An x-ray absorption spectroscopy study of Cd binding onto a halophilic archaeon

Allison R Showalter\(^1\), Jennifer E S Szymanowski\(^2\), Jeremy B Fein\(^2\) and Bruce A Bunker\(^1\)

\(^1\)Department of Physics, University of Notre Dame, Notre Dame, IN 46566, USA
\(^2\)Department of Civil and Environmental Engineering and Earth Sciences, University of Notre Dame, Notre Dame, IN 46566, USA

Email: ashowal1@nd.edu

Abstract. X-ray absorption spectroscopy (XAS) and cadmium (Cd) isotherm experiments determine how Cd adsorbs to the surface of halophilic archaeon *Halobacterium noricense*. This archaeon, isolated from the Waste Isolation Pilot Plant (WIPP) near Carlsbad, New Mexico could be involved with the transport of toxic metals stored in the transuranic waste in the salt mine. The isotherm experiments show that adsorption is relatively constant across the tolerable pH range for *H. noricense*. The XAS results indicate that Cd adsorption occurs predominately via a sulfur site, most likely sulfhydryl, with the same site dominating all measured pH values.

1. Introduction
Nuclear activity from energy and weapons production generates radioactive waste, which must be stored for thousands of years with storage in deep geological formations as the current method of disposal. These waste products may result in environmental contamination [1]. The U.S. Department of Energy commissioned the Waste Isolation Pilot Plant (WIPP) in a salt mine near Carlsbad, New Mexico for the U. S. to store transuranic (TRU) waste, made up of items such as tools or debris contaminated with radioactive elements higher than uranium. Microorganisms can increase the mobility of metals, since metal adsorption onto biological surfaces ties transport of the metal to transport of the microorganism [2]. This research project seeks to characterize heavy metal adsorption at high ionic strength on halophilic archaeon *Halobacterium noricense* isolated from the WIPP halite.

Determination of the binding environment of cadmium (Cd) to *H. noricense* will characterize the transport capacity of the archaea. Cd is a toxic metal pollutant present in the TRU waste. Furthermore, Cd adsorption to bacteria has been well-studied, for comparison of Cd adsorption to environmental microorganisms [3]. Previous studies showed that manganese, neptunium and uranium adsorption onto different halophilic archaea or bacteria was rapid (within 120 minutes) and depended on temperature, pH and salinity of the solution [4]. Other studies on thermophiles exhibit similar properties, with Cd adsorption depending on biomass concentration and pH [5]. For this study, isotherm experiments and x-ray absorption spectroscopy (XAS) were used to determine the behavior of Cd adsorption onto *H. noricense*. XAS provides the identity and distance of elements in the local environment surrounding a central atom, can differentiate between elements, requires no long range order and is sensitive to small concentrations of the element of interest [6], making it an ideal technique for this system.
2. Materials and methods
The _H. noricense_ archaeon was isolated from the halite at the WIPP following standard biological identification, preparation, storage and culture methods [7]. All experiments were performed using 100 g archaea/L in 3 M NaCl solution with varying Cd concentrations. Kinetics experiments used 7.5 ppm Cd and isotherm experiments ranged from 7.5 ppm-100 ppm Cd with a pH range of 5.5-7.5. Analysis made use of inductively coupled plasma-optical emission spectroscopy (ICP-OES) with matrix-matched Cd standards. XAS measurements used 10 or 20 ppm Cd. Standards for the XAS measurements include commercial powders and aqueous standards for including 10 mM Cd in a 100 mM solution of the complexation ligand (perchlorate, acetate, or EDTA) performed in both pure water and in 3 M NaCl solution. Cd K-edge XAS measurements were performed at the MRCAT Sector 10-ID [8] beam line located at the Advanced Photon Source of Argonne National Laboratory (USA). Data analysis was completed using the methodology of the UWXAFS package. Theoretical models were generated using FEFF6 and data analysis was completed using the ARTEMIS graphical interface [9]. Simultaneous \( k, k^2, \) and \( k^3 \) weighting was applied over a fitting range of 1.2-3.0 Å with a Fourier transform range of 2.1-8.6 Å\(^{-1}\). Solid metal oxide powders (CdO and CdCO\(_3\)) were analyzed to determine the passive electron reduction factor, \( S_0^2 \), with the resulting empirical \( S_0^2 \) value of 1.1 ± 0.1.

3. Results and discussion

3.1. Kinetics experiments
Kinetics experiments were performed in order to determine the equilibration time. The results, shown in figure 1, indicate that full adsorption occurs after about 8 hours. This is in contrast to other systems, including Cd-bacterial systems, which achieve equilibrium in 2 hours or less [3, 4].

3.2. Isotherm experiments
Isotherm experiments determined adsorption dependence on such factors as biomass-to-Cd concentration ratio and pH. The isotherm results show that adsorption seems to be relatively independent of the starting cadmium concentration or pH, as shown in figure 2. The _H. noricense_ has a small pH tolerance window of about 5.5-9.7 and all isotherm measurements were performed from 5.5-7.5. This would indicate that adsorption occurs at select sites available in limited quantities on the archaeal surface.

![Figure 1: Kinetics experimental data for 100 g/L archaea in 3 M NaCl with 7.5 ppm Cd at pH 6.7.](image)

![Figure 2: Isotherm experimental data for 100 g/L archaea in 3 M NaCl with a) varying biomass-to-Cd concentration ratio at pH 6.7 and b) varying pH. Note for figure 2a that the amount of Cd adsorbed plateaus, indicating a limited number of Cd adsorption sites for a given amount of biomass. In figure 2b, for all pH values the % of Cd adsorbed vs. the starting Cd concentration shows similar trending.](image)
3.3. X-ray absorption spectroscopy

The x-ray absorption near edge spectroscopy (XANES) data show significant differences between the standards used for comparison, but strong similarity between the archaeal samples. Figure 3 shows a comparison between selected standards and the Cd-archaea data. A qualitative assessment of the data indicates that the Cd-archaea adsorption is most similar to the CdS powder standard, indicating a primary binding environment of Cd to sulfur on the archaea surface.

![Figure 3: XANES data of 10 ppm Cd on archaea and 20 ppm Cd on archaea with standards. Note that the Cd ion in water is different than the Cd ion in 3 M NaCl solution and that there are differences between the S environment standards and Cl environment standards. Both Cd on archaea samples show similar characteristics to the CdS powder standard.](image)

The extended x-ray absorption fine structure (EXAFS) spectroscopy data show similar results to the XANES data, that the Cd-archaea samples most closely resemble the CdS standard. However, in the EXAFS region, the samples also show some similarity to the hydrated Cd$^{2+}$ ion in 3 M NaCl, as seen in figure 4a. This would indicate that though the Cd-S bond is the primary attachment to the surface, there may be some shorter Cd-O or Cd-Cl bonds present, indicating some other minor surface site on the archaeal surface or a Cd-Cl or Cd-O complex being bound to the surface. In order to determine whether the Cd-S bond is the result of precipitation or a sulfhydryl bond, the CdS powder standard is closely compared with the Cd-archaea sample, as shown in figure 4b. Since the Cd-archaea sample is lacking the Cd-Cd peak at about 4.0 Å that is seen in the CdS powder standard, the Cd-S bond in the sample is most likely a sulfhydryl bond.

![Figure 4: The EXAFS data for Cd-archaea samples showing a) the real part with many standards for comparison and b) the magnitude comparing just the Cd-archaea samples with the CdS standard.](image)

4. Conclusions

Our results for Cd binding on archaeon *H. noricense* show small variations in adsorption for the moderate pH range where archaea can survive and that the amount of cadmium adsorbed plateaus, indicating adsorption is site-limited and only occurs at selected sites. Both near edge and extended fine
structure XAS results show most similarity to the cadmium sulfide standard, indicating a sulfhydryl bond or cadmium sulfide precipitation. The analysis is inconsistent with a Cd-Cd bond at the correct distance for a cadmium sulfide precipitate, so cadmium binding to the archaeal surface is most likely sulfhydryl. This differs from cadmium adsorption to bacteria in that cadmium adsorption to bacteria shows strong pH dependence with a variety of sites, including sulfhydryl, carboxyl, and phosphoryl, with sulfhydryl only appearing in x-ray absorption spectroscopy fitting at low cadmium adsorption. Cadmium adsorption on archaea shows only a sulfhydryl bond at all measured concentrations. Sulfhydryl bonds are rather strong, but site limited on the archaea, so cadmium ions could attach to the archaea in limited quantities at the transuranic waste storage site.

Acknowledgements
MRCAT is supported by the U.S. Department of Energy and the member institutions. Use of the APS was supported by the U. S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract No. DE-AC02-06CH11357. A.R.S. wishes to thank the staff at MR-CAT and K. Werellapatha for beamline support and useful conversations. Some experiments and analyses used instrumentation at the Notre Dame Integrated Imaging Facility (NDIIF) and the Center for Environmental Science and Technology (CEST) at the University of Notre Dame. This research was supported, in part, by a CEST/Bayer Predoctoral Research Fellowship to A.R.S.

References
[1] Hu Q-H, Weng J-Q and Wang J-S 2010 J. Environ. Radioact. 101 426-37
[2] Yee N and Fein J B 2002 Chem. Geo. 185 303-19
Vijayaraghavan K and Yun Y-S 2008 Biotech. Adv. 26 266-91
[3] Fein J B, Daughney C J, Yee N and Davis T A 1997 Geochim. Cosmochim. Acta 61 3319-28
Burnett P-G G, Daughney C J and Peak D 2006 Geochim. Cosmochim. Acta 70 5253-69
Mishra B, Boyanov M, Bunker B A, Kelly S D, Kemner, K M and Fein J B 2010 Geochim. Cosmochim. Acta 74 4219-33
[4] Naik S and Furtado I 2014 Geomicrobiology Journal 31 708-15
Ams D A, Swanson J S, Szymanowski J E S, Fein J B, Richmann M and Reed D T 2013 Geochim. Cosmochim. Acta 110 45-57
Francis A J, Gillow J B, Dodge C J, Harris R, Beveridge T J and Papenguth H W 2004 Radiocim. Acta 92 481-8
[5] He Z, Yang Y, Zhou S, Zhong H and Sun W 2013 Colloids Surf., B 102 667-673
Daughney C J, Hetzer A, Heinrich H T M, Burnett P-G G, Weerts M, Morgan H, Bremer P J and McQuillan A J 2010 Chem. Geo. 273 82-90
[6] Bunker G 2010 Introduction to XAFS: A practical guide to x-ray absorption fine structure spectroscopy (New York: Cambridge University Press)
[7] Swanson J, Simmons K, Norden D and Reed D 2013 Microbial characterization of halite and groundwater samples from the WIPP Report for Los Alamos National Laboratory
[8] Segre C U, et al.2000 AIP Conf. Proc. 521 419-22
[9] Stern E A, Newville M, Ravel B, Yacoby Y and Haskel D 1995 Physica B 117-20
Newville M, Livins P, Yacoby Y, Rehr J J and Stern E A 1993 Phys. Rev. B 47 14126-31
Ravel B and Newville M 2005 J. Synchrotron Rad. 12 537-41
Zabinsky S I, Rehr J J, Ankudinov A, Albers R C and Eller M J 1995 Phys. Rev. B 52 2995-3009