

Microbial Reduction of Weakly Crystalline Iron (III) Oxides and Suppression of Methanogenesis in Paddy Soil

D. Qu,¹ S. Ratering,² S. Schnell²

¹ Max-Planck-Institute for Terrestrial Microbiology, Karl-Frisch-Strasse, 35043 Marburg, Germany

² Justus-Liebig University Giessen, Institute of Applied Microbiology, IFZ, Heinrich-Buff-Ring 26-32, D-35392 Giessen, Germany

Received: 31 August 2002/Accepted: 10 March 2004

Due to the fact that iron is among the most stable elements formed by nuclear fusion it has a high cosmic and terrestrial abundance. The iron(III) oxides, one important group of iron minerals, differ in the arrangement of the atoms in the crystal structure (Schwertmann and Taylor, 1989). The degree of structural order and the crystal size depend on the conditions under which the crystals were formed and a range of crystallinity is characteristic of these compounds (Cornell and Schwertmann, 1996). Since several years it is well known that iron(III) oxides can serve as electron acceptor for microorganisms (Lovley and Phillips, 1988; Myers and Neelson, 1988). The iron(III) oxides of low crystallinity seem to be reduced predominantly by microorganisms (Munch and Ottow, 1980; Phillips et al., 1993; Wahid and Kamalam, 1993). Crystalline iron(III) oxides like goethite or hematite seem to be not or only to a smaller fraction available for microbial reduction. Few reports are available about the reduction of crystalline iron(III) oxides in nature (Macedo and Bryant, 1989). The mechanism how bacteria reduce solid iron(III) oxides is still unknown. Studies with pure cultures showed that the direct contact between some bacteria and iron(III) crystal is necessary for the reduction of the oxides (Munch and Ottow, 1980; Tugel et al., 1986; Nevin and Lovley, 2000). Other reports showed the release of a small menaquinone related molecule into the growth medium of *Shewanella putrefaciens* which possibly transfers electrons to the poorly soluble iron(III) oxides (Newmann and Kolter 2000). Also for *Geothrix fermentans* quinone as electron shuttle is assumed (Nevin and Lovley, 2002). Beside crystallinity also the surface area seem to influence microbial reducibility of iron oxides (Roden and Zachara, 1996). In addition to removal of dissolved iron(II) from the surface of iron(III) oxides promoted microbial reduction of crystalline iron(III) oxides by *Shewanella alga* strain BrY (Roden and Urrutia, 1999; Urrutia et al., 1999; Roden et al., 2000). An other characteristic of iron(III) oxides discussed to influence the reduceability of crystalline iron(III) oxides is the Al-substitution of Fe-atoms in the crystal. While Bousserhine et al. (1998; 1999) demonstrated that the dissolution of Al-substituted goethite by a cometabolic iron-reducing *Clostridium spec.* was slower compared to non substituted goethite,

Correspondence to: D. Qu, Northwest Sci-Tech University of Agriculture and Forestry, College of Resources and Environment, 712100 Yangling, People's Republic of China

Maurice et al. (2000) showed that the initial dissolution by an aerobic *Pseudomonas mendocina* var. increased with increasing Al-content. Soil iron(III) oxides like goethite are rarely found in pure state and Al-substitution can result in up to 12.7 mole% in seasonally waterlogged paddy soil (Karim, 1984).

In waterlogged paddy soils the availability of the iron oxides for microbial reduction is of interest because it was shown that iron reduction suppressed the emission of the greenhouse gas methane from paddy fields (Jäckel and Schnell, 2000a). Iron reduction was found to be the second most important anaerobic processes of organic matter mineralization after methanogenesis in paddy fields (Jäckel and Schnell, 2000b; Yao and Conrad, 2000). High concentrations of available iron(III) further increased the role of iron(III) reduction in anaerobic degradation. The successful competition of iron reducers for electron donors with methanogenes can be explained by thermodynamics resulting in lower substrate threshold concentrations for processes with more favorable thermodynamics. Iron-reducing bacteria were able to lower the hydrogen partial pressure below values that can be used by methanogens (Lovley and Phillips 1987, 1998; Achnich et al., 1995a, 1995b). The objective of this study was to examine the suppression extend of methanogenesis by amendments of various iron(III) oxides differing in crystallinity and Al-content.

MATERIALS AND METHODS

The paddy soil was obtained in November 1995 from a rice field located in the Po river valley near Arborio, Italian. The soil was air-dried and stored in polyethylene containers. Before the experiments dry soil lumps were crushed using a mechanical grinder and sieved through a 1 mm mesh size. The soil characteristics was described by Ratering and Schnell (2000). Fresh anoxic paddy soil slurries were prepared by suspending sieved soil in demineralised water (200 g dry soil plus 200 ml water in 1 L glass bottles, the headspace was flushed with N₂ and the slurries were incubated at 25°C). CH₄, CO₂ and H₂ concentrations were measured by GC analysis (Conrad et al., 1987). Porewater samples of slurries were analyzed by HPLC and IC for fatty acids (Krumboeck and Conrad, 1991), sulfate, and nitrate (Bak et al., 1991). Fe(III) and Fe(II) concentrations were determined by IC after 24 hours extraction at room temperature with 0.5 M HCl (Schnell et al., 1998). Different iron oxides including Al-substituted iron oxides were prepared after Schwertmann and Cornell (1991). All iron(III) oxides were added as an aqueous suspension at the beginning of the experiment.

RESULTS AND DISCUSSION

Experiments with anoxic soil slurries with and without addition of iron(III) oxides

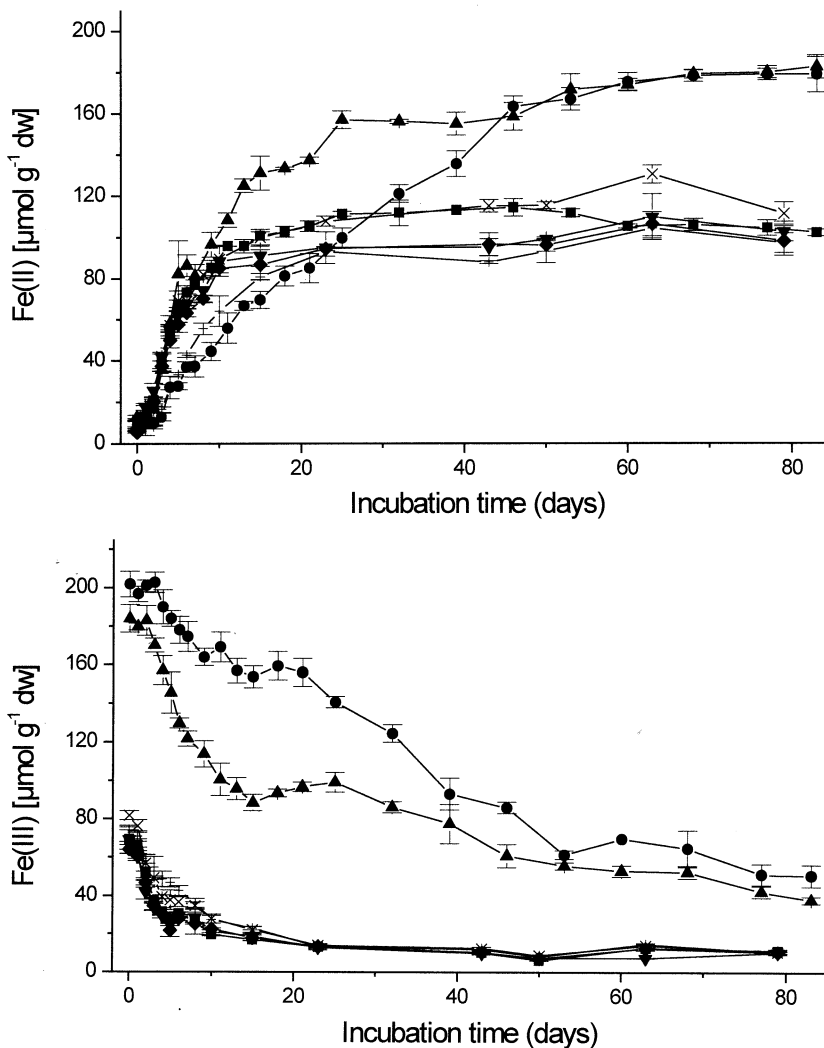


Figure 1. The time course of iron(II) and iron(III) concentration in anoxic incubated slurries of paddy soil. Data are means of triplicates \pm SE (Curve symbol \blacksquare : control, \bullet : ferrihydrite, \blacktriangle : lepidocrocite, \blacktriangledown : goethite, \blacklozenge : Al-goethite, $+$: hematite, \times : Al-hematite)

showed that both amended ferrihydrite and lepidocrocite were reduced during the incubation. After a short lag-phase the iron(II) concentration increased to 100 $\mu\text{mol g}^{-1} \text{ dw}$ at day 15 in the control experiments without addition and stayed constant until the end of experiment (Figure 1). Experiments with addition of ferrihydrite and lepidocrocite showed higher iron(II) concentration up to 170 $\mu\text{mol g}^{-1} \text{ dw}$ at the end of the experiment. Reduction of amended lepidocrocite and ferrihydrite was completed after 25 days and 45 days, respectively.

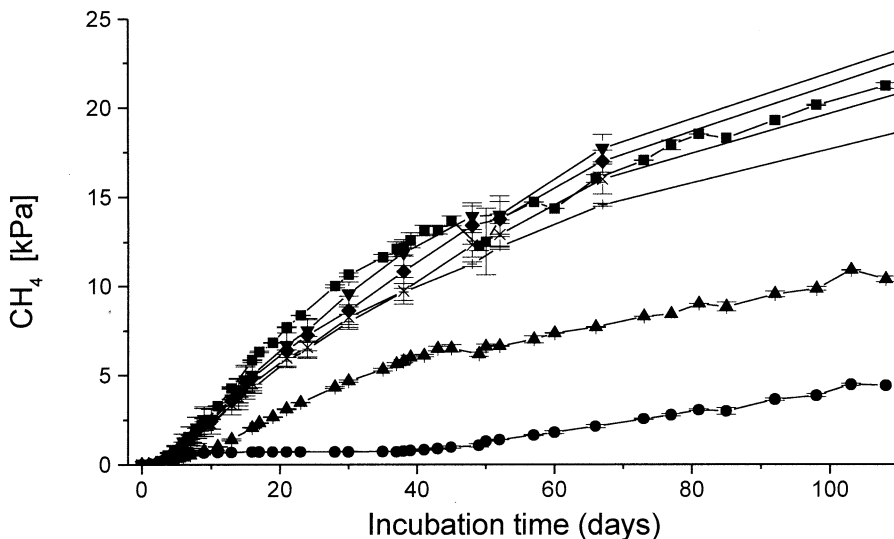


Figure 2. Effect of iron oxide addition on CH₄ partial pressure in anoxic incubated slurries of paddy soil. Data are means of triplicates +/- SE. (Curve symbol ■ : control, ● : ferrihydrite, ▲ : lepidocrocite, ▼: goethite, ◆ : Al-goethite, + : hematite, x : Al-hematite)

Measurement of iron(III) concentrations showed that only 70% of the amended lepidocrocite and ferrihydrite were reduced during the experiment (Figure 1). In contrast to the experiment with ferrihydrite and lepidocrocite, all other iron(III) oxides (goethite, Al-goethite, hematite and Al-hematite) showed no effect on the iron(II) concentrations compared to the control experiment indicating no microbial reduction of those iron(III) oxides. Iron(III) concentrations of experiments with hematite and goethite amendment were similar to those of the control because extractions with 0.5 M HCl did not dissolve crystalline iron(III) oxides.

The time course of methane production after addition of different iron oxides in slurries of paddy soil is shown in Figure 2. The addition of various iron oxides inhibited methane production to different extents. Ferrihydrite and lepidocrocite reduced methane production to 44 and 65% of the non amended control (Table 1). Hematite and Al-substituted hematite inhibited methane production only slightly, whereas goethite and Al-substituted goethite showed no effect.

To evaluate the mineralization performance in the experiment with iron oxides amendments the carbon dioxide partial pressure was measured in the headspace of the bottles. The CO₂ partial pressures increased from the experimental start to 40 days (Figure 3). The CO₂ increase in assays with ferrihydrite and lepidocrocite amendments were lower than those of the control and all other iron(III) oxide

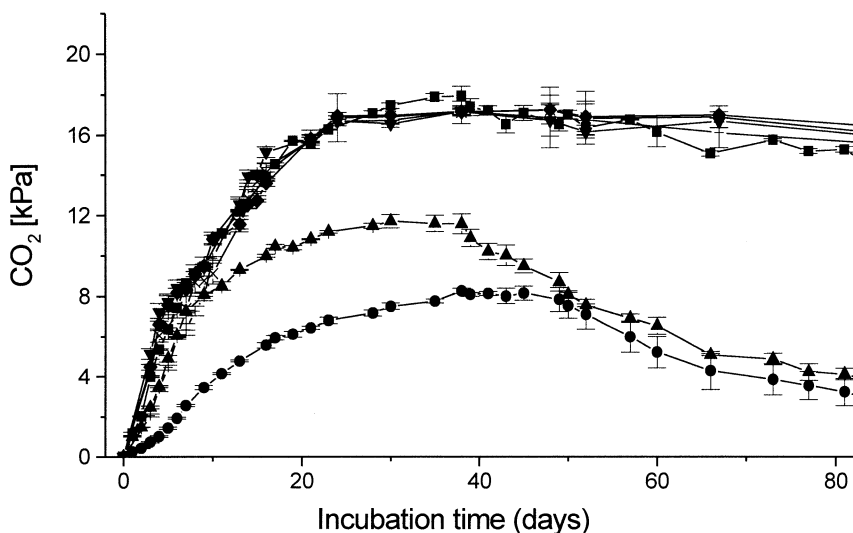


Figure 3. Effect of iron oxide addition on CO₂ partial pressure in the headspace of anoxic incubated slurries of paddy soil. Data are means of triplicates +/- SE. (Curve symbol ■ : control, ● : ferrihydrite, ▲ : lepidocrocite, ▼ : goethite, ◆ : Al-goethite, + : hematite, x : Al-hematite)

additions. After 40 days the CO₂ partial pressure decreased again in the presence of ferrihydrite or lepidocrocite which can be explained by precipitation of siderite in the assays. Siderite was reported to be the dominant speciation of Fe(II) in natural waters with carbonate alkalinity greater than 1 mM (King 1998). In the presence of increasing iron(II) concentrations as a result of microbial iron reduction the equilibrium of hydrogen carbonate and carbon dioxide was shifted by the precipitation of FeCO₃. At the end of the experiment the dissolved and solid fraction of hydrogen carbonate and carbonate was calculated after addition of sulfuric acid which expelled all fractions as gaseous carbon dioxide (Table 2). CO₂ released from organic substances by sulfuric addition was negligible. The total amount of carbon dioxide calculated as the sum of gaseous carbon dioxide after sulfuric acid treatment and the methane concentration were similar all assays indicating that mineralization was similar as well.

Due to very low concentrations of nitrate and sulfate (below 1 μmol gdw⁻¹ soil) as alternate electron acceptors their contribution for anaerobic mineralisation is negligible (below 1%) and iron reduction and methanogenesis are the predominant processes of anaerobic organic matter degradation. The competition between iron reduction and methanogenesis for electron donors becomes obvious following the hydrogen and acetate concentrations during the time course of the experiments. The contribution of iron reduction in non amended paddy soil was 19% and that of

methanogenesis was 81%. The contribution of iron reduction in non amended paddy soil was 19% and that of methanogenesis was 81%. The addition of ferrihydrite and lepidocrocite increased the contribution of iron reduction to 63% and 47%, respectively. Hematite and Al-substituted hematite addition increased the contribution of iron reduction only slightly; goethite and Al-substituted goethite showed no effect (Table 2).

Table 1. Contribution (%) of iron(III) reduction and methanogenesis to anaerobic degradation of organic matter in slurry experiments with paddy soil calculated as electron balance assuming hydrogen as electron donor.

Treatment	Contribution to anaerobic degradation (%)	
	<i>Fe(III) reduction</i>	Production of methane
control	18.3 ± 0.5	80.9 ± 0.1
ferrihydrite	63.3 ± 3.5	35.8 ± 0.2
lepidocrocite	46.9 ± 2.2	52.3 ± 0.3
goethite	16.2 ± 0.8	83.1 ± 0.1
Al-goethite	15.3 ± 0.9	84.0 ± 1.6
hematite	20.2 ± 1.0	78.9 ± 0.7
Al-hematite	20.7 ± 1.0	78.5 ± 0.5

Due to low concentrations of nitrate and sulfate in paddy soil (data not shown) these electron acceptors contribute to a minor content (less than 1 %). For control, ferrihydrite, and lepidocrocite the data were used after 190 days, for goethite, Al-goethite, hematite, and Al-hematite after 136 days. The amount of iron oxide added was about 100 mM Fe(III) in all assays. Means of three replicates.

Table 2. Carbon balance of anoxic incubated slurries of paddy soil after 105 days. Data are means of triplicates +/- SE ($\mu\text{mol g}^{-1}$ dw soil).

Treatment	CH ₄ -C	CO ₂ -C	CO ₃ ²⁻ -C	total-C
control	26.96	17.27	28.11	72.34 ^a
ferrihydrite	6.39	3.26	69.73	79.38
lepidocrocite	15.56	4.31	60.52	80.39

a: Two deflation during the incubation prevented over press in the bottles, therefore the total-C value is underestimated.

In the non amended control assays iron reduction was completed after 20 days due to limitation of iron(III) and then methanogenesis was the predominate process. At this time the hydrogen partial pressure was 2.5 Pa (Figure 4) which is the typical

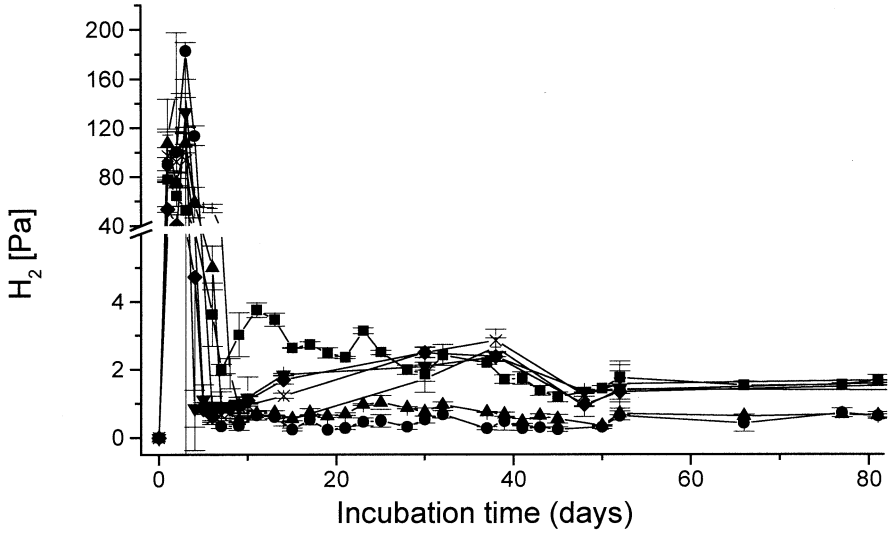


Figure 4. Effect of iron oxide addition on H₂ partial pressure in the headspace of the slurries. Data are means of triplicates +/- SE. (Curve symbol ■ : control, ● : ferrihydrite, ▲ : lepidocrocite, ▼ : goethite, ◆ : Al-goethite, + : hematite, x : Al-hematite)

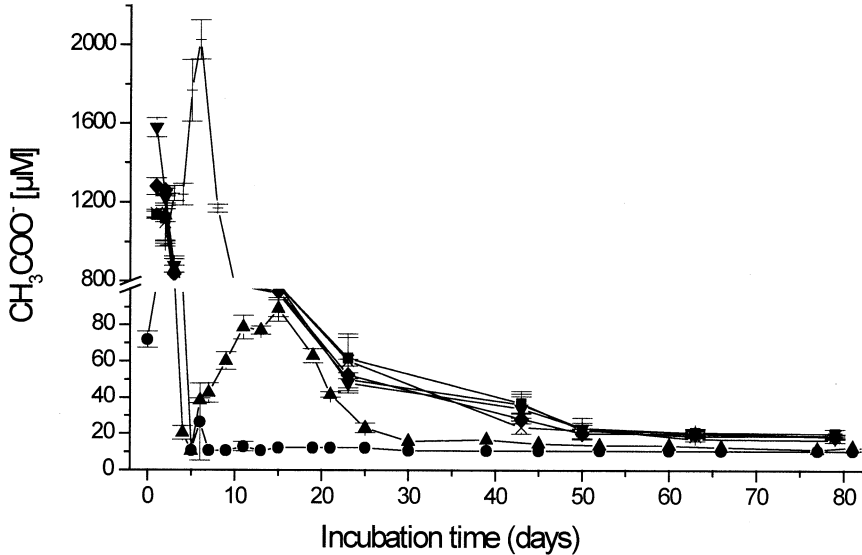


Figure 5. Effect of iron oxide addition on acetate concentration in anaerobic incubated slurries. Data are means of triplicates +/- SE. (Curve symbol ■ : control, ● : ferrihydrite, ▲ : lepidocrocite, ▼ : goethite, ◆ : Al-goethite, + : hematite, x : Al-hematite)

threshold value for methanogenesis. In contrast to the control the hydrogen partial pressure in assays with ferrihydrite and lepidocrocite amendment was below 1 Pa during the time when iron reduction was the predominate process and methanogenesis consequently was suppressed according to the thermodynamic theory (Zehnder and Stumm, 1988). Similarly the acetate concentrations were in assays with ferrihydrite and lepidocrocite amendment lower during the whole time course (Figure 5). High initial acetate concentrations in all assay can be explained with primary fermentation activity. Later acetate consumption by anaerobic respiration processes overbalanced acetate production by fermentation. In the control assays acetate concentrations remained above 20 μM until the end of the experiment whereas in assays amended with ferrihydrite or lepidocrocite acetate concentrations reached values below the detection limit (5 μM) (Figure 5). The results of this study demonstrate that ferrihydrite and lepidocrocite as iron oxides of low crystallinity can be used as electron acceptor by iron reducer of electron acceptor by iron reducer of italian paddy soil and suppressed methanogenesis effectively. Iron oxides of higher crystallinity e.g goethite, Al-substituted goethite, hematite, and Al-substituted hematite were reduced to no or only a small extent and showed no or only little suppression of methanogenesis.

Acknowledgments. We thank Ralf Conrad, Max-Planck-Institute for Terrestrial Microbiology, Marburg, for financial support and for helpful discussions. Further on we thank Udo Jäckel for introduction to analytic methods and instruments. Presented at “First International Conference on Pollution Eco-chemistry and Processes”, Aug. 26-31, 2002, Shenyang, China.

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