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Influence of pH and natural organic matter on zinc biosorption in a model lignocellulosic biofuel biorefinery effluent

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HIGHLIGHTS

- Zn biosorption was tested in a biorefinery wastewater rich with organic matter.
- Effects of pH and NOM were first characterized in batch culture.
- Zn removal in membrane bioreactors at pH 6.5 and 8 was 26% and 91%, respectively.
- Natural organic matter inhibited Zn removal.
- pH manipulation dramatically increased Zn removal.

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ABSTRACT

The effect of dissolved natural organic matter (NOM) and pH on microbial biosorption of Zn was evaluated in a model lignocellulosic biofuel refinery effluent rich in NOM. Batch culture experiments conducted with two model microorganisms (yeast, *Candida tropicalis*; bacteria *Novosphingobium nitrogenifigens* Y88^T), showed an inhibitory effect of NOM, and an optimum pH for Zn removal at 7.5–8.0. Membrane bioreactors with mixed autochthonous organisms were operated at pH 6.5 and pH 8.0 to better simulate real-world remediation scenarios. More Zn was removed at the high (91%) than at the low (26%) pH, presumably because the higher pH freed negatively-charged functional groups on the cellular biomass for passive Zn binding. Manipulating the pH of bioreactors can significantly improve metal removal in NOM rich wastewater. Such reactors could maintain water quality for closed-cycle biorefineries, leading to reduced water consumption, and a more sustainable biofuel.

1. Introduction

Biofuels are gaining popularity as a sustainable alternative to transportation fossil fuels (Hill et al., 2006). However, biofuel biorefineries are likely to consume large amounts of water (Vörösmarty et al., 2000), a factor that will threaten their sustainability. By analogy with other lignocellulosic biorefineries (e.g. pulp and paper mills), biofuel refineries will most likely recycle process water. Unfortunately, continual water recycling leads to problematic build-up of metals, salts and organic material that will limit the extent of water recycling and interfere with in-plant processes (e.g. metal inhibition of key enzymatic processes; Tejirian and Xu, 2010). Another reason for concern is the toxicity of metals to aquatic wildlife in the receiving environment. Metals can be removed with nanofiltration or reverse osmosis (Manttari and Nystrom, 2007), but these are expensive processes. Microbial metal sorption, on the other hand, has been recognized as a simple, low cost bioremediation strategy for waters contaminated with heavy metals (Gadd, 2009; Vijayaraghavan and Yun, 2008), and could be used to control metal accumulation in biorefineries.

Metal sorption by microbes is also of fundamental scientific interest because the interactions of metals with microorganisms can determine the speciation, mobility and therefore toxicity of metals in the natural environment (Chapelle, 2000; Moreau et al., 2007). Most studies exploring microbial metal sorption do so in simple aqueous solutions. However, natural organic matter (NOM) can also bind metals (de Schamphelaere et al., 2004; Tipping and Hurley, 1992) and can potentially compete with organisms for metal binding. Indeed, the role of NOM in metal biosorption has been integrated into aquatic toxicity assessments, in which NOM-bound metals are regarded as not bioavailable

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(Di Toro et al., 2001). Lignocellulosic biofuel biorefinery wastewaters are estimated to have chemical oxygen demands (COD) in the range of 3000–7000 mg/L, sourced mainly from the feedstock (Merrick and Company, 1998). Much of that organic material will be recalcitrant NOM that is likely to influence metal removal, although the nature and extent of this binding, and its impact on metal remediation is not well understood. The effect of NOM on metal removal from wastewater has been the focus of a few investigations, mainly concerning municipal wastewaters (Crane et al., 2010; Prigione et al., 2009; Santos et al., 2010).

Previous work in our laboratory has shown that zinc (Zn) is one of the most abundant heavy metals in pulp mill effluent and it is also likely to be abundant in lignocellulosic biofuel biorefinery effluent (Palumbo et al., 2012). Zn is a concern because of the possible impact to freshwater ecosystems receiving discharge from a biorefinery. For example, some algae are very sensitive to Zn, with levels as low as 0.039 mg/L causing a 50% inhibitory effect on *Selenastrum capricornutum* cell division (EC_{50}) after 48 h (Franklin et al., 2001). This level is an order of magnitude lower than that measured in lignocellulosic biorefinery wastewaters (Palumbo et al., 2012).

The present study sought to characterize the relationship between metal remediation, NOM concentration and pH (an important factor controlling metal speciation and metal-NOM association) in a model lignocellulosic biofuel refinery wastewater. We hypothesized that pH and NOM concentration can be manipulated in bioreactors to optimize Zn removal. Therefore, the effects of these parameters were characterized in batch studies using two model organisms identified from our previous research: *Novosphingobium nitrogenifigens* $Y88^T$ and *Candida tropicalis*, (Palumbo et al., 2012). The resulting information was integrated into the second part of this work, in which bench scale bioreactors were used to simulate Zn removal in a full-scale lignocellulosic biorefinery bioreactor. The results show the potential of microbial sorption as a water treatment technology that can enable greater water reuse in biorefineries.

2. Methods

2.1. Model wastewater (MW)

Because no full scale lignocellulosic ethanol biorefinery currently exists, a model wastewater (MW) was previously created (Palumbo et al., 2012) using pilot plant data (Merrick and Company, 1998) to modify a pulp mill effluent to resemble a lignocellulosic ethanol biorefinery effluent. This was achieved mainly by addition of acetic acid (400 μL/L) and ethanol (127 μL/L). The solution was 0.45 μm syringe-filtered to provide a homogeneous MW and to confine batch experiments to the dissolved phase only. Tests were conducted under mesophilic conditions (36 °C; see Palumbo et al. (2012) for more details). The final NOM content in the MW from the pulp mill effluent was measured as 837 mg/L dissolved organic carbon (DOC). This figure excludes the DOC contribution of the added ethanol and acetic acid.

2.2. Base salts medium

A base salts (BS) medium was created to enable testing of Zn sorption under various NOM concentrations. The medium was designed for use as a diluent of the MW and had similar N and P concentrations and salt composition as the MW, but lacked the NOM. BS consisted of Difco yeast extract (BD Biosciences, 50 mg/L), KCl (71 mg/L), NaCl (152 mg/L), $CaCl_2$ $2H_2O$ (198 mg/L), $MgSO_4$ $7H_2O$ (94 mg/L), K_2HPO_4 (18 mg/L). Also, acetic acid, ethanol, NH_4Cl, K_2HPO_4 and $NaHCO_3$ were added from the same stock solution as that used to make the MW. The final pH was 5.4, identical to that of the MW.

2.3. Model microorganisms

A N_2-fixing bacterium, *N. nitrogenifigens*, was previously isolated on solid media with 5 mM NiCl from a bioreactor digesting pulp and paper mill effluent (Addison et al., 2007, Genbank nucleotide database accession No. DQ448852 and DQ660368). A yeast, *C. tropicalis*, was isolated from a bioreactor digesting pulping effluent supplemented with 11 mg/L Mn and 13 mg/L Zn (Palumbo et al., 2012, Genbank accession No. JQ640572).

2.4. Zn uptake over time

A batch culture experiment using the two model organisms was conducted to monitor Zn uptake by growing cells over 96 h in the MW. To construct the 96 h time series, the contents of 36 flasks were sacrificed for sampling at 0, 6, 9, 12, 15, 18, 24, 48, and 96 h, with two replicate flasks per time point.

Two sets of un-inoculated flasks served as controls which were sampled at 24, 48, and 96 h. The initial pH of the MW and the pH of the first set of controls was pH 5.4. A second set of un-inoculated controls was adjusted to pH 9.0 by adding 310 μL 1 M NaOH. This second set of controls was used to check for abiotic precipitation due the increase in pH from microbial metabolism typically seen in inoculated flasks. The pH of the second controls drifted down from 9.0 to about 8.0 so approximately two drops of NaOH were added an hour before sampling to maintain the pH.

Each experiment was initiated by making a new aliquot of MW. An aliquot of this solution (50 mL) was added to each acid-washed 250 mL Erlenmeyer flask in a biohazard hood. Metal stock solution (0.5 mL) was added to achieve a concentration of 13 mg/L Zn (0.20 mM, as $ZnCl_2$; Sigma–Aldrich, Fluka, St. Louis, Mo., USA), based on Palumbo et al. (2012). The inoculum (0.5 mL) of either organism (prepared as in Palumbo et al., 2012) was added to each flask. Separate flasks containing un-inoculated MW served as no-organism controls. All flasks were covered with Breathseal plate sealer (Greiner Bio-one, Frickenhausen, Germany) and then placed in a shaking (100 rpm) incubator maintained at 36 ± 1 °C.

Flask sampling began by taking a 3 mL aliquot of culture used for OD measurement at 600 nm. The remaining solution was weighed and then centrifuged at 4500*g* for 10 min to pellet biomass. Supernatant (25 mL) was taken for DOC analysis and a 10 mL aliquot was acidified to pH < 2.0 with HNO_3 for Zn analysis (see Section 2.9). The pH of the remaining supernatant (including the biomass pellet) was measured. This experiment was repeated once more in its entirety for a total of four replicates per treatment.

2.5. Zn uptake with varying NOM concentrations

A batch culture experiment using the two model organisms was conducted to monitor Zn uptake by growing cells in media with decreasing NOM concentrations. MW was serially-diluted with BS to make media with decreasing concentrations of NOM. Treatment groups consisted of 1× NOM (undiluted MW), 0.5× NOM, 0.25× NOM, 0.125× NOM and 0× NOM (BS medium only), resulting in average measured concentrations of: <15, 104, 212, 432, and 837 mg/L dissolved organic carbon (DOC) in the media mixtures, excluding 212 mg/L DOC from the acetic acid and ethanol that was added as described in Section 2.1.

This experiment included duplicate flasks inoculated with one of the two model organisms or the un-inoculated control for each of the 5 different NOM treatments (30 total flasks), sampled at 24 h (the time of maximal uptake from the times series). The