

Thermodynamics of Reversible Proton Exchange and Metal Sorption Onto *Shewanella putrefaciens*

Johnson Haas (johnson.haas@wmich.edu)¹ &
Thomas DiChristina (thomas.dichristina@biology.gatech.edu)²

¹ Dept of Chemistry, Western Michigan University, Kalamazoo, Michigan, USA

² School of Biology, Georgia Institute of Technology, Atlanta, Georgia, USA

Surface complexation reactions occurring on bacterial cell membranes represent a potentially major process affecting the geochemical behaviour of dissolved chemical species in sediments and the water column. Specifically, adsorption onto bacterial cells may strongly influence the speciation and transport of heavy metals (e.g. Pb, Cd, Cu), transuranic radionuclides (U, Th, Pu, Am, Cm) and fission products associated with transuranic elements, such as the lanthanides. Anaerobic metal-reducing bacteria (MRB) such as *Shewanella* and *Geobacter* derive metabolic energy from the enzymatic reduction of metallic chemical species in solution or as solid phases. Thus, for MRB metal sorption represents a major obstacle for anaerobic growth, and these microbes may possess unique metal-binding qualities that have evolved to optimise the efficiency of ion acquisition or cell-mineral attachment. We have conducted a series of laboratory experiments measuring the proton exchange and metal sorption properties of the facultatively-anaerobic MRB *Shewanella putrefaciens* strain 200R (Obuekwe et al., 1981; MacDonell & Colwell, 1985). We have chosen *S. putrefaciens* as a model organism representing gram-negative MRB found in a wide variety of marine, estuarine and freshwater settings. In this study bacteria were cultivated in sealed batch reactors on Luria Bertani (LB) medium to late logarithmic-stage growth, calibrated by UV-visible spectrophotometry of harvested cell suspensions. Cells were inactivated by treatment with 25 µg/L of chloramphenicol and were washed, centrifuged and resuspended through three repeats in 0.1 M NaCl. Final cell densities were direct-counted using epifluorescence microscopy. Acid-base titrations on *S. putrefaciens* were performed under positive pressure of N₂(g) and in a background electrolyte solution of 0.1 M NaCl. Cell suspensions were titrated against 1.0 M NaOH or HCl. The results indicate that *S. putrefaciens* exhibits a net positive surface charge at pH conditions below ~5, and a net negative surface charge at more basic pH values. Titration results were used to constrain an optimal model of the cell surface in terms of the number of discretely separable types of ionisable proton-exchange sites on the cell surface, the density of ionisable sites, and their thermodynamic properties, within the framework of a Stern surface-complexation model. Calculations were performed using the algorithm FITEQL (Westall, 1982). Bacterial specific surface area was estimated geometrically at 100 m²/g, using the volume to surface area relationship for bacterial cells given by He & Tebo (1998). An optimal fit to the

experimental data was obtained for a two-site model involving an acid or proton-yielding site having a pK of ~4.7 and a site density of ~0.57 sites/nm², and a base or proton-accepting site having a pK of ~8.2 and a site density of ~0.40 sites/nm². Site identification is not possible through titration studies alone, but the value of the predicted acid-site pK is consistent with either γ-carboxyl groups on amino acids of wall proteins or carboxyl groups of outer-membrane core lipopolysaccharide (LPS) components. The predicted base site most likely represents α-amino groups of wall proteins or amine groups of glucosamine associated with the LPS layer. Metal sorption studies were also conducted to quantify the properties of metal binding to *S. putrefaciens*. Sorption studies involved incubation of inactivated cell suspensions for approximately one hour in solutions containing 1 µmol/L of U, Pb, Cu, Cd or Zn, as a function of pH and bacteria-to-metal concentration ratio. Results of sorption studies demonstrate that reversible metal uptake by *S. putrefaciens* exhibits sorption-edge behaviour as a function of pH, with minimal sorption occurring at pH values < 2 and maximal sorption of Pb(II), Cu(II), Cd(II) and Zn(II) at more basic pH values. U(VI) sorption displays double-sorption-edge behaviour, resulting from competition with hydroxide and carbonate anions at basic pH values, similar to U(VI) sorption onto mineral surfaces (e.g. Ho & Doern, 1985; Waite et al., 1994). Results of titration and sorption experiments will be presented, along with model predictions for thermodynamic properties of metal-sorption reactions. Our results demonstrate that surface complexation onto MRB can strongly influence the speciation of metals in solution, and may therefore govern rates of bacterial metal reduction in nature.

Obuekwe CO, Westlake DWS & Cook FD, *Can.J.Microbiol.*, **27**, 692-697, (1981).

MacDonell MT & Colwell RR, *System.Appl.Microbiol.*, **6**, 171-182, (1985).

Westall JC, *Report 82-02, Oregon State Univ.*, (1982).

He LM & Tebo BM, *Appl.Env.Microbiol.*, **64**, 1123-1129, (1998).

Ho CJ & Doern DC, *Can. J. Chem.*, **63**, 1100-1104, (1985).

Waite TD, Davis JA, Payne TE, Waychunas GA & Xu N, *Geochim. Cosmochim. Acta*, **58**, 5465-5478, (1994).