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Microbial reduction and precipitation of vanadium by mesophilic and thermophilic methanogens

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ABSTRACT

Vanadium is a trace element required by microorganisms, but it is toxic at high concentrations. Some microorganisms are able to reduce vanadium as a way of detoxification or to utilize it as an electron acceptor in respiration. To date, all reported vanadium-reducing microbes belong to either bacteria or eukaryote and no archaeal strain has been reported to possess this capability. To explore the potential of archaea in vanadium reduction, we studied microbial reduction of vanadate (V^{5+}) by a mesophilic (*Methanosarcina mazei* with the optimal temperature of 37 °C) and a thermophilic (*Methanothermobacter thermautotrophicus*, with the optimum temperature of 65 °C) methanogen in both growth and non-growth media. These two methanogens reduced up to 10 mM and 5 mM of V^{5+}, respectively, in a growth medium. However, in a non-growth medium both methanogens did not even reduce 2 mM V^{5+}. Methanogenesis was inhibited by V^{5+} bioreduction, possibly due to diversion of electrons from methanogenesis to vanadate reduction. Bioreduction of V^{5+} in a growth medium induced biogenic solid precipitation. Scanning electron microscopy (SEM) observations revealed the relationship between methanogenic cells and solid precipitates. Transition electron microscopy (TEM) with electron energy loss spectroscopy (EELS) and electron paramagnetic resonance (EPR) spectroscopy determined that the oxidation state of reduced vanadium in biogenic precipitates was +4. These data collectively demonstrated that both mesophilic and thermophilic methanogens were capable of reducing V^{5+} to vanadyl under a variety of conditions.

1. Introduction

Vanadium is the second most abundant transition metal in seawater, with an average concentration of 30 nM (Butler, 1998). Vanadium also widely exists in the Earth's crust (Rehder, 1991), so vanadium contamination in groundwater can result from either natural or industrial sources (Ortiz-Bernad et al., 2004). In natural systems, vanadium can be found in titanomagnetite deposits (Evans and White, 1987), uranium-bearing minerals (Crans et al., 1998), crude oils, coal, and carbonaceous fossil fuels (Baroch, 1983; Rehder, 1995). In industrial systems, vanadium is a valuable metal that is widely used in modern technologies, including metallurgy, the atomic energy industry, space technology, pharmaceutical industrial processes, and other high-tech industries (Nriagu, 1998). In addition, vanadium compounds are widely used as catalysts in petroleum refining, catalytic reduction of NO_X, sulfuric acid production, and production of phthalic anhydrides (Bosch et al., 1989; Yoo, 1998).

Vanadium is required by microorganisms as a trace nutrient, but it becomes toxic at high concentrations. Since the anionic form of vanadium (VO_3^-) has a similar structure to phosphate (PO_3^-), biological uptake of vanadium may adversely affect phosphate metabolism (Mannazzu et al., 1997), such as inhibition of many phosphate-related enzymes including ATPhases (Henderson et al., 1989). The toxicity of vanadium increases with its valence state and solubility; therefore, pentavalent vanadium is considered as the most toxic form (Crans et al., 1998). Vanadium (IV) is less toxic and insoluble at neutral pH (Patel et al., 1989). Therefore, reduction of vanadate (V^{5+}) to lower oxidation states (such as V^{4+}) is considered as a remediation method to remove vanadium from contaminated groundwater (Ortiz-Bernad et al., 2004).

Some microorganisms are able to reduce metal compounds (Lovley et al., 1993; von Canstein et al., 1999; Lovley, 2000; Niggemyer et al., 2001) and some reduced and immobilized metals, can be recovered at industrial scale (Krebs et al., 1997). Since 1970s, many researchers have reported bioreduction of vanadium by various microorganisms (Bautista and Alexande, 1972; Yurkova and Lyalikova, 1990; Bisconti et al., 1997; Ortiz-Bernad et al., 2004; Van Marwijk et al., 2009); these studies have opened a possibility of using a microbial approach to remove toxic vanadium from drinking water sources. Several *Pseudomonas*

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strains have been reported to be capable of reducing vanadate (V^{5+}) to lower oxidation states (V(III) and V(IV)). One of these bacteria, *Pseudomonas vanadiumreductans*, formed a vanadium deposit similar to sherwoodite (Lyalikova and Yurkova, 1992). Metal-reducing bacteria *Shewanella oneidensis* and *Geobacter metallireducens* are also capable of growth with V^{5+} as the sole electron acceptor (Carpentier et al., 2003; Ortiz-Bernad et al., 2004; Carpentier et al., 2005). Moreover, one eukaryotic strain, *Saccharomyces cerevisiae*, was capable of reducing V^{5+} to vanadyl (Bisconti et al., 1997). To date, these vanadium-reducing microorganisms belong to either the bacterial or the eukaryotic domain, and none from the archaeal domain has been reported to possess this capability (Rehder, 2008).

Methanogens, as cosmopolitan *archaea*, occur both in modern anoxic environments and on the ancient earth. Recent studies have shown that methanogens have the ability to reduce structural Fe(III) in iron oxides (Van Bodegom and Stams, 1999) and clay minerals (Zhang et al., 2012), yet it remains unclear if methanogens can also reduce vanadium, and if so, what is the relationship between vanadium reduction and methanogenesis?

The aim of this study was to investigate the capability of two methanogens to reduce V^{5+}, including mesophilic and thermophilic strains. We conducted laboratory experiments to address the following questions: (i) Are mesophilic and thermophilic methanogens capable of reducing V^{5+}? If so, what are the extents and rates of V^{5+} reduction with different substrates and medium compositions? (ii) What is the relationship between V^{5+} reduction and methanogenesis? (iii) Are methanogens able to remove vanadium from contaminated groundwater through redox reactions? To address these questions, mesophilic *Methanosarcina mazei* (optimal temperature of 37 °C), and thermophilic *Methanothermobacter thermautotrophicus* (optimum temperature of 65 °C) were used to reduce vanadate at different concentrations. Various laboratory techniques were used to examine the reduction progress and to characterize mineralogical changes. Our results demonstrated that both mesophilic and thermophilic methanogens were able to reduce V^{5+}. This result has important implications for remediation of vanadium-contaminated water bodies and for the understanding of the biochemical importance of vanadium.

2. Materials and methods

2.1. Cell culturing

M. mazei and *M. thermautotrophicus* were kindly provided by Dr. Xiuzhu Dong (Institute of Microbiology, Chinese Academy of Sciences, Beijing, China). These strains are routinely cultured in a sulfate-free culture medium (revised from Zehnder and Wuhermann, 1977) under strictly anoxic condition. In brief, this medium consists of (per liter) 0.54 g KH_2PO_4, 1.33 g $Na_2HPO_43H_2O$, 1.8 g yeast extract, 0.5 g tryptone, 0.5 g peptone, 4 g $NaHCO_3$, 0.29 g NH_4Cl, 0.096 g $MgCl_26H_2O$, 0.0096 g $CaCl_22H_2O$, 0.29 g NaCl, 1 mL vitamin solution (Kenealy and Zeikus, 1981), 1 mL trace mineral solution (Zehnder and Wuhermann, 1977), and 1 mL 0.1% resazurin solution (redox indicator). The medium was made anoxic in serum bottles (100 mL volume) with O_2-free N_2/CO_2 (80:20) mix gas via passing through a hot copper column and sterilized via autoclaving. Sterilized methanol (final conc. 0.5%) was added to support the growth of *M. mazei* as a substrate (Boone and Mah, 1987), whereas for *M. thermautotrophicus* H_2/CO_2 (80:20) mix gas was provided as a substrate (Zhang et al., 2013). In the microcosms with H_2/CO_2 as a substrate, the gas mix was injected into the headspace of the serum bottles until a pressure of 140 kPa was reached. Before cell inoculation, all the serum bottles were stored at 65 °C overnight to allow the sulfur functional group of yeast extract to react with any residual O_2. The final pH of the medium was adjusted to 7.0 with 0.1 N HCl. *M. mazei* and *M. thermautotrophicus* were cultured in this medium at 37 °C and 65 °C, respectively. They were transferred three times before bioreduction experiments were initiated.

2.2. Vanadium reduction experiment

2.2.1. Reduction in growth medium

Sodium metavanadate (Sigma-Aldrich, USA, cat# 590088) was added to the sulfate-free culture medium to achieve three different final concentrations of V^{5+} (2 mM, 5 mM, and 10 mM) in Balch culture tubes (total volume, 20 mL). The tubes were purged with N_2/CO_2 (80:20), and sealed with thick butyl rubber stoppers and aluminum caps. After autoclaving, three types of filter-sterilized substrates, H_2/CO_2 (80:20 gas mix), methanol (final conc. 0.5%), and sodium acetate (final conc. 30 mM) were added to duplicate tubes for the experiments with *M. mazei*. Filter-sterilized H_2/CO_2 (80:20 gas mix) was added to duplicate tubes for the experiments with *M. thermautotrophicus*. For the tubes containing H_2/CO_2, the gas pressure in the headspace was 140 kPa, the initial amount of H_2 in the Balch culture tube was approximately 0.1 mmol, and for the other tubes (methanol and acetate), the headspace gas pressure was adjusted to 140 kPa with ultrapure N_2. All tubes were stored at 65 °C overnight to achieve an anoxic condition. The results (below) showed that the amount of abiotic reduction of V^{5+} by the sulfur functional group of yeast extract was insignificant in comparison with the amount of bioreduction.

M. mazei and *M. thermautotrophicus* cells in their exponential phase were injected into the experimental tubes, with a final concentration of 10^8 cells/mL for both strains based on acridine-orange direct counts (AODC). There were two control groups in this experiment. Control A group was identical to the experimental tubes except that cells were replaced with an equal amount of culture medium (abiotic control), whereas Control B group was identical to the experimental tubes but without any V^{5+} (growth control). All solutions and cultures were transferred using sterile needles and syringes inside an anaerobic chamber (Coy Laboratory Products, Grass Lake, MI, USA). The experimental tubes with 10 mL V^{5+} solution (and 10 mL headspace) were incubated at 37 °C for *M. mazei*, and 65 °C for *M. thermautotrophicus*.

2.2.2. Reduction in non-growth medium

M. mazei and *M. thermautotrophicus* cells were harvested at the same time as those for the growth experiments by centrifugation (5000 ×*g*, 10 min), but the sulfate-free growth medium was replaced with anoxic sodium bicarbonate buffer (4 g/L, pH 7.0) by repeated washes (3 times). At the end of the final wash, cell pellets were re-suspended in the buffer for the non-growth experiments.

Sodium metavanadate was added to the sodium bicarbonate buffer (4 g/L, pH 7.0) to achieve three final concentrations of vanadium (2 mM, 5 mM, and 10 mM) in Balch culture tubes. The tubes were treated the same way as for the growth experiment, and different substrates (H_2/CO_2, methanol, and acetate) were added as previously described. Instead of storing at 65 °C overnight to remove the residual O_2 by yeast extract, Na_2S solution (final conc. 0.13 mM) was added to these experimental tubes to achieve an anoxic condition. With such a low concentration, this reducing agent only reduced a very small amount of V^{5+} (about 0.2–0.4 mM).

The same amounts of washed cell suspensions (*M. mazei* or *M. thermautotrophicus*) as for the growth experiments were added to each experimental tube. Again there were two control groups for this experiment (abiotic control and growth control). The experimental tubes containing 10 mL aqueous medium with vanadate (and 10 mL headspace) were incubated at 37 °C for *M. mazei*, and 65 °C for *M. thermautotrophicus*.

2.3. Analytical methods

2.3.1. Chemical analyses

The progress of V^{5+} bioreduction was monitored by measuring aqueous concentration of V^{5+} over the course of the experiment by a modified perphenazine method (van Marwijk et al., 2009). At the end of the experiments, solid precipitates and aqueous solutions were