

## Role of Syntrophy in the Microbial Reduction of Crystalline Iron Oxides

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### Background

Syntrophic microbial consortia composed of anaerobic bacteria are able to catalyze electron transfer reactions that a single organism cannot [1]. Syntrophy is a community-level process that involves interspecies exchange of metabolites between organisms. Cooperative exchange of electrons and hydrogen in microbial communities is known to play an important role in the fermentation of complex natural organic matter to CO<sub>2</sub> and CH<sub>4</sub>. Currently, the role of syntrophy in the the reduction of crystalline iron oxides is poorly understood. Here we examined the reduction of crystalline iron oxides by an anaerobic microbial consortium during fermentative growth. We present evidence that the rapid iron reduction rates are enabled by syntrophic interactions between the bacteria that form this consortium.

### Materials and Methods

A consortium composed of two microorganisms was cultivated from a sediment core collected from the United States Department of Energy Field Research Center (FRC) in Oak Ridge Tennessee. The consortium consists of a fermenting *Clostridium* species and a hydrogen-oxidizing bacterium belonging to the family *Vellionellaceae*. Iron reduction experiments were conducted to evaluate the ability of this consortium and pure culture to reduce goethite, hematite and ferrihydrite during fermentation of peptone. At periodic time points, samples were collected and analysed for Fe(II) and hydrogen production by ferrozine assay and gas chromatography, respectively. PCR amplification of the 16S rRNA genes and denaturing gradient gel electrophoresis (DGGE) analysis were performed to determine the dominant member of the microbial community.

### Results and Discussion

When grown on fermentable substrates, the microbial consortium reduced goethite ( $\alpha$ -FeOOH) and hematite (Fe<sub>2</sub>O<sub>3</sub>) with specific log reduction rates of -3.1 and -3.3 pmol m<sup>-2</sup> h<sup>-1</sup> cell<sup>-1</sup>, respectively. DGGE analyses indicated that the *Clostridium* species was the dominant member in the consortium during the reductive dissolution of crystalline iron oxides. In pure culture, the *Clostridium* species was able to reduce ferrihydrite, but it reduced goethite and hematite at 4 to 5 times lower rates than the intact consortium. Finally, we show that the *Clostridium* species produces hydrogen during fermentation, and that the growth of the *Vellionellaceae* partner is stimulated by the presence of H<sub>2</sub> gas. These results suggest that interspecies hydrogen transfer may play a key role in controlling the rates of crystalline iron oxide reduction by fermentative microbial communities.

[1] Summers *et al.*, (2010) *Science*, **330**, 1413-1415.

## Profile of sulfate isotopic composition of Lake Matano, Indonesia

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Lake Matano, Indonesia, is one of the ten deepest lakes in the world and the largest modern ferruginous basin [1,2]. Despite a low thermal gradient, a persistent chemocline at 100 m isolates surface waters with low sulfate concentrations (<25  $\mu\text{mol.l}^{-1}$ ) and sulfate-free deep waters [1]. The chemical properties of Lake Matano make it a prospective analogue for the ferruginous Archean ocean and it has been used to study the potential role of anoxygenic phototrophic Fe(II)-oxidizing bacteria in the synthesis of Banded Iron Formations [1] as well as carbon cycling in ferruginous settings [2]. Dissolved sulfate is completely removed between 100 and 150 m by sulfate reduction. However, very low concentrations of sulfide are observed in the Fe(II)-rich chemocline because of efficient removal by precipitation of FeS [2].

To explore the biogeochemical cycling of sulfur in Lake Matano, we investigate the isotopic composition of sulfur in sulfate ( $\delta^{34}\text{S}_{\text{SO}_4}$ ) from a water profile spanning the surface and chemocline of the lake. Because of the low concentrations of sulfate make the analysis of the sulfur isotopic composition of SO<sub>4</sub> with conventional BaSO<sub>4</sub> precipitation is nearly impossible because it would require a huge amount of sample. Here, we use a recently developed method for  $\delta^{34}\text{S}_{\text{SO}_4}$  analysis using the ThermoScientific 'Neptune' multicollector inductively-coupled plasma mass spectrometer (MC-ICPMS). This method allows us to measure the isotopic composition of sulfur from trace sulfates in natural samples. Sulfate was extracted from lake waters using a cation-exchange resin (Dionex AG50X8) and analyzed on the Neptune as Na<sub>2</sub>SO<sub>4</sub>. Furthermore, using a Cetac 'Aridus' as an introduction system decreases isobaric interference due to O<sub>2</sub> isotopologues on the isotopes <sup>32</sup>S, <sup>33</sup>S and <sup>34</sup>S and <sup>32</sup>S-H on <sup>33</sup>S. We will also explore the triple isotope variability of this unique system using our new MC-ICPMS method.

Reproducibility of  $\delta^{34}\text{S}$  better than 0.2 ‰ (1 $\sigma$ ) is achieved by running 5 to 20 nmol of sulfate (1 to 10 ml of waters from Lake Matano). Results will provide new constraints on the fractionation of sulfur isotopes in sulfate-poor water, thus helping to constrain our understanding of sulfur cycling during Archean times in a ferruginous ocean.

[1] Crowe, Jones, Kastev, Magen, O'Neill, Sturm, Canfield, Haffner, Mucci, Sundby and Fowle (2008), *Proceedings of the National Academy of Science* **105**, 15938-15934

[2] Crowe, Kastev, Leslie, Sturm, Magen, Nomosatryo, Pack, Kessler, Reesburgh, Roberts, Gonzalez, Douglas, Haffner, Mucci, Sundby and Fowle (2011), *Geobiology* **9**, 61–78