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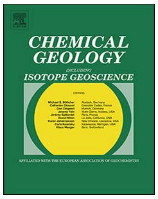

Reduction of structural Fe(III) in nontronite by thermophilic microbial consortia enriched from hot springs in Tengchong, Yunnan Province, China

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ABSTRACT

Iron redox cycling provides one of the most important energy sources supporting microorganisms in terrestrial hot springs. In order to understand microbial Fe(III) reduction processes in hot springs, Fe(III)-reducing bacterial consortia were successfully retrieved from seven acidic hot springs in Tengchong County, Yunnan Province, China (a temperature range from 40.6 °C to 74.7 °C and a pH range from 2.64 to 4.72). These Fe(III)-reducing consortia were mainly composed of sulfate reducing bacteria (SRB, including *Desulfotomaculum*, *Thermodesulfobacterium* and *Desulfomicrobium*) and organic matter fermenting bacteria (*Thermoanaerobacterium* and *Thermoanaerobacter*), with each consortia having specific composition depending on the physio-chemical conditions of the studied hot springs. The obtained consortia were capable of dissimilatory reduction of Fe(III) in nontronite NAu-2 (ferruginous smectite) with 20.8% to 29.8% reduction extent and initial reduction rate of 0.011–0.023 mM Fe(III)/g NAu-2/h. These extents and rates were comparable to those by mesophilic Fe-reducers in the presence of electron shuttles. Both high temperature and the possible presence of electron-shuttles in hot springs may have accounted for these observed high reduction extents and rates. Various secondary minerals formed during NAu-2 bioreduction, including Fe sulfides and oxides, silicates, and silica. In one spring, illitization and kaolinization of smectite were observed, suggesting that Fe-reducers play important roles in mineral transformation in geothermal environments.

1. Introduction

Iron is the second most abundant, redox-active element in the Earth's crust. In circumneutral, oxic environments, iron exists primarily as Fe(III) minerals such as oxides and phyllosilicates. Transformation between Fe(III) and Fe(II) in natural environments can be promoted by various microbial activities. Mineral-associated iron in soils and sediments is sufficiently abundant to support the growth and metabolism of Fe(II)-oxidizing and Fe(III)-reducing microbial communities, which play important roles in elemental cycling and bioremediation (Lovley et al., 2004; Taylor and Konhauser, 2011). For example, bioreduction of Fe(III) can be coupled with mineralization of organic matter (including organic contaminants) (Stucki, 2013), and resulting biogenic Fe(II) can be employed to immobilize heavy metals (Bishop et al., 2014; Dong and Lu, 2012) and radioactive metals (e.g., technetium, uranium) (Bishop et al., 2011; Zhang et al., 2009).

Clay minerals (interchangeably called phyllosilicates) are ubiquitous in surficial environments and they are derived from weathering and alteration of primary rocks (e.g., basalts, granite, and volcanic ash). Extensive studies have demonstrated that many functional groups of anaerobic microorganisms, including dissimilatory Fe(III)-reducing bacteria (DIRB), sulfate-reducing bacteria (SRB) and methanogens, can efficiently reduce structural Fe (III) in clay minerals and thus change their physicochemical characteristics (Childers et al., 2002; Kappler and Straub, 2005). For instance, *Shewanella* spp. and *Geobacter* spp., two well-documented mesophilic DIRB that are common in soils and sediments, can reduce structural Fe(III) in many clay minerals (e.g., smectite, illite, chlorite), resulting in mineral dissolution and/or phase transformation (Dong et al., 2003b; Kim et al., 2005; Shelobolina et al., 2012; Zhang et al., 2011). Under microbial Fe(III) reduction, Fe (oxyhydr)oxides are usually transformed from poorly ordered into highly crystalline oxides (e.g., ferrihydrite to magnetite and green rust) and/or other Fe-containing mineral forms such as vivianite and siderite depending on the medium composition (Fredrickson et al., 1998; Glasauer et al., 2003). In contrast, clay structural Fe(III) bioreduction generates more

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complex biogenic minerals. For example, smectite can be illitized in a few weeks after bioreduction of structural Fe(III) by various bacteria, with concomitant formation of new minerals such as vivianite, siderite, feldspar, carbonate, and silica (Kim et al., 2004; Liu et al., 2012; Zhang et al., 2007; Zhang et al., 2012). Mineral transformation during clay Fe(III) bioreduction could be mainly influenced by bacterial type, media composition (e.g., K, Al, CO_3^{2-}, PO_4^{3-}) and incubation condition (e.g., temperature, pH) (Koo et al., 2014; Zhang et al., 2007). For example, amendment of K and Al can promote illitization of smectite as illite requires more K and Al in its structure than smectite does (Koo et al., 2014). Thus, considerable debate exists on whether Fe reduction products in artificial defined media can represent clay mineral fraction under environmental conditions (Weber et al., 2006).

In geothermal ecosystems, microbially mediated Fe reduction is an important process because of abundant Fe available in hydrothermal fluids (Pester et al., 2014; Wu et al., 2013), limited O_2 solubility, and abundance of reduced gases (Kashefi et al., 2004; Vargas et al., 1998). High temperature fluids are effective in altering primary Fe-bearing silicates into secondary clay minerals, which usually develop into clay belts along fluid channels or around spring pools (McMurtry and Yeh, 1981; Zhu and Tong, 1987). These secondary clay minerals represent an important Fe(III) source for thermophiles in these ecosystems. Recent studies found that diverse thermophiles, especially those hyperthermophiles that are closest to the common ancestor, possess the capability to reduce Fe(III) in clays and oxides (Kashefi and Lovley, 2003; Kashefi et al., 2008; Manzella et al., 2013; Vargas et al., 1998). As a result, these studies have raised a hypothesis that bioreduction of solid-form Fe(III) is one of the most important microbial metabolisms for early life, because Fe(III)-containing minerals were abundant on the early “hot” and hypoxic Earth.

Although recent efforts have isolated novel hyperthermophiles from geothermal habitats with mineral structural Fe(III) as electron acceptor (Kashefi et al., 2002; Kashefi et al., 2008; Manzella et al., 2013; Tor et al., 2001; Yamada et al., 2014), these isolates only represent a small percentage of thermophilic population in hot springs. Furthermore, many studies have primarily focused on a single thermophilic strain to reduce Fe(III) in clays and oxides using a defined medium [(Slobodkin, 2005; Weber et al., 2006) and therein]. However, in natural geothermal environment, bioreduction process may be carried out by a mixed microbial consortia and different functional groups may work together and benefit from one another (e.g., Fe reduction) (McGlynn et al., 2015). For example, electron transfer between species during Fe(III) reduction alleviates the requirement of substrate and energy and thus supports syntrophy of microbial consortia (Nagarajan et al., 2013). Besides, the mineralogical transformation by Fe(III) bioreduction may be significantly affected by the water quality of different hot springs. Therefore, many open questions remain with respect to Fe(III) bioreduction by thermophiles. For example, it remains unclear if certain geochemical conditions favor certain Fe(III)-reducing microbial population. It is also poorly known about how bioreduction process impacts clay mineral transformation in geothermal environment.

The Rehai Geothermal Field (RGF), Tengchong County of Yunnan Province, western China, is at the southeastern margin of the Tibet-Yunnan geothermal (TYG) zone. The TYG zone located on the fault zone resulting from the collision between the India and Eurasia plates, which formed (Wang et al., 2008).The RGF possesses numerous hot springs and geothermal pools, which exhibit a wide range of physicochemical conditions (e.g., pH ranging from < 1.8 to > 9.3, temperature from ambient to near boiling) (Liao and Guo, 1986). Intensive hydrothermal alteration of basalt and andesite occurs along fluid channels, which results in the formation of secondary silicate minerals (e.g., kaolinite, illite, smectite, feldspar, mica) (Zhu and Tong, 1987; Zou et al., 2010). Recent PhyloChip- and 454 pyrosequencing-based microbial studies revealed that potentially thermophilic dissimilatory Fe(III) reducers such as *Thermoanaerobacter*, *Desulfotomaculum* and *Thermotoga* are present in these hot springs (Briggs et al., 2014; Hedlund et al., 2012; Hou et al., 2013; Song et al., 2013). A close association between these highly diverse thermophiles and clay minerals favors their interaction, especially in shallow, clay-rich muddy pools. Thus, the RGF hot springs are ideal sites for studying clay mineral-thermophile interactions.

The objective of this study was to study microbial Fe(III) reduction process in nontronite NAu-2, a model Fe(III)-rich clay mineral smectite by thermophilic Fe(III)-reducing consortia retrieved from hot spring water. Specifically, the study was mainly to answer three following questions: 1) what microbial species participate in the Fe(III) reduction in different hot springs? 2) how does structural Fe(III) bioreduction affect clay mineralogical properties in hot springs? and 3) how do microbial type and hot spring water geochemistry influence Fe(III)-reducing products? Hot springs in RGF were sampled for this study. An integrated approach was employed including geochemistry, mineralogy, and microbiology. The results of this study improve our understanding of Fe biogeochemical cycling in geothermal environments.

2. Materials and methods

2.1. Field measurements and sampling

Nineteen hot springs in the RGF were selected for field measurements and sampling in July 2014 (Table 1). Water temperature and pH were measured using a portable meter (LaMotte, MD, USA). Water chemistry (S^{2-}, Fe^{2+}, and NH_4^+) was measured by using Hach kits (Hach Company, CO, USA). After these field measurements, water/sediment slurry was aseptically collected from these hot springs. Approximately 500 mL water was collected from each spring, frozen in ice, and transported to lab for preparation of various growth media.

2.2. Clay preparation

Nontronite NAu-2 was purchased from the Source Clays Repository of the Clay Minerals Society (West Lafayette, IN, USA) (Gates et al., 2002) and was employed as the sole Fe(III) source for enriching thermophilic DIRB in the Tengchong hot springs. Clay separation method and mineralogical characteristics of 0.02–0.5 μm fraction of NAu-2 were described in detail elsewhere (Jaisi et al., 2005; Keeling et al., 2000). Anaerobic and sterile NAu-2 stock solution was created as follows: the obtained NAu-2 fraction was suspended in distilled water to reach a final concentration of 50 g L^{-1}, autoclaved, purged with N_2 gas (to remove O_2), and sealed with thick butyl rubber stoppers for later use.

2.3. Enrichments of Fe(III) reducing consortia

Prior to field work, Balch glass tubes (total volume, 25 mL) were filled with N_2 gas, sealed with thick butyl rubber stoppers, and autoclaved. In the field, about 9 mL of sediment slurry (v:v, spring water:sediment = 9:1) was collected with a sterile needle from each selected hot spring and aseptically injected into the prepared N_2 gas-filled tubes. These tubes were amended with 1 mL NAu-2 stock solution (final NAu-2 conc. = 5 g L^{-1}) and lactate (final conc. = 20 mM) to serve as the sole electron acceptor and donor, respectively. The inoculated tubes were incubated in situ for 5 days and were subsequently transported to laboratory at ambient temperature. In the lab the tubes were incubated at in-situ spring temperatures (Table 1).

For subsequent transfers of enrichment from each spring, in-situ hot spring water was used. To minimize any abiotic reduction, hot spring water used for medium preparation was first purged with air to remove any H_2S (Cleceri et al., 1998), and then was filtered through a 0.22 μm filter. The spring water was slightly pH-adjusted to match the in-situ value of each corresponding hot spring and then purged with N_2 to remove oxygen. Those enrichments showing positive Fe(III) reduction, as indicated by a color change of NAu-2 from brown to dark green, were transferred in a glove box to a new medium (1:9 v:v ratio), which