

Reduction of structural Fe(III) in nontronite by humic substances in the absence and presence of *Shewanella putrefaciens* and accompanying secondary mineralization

HONGYAN ZUO¹, LIUQIN HUANG², ROSALIE K. CHU³, NIKOLA TOLIC³, NANCY WASHTON³, ZIHUA ZHU³, RICHARD E. EDELMANN⁴, SAMAR ELAGAMY⁵, ANDRE SOMMER⁵, FUBO LUAN⁶, QIANG ZENG⁷, YU CHEN⁷, DAFU HU⁷, DI ZHAN⁷, JINGLONG HU⁷, AND HAILIANG DONG^{1,7,*}

¹Department of Geology and Environmental Earth Science, Miami University, Oxford, Ohio 45056, U.S.A.

²State Key Laboratory of Biogeology and Environmental Geology, China University of Geosciences, Wuhan 430074, China

³Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, Washington 99354, U.S.A.

⁴Center for Advanced Microscopy & Imaging, Miami University, Oxford, Ohio 45056, U.S.A.

⁵Molecular Microspectroscopy Laboratory, Department of Chemistry and Biochemistry, Miami University, Oxford, Ohio 45056, U.S.A.

⁶Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

⁷State Key Laboratory of Biogeology and Environmental Geology, China University of Geosciences, Beijing 100083, China

ABSTRACT

Studies have shown the electron shuttling role of humic substances (HS) in enhancing microbial reduction of solid-phase Fe(III), but it is unknown if native HS can reduce structural Fe(III) in clays and how their chemical properties affect this process and secondary mineralization. The objective of this study was to evaluate the role of natural HS, Leonardite humic acid (LHA), and Pahokee Peat humic acid (PPHA) in reducing structural Fe(III) in nontronite with or without *Shewanella putrefaciens*. The extent of Fe(III) reduction was determined with a wet chemical method. Electrochemical methods, spectroscopy, and mass spectrometry were used to determine the changes of HS electrochemical and molecular composition after bioreduction. X-ray diffraction and electron microscopy were used to observe mineralogical transformations. The results showed that natural HS not only served as an electron donor to abiotically reduce Fe(III) in nontronite but also served as an electron shuttle to enhance Fe(III) bioreduction by *S. putrefaciens*. In the presence of CN32 cells, both the rate and extent of Fe(III) reduction significantly increased. Between the two HS, PPHA was more effective. The final bioreduction extents were 12.2 and 17.8% with LHA and PPHA, respectively, in bicarbonate buffer. Interestingly, when CN32 cells were present, LHA and PPHA donated more electrons to NAu-2, suggesting that CN32 cells were able to make additional electrons of LHA and PPHA available to reduce structural Fe(III). Although LHA reduced less Fe(III), it induced more extensive mineral transformation. In contrast, PPHA reduced more Fe(III) but did not induce any mineralogical change. These contrasting behaviors between the two humic acids are ascribed to their differences in electron-donating capacity, reactive functional group distribution, and metal complexation capacity. A unique set of secondary minerals, including talc, illite, silica, albite, ilmenite, and ferrihydrite formed as a result of reduction. The results highlight the importance of coupled C and Fe biogeochemical transformations and have implications for nutrient cycling and contaminant migration in the environment.

Keywords: LHA, mineralization, nontronite, PPHA, *Shewanella putrefaciens*