A Linked Iron Reduction and Oxidation System: Investigating Biofilm Development and Fe Oxyhydroxide Precipitation-Dissolution Reactions.

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Abstract

Fe reduction was linked to to Fe re-oxidation in a two column laboratory experiment using inoculum from Fe mat collected from the Great Sippewissett Salt Marsh, Woods Hole, MA. Fluorescent in situ hybridization (FISH) analyses of biofilms isolated on slides precoated with Fe oxides, identified several proteobacterial groups (α,β,γ, and δ) in the Fe reducing column. Fe^{2+} generation was observed to occur in this column measuring 720 μM at the sediment water interface. FISH analyses of the biofilm communities established on blank slides placed in the second, Fe oxidizing column identified γ proteobacteria; the same phylogenetic group was identified by Emmerson and Moyer (1997) for two neutrophilic, motile Fe oxidizing bacterial strains isolated from Fe oxidizing mats. FISH results for Fe oxidizing column agreed with FISH results for Fe oxidizer enrichment agar gradient tubes inoculated with the same Fe mat used to seed the two columns. Light and scanning electron microscopic inspection indicated biofilm establishment on slides in both columns; Fe oxide degradation and diagenesis in the reducing column; and deposition of Fe oxide like particles in the Fe oxidizing column.

Introduction

Microbial catalysis of iron redox reactions contributes to the dynamic nature of iron cycling in natural aqueous environments and thus influences a wide range of important geochemical processes such as nutrient dynamics (e.g. phosphorus cycling) and trace element transport and ultimate fate (e.g. Fe oxide formation and dissolution and associated

capture or release of inorganic contaminants). While these two two microbially driven iron redox processes are spatially separated according to oxygen gradients within aqueous sedimentary systems; from a geochemical perspective these processes can be thought of as a linked iron cycle that in the process of shunting electrons back and forth between iron and organic compounds influences a variety of important geochemical reactions (Fig.1).

The objectives of this project were to mimic such a linked microbially catalyzed Fe cycle in the laboratory, using a two column flow through system; to characterize the bacterial biofilms that developed under the two regimes of Fe reduction and oxidation; and to examine potential Fe oxyhydroxide formation and diagenesis. Column 1 was set up to be an Fe^{3+} reduction column which would generate an Fe^{2+} feed for column 2 where subsequent reoxidation of Fe^{2+} to Fe^{3+} would occur.

Methods

The linked column system (Fig. 2) was established in the laboratory and left to run for approximately two weeks. The Fe reduction column (column 1) was seeded with a mixture of sand from Sippewissett Salt marsh and approximately 75g (750mM of $Fe(OH)_{3s}$) of synthetically prepared amorphous ferric oxide (hydrous ferric oxide; HFO; Warren and Ferris 1998); and a small amount of Fe mat from the Sippewissett. This mixture was placed in the bottom one third of the column and anoxic synthetic, SO_4^{2-} free basal salts media was pumped through at a very low flow rate (0.1ml/sec) for 24 hours to purge any SO_4^{2-} from the column. The media was prepared using a modified version of Widdel's recipe from The Prokaryotes (p.3359) as follows:

Basal Salts Media (Widdel)

Distilled H_2O	1 litre	
NaCl	20g	
$MgCl_2 \cdot 6H_2O$	3g	
$CaCl_2 \cdot 2H_2O$	0.15g	
Na_2SO_4	4g	
NH_4Cl	0.25g	
KH_2PO_4	0.2g	(separate solution)
KCl	0.5g	

For our purposes we left out the Na_2SO_4 to discourage sulphate reducers and added 25g of NaCl to compensate for any ionic strength changes.

After 24 hours of purging the first, Fe reduction column with the anoxic media, the prepared HFO coated slides were placed into the column so that one slide was completely submerged in the sediments and the other two slides were at two different depths in the water column to allow for biofilm development and evaluation of diagenetic changes in the HFO's. The precoated HFO slides were prepared by smearing synthetic HFO in a uniform layer across the slides and allowing them to air dry for 3 days prior to insertion into the column. A control slide was kept for comparison purposes.

Column 2 (Fe oxidation) was seeded initially with a little sand and selected Fe mat from the Sippewissett. We selected Fe mat that had no visible green phototrophic matter associated with it to reduce the likelihood of phototrophic growth in the column. The outflow of column 1 fed into the bottom of column 2 which was left open to the atmosphere to establish an oxygen gradient in the system. A slide holder was also placed in column 2 with blank slides to allow for both biofilm development and Fe oxide precipitation (see Fig. 2).

The columns were fed slowly with continuously N_2 bubbled SO_4^{2-} free media for a period of 2 weeks. An initial 10 mmoles of acetate was added to the media to help establish the Fe reducers in column 1 (1 litre of 10mM acetate). Acetate was chosen as it has been shown to be oxidized in the presence of Fe oxides (Lovley, 1991). According to equation 1, 10mM of acetate would require 800 mM of $Fe(OH)_{3s}$ to completely oxidize the acetate to CO_2; slightly more than the total mass of $Fe(OH)_{3s}$ initially added (750mM).

$$\mathbf{1}CH_3COO^- + \mathbf{8}Fe(OH)_{3s} + 7H^+ \leftarrow \quad 2HCO_3^- + 8FeOH^+ + 12\ H_2O \qquad (1)$$

Once set up, the columns were kept in the dark to discourage phototrophic growth and fresh anoxic media was prepared every other day to discourage external microbial growth that would be introduced into the system.

After two weeks, the media was shut off and the columns were allowed to equilibrate overnight. From column 1, water samples for Fe^{2+} concentrations were taken from just above the sediment water interface, the middle of the H_2O column and the top of the column where the feed to column 2 occurred. Fe^{2+} concentrations were measured using the ferrozine assay.

Biofilm development on these slides was examined using light and, scanning electron (SEM) microscopies; as well as with available molecular biological tools (e.g. fluorescence in situ hybridization; FISH). Diagenetic mineral transformations (column 1) and development (column 2) were qualitatively examined using SEM and light microscopies.

Fe Gradient Tubes

An enrichment was set up for Fe oxidizers using an Fe agar gradient tube method modified from Emmerson and Moyer (1997) and Hallbeck *et al* (1993). Briefly, synthetic siderite (ferrous carbonate) was prepared and then mixed with a modified Wolfe's mineral media (MWMM) and 1% agarose. This mixture was placed in the bottom of roll tubes and left to harden. Once hardened a solution of MWMM and 0.15% agarose was then poured on top of the reduced iron mineral plug. These tubes were inoculated with Fe mat organisms by pipetting approximately 100 µls slowly from the top of the $FeCO_3$ plug surface up through the agar overlayer. The tubes were then incubated in the dark at 22˚C, checked every 2 days and transferred to fresh tubes when growth was evident. Communities from the fresh Fe oxide precipitating layer were examined using light microscopy and FISH.

Evaluation of the bacterial communities using FISH followed the protocol of the Microbial Diversity course (1999). We selected a variety of proteobacterial probes (Fe reducers: $\alpha,\beta,\gamma,\delta$; Fe oxidizers: γ ,δ)as both Fe reducing and Fe oxidizing bacteria have been shown to fall into a number of these phylogenetic categories. We specifically selected the γ proteobacteria as Emmerson and Moyer describe two new strains of iron oxidizing bacteria that grow at circumneutral pH, in association with *Leptothrix* and *Gallionella* Fe mats that were more important in contributing to Fe oxidation. In addition we used a low G+C Gram + probe as *Clostridium* has recently been shown to use Fe^{3+} in a dissimilatory fashion during the fermentation of glucose (Dobbin et al 1999).

Results

Evident iron reduction occurred in column 1 by the presence of Fe^{2+} in solution. At the sediment water interface, an Fe^{2+} concentration of 720 µM was measured, decreasing

up through the water column to 330 μM and 230 μM at the feed into column 2. Further the clear gradient that established once the media flow was shut off indicated that the Fe^{2+} was being generated in the sediment and then diffusing up out of the sediments. The slide that was buried in the sediments of column 1 had the greatest amount of cleared slide (i.e. reduction of Fe^{3+} and associated dissolution of the HFO) compared to the two slides that were suspended in the water column. Diagenetic changes to the precoated HFO slides was qualitatively evident by visual inspection of colour changes (darker colouration) and morphological changes (weathering of the oxide surface) using light and SEM microscopies, as was the presence of clear biofilm communities on the HFOs (Fig. 3).

In column 2, no visually apparent Fe oxide precipitation occurred on the slides, however inspection by light microscopy indicated amorphous orange coloured precipitates on the slides that resembled Fe oxide particles (Fig. 4). Further, yellowish material deposited on the sides of the column near the top (where oxygen was diffusing in) dissolved completely in concentrated HCl, consistent with an amorphous Fe precipitatel.

The Fe oxidizing agar gradient tubes showed white veils of bacteria associated with clear Fe oxides within a couple of days. Subsequent transfers of these bacteria to new tubes continued to produce white veils of bacteria with associated Fe oxide precipitates. Control tubes that were not inoculated showed an Fe oxide band corresponding to the oxic-anoxic boundary in the tube (i.e. Fe^{2+} diffusing up from the $FeCO_3$ plug is oxidized to Fe^{3+} upon hitting oxygen and precipitates as Fe oxides). However this band of Fe oxide was much more diffuse and uniform in comparison to the inoculated tubes where new bands of Fe oxides always associated with white veils of bacteria occurred below the abiotic Fe^{2+} - Fe^{3+} boundary. The bacteria in these white veils were short motile rods when viewed under the microscope.

Evaluation of the biofilm communities on the HFO slides from column 1 using FISH indicated the presence of all proteobacteria groups probed for (Table 1) but no low G+C Gram + bacteria. In column 2, where putative evidence at least, suggested Fe oxidation was occurring, FISH analyses indicated γ proteobacteria but no δ proteobacteria on the slides. Similarly, FISH analyses of the Fe oxidizing agar gradient tubes indicated the presence of γ proteobacteria but no δ proteobacteria. As is evident in Fig. 4, FISH analyses using the γ proteo bacteria probe clearly shows the presence of these bacteria associated with the suggested Fe oxide particle. Since both the column and the gradient tubes were inoculated with the same Fe mat material from the Sippewissett and we find γ proteobacteria in both these systems like the neutrophilic Fe oxidizing bacteria described by Emmerson and Moyer (1997); our data are at least consistent with the hypothesis that Fe oxidizers were present within the second column.

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Table 1: Results of FISH analyses of column 1 (HFO reduction), column 2 (Fe oxidation, and $FeCO_3$ agar gradient tubes (Fe oxidation).

PROBES	Column 1	Column 2	$FeCO_3$ tubes
proteobacteria			
α- (*e.g. Rhodobacter*)	✓		
β- (*e.g. Gallionella, Leptothrix*)	✓		
γ- (*e.g. Shewanella*, strains ES-1*, ES-2*)	✓	✓	✓
δ- (e.g. *Geobacter, Desulfovibrio*)	✓	–	–
low G+C Gram + (*e.g. Clostridium*)	–		

* strains ES-1 and ES-2 are neutrophilic γ proteobacteria isolated by Emmerson and Moyer (1997).

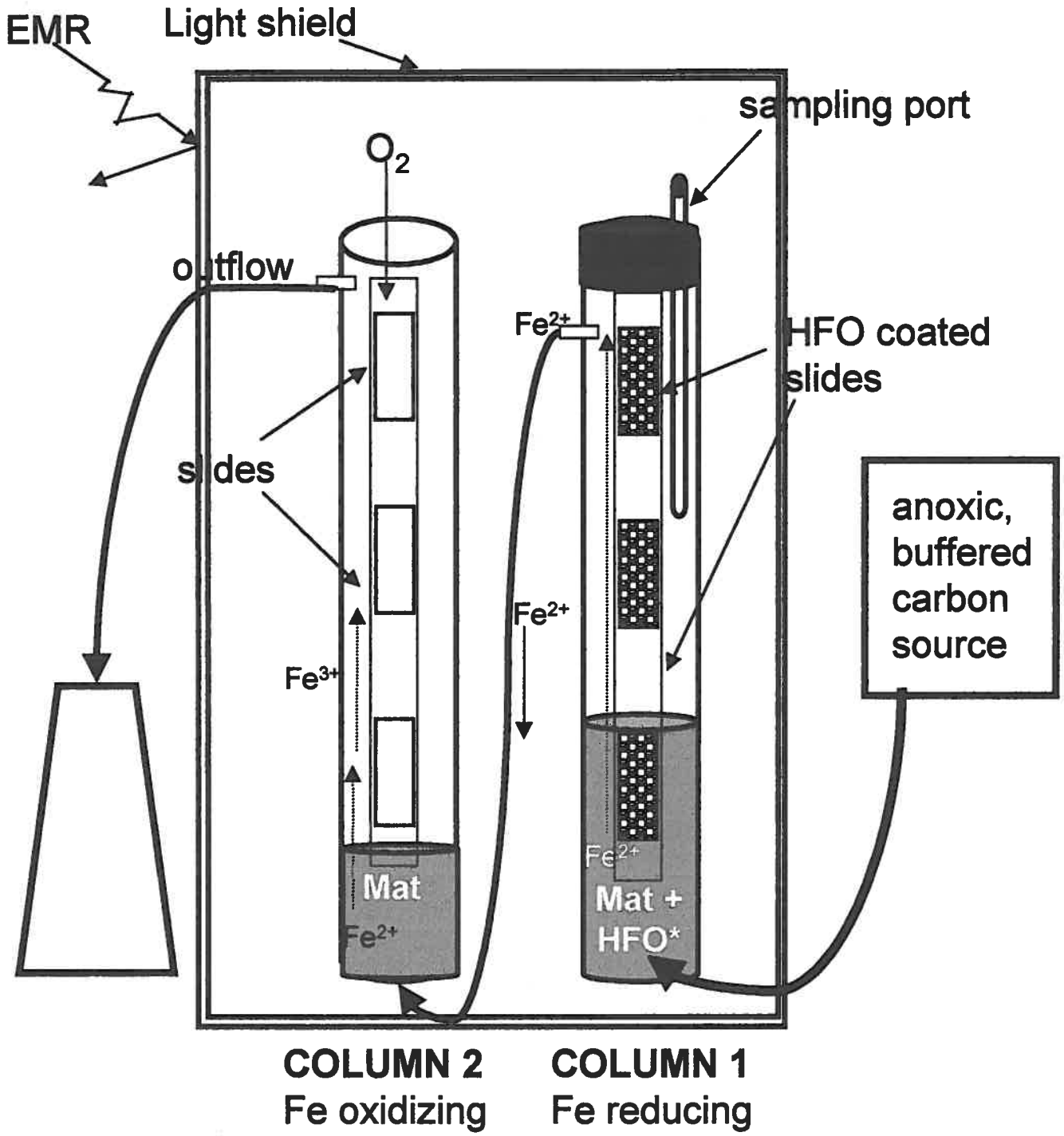


Fig. 1. Schematic of experimental flow through column design for microbially catalyzed Fe reduction (column 1) and Fe oxidation (column 2) using Sippewissett marsh mat.

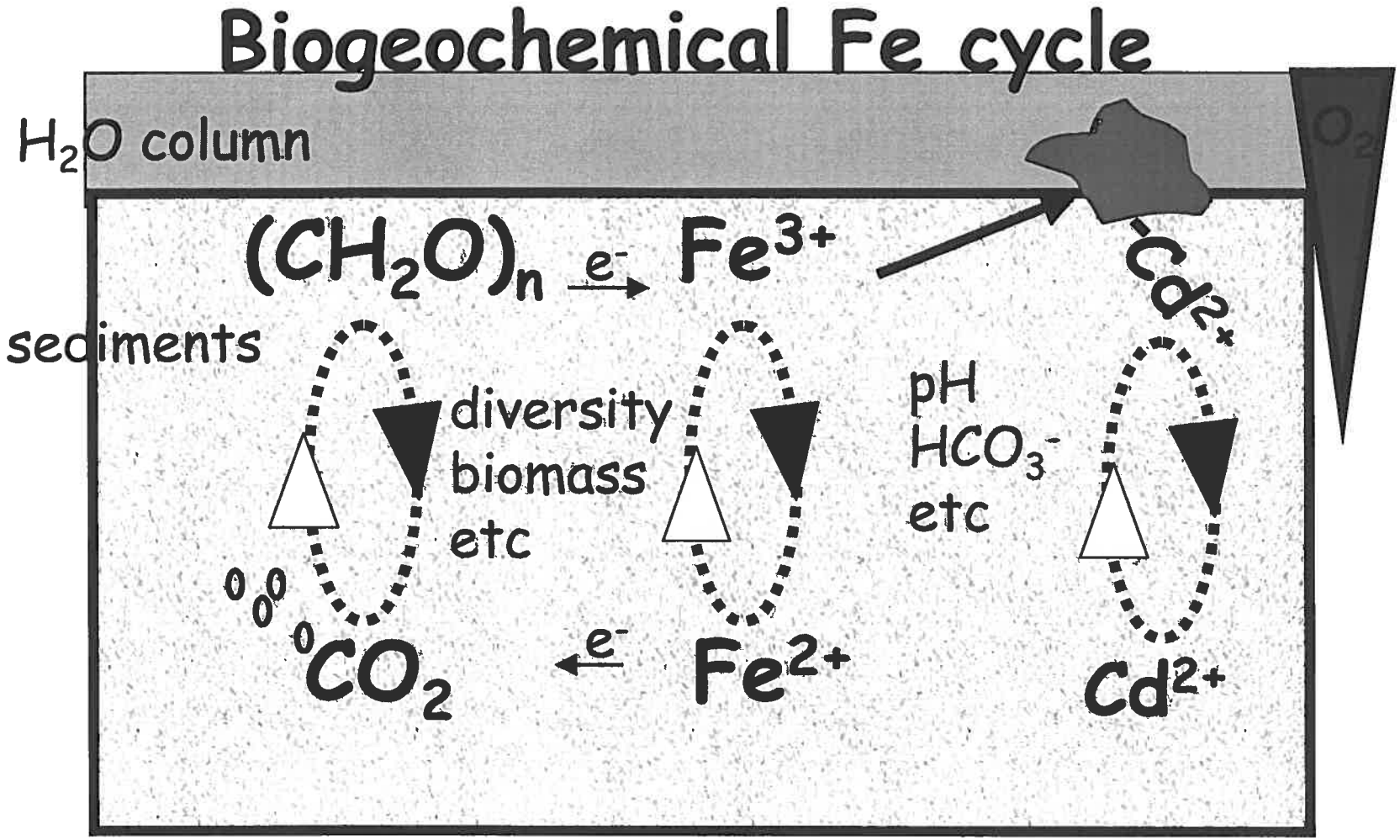
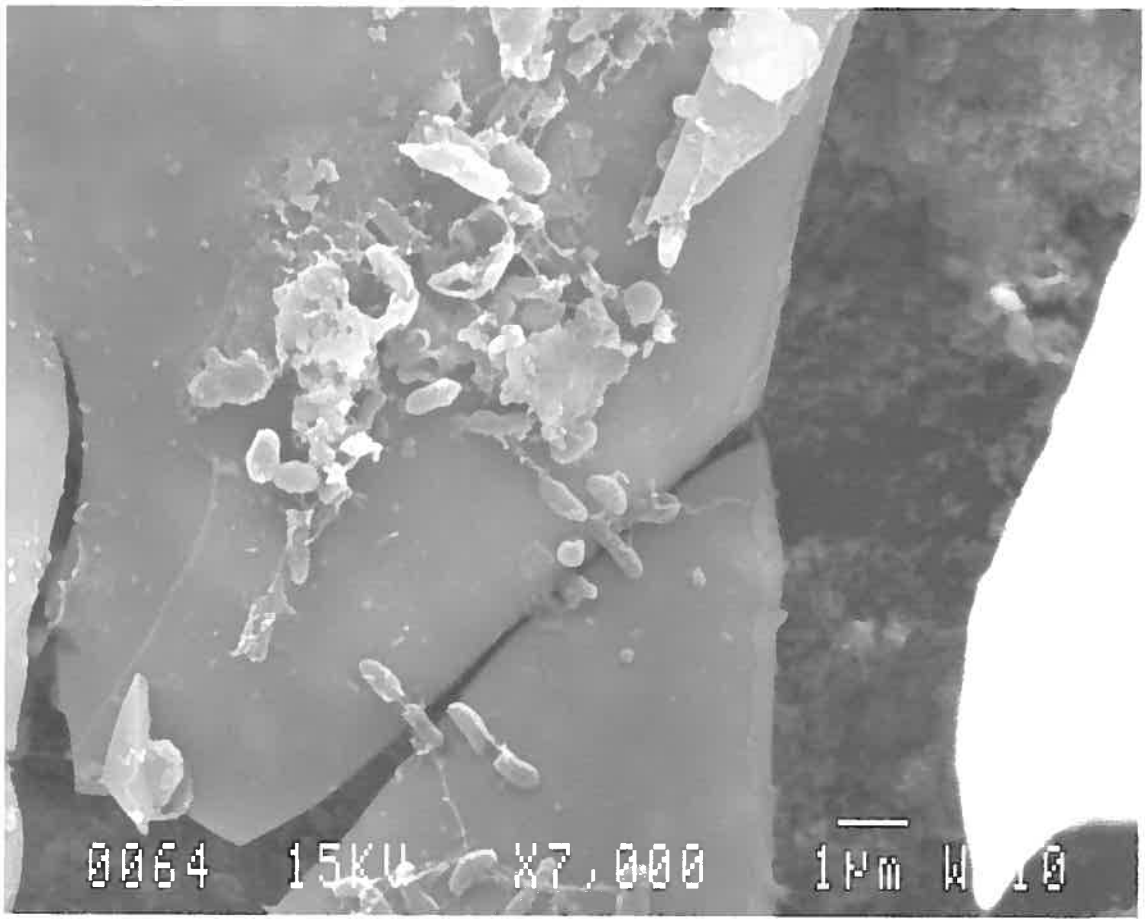
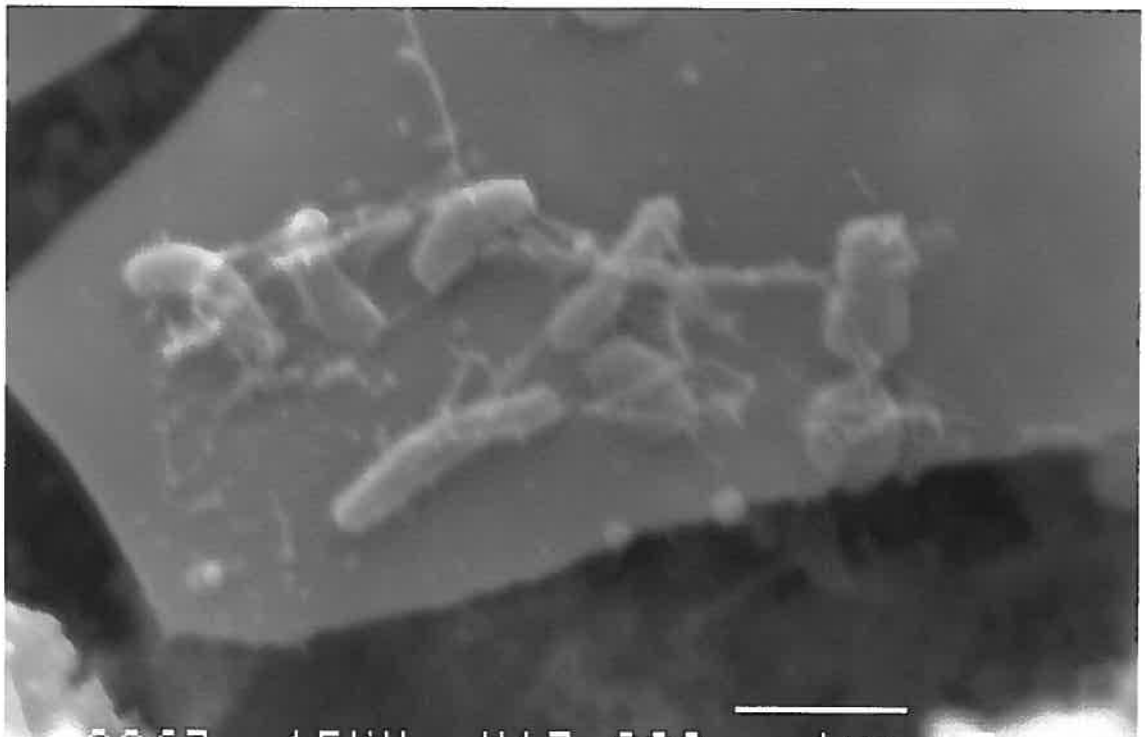


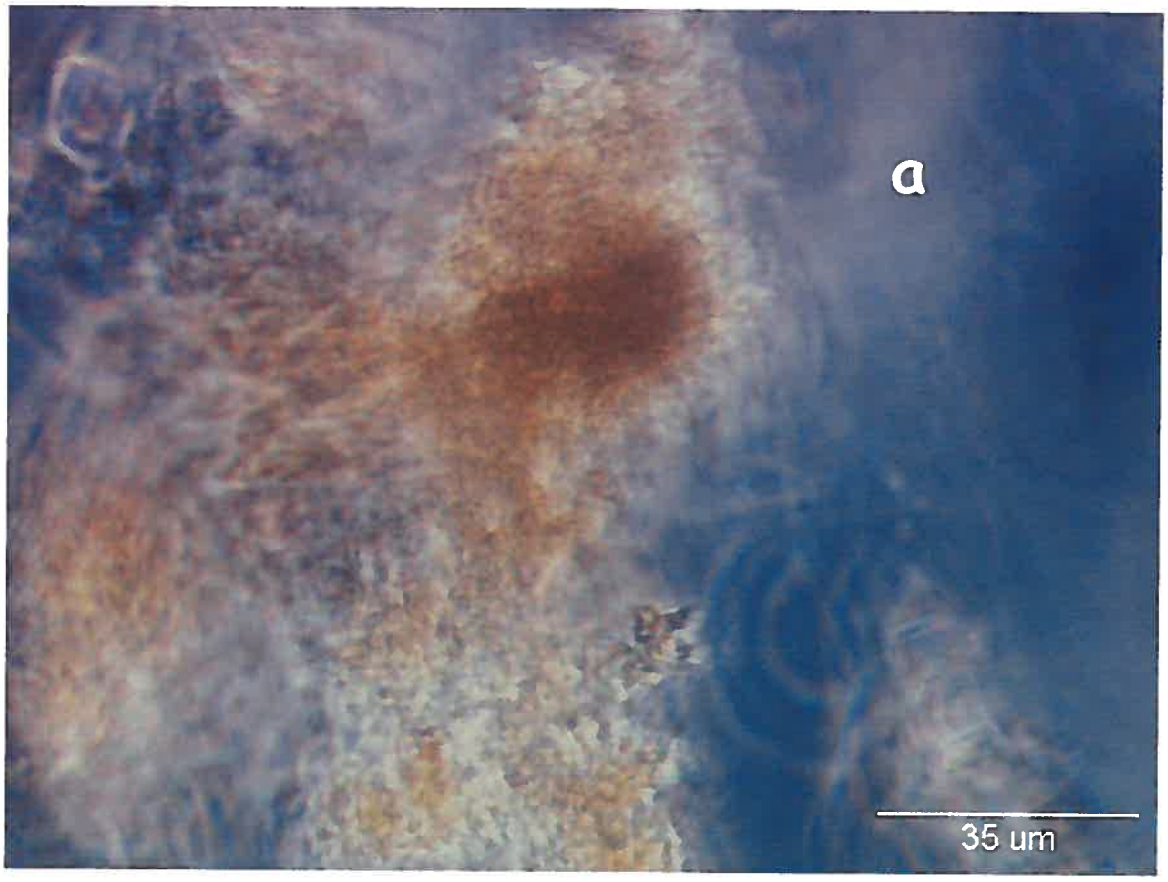
Fig. 2. A biogeochemical diagram of a linked Fe cycle occurring within sediments. Fe^{3+} is reduced in anoxic lower sediments to Fe^{2+}, usually coupled to oxidation of organic matter. This Fe^{2+} diffuses up in the sediments where it can be reoxidized by bacteria coupling this oxidation step to chemolithotrophy. Implications for geochemically relevant questions such as contaminant capture by solid phase oxidized iron or release by reduced soluble iron are also shown.





ig. 3 SEM of biofilms formed on
n Fe reduction column

Fe oxide particle(?) from "Fe oxidizing column



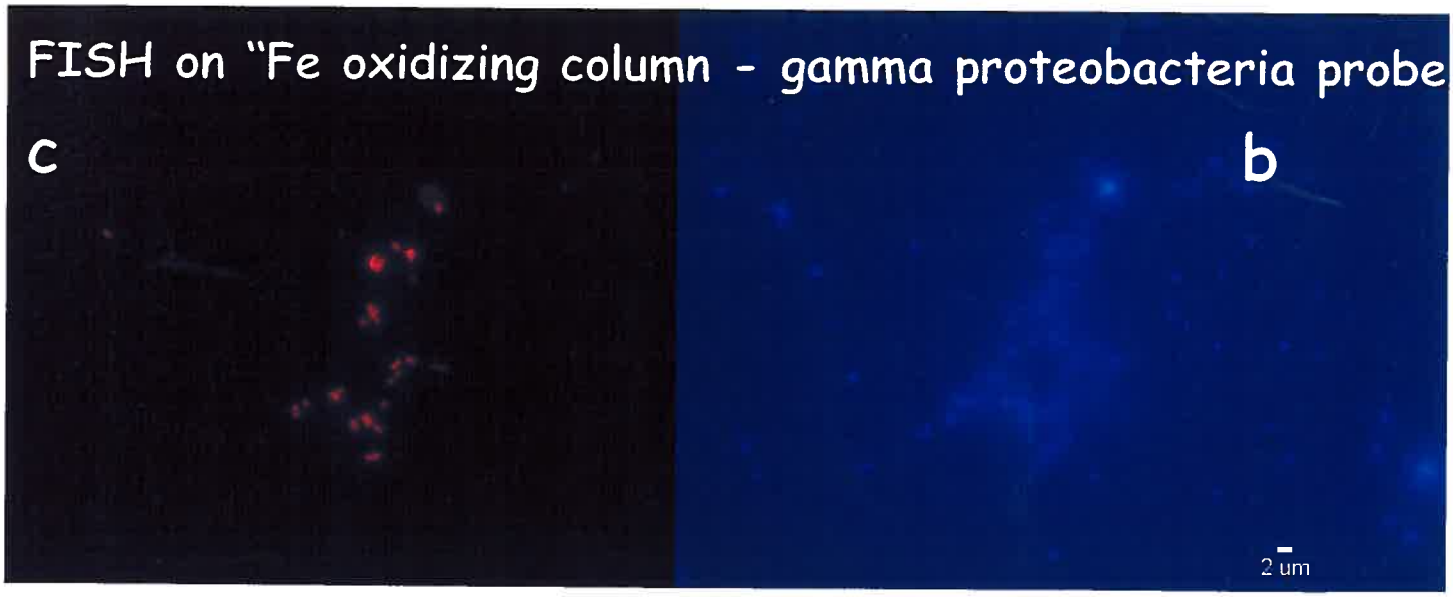


Fig. 4 phase contrast image (a) and FISH results (b DAPI; c gamma proteobacteria probe) on slide inserted in the Fe oxidizing column