HAL open science

Dissimilatory iron reduction in the presence of hydrogen: a case study of microbial activity and nuclear waste disposal

Marta K Schütz, Marie Libert, Michel Schlegel, Jean-Eric Lartigue, Olivier Bildstein

To cite this version:

Marta K Schütz, Marie Libert, Michel Schlegel, Jean-Eric Lartigue, Olivier Bildstein. Dissimilatory iron reduction in the presence of hydrogen: a case study of microbial activity and nuclear waste disposal. Procedia Earth and Planetary Science, 2013, Proceedings of the Fourteenth International Symposium on Water-Rock Interaction, WRI 14, 7, pp.409 - 412. 10.1016/j.proeps.2013.03.177 . hal-02865971

HAL Id: hal-02865971

https://hal.science/hal-02865971v1

Submitted on 12 Jun 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Available online at www.sciencedirect.com

SciVerse ScienceDirect

Procedia Earth and Planetary Science 7 (2013) 409 – 412

Procedia
Earth and Planetary Science

Water Rock Interaction [WRI 14]

Dissimilatory iron reduction in the presence of hydrogen: a case study of microbial activity and nuclear waste disposal

Marta K. Schütz[a,c]*, Marie Libert[a], Michel L. Schlegel[b], Jean-Eric Lartigue[a], Olivier Bildstein[a]

[a]*CEA Cadarache, DEN, DTN/SMTM/LMTE, 13108 Saint Paul lez Durance, France*
[b]*CEA Saclay, DEN, DANS/DPC/SCP/LRSI, F91191 Gif-sur-Yvette, France*
[c]*Aix-Marseille Université, Sciences de l'Environnement, 13545 Aix en Provence, France*

Abstract

Long-term corrosion in water-saturated conditions is the main factor responsible for the alteration of nuclear waste packages. However, the formation of passive layers (e.g. magnetite) on the metal surface can generally be considered a protective mechanism against corrosion. Understanding the impact of living microorganisms on the long-term durability of metallic packages is still an open issue, especially with regard to microbiologically influenced corrosion (MIC) processes. This study examines the impact of microbial activity on the reduction of structural Fe(III) in magnetite. The results demonstrate that such Fe oxides are available as electron acceptors using H_2 as electron donor for microbial metabolism, which may have a direct effect on the rates of corrosion.

© 2013 The Authors. Published by Elsevier B.V. Open access under CC BY-NC-ND license.
Selection and/or peer-review under responsibility of the Organizing and Scientific Committee of WRI 14 – 2013

Keywords: hydrogen; magnetite; iron-reducing bacteria; anoxic metallic corrosion; nuclear waste geological disposal.

1. Introduction

The French concept of high-level radioactive waste disposal relies on a multi-barrier system. The waste is confined in a glass matrix packaged into multiple metallic envelopes buried at 500 m depth in natural clay formations. Such system has been designed in order to prevent the migration of radionuclides as well as to prevent water penetration into the radioactive waste repository [1].

The environmental conditions within the repository will change with time. After the closure, the conditions will be warm and oxidizing (10 to 100 years). The temperature will then decrease and the

* Corresponding author. Tel.: +33 4 42254942; fax: +33 4 42256272.
E-mail address: martakerber@gmail.com.

1878-5220 © 2013 The Authors. Published by Elsevier B.V. Open access under CC BY-NC-ND license.
Selection and/or peer-review under responsibility of the Organizing and Scientific Committee of WRI 14 – 2013
doi:10.1016/j.proeps.2013.03.177

conditions will change to anoxic because the radiation rate is reduced and oxygen is consumed, for example, by aerobic corrosion and microbial activities. A re-saturation of the repository by interstitial water of the geological formation will ultimately occur [1], with concomitant corrosion of the metallic envelopes. A better understanding about these processes, especially over a long time, is needed to ensure a safe disposal.

Several studies have demonstrated that bacteria and archaea are present in most of the deep geological formations already investigated, surviving even under extreme conditions [2,3]. In addition, introduction of microorganisms into disposal repository is also expected as a consequence of the human activities during the construction and operational phases. Within nuclear waste repository, metallic corrosion can be considered as a source of nutritional and energetic substrates for microbial growth. Under anoxic conditions, hydrogen will be produced and iron (hydr)oxides (e.g. magnetite, Fe_3O_4) will be formed on the metal surface, which can act as a protective way against corrosion (as passive layer). Therefore, the activities of hydrogen-oxidizing bacteria (HOB) and iron-reducing bacteria (IRB) become parameters that also need to be evaluated regarding the safety of the current disposal concept.

This paper investigates the microbial activity in presence of hydrogen (as electron donor) and magnetite (as electron acceptor) through a series of experiments. The goal is to evaluate if *Shewanella oneidensis* strain MR-1 (IRB and HOB model organism) is capable of reducing the structural Fe(III) from magnetite coupled to H_2 oxidation for respiration. Such Fe(III) bioavailability in iron (hydr)oxides can likely alter the protective properties of passive layers, which could enhance the anoxic corrosion rate.

2. Materials and Methods

All experiments were performed under strictly sterile conditions in batch reactors at 30°C under an anaerobic $H_2/N_2/CO_2$ (60:30:10%, respectively) atmosphere. Both abiotic and biotic conditions were carried out.

2.1. Bacteria

Shewanella oneidensis strain MR-1 (ATCC 700550™) was chosen as model of IRB and HOB. Cultures were grown aerobically to a stationary growth phase in Luria Bertani Broth (LB) medium (5 $g.L^{-1}$ NaCl, 10 $g.L^{-1}$ tryptone, 5 $g.L^{-1}$ yeast extract) for 24 h at 30°C. Bacterial cells were harvested from the LB medium by centrifugation (4000 rpm/20 min), washed once with sterile minimal medium (M1) and then inoculated into the reactors (initial concentration $8x10^7$ $cells.mL^{-1}$ counted by epifluorescence method with LIVE/DEAD® *Bac*Light™ kit).

2.2. Experimental medium

A chemically defined minimal medium (M1) was prepared according to Kostka and Nealson (1998) [4]. However, minor modifications were made to the composition [5] in order to obtain a representative solution of the groundwater found in the clay formations for geological disposal in France. The final composition was the following: 9 mM $(NH_4)_2SO_4$, 0.5 mM K_2HPO_4, 0.3 mM KH_2PO_4, 2 mM $NaHCO_3$, 0.8 mM $MgSO_4.7H_2O$, 0.4 mM $CaCl_2.2H_2O$, 45 µM H_3BO_3, 10 µM NaCl, 4 µM $FeSO_4.7H_2O$, 5 µM $CoSO_4.7H_2O$, 5 µM $NiSO_4.6H_2O$, 3 µM $Na_2MoO_4.2H_2O$, 11 µM Na_2SeO_4, 1 µM $MnSO_4.H_2O$, 0.8 µM $ZnSO_4.7H_2O$, 0.2 µM $CuSO_4.5H_2O$, 17 mM HEPES buffer; amino acids (0.11 M arginine, 0.13 M glutamate, 0.19 M serine); and vitamins (0.08 mM nicotinic acid, 0.01 mM thiamine-HCl, 0.40 µM biotine). The pH was adjusted to approximately 7 with NaOH and then the medium was sterilized by

autoclaving (120°C for 20 min) or filtration (0.22 µm), depending on the presence of components that cannot resist to heating (e.g. amino acids).

2.3. Magnetite

Magnetite ($FeO:Fe_2O_3 = Fe_3O_4$) is an iron oxide of mixed oxidation state [Fe(II), Fe(III)]. Samples were prepared using the synthesis method described by Schwertmann and Cornell (1991) [6]. A solution of 0.3 M $FeSO_4.7H_2O$ (560 mL) was heated to 90°C and then a mixture (240 mL) of 0.27 M in KNO_3 and 3.33 M in KOH was added dropwise. The suspension was maintained at 90°C for 1 h with continuous stirring and cooled overnight. All synthesis was carried out under N_2 atmosphere. At the end, a dark black solid was obtained, which was washed several times with degassed Milli-Q water followed by freeze drying. The samples were sterilized by UV irradiation for 30 min under anaerobic conditions and an amount of 7 $g.L^{-1}$ was added into the reactors.

2.4. Fe(II)/Fe(III) speciation

Fe(II)/Fe(III) speciation in solution was analyzed colorimetrically using the Ferrozine assay [7], which was extensively described by Viollier et al. (2000) [8]. Sampled suspensions were filtered through 0.02 µm into 0.5 N HCl (50:50 v/v). This filtrate was considered as the soluble Fe(II) fraction. Since Fe(III) cannot be analyzed directly, a reducing reagent (hydroxylamine) was used under strongly acidic conditions in order to obtain the total Fe concentration. Consequently, Fe(III) concentrations were obtained using the difference between total Fe and Fe(II) concentrations. The absorbance of the samples was determined by spectroscopy at 562 nm (Tecan, Infinite M1000).

3. Results and Discussion

Our results demonstrate the ability of *Shewanella oneidensis* to reduce Fe(III) from magnetite in presence of H_2 as the sole electron donor. Figure 1 shows total Fe concentration with Fe(II)/Fe(III) speciation for abiotic (Fig. 1a) and biotic (Fig. 1b) conditions.

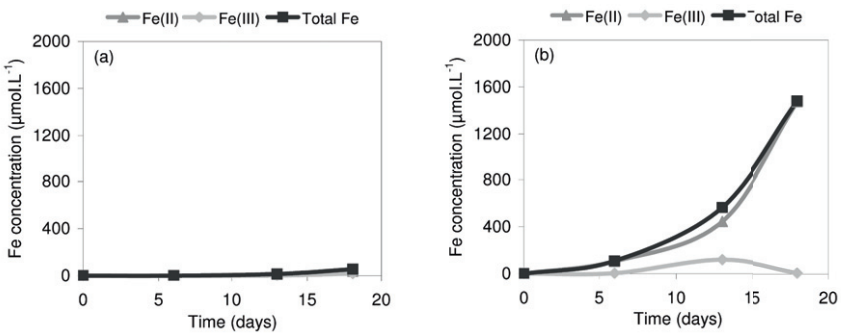

Fig. 1. Magnetite bioreduction: Fe(II) (▲), Fe(III) (◆) and total Fe (■) concentrations; (a) abiotic and (b) biotic conditions.

No magnetite dissolution/reduction was observed in abiotic conditions (Fe concentrations remain constant over 18 days). In contrast, an effect of the bacterial activity was evidenced with respect to Fe(II)

production. The rate of magnetite reduction was initially slow followed by a rapid increase after 10 days, showing an increase with time of soluble Fe(II). The bioreduction was followed by a slight increase in the pH (results not shown), which is in agreement with the literature [9,10]. The pH reached a final value of 6.8 with a starting pH of 6.6 (pH remains constant for abiotic conditions).

The results showed that 1500 $\mu mol.L^{-1}$ of Fe(III) was reduced over 18 days, assuming that 1 mol of Fe(II) was produced for each mol of Fe(III) reduced. Therefore, 2.4% of the total amount of Fe(III) was reduced from magnetite (initial amount 62 $mmol.L^{-1}$). H_2 was not completely consumed by *Shewanella oneidensis* (results not shown), suggesting that at higher concentrations (in this study 80 $mmol.L^{-1}$), the efficiency of H_2 oxidation coupled to Fe(III) reduction is significantly decreased. Similar results were reported by Fredrickson et al. (2003) [10] using lactate (20 $mmol.L^{-1}$) as the sole electron donor; an amount of 52% of lactate oxidation was observed for 50 $mmol.L^{-1}$ of 2-line ferrihydrite. Lactate oxidation was greater at low concentrations (e.g. 93% for 1 $mmol.L^{-1}$ lactate).

4. Conclusion

The activity of iron-reducing bacteria has implications for natural biogeochemical processes as well as for fate and transport of multivalent metals and radionuclides. The results presented herein demonstrate that a crystalline Fe oxide of mixed oxidation state is available as electron acceptor for bacterial metabolism. Regarding the issues related to metallic corrosion within nuclear waste repository, it is evidenced that such Fe(III) bioavailability in addition to the presence of H_2 can potentially alter the protective properties of passive layers, which may have a direct effect on anoxic corrosion rate. The new mineral phases formed by magnetite reduction have still to be investigated in order to better understand the biomineralization processes involved.

Acknowledgements

This research was funded by a grant (n° 238579) from the European Community's Seventh Framework Programme (FP7/2007-2013).

References

[1] Andra. Dossier Argile: Synthesis - Evaluation of the feasibility of a geological repository in an argillaceous formation. Reference 266VA, 2005.

[2] Pedersen K. Microbial processes in the disposal of high level radioactive waste 500 m underground in Fennoscandian Shield rocks. In: *Interactions of Microorganisms with Radionuclides*, Elsevier; 2002, p. 279–311.

[3] Stroes-Gascoyne S, Hamon CJ, Dixon DA, Martino JB. Microbial analysis of samples from the tunnel sealing experiment at AECL's Underground Research Laboratory. *Phys Chem Earth* 2007; **32**: 219–231.

[4] Kostka JE, Nealson KH. Isolation, cultivation, and characterization of iron- and manganese-reducing bacteria. In: *Techniques in Microbial Ecology*, Oxford University Press, New York, 1998, p. 468.

[5] Esnault L, Jullien M, Mustin C, Bildstein O, Libert M. Metallic corrosion processes reactivation sustained by iron-reducing bacteria: Implication on long-term stability of protective layers. *Phys Chem Earth* 2011; **36**: 1624–9.

[6] Schwertmann U, Cornell RM. *Iron Oxides in the Laboratory: Preparation and Characterization*. New York: Weinheim; 1991.

[7] Stookey LL. Ferrozine - A New Spectrophotometric Reagent for Iron. *Anal Chem* 1970; **42**: 779–781.

[8] Viollier E, Inglett PW, Hunter K, Roychoudhury AN, Van Cappellen P. The Ferrozine method revisited: Fe(II)/Fe(III) determination in natural waters. *Appl Geochem* 2000; **15(6)**: 785–790.

[9] Dong H, Fredrickson JK, Kennedy DW, Zachara JM, Kukkadapu RK, Onstott TC. Mineral Transformation Associated with the Microbial Reduction of Magnetite. *Chem Geol* 2000; **169**: 299–318.

[10] Fredrickson JK, Kota S, Kukkadapu RK, Liu C, Zachara JM. Influence of Electron Donor/Acceptor Concentrations on Hydrous Ferric Oxide (HFO) Bioreduction. *Biodegr* 2003; **14**: 91–103.