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Cr(VI) reduction coupled with anaerobic oxidation of methane in a laboratory reactor

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ABSTRACT

The process of anaerobic oxidation of methane (AOM) is globally important because of its contribution to the carbon cycle in the environment. Besides, microorganisms play important roles in the environmental fate of chromium. However, there have been no studies to date on the interaction between methane and chromium in batch reactor systems. In this study, biological Cr(VI) reduction was investigated using methane as the sole electron donor. Isotopic $^{13}CH_4$ in the batch experiments and long-term performance in the reactor demonstrated that Cr(VI) reduction is coupled with methane oxidation. High-throughput sequencing of the 16S rRNA genes demonstrated that the microbial community had changed substantially after Cr(VI) reduction. The populations of ANME-2d archaea were enhanced, and they became the only predominant AOM-related microbe. Interestingly, other bacteria with significant increases in abundance were not reported as having the ability to reduce Cr(VI). According to these results, two mechanisms were proposed: 1) Cr(VI) is reduced by ANME-2d alone; 2) Cr(VI) is reduced by unknown Cr(VI)-reducing microbes coupled with ANME-2d. This study revealed the potential relationship between Cr(VI) reduction and CH_4 oxidation, and extended our knowledge of the relationship between the AOM process and biogeochemical cycles.

1. Introduction

Hexavalent chromium (Cr(VI)) is highly toxic to all living organisms and carcinogenic and mutagenic in humans (Kathiravan et al., 2011). It is an important raw material in the metallurgical, electroplating, printing, ceramics, wood preservation and leather manufacturing industries and commonly present in their effluent streams (Testa et al., 2004). Cr(VI) is toxic because of its high solubility and, as a result, it can be readily transported through groundwater (Faybishenko et al., 2008). The reduction of Cr(VI) to Cr(III) is a key process for the removal of Cr(VI) contamination in water and wastewater. When Cr(VI) is reduced to Cr(III), its solubility and mobility decrease (Pan et al., 2014). Chromium is also an environmental background element in soils on a global scale (Adriano, 2001). The two common oxidation states of Cr present in the environment, i.e. Cr(III) and Cr(VI), are interchangeable through oxidation and reduction reactions. Microbial reduction of Cr(VI) is seemingly ubiquitous in both Cr(VI)-contaminated and uncontaminated environments (Fuller et al., 2015; Katsaveli et al., 2012). Many genera of bacteria and archaea, common to different environments, are able to reduce Cr(VI) (He et al., 2015; Kashefi and Lovley, 2000; Miao et al., 2015). Under anaerobic conditions, there are two main chromate-reducing mechanisms: 1) indirect reduction through a non-enzymatic reduction pathway such that the products of microbial metabolism and/or decomposition, such as Fe^{2+} or H_2S, can mediate the reduction of Cr(VI) to Cr(III) (Kamaludeen et al., 2003); 2) direct reduction through an enzymatic reduction pathway (Ackerley et al., 2004).

A variety of organic compounds can be used as the electron donor for Cr(VI) reduction. Bartlett et al. reported that Cr(VI) was reduced by natural organic matter (NOM) in the soil and also humic substances in water (Bartlett, 1991). Similar to NOM, methane is widely distributed in the environment. However, the question is whether methane can serve as an electron donor for Cr(VI) reduction.

Among natural environments, wetland soils are the largest single source of greenhouse gas methane. The anaerobic oxidation

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of methane (AOM), using electron acceptors such as sulfate, manganese/iron and nitrite/nitrate, has been identified as an important sink for methane in wetland soils (Katrin and Antje, 2009; Raghoebarsing et al., 2006; Siegert et al., 2011). Besides, in natural soils, such as aquatic sediments and soils, generally contain detectable levels of chromium (Richard and Bourg, 1991). Therefore, we can infer the coexistence of methane and chromium in environmental soils, but the relationship between them has seldom been studied. A characterized model methanotroph *Methylococcus capsulatus (Bath)* was reported to be able to reduce Cr(VI) with methane aerobically (Al Hasin et al., 2010). However, the connection between Cr(VI) and methane under anaerobic condition needs to be investigated. There are probable connections between the AOM process and chromium. By comparing the changes in Gibbs free energy (ΔG), it is evident that the process of methane oxidation coupled with Cr(VI) reduction ($\Delta G^{0\prime} = -878.8$ kJ mol^{-1} CH_4; Eq. (1)) is much more thermodynamically favorable than the process of methane oxidation coupled with nitrate reduction ($\Delta G^{0\prime} = -519.8$ kJ mol^{-1} CH_4; Eq. (2)). In theory, the bioreaction involving Cr(VI) (Eq. (1)) must exist, although scientists have not found any evidence for it thus far.

$$CH_4 + 4/3Cr_2O_7^{2-} + 32/3H^+ \rightarrow 8/3Cr^{3+} + CO_2 + 22/3H_2O \quad (\Delta G^{0\prime} = -878.8 \text{ kJ mol}^{-1} CH_4) \quad (1)$$

$$CH_4 + 4NO_3^- \rightarrow CO_2 + 4NO_2^- + 2H_2O \ (\Delta G^{0\prime} = -519.8 \text{ kJ mol}^{-1} CH_4) \quad (2)$$

Experimental evidence for denitrifying anaerobic methane oxidation (DAMO) has been obtained using sludge from bioreactors, in which the DAMO process involves both DAMO archaea and DAMO bacteria (Islas-Lima et al., 2004) or solely DAMO bacteria (Haroon et al., 2013). A follow-up study found that the DAMO archaea belong to the ANME-2d family and are related to the NC10 phylum (Shi et al., 2013). Although the enriched DAMO cultures have been documented and characterized thoroughly, the impact of Cr(VI) on them have never been studied seriously. It has been reported that Cr(VI) intervention was able to affect the denitrification performance and alter the microbial community structure and function in an activated sludge system (Miao et al., 2015). But how the community structure of DAMO organisms shifts under Cr(VI) stress remains unknown.

The main aim of this study was to investigate the effects of Cr(VI) intervention on an AMO process inoculated with DAMO cultures. ^{13}C-labeled CH_4 was used to investigate the possible involvement of methane oxidation in Cr reduction. Meanwhile, high-throughput sequencing, metagenomic analysis and quantitative PCR (qPCR) were used to evaluate the changes in microbial consortia structure after Cr reduction. Finally, a mechanism for Cr(VI) reduction and methane oxidation was proposed. It is anticipated that these findings may extend our knowledge of the AOM process in biogeochemical element cycles.

2. Materials and methods

2.1. Inocula and cultivation conditions

A parent reactor culture containing DAMO archaea and DAMO bacteria was enriched with methanogenic sludge (1.18 mmol dissolved CH_4/L and 0.06 μmol Cr(VI)/L) and activated sludge from a wastewater treatment plant (1.36 μmol dissolved CH_4/L and 0.39 μmol Cr(VI)/L) in a 3-L glass reactor with a working volume of 2 L that had been running for more than 2 years. The temperature was controlled at 35 °C and the pH was controlled between 7.0 and 8.5. Nitrate was added by injection of a concentrated stock solution to maintain the concentration of nitrate between 0.7 and 14 mmol/L. A gas mixture (95% CH_4, 5% CO_2) was used to flush the reactor to provide methane. Each month, the reactor contents were allowed to settle and 500 mL of supernatant were exchanged for fresh medium in which the dissolved oxygen concentration was below the detection limit. After enrichment over a long time period, the consumption rate of NO_3^-–N was stable at around 10.0 mg/L per day.

2.2. Mineral salts medium

The mineral salts medium contained (per L) $KHCO_3$ 0.5 g, KH_2PO_4 0.05 g, $MgSO_4 \cdot 7H_2O$ 0.2 g, $CaCl_2$ 0.2265 g, NH_4Cl 0.12 g (not present in the medium of the parent reactor), acidic trace element solution 0.2 mL and alkaline trace element solution 0.5 mL. The acidic trace element solution contained (per L) $FeSO_4 \cdot 7H_2O$ 2.085 g, $ZnSO_4 \cdot 7H_2O$ 0.068 g, $CoCl_2 \cdot 6H_2O$ 0.12 g, $MnCl_2 \cdot 4H_2O$ 0.5 g, $CuSO_4$ 0.32 g, $NiCl_2 \cdot 6H_2O$ 0.095 g, H_3BO_3 0.014 g and HCl 100 mmol. The alkaline trace element contained (per L) $Na_2WO_4 \cdot 2H_2O$ 0.05 g, Na_2MoO_4 0.242 g and NaOH 0.4 g. The medium was sparged with N_2–CO_2 (95:5, v/v) for more than 30 min to obtain anaerobic conditions, and the pH was adjusted to 7.3–7.6 by manual addition of 1 M HCl or 1 M NaOH.

2.3. Isotope tracer experiments

Potential methane oxidation in the experimental vials was evaluated using ^{13}C stable isotope labelling. Two identical 100-mL glass vials with 50-mL working volumes were used: one as the control without CH_4, named the control vial, and the other for CH_4 addition, named the methane vial. Both vials contained 40 mL of N_2-purged mineral salt medium and 10 mL of nitrate-free inocula from the parent reactor. The methane vial was continuously flushed with CH_4–CO_2 (95:5, v/v) and the control vial was continuously flushed with N_2–CO_2 (95:5, v/v). After about 30 min of flushing, the vials were covered with butyl rubber stoppers. Subsequently, Cr(VI) stock solution was injected into each vial, resulting in a final concentration of 0.01 mM Cr in each vial. From the methane vial, 10 mL of headspace gas was removed and replaced with an equal volume of $^{13}CH_4$ (>99%, Sigma Aldrich), resulting in a final concentration of 20% $^{13}CH_4$ (by volume). The vials were stirred at 180 rpm in an incubator shaker, and the temperature was maintained at 35 °C. All experiments were performed in duplicate.

2.4. Long-term Cr(VI) reduction with methane oxidation in the bioreactor

A 450-mL bottle with 300 mL of working volume was used to determine the long-term performance of Cr(VI) reduction. The inocula were obtained from the parent reactor after NO_3^-–N was exhausted (the consumption rate of NO_3^-–N was 10.15 mg/L/day). The inocula were diluted with mineral salts medium to a biomass concentration of approximately 0.11 g volatile suspended solids (VSS)/L. Every 5–10 days, the bioreactor was sparged with CH_4–CO_2 (95:5, v/v) and injected with Cr(VI) stock solution to a final concentration of approximately 10 mg Cr/L. After all the Cr(VI) was reduced, the reactor contents were allowed to settle and 100 mL of the supernatant were removed and replaced with fresh mineral salts medium and sparged with CH_4–CO_2 (95:5 v/v) for 15 min. Cr(VI) was supplied via the Cr(VI) stock solution.

2.5. Valence state analysis of reduced chromium

After reacting with Cr(VI), Cr-loaded microbes were centrifuged at 5000 g for 10 min and collected. They were then washed three