Chemical Geology 481 (2018) 155–164

Contents lists available at ScienceDirect

Chemical Geology

journal homepage: www.elsevier.com/locate/chemgeo

# Variations of stable isotope fractionation during bacterial chromium reduction processes and their implications

Qiong Zhang[a,*], Ken Amor[a], Stephen J.G. Galer[c], Ian Thompson[b], Don Porcelli[a]

[a] Department of Earth Sciences, University of Oxford, Oxford, United Kingdom
[b] Department of Engineering Science, University of Oxford, Oxford, United Kingdom
[c] Max Planck Institute for Chemistry, Climate Geochemistry Department, Mainz, Germany

## ARTICLE INFO

Editor: Michael E. Böttcher

*Keywords:*
Cr
Bacterial reduction
Biogeochemistry
Stable isotope fractionation

## ABSTRACT

Many chemical processes generate subtle but readily measured changes in isotope compositions of elements across the periodic table. The elements involved therefore carry diagnostic information about their chemical histories in complex geochemical or biochemical environments. Distinctive Cr isotope signatures can be used to identify immobilization processes of Cr in the environment, such as microbial Cr(VI) reduction, abiotic Cr(VI) reduction, and adsorption. Here we demonstrate that under well-controlled conditions, Cr isotopes can also be used to distinguish between different biological Cr(VI) reduction pathways. The reduction of Cr(VI) by two facultative anaerobic bacteria, *Pseudomonas fluorescens* LB 300 and *Shewanella oneidensis* MR 1, was investigated to determine the conditions under which Cr(VI) is reduced and to quantify the corresponding isotope signatures. The present study considers the effects of a broad range of parameters on Cr isotope fractionation, including bacterial species, electron donors, pH, and respiration pathways (aerobic vs. anaerobic) that must be considered for understanding Cr isotope variations under different experimental and environmental conditions.

In the bacterial Cr(VI) reduction experiments, the $^{53}Cr/^{52}Cr$ isotope ratio of the remaining Cr(VI) increased by up to + 8‰, indicating that lighter isotopes of Cr were preferentially reduced. In aerobic experiments, although Cr reduction rates increased as pH increased from 4 to 8, the fractionation factor did not vary significantly ($\varepsilon = -3.21 \pm 0.18$‰). Experiments using different electron donors demonstrated that citrate promoted the greatest Cr reduction rate compared with glucose, acetate, and propionate. Under aerobic conditions, although the Cr(VI) reduction rates varied substantially between different experimental settings, the isotope fractionation factors were indistinguishable between all the environmental conditions examined ($\varepsilon = -3.1$‰), with the exception of when citrate was the electron donor ($\varepsilon = -4.3$‰).

Cr reduction rates were generally much faster under anaerobic conditions for both bacteria investigated. The utilisation of different electron donors resulted in the same Cr reduction rates by the bacteria, but fractionated Cr with a broad range of isotope fractionation factors, from − 1.58 ± 0.16‰ to − 4.93 ± 0.36‰. Although it has been proposed in many previous studies that there is an inverse relationship between reduction rates and the fractionation factors, no clear relationship between the reduction rates and fractionation factors was observed in this study.

The Cr isotope fractionation factors $\varepsilon$ were insensitive to pH and electron donor concentration, but dependent on the type of electron donors and redox conditions in the cultures. This indicates that isotope variations may be used to identify when different biological pathways are involved, and so to investigate metabolic processes. The $\varepsilon$ value from all experimental conditions examined ranged between − 1.58 and − 4.93‰, with a mean value at − 3.3‰. While Cr isotopes might be used to separate the effects of abiotic and microbially mediated reduction in environmental sites, the fractionation factors from reduction by individual bacterial species overlap with those from several individual abiotic reduction processes, suggesting that site-specific data (*e.g.,* fractionation factors associated with indigenous bacterial populations and local groundwater chemistry) are required in order to use Cr isotopes to distinguish between different reduction mechanisms.

* Corresponding author.
*E-mail address:* joan.zhang@earth.ox.ac.uk (Q. Zhang).

https://doi.org/10.1016/j.chemgeo.2018.02.004
Received 23 August 2017; Received in revised form 30 January 2018; Accepted 1 February 2018
Available online 06 February 2018
0009-2541/ 

## 1. Introduction

Isotope compositions of many elements change during chemical reactions and therefore these compositions carry diagnostic information about the chemical histories of the elements in complex geochemical or biochemical environments. Techniques for chemical separation and high precision mass spectrometry can now achieve the necessary resolution for resolving the range of naturally occurring isotope ratios, typically in a few micrograms or less of the element of interest. However, the isotope fractionation factors for the different relevant processes must be documented. Within the last decade, variations in chromium isotope ratios have been documented and can be used to understand the complex processes affecting the behaviour of Cr in the environment. This provides a powerful diagnostic tool for studies of environmental contamination, of the chemical cycles of redox-sensitive elements, and of paleoclimate. In particular, microbial processes reduce Cr(VI) using a range of mechanisms that are associated with different fractionation factors, indicating that Cr isotopes can be used not only to identify different microbial processes in the environment, but also to explore how microbes reduce Cr. These mechanisms are dependent on the environmental conditions under which the reduction processes occur. Therefore, information about the impacts of different environmental factors on microbial activity and corresponding isotope fractionation process would be enormously valuable in establishing isotopic fractionation as an effective indicator of prevailing conditions such as redox, pH, and concentration.

Chromium is relatively abundant among trace metals in the earth's crust with an average of 1.80 mmol/kg (Rudnick and Gao, 2003) and is naturally released to the environment by weathering and leaching from rocks. Cr exists in many different oxidation states (0 to VI), but only two of them, trivalent and hexavalent Cr, are stable in the environment (Kotaś and Stasicka, 2012).

The predominant oxidation state in igneous and metamorphic rocks is Cr(III), which is generally insoluble and surface-reactive, and therefore relatively immobile in the environment. When Cr is oxidized to Cr (VI), its solubility increases significantly, and, as a consequence, so does its mobility. Cr naturally occurs in rivers and lakes at concentrations of 0.5 to 100 nM and about 0.1 to 16 nM in seawaters (Bradl, 2005). In addition to inputs from natural sources, the presence of high levels of Cr can also be a consequence of its widespread use in industrial processes such as tanning leather, making stainless steel, and preventing engine corrosion.

Because Cr is spread widely in the environment, has high toxicity, and exhibits distinctive physico-chemical characteristics under different redox conditions, it is important to effectively monitor Cr distributions and identify the abiotic and biotic conditions that determine its chemical states. Cr stable isotopes can be of aid, since Cr has 4 of them, and redox reactions result in products that are significantly enriched in the heavier isotopes (Ellis et al., 2002). Thus, changes in isotopic composition may be used as indicators of redox conditions in the environment. Likewise, isotopic compositions enable differentiation between natural and anthropogenic local sources of Cr (Ellis et al., 2002; Izbicki et al., 2012; Johnson and Bullen, 2004), and assessment of the effectiveness of remedial interventions.

Cr isotope data are usually reported as relative deviations from the standard (NIST SRM 979) in parts per thousand (per mil or ‰):

$$\delta^{53}Cr = \left[ \frac{(^{53}Cr/^{52}Cr)_{sample}}{(^{53}Cr/^{52}Cr)_{standard}} - 1 \right] \times 1000‰ \quad (1)$$

The isotope fractionation factor is:

$$\alpha = \frac{(^{53}Cr/^{52}Cr)_{product}}{(^{53}Cr/^{52}Cr)_{reactant}} \quad (2)$$

which can also be expressed using $\varepsilon$ notation:

$$\varepsilon \approx (\alpha - 1) \times 1000‰ \quad (3)$$

An understanding of the mechanisms by which the isotopic ratios of Cr are modified may enable the monitoring and predictions of the fate of Cr(VI) in contaminated sites (Berna et al., 2010) or in the natural environment (Ellis et al., 2002; Gao and Schulze, 2010; Raddatz et al., 2010; Wanner et al., 2012). For instance, Cr(VI) reduction reactions, such as those performed by microbial communities, result in reaction of lighter isotopes at a faster rate than heavier ones, leaving diminished concentrations of Cr(VI) that is isotopically heavy. In contrast, if Cr(VI) in a contaminant plume were only diluted in the environment, the $\delta^{53}$Cr value would not change.

Cr isotopes are not only useful as indicators of redox conditions prevailing in modern environments, but may also be used as a proxy of prevailing oxygenation conditions over geological time. The isotopic composition of Cr in sedimentary deposits can reflect oxygen levels in the hydrosphere and atmosphere in the past (Frei and Polat, 2013; Frei et al., 2013; Rotaru et al., 1992).

The dominant environmental processes that control Cr cycling include both biotic and abiotic reactions. Once data are available for isotope signatures generated from biotic and abiotic reactions, these signatures can be used to identify which reactions are the dominant controls on Cr reduction in the environment. Although isotopic data on abiotic Cr(VI) reduction are still limited, it is clear that the values of the fractionation factors depend on the reduction mechanism. For example, Zink et al. (2010) reported the isotope fractionation factors to be − 3.5 ‰ and − 5.0 ‰ for Cr(VI) reduction by $H_2O_2$ in aqueous media under highly acidic and circum-neutral conditions, respectively. Kitchen et al. (2012) conducted Cr(VI) reduction experiments with aqueous Fe(II) and reported fractionation factors to be − 4.2 ‰ at pH values ranging between 4 and 5.3. Dossing et al. (2011) reported values of − 3.0‰ to − 4.4‰ for reduction by aqueous Fe(II) at circum-neutral pH, and of − 1.5‰ when Cr was reduced by dissolved Fe(II) and Fe(II) + Fe(III) green rust together. Basu and Johnson (2012) found $\varepsilon$ values from − 2.1 ‰ to − 3.9 ‰ in Cr(VI) reduction experiments with different forms of iron. Oxidation of Cr(III) by Mn oxides is also a dominant inorganic control on Cr behaviour in oxic environments (Feng et al., 2006; Landrot et al., 2012). In addition to redox reactions, it is possible that adsorption may induce Cr isotope fractionation. However, Ellis et al. (2004) investigated the effects of sorption of Cr(VI) onto goethite and $\gamma - Al_2O_3$, and found that equilibrium fractionation of Cr stable isotopes during adsorption on these phases is negligible. Cr(VI) isotope fractionation during adsorption onto other phases has not been determined.

Shifts in isotope fractionation due to microbial activity also provide valuable insight into the mechanisms of metal cycling and can be used to quantify biogeochemically mediated redox processes in the environment (Basu et al., 2014; Han et al., 2012; Sikora et al., 2008). Sikora et al. (2008) were the first to investigate the impact of microbial factors on Cr isotope fractionation. They conducted anaerobic reduction experiments with *Shewanella oneidensis* MR 1 and determined that fractionation factors ranged from − 4.0 to − 4.5 ‰ when reduction rates were low, but was − 1.8 ‰ at greater reduction rates. Han et al. (2012) reported Cr isotope data during reduction by *Pseudomonas stutzeri* RCH2 under both aerobic ($\varepsilon = -2$ ‰) and denitrifying conditions ($\varepsilon = -0.4$ ‰). Basu et al. (2014) reported Cr(VI) reduction by a metabolically diverse group of bacteria, with Cr isotope fractionation factors ranged from − 2.17 to − 3.14‰, and suggested that stronger isotopic fractionation was induced during Cr(VI) reduction under electron-donor-poor conditions.

There are many factors that can impact the microbial processing of Cr, such as the presence of specific electron donors (Brodie et al., 2011) and Cr concentration. Such factors have been shown to have an impact with S, where the extent of S microbial isotope fractionation is dependent on S concentration and which electron donors are present (Habicht and Canfield, 1997; Kaplan and Rittenberg, 1964). Similar effects may be reasonably expected for Cr isotope fractionation. However, such knowledge is still limited. Therefore, the aim of this study was to fill this knowledge gap by determining Cr isotope fractionation