

Comparison of Reduction Extent of Fe(III) in Nontronite by *Shewanella putrefaciens* and *Desulfovibrio vulgaris*

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INTRODUCTION

Clay minerals, the main constituents of most marine sediments, play a fundamental role in organic matter preservation, due to their high surface area and strong adsorption capability (Bock and Mayer, 2000). Indeed, adsorbed organic matter can aggregate clay particles and result in the formation of organic-mineral complex (Arnarson and Keil, 2001), which protects the organic matter from further microbial degradation (Yu et al., 2009). However, dissimilatory iron reducing bacteria (DIRB) as well as sulfate reducing bacteria (SRB) can re-mobilize the structural ferric iron in clay minerals and partly lead to release of adsorbed organic matters (Dong et al., 2009; Zhang et al., 2007; Li et al., 2004). Therefore, the geochemical iron cycle in clay minerals can impact the fate of adsorbed organic matter. In this study the reduction extents of Fe(III) in clay minerals by DIRB *Shewanella putrefaciens* and

SRB *Desulfovibrio vulgaris* were compared to further probe the organic matter fate in sediments.

MATERIALS AND METHODS

Nontronite (sample NAu-2), an iron-rich smectite, was selected for experiment due to its importance in organic carbon preservation in black shales (Kennedy et al., 2002). Glycine was intercalated into the inter-layer of NAu-2 (referred to Gly-NAu-2 hereafter) following a previous procedure (Benincasa et al., 2000) and successful intercalation was confirmed by X-ray diffraction (XRD). Fe(III) content of Gly-NAu-2 was measured by direct current plasma (DCP) emission spectroscopy (for details, see Zhang et al., 2007).

S. putrefaciens CN32, a typical DIRB, was routinely cultured aerobically in tryptic soy broth (TSB) and *D. vulgaris*, a sulfate reducing bacterium, was incubated in strictly anaerobic growth medium. Cells in the log phase were washed with anaerobic bicarbonate buffer (pH=7.0) three times, and then respectively injected into anaerobic bicarbonate buffer with 5 g/L NAu-2 or 5 g/L Gly-NAu-2 to get a final concentration of 10^8 cells/mL. All experiments were run in duplicates and lactate (20 mM) was used as the sole electron donor. In *D. vulgaris* inoculated system sodium sulfate (50 mM) and 0.1 mM anthra-quinone-2, 6-disulfonate (AQDS) were added as electron acceptor and electron shuttle, respectively in parallel experimental sets. Reduced Fe(II) content was monitored by the ferrozine assay using 0.5 N HCl extraction (Jaisi et al., 2005).

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RESULTS

The increase in the d_{001} spacing from 10.91 (at $2\theta=8.09$) for NAu-2 to 16.92 (at $2\theta=5.20$) for Gly-NAu-2 suggests that a certain amount of glycine was intercalated into the interlayer of the NAu-2 structure and the spacing was increased (Fig. 1). Noticeably, the total iron in Gly-NAu-2 was 18.7%, about 4.7% lower than the initial iron content in NAu-2. The decrease of iron content due to the intercalation of NAu-2 was also reported by Zhang et al. (2007) with cysteine.

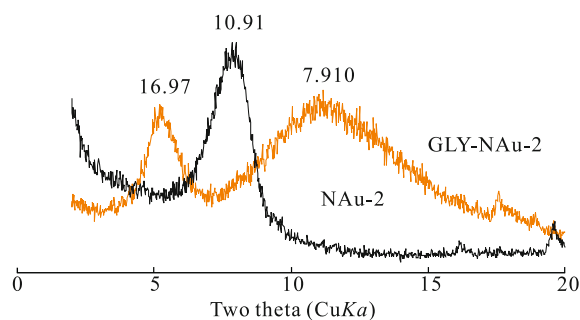


Figure 1. XRD patterns for NAu-2 and Gly-NAu-2.

Both *S. putrefaciens* and *D. vulgaris* could reduce Fe(III) in both NAu-2 and Gly-NAu-2, however, the reduction extent was significantly different under diverse conditions, and also varied with strains under similar conditions (Fig. 2). The extents of reduction in controls without cells were less than 3% (data not shown). In *S. putrefaciens* inoculated systems with AQDS the reduction extent of NAu-2 sharply increased within 2 d and reached the maximum value of 35% in 8 d (Fig. 2a, I-a)). Without AQDS the final reduction extent of NAu-2 (Fig. 2a, I-b) was only around 21%. The structural Fe(III) of Gly-NAu-2 seemed refractory to microbial reduction even with the facilitation of AQDS since a low reduction rate and a reduction extent were observed in the Gly-NAu-2 system (Fig. 2a, II).

While in the *D. vulgaris* inoculated systems, reduction extents were greatly enhanced by the addition of AQDS and/or sulfate with NAu-2 as electron acceptor (Fig. 2b, I-a, I-b, III-a, III-b). Much higher reduction extents of NAu-2 were observed in AQDS amended systems which were around 26% (Fig. 2b, I-a, I-b). The lowest reduction extent was about 9.5% without the addition of SO_4^{2-} and AQDS (III-b). The final reduction extent of Gly-NAu-2 was ca. 16%.

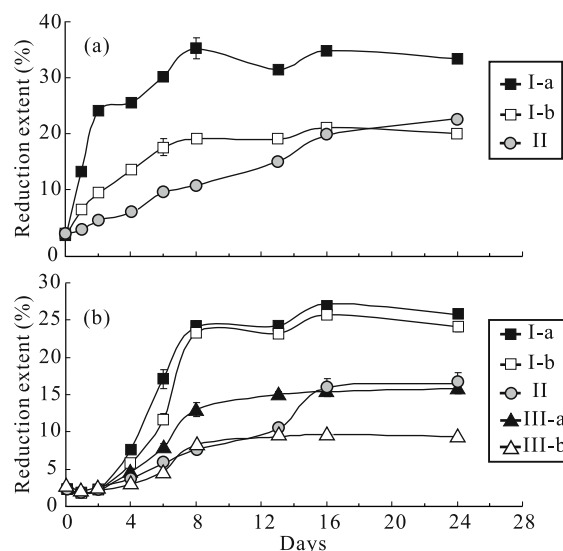


Figure 2. Reduction extent of NAu-2 or Gly-NAu-2 by *S. putrefaciens* (a) and *D. vulgaris* (b) with time in different conditions. (a) I-a. AQDS+NAu-2; I-b. NAu-2; II. AQDS+Gly-NAu-2; (b) I-a. SO_4^{2-} +AQDS+NAu-2; I-b. AQDS+NAu-2; II. SO_4^{2-} +AQDS+Gly-NAu-2; III-a. SO_4^{2-} +NAu-2; III-b. NAu-2.

DISCUSSION

In both inoculated systems (Fig. 2), AQDS, the artificial analog of humics, enhanced structural Fe(III) reduction in NAu-2 which is consistent with previous studies (Lovley et al., 1996). This might suggest an important role that natural humics might play in the burial of organic matter. It was unexpected that the reduction extents of Fe(III) in Gly-NAu-2 were much lower than those of NAu-2 under the same conditions. Two reasons might account for this. Firstly, a certain amount of active Fe(III) in NAu-2 had been released during the glycine intercalation which would leave refractory structural ferric for bacterial respiration. Secondly, during the respiration of lactate by microorganisms glycine in the interlay of NAu-2 could compete against Fe(III) for electrons (Myers and Nealson, 1988) and thus would lower the ferric iron reduction extent. Furthermore the presence of organic matter in the interlayer of smectite might physically block the electron transfer, thus decreasing the reduction extent (Zhang et al., 2007).

Bioreduction rates and extents of NAu-2 by *D. vulgaris* were closed to each other in the presence of AQDS and only minor differences resulted from the

presence and absence of sulfate. Without the facilitation of AQDS, sulfate could enhance the bioreduction of structural ferric in NAu-2 even though more than 10 d might be taken to show the enhancement.

In our experiments higher reduction extents of structural iron were achieved by *S. putrefaciens* than by *D. vulgaris* under the same situation. *D. vulgaris* was reported to reduce iron via c_3 cytochrome (Lovley and Phillips, 1994). However, besides the membrane enzyme, *S. putrefaciens* also possesses conductive pili and secreted compounds which could serve as electron shuttle and enhance iron reduction (Gorby et al., 2006). Moreover, our results show a more important role of DIRB in controlling the fate of adsorbed organic matter than it was previously reorganized.

CONCLUSIONS

Both *S. putrefaciens* and *D. vulgaris* reduced the structural Fe(III) in NAu-2. But a higher reduction extent was observed in the *S. putrefaciens* inoculated system under the same conditions. AQDS enhanced the reduction extent, whereas, organic matter (Glycine) in the interlayer of NAu-2 decreased the reduction extent. In the *D. vulgaris* inoculated systems, sulfate played a positive role in iron reduction in the absence of AQDS, but had less effect with AQDS. Therefore DIRB as well as SRB could play an important role in the fate of adsorbed organic matter through the structural iron reduction in clay minerals.

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