

## Role of Extracellular Polymeric Substance Sulfhydryl Sites in Bacterial Detoxification of Cadmium

QIANG YU<sup>1,\*</sup>, BHOOPESH MISHRA<sup>2</sup>,

JEREMY B. FEIN<sup>1</sup>

<sup>1</sup>Department of Civil and Environmental Engineering and Earth Sciences, University of Notre Dame, Notre Dame 46556, USA (presenting author, email: qyu@nd.edu)

<sup>2</sup>School of Chemical and Process Engineering, University of Leeds, Leeds LS29JT, United Kingdom

Bacteria can adsorb a variety of metals via their surface binding sites that are present within cell envelopes and on extracellular polymeric substances (EPS). These adsorption reactions affect not only the transport of metals but also the bioavailability and toxicity of metals towards bacterial cells, therefore strongly affecting the fate of metals in the environment. However, the mechanisms of how adsorption reactions affect metal toxicity are still poorly understood.

In this study, we explore the role of EPS sulfhydryl sites in bacterial detoxification of Cd, using *Pseudomonas putida* that has surface sulfhydryl sites mostly on its EPS molecules, as a model bacterial species. Our results show that the presence of 20 ppm Cd in LB medium causes an extended lag phase during the growth of *P. putida* cells compared to cells grown in Cd-free controls, but the Cd does not affect the overall extent of growth of the cells, suggesting that *P. putida* cells have the ability to detoxify Cd. The analysis of the biomass samples using extended X-ray absorption fine structure (EXAFS) spectroscopy indicates that the adsorbed Cd on the biomass is present predominantly bound to sulfhydryl sites on the EPS of *P. putida* cells, suggesting that Cd adsorption onto EPS sulfhydryl sites represents a strategy adopted by *P. putida* for keeping toxic Cd away from cells. We conducted Cd toxicity experiments by exposing cells that were harvested at different growth stages, and that contained different concentrations of sulfhydryl sites, to LB media with and without Cd. The extent of cell growth is positively correlated to the measured sulfhydryl concentration on the EPS of the different biomass samples, but is independent of the measured total binding site concentration on the cell surfaces. This study suggests that the sulfhydryl sites on bacterial EPS can play an important role in binding and detoxifying toxic metals. Therefore, it is crucial to quantify the concentration and distribution of sulfhydryl sites on the EPS of other bacterial species and natural samples in order to determine whether the strategy employed by *P. putida* to combat metal toxicity is representative of bacterial cells in general.