent with a controlled substrate and copper concentration.

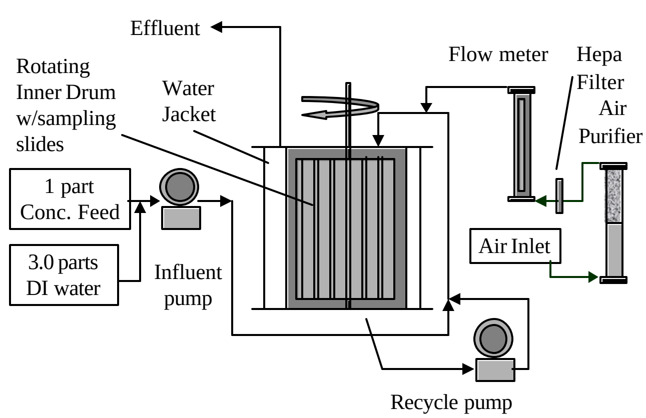

Figure 1. Experimental flow chart of biofilm reactor.

Influent Design: The substrate 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 join producing 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

The concentrated feed is prepared according to the observed ratio described above. Table 1 shows the desired concentrations entering the reactor. A fresh twenty liters of concentrated feed is prepared weekly. The organics (except urea) and approximately eighteen liters of DI water are autoclaved to prevent the feed from fouling (fouling has 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; initial, third day, and seventh day. 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 (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), Total Suspended Solids (TSS), and Volatile Suspended Solids (VSS) (APHA, 1998) are determined for the biofilm as well. TS is used to represent the total mass of the biofilm; TSS as the total biomass; and VSS as the viable biomass.

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.

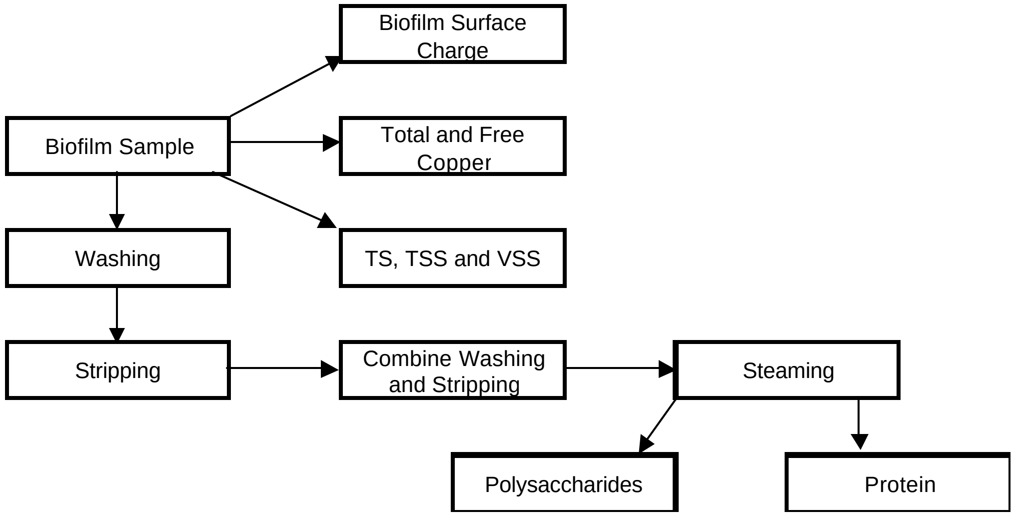

Figure 2. Flowchart of biofilm and EPS analyses.

Principal Findings and Significance

The initial reactor running conditions we studied were: a mimic wastewater influent containing 100ppb Cu^{2+} and 150 mg/L of COD. This report describes the principal findings for these running conditions.

Copper Removal: The total influent copper concentration was maintained at 104 (± 5) ppb, and the resulting total effluent copper concentration was 98 (± 5) ppb. The free copper concentration of the influent was 66 (± 7) ppb and the effluent 42 (± 9) ppb. Although only 5.8% of the total copper was removed, 36% of the free copper was removed from the influent, either by sorption or forming complexes. The toxicity of copper mainly comes from free copper, therefore, this biofilm reactor is effective in removing the free copper and reducing copper toxicity.

Biofilm: On each sampling slide, 54.0 (± 4.1) mg TS and 43.1 (± 0.4) mg TSS of biofilm were obtained from the reactor. The biofilm was found to have net surface charge of -0.51 (±0.06) μequivalents/mg TS and total copper of 1.10 (±0.06) mg Cu/g TS (see Table 2). The negative surface charge on the biofilm suggests that more copper sorption can be expected with longer reactor running time. The mass of copper contained within the biofilm is an important factor for determining the threshold of copper sorbed by biofilm and consequently the effectiveness of a biofilm system in removing copper from wastewater; and this will be further determined after more running conditions (controlled substrate and copper concentration) are studied.

Biofilm EPS Production: The EPS polysaccharides to protein ratio was 0.785 (see Table 2). This is quite different compared to the data collected by Ramasamy and Zhang (2004) under the reactor influent conditions of 0 ppb Cu^{2+} and 150 ppm COD, which showed EPS polysaccharide concentrations more than twice that of protein. This clearly indicates that this could be the result of cellular response to copper contamination. The response is expressed by producing less polysaccharides.

Table 2. Analyses for biofilm and biofilm EPS

	Parameter	Value
Biofilm	TS, mg/sampling slide	54.0 ± 4.1
	TSS, mg/sampling slide	43.1 ± 0.4
	Total copper sorbed by the biofilm, mg Cu/g TS	1.10 ± 0.06
	Biofilm surface charge, μequivalents/mg TS	-0.51 ± 0.06
Biofilm EPS	EPS-carbohydrate, mg/g TS	15.7 ± 2.2
	EPS-protein, mg/g TS	20.0 ± 1.6

Note: VSS of the biofilm didn't work out.

References

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Zhang, X., Bishop, P. L., Kinkle, B. K. (1999) Comparison of extraction methods for quantifying extracellular polymers in biofilms. *Wat. Sci. Tech*. **39,** 211-218.

Publications

Water Resources Research Institute Annual Report (this report)
Brussee, K. (2004) Copper removal by biofilms. Abstracts for the University of Massachusetts Lowell 7th Annual Student Research Symposium, #10.

Students Supported (number and level)

One Master's student is being supported.
A Master's thesis is being written as a result of this project.

Future Funding

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