TO: Texas Hazardous Waste Research Center

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SUBJECT: Final Report

PROJECT NUMBER: 513UTA0038H

PROJECT TITLE: Saving energy in drinking-water biofilter operation: A fundamental study of the relationship among nutrient conditions, EPS production, and biofilter headloss

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I. Description and Objective of Research Project

The overall goal of the project was to better understand the relationship among nitrogen and phosphorus concentrations, EPS production, and headloss in drinking-water biofilters so that filter run times might be lengthened and energy demands for filter backwashing might be reduced. The specific objectives of the project were as follows:

1. Optimize an EPS extraction protocol for granular biofilter media.
2. Compare the EPS concentration and headloss in bench-scale biofilters under carbon-, nitrogen-, and phosphorus-limited conditions.

II. Methodology, Results, and Accomplishments

The methods, results, and accomplishments are described below, as they relate to the objectives of the project.

Objective 1. Optimize an EPS extraction protocol for granular biofilter media.

The protocol was developed using samples from granular activated carbon (GAC) collected from full-scale biofilters. Six extraction methods were tested:

1. Formaldehyde (Liu & Fang 2002)
2. Formaldehyde + NaOH (Liu & Fang 2002)
3. Tris, ethylenediaminetetraacetic acid (EDTA), 2.5% NaCl, pH 8 (Lauderdale et al. 2012)
4. Tris, EDTA, 2.5% NaCl, pH 11
5. 2% NaCl (Xu & Chellam 2005)
6. Cation exchange resin (CER, Frølund et al. 1996)

In general, 2 g of wet media were mixed with 10 mL of buffer; for the CER extraction, 80 g of GAC, 20 g (wet weight) of washed CER (Dowex® Marathon^{TM} Na^+ form 20x50 mesh, Sigma-Aldrich, St. Louis, MO), and 400 mL of buffer (Frølund et al. 1996) were stirred using an overhead stirrer. The extraction was finished by centrifuging samples at 10,000×g for 10 min. The supernatant (containing the EPS) was filtered through a 0.45-μm filter and saved for subsequent analysis. The solids were retained for total solids (TS) analysis. EPS was quantified by measuring the bulk polysaccharide and protein concentrations. Polysaccharides were quantified using a modified phenol-sulfuric acid assay (DuBois et al. 1956, Masuko et al. 2005). Proteins were quantified using a modified Lowry assay with the Pierce bicinchoninic acid (BCA) protein kit with bovine serum albumin (BSA) as the standard (Thermo Fisher Scientific, Rockford, IL).

- The extraction method using 10 mM Tris, 10 mM EDTA, 2.5% NaCl, and pH 8 yielded a higher concentration of polysaccharides and proteins than did the other tested methods.

Formaldehyde was found to interfere with polysaccharide and protein analyses.

The extraction method using 10 mM Tris, 10 mM EDTA, 2.5% NaCl, and pH 8 was then optimized by varying the initial physical treatment, shaking intensity, temperature, and time.

- All four parameters significantly affected the EPS yield (1-way ANOVA, $p<0.002$). The EPS quantities increased with increasing temperature, time, and shaking. Our results suggest that vortexing the filter media for 1 min at the outset, and incubating at 35 °C for 8 h with shaking at 200 rpm maximizes the recovered concentrations of polysaccharides and proteins.

It was important to assess the extent of contamination of the EPS pool by intracellular compounds released from cell lysis during the extraction process. We evaluated this contamination by quantifying the polysaccharides and proteins released by a planktonic culture of *Escherichia coli* (10^7 colony forming units/mL) when subjected to the extraction protocol.

- The lysis tests indicated that intracellular proteins could constitute approximately 11, 20, or 23 percent of the total protein pool for EPS extractions lasting 4, 8, or 24 hours, respectively. We suggest an extraction time of 4 h to minimize contamination from cell lysis.

To extract EPS from granular biofilter media, we recommend using a 10 mM Tris, 10 mM EDTA, 2.5% NaCl, pH 8 buffer with vortexing for 1 min followed by a 4-h incubation at 35 °C with shaking at 200 rpm. This method accomplishes the goal of maximizing the concentrations of polysaccharides and proteins while minimizing contamination from intracellular compounds.

Objective 2. Compare the EPS concentration and headloss in bench-scale biofilters under carbon-, nitrogen-, and phosphorus-limited conditions.

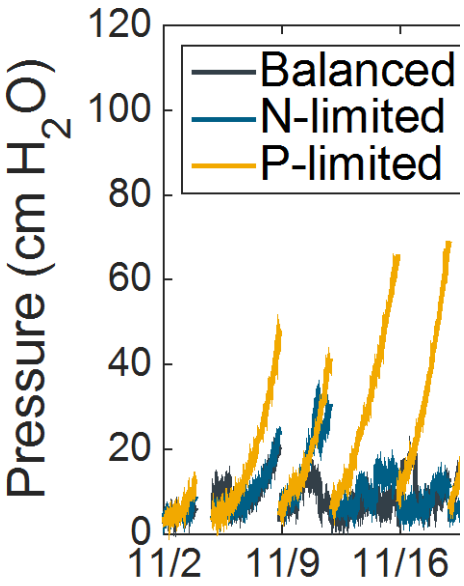
Three parallel trains of two columns in series were operated, with each train subject to a different nutrient limitation. The first column in each train was the primary column being interrogated in this study. It had an inner-diameter of 2.5 cm, a bed-depth of 15 cm, and a theoretical empty bed contact time (EBCT) of 3 min. The second column in series provided acclimated GAC to replace GAC in the primary column after core samples were removed. The columns were packed with 0.5-mm diameter GAC that was obtained from a full-scale biofilter. The columns were seeded with bacteria from Lake Austin surface water.

The columns were fed a synthetic water consisting of a 1 mM carbonate buffer at pH 7.4, salts, vitamins, and trace metals at a hydraulic loading rate of 3 m/h. The carbon sources (i.e., acetate, formate, formaldehyde, and glyoxal) simulate ozonated water and provided a biodegradable organic carbon concentration of 400 μg/L (modified from Elhadi et al. 2006). Nitrogen was provided as NH_4Cl, and phosphorus was provided as KH_2PO_4.

Each column was fed a different nutrient ratio. A C:N:P molar ratio of 100:10:1 is considered a balanced condition in which no nutrient is limiting (LeChevallier et al. 1991), and a nutrient limitation is achieved by decreasing the relative amount of nitrogen or phosphorus provided. One

column was operated under the balanced condition (100:10:1), one under a nitrogen limitation (100:0.1:1), and one under a phosphorus limitation (100:10:0.01).

The performance of each column was assessed by measuring the dissolved oxygen (DO) consumption across the filter, the headloss across the filter, and the EPS concentrations on the filter media.



- The median change in DO across each filter was 0.9 mg/L, which is slightly greater than the theoretical oxygen demand of 0.77 mg/L. DO consumption did not differ among the balanced, N-limited, and P-limited columns.
- Figure 1 shows several example headloss profiles in the primary column of each biofilter train. The P-limited column routinely developed headloss more rapidly than did the N-limited and balanced columns.
- The EPS concentrations appear similar across all three biofilter trains, with protein concentrations being over an order of magnitude greater than polysaccharide concentrations. This trend matches what we have observed in media samples from full-scale drinking water biofilters.

Figure 1. Headloss in the primary column of each biofilter train as a function of time. Five events of backwashing and headloss accumulation are shown.

The main accomplishment associated with this objective is that we showed increased headloss in a biofilter under P-limited conditions. Increased EPS content was not the reason for the increase in headloss, so the underlying mechanism must continue to be investigated.

III. Future Activities

This project is continuing. First, we are assessing the reason why headloss is highest in the P-limited biofilter train as compared to the other biofilter trains without substantial differences in EPS concentrations among the biofilter trains. Second, we testing other nutrient ratios (i.e., a 10x limitation rather than a 100x limitation) to determine the nutrient threshold that triggers faster headloss accumulation.

IV. Acknowledgements

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V. References

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VI. Publications/Presentations

Publications

Keithley, S. and M. J. Kirisits. (2015) "Extracellular Polymeric Substances in Drinking Water Biofilters: Role of Nutrients and Impact on Headloss." *Proceedings of the American Water Works Association Annual Conference and Exposition*. Anaheim, California. 5 pp.

Keithley, S. and M. J. Kirisits. (2015) "Nutrients, Extracellular Polymeric Substances, and Headloss in Biofilters." *Proceedings of Texas Water 2015 - The Joint Conference of the Texas Section American Water Works Association and the Water Environment Association of Texas*. Corpus Christi, Texas. 5 pp.

Presentations

Keithley, S., M. J. Kirisits. "Extracellular polymeric substances in drinking water biofilters: Role of nutrients and impact on headloss." American Water Works Association Annual Conference and Exposition. Anaheim, CA. June 7-10, 2015.

Keithley, S. "Nutrients, extracellular polymeric substances, and headloss in drinking water biofilters." Poster presentation. World Environmental and Water Resources. Austin, TX. May 17-21, 2015.

Keithley, S., M. J. Kirisits. "Nutrients, extracellular polymeric substances, and headloss in biofilters." Texas Water 2015. Corpus Christi, TX. April 14-17, 2015.