Influence of hydrogen in the presence of organic matter on bacterial activity under radioactive waste disposal conditions

Camille Chautard, A. Ritt, M. Libert, Laurent de Windt

Influence of hydrogen in presence of organic matter on bacterial activities under radioactive waste disposal conditions

C. Chautard[a,c,*], A. Ritt[b], M. Libert[c], L. De Windt[d]

[a] *IRSN, PRP-DGE/SEDRAN/BERIS, B.P. 17, 92262 Fontenay-aux-Roses Cedex, France*
[b] *IRSN, PRP-DGE/SRTG/LAME, B.P. 17, 92262 Fontenay-aux-Roses Cedex, France*
[c] *CEA, DEN/DTN/SMTM/LMTE, bât 307, 13108 Saint Paul Lez Durance Cedex, France*
[d] *Mines-ParisTech, Geosciences Dpt., 77305 Fontainebleau Cedex, France*

Abstract

According to the French design for the disposal of high level radioactive waste (HLW), waste will be emplaced in an environment involving metallic materials into a geological clay formation. The presence of microorganisms has recently been evidenced in such environments. Therefore, at this state of knowledge, the introduction of microbial species during the construction and operational phases as well as the survival of bacteria after the disposal closure are to be accounted for in safety assessment context. Sulfate-reducing bacteria (SRB) activities are notably expected to have an impact on corrosion processes and thus to influence the evolution of metallic and clayey materials involved in a HLW disposal cell. The present work investigates the potential development of a SRB, Thermodesulfovibrio hydrogeniphilus, in order to better assess its metabolism in the presence of dissolved organic matter (DOM), representative of the DOM present in an argillaceous pore water, and hydrogen which will be produced by the anaerobic corrosion of metallic materials. After 49 days of batch experiments, hydrogen enhances the bacterial development in presence of a low amount of DOM, whereas the DOM alone does not seem to sustain bacteria activities.

Keywords: Radioactive waste, Sulfate reducing bacteria, Hydrogen, Organic Matter

1. Introduction

The facility design developed by Andra in France for HLW disposal into a geological argillaceous formation involves metallic materials (containers, overpacks, liner) [1]. The anoxic and saturated conditions prevailing in this geological medium will lead, after a transient period, to the anoxic aqueous corrosion of these metallic materials and as a consequence to the release of aqueous iron and the production of hydrogen.

* Corresponding author. Tel.: +3-344-225-3039; fax: +3-344-225-6272;
E-mail address: camille.chautard@irsn.fr.

The presence of microorganisms has recently been evidenced in deep clayey environment [2, 3]. Besides, microbial species will be introduced during the construction and operational phases of the repository. Bacteria will be able to tolerate an environment with few nutrients to sustain life under dry, highly radioactive, low porosity and high temperature conditions [4]. SRB activities are expected to influence the corrosion processes and may consequently impact the integrity of the overpack. Thus, their potential development must be investigated in order to better assess their metabolism, which may influence the evolution of metallic materials involved in a HLW disposal cell.

Microorganisms need energetic substrates for their metabolic activities (electron donor and acceptor), as well as carbon, phosphate and nitrogen sources for the cell synthesis. Sulfates used by SRB as electron acceptor will not limit the metabolism reaction because of their presence in significant amount in argillaceous pore water [5]. The reduction of a sulfate molecule by SRB consumes eight electrons that are provided by an electron donor from the environment. Deep argillaceous formations generally contain low amounts of biodegradable OM (electron donor) in pore water [6] and are, therefore, nutrient poor for microbial development. However, the radiolysis of pore water and the corrosion of metallic components of HLW disposal cell in anoxic conditions will lead to the production of hydrogen, which may also be used as an electron donor for microbial activity [7].

To study bacteria impact on metallic materials, an experiment was carried out using a percolation cell in conditions that may occur in HLW vaults [8]. SRB survival was observed after twelve months of experiment. The purpose of the present work is to quantify in batch experiments the potential for bacterial growth stimulation due either to the production of hydrogen or to the presence of OM in the percolation cell.

2. Materials and methods

2.1. Batch systems

Experiments were performed in batch reactors (volume solution 150 ml). Series 1 and 2 (Table 1) were conducted under hydrogen atmosphere (9 mmol, H_2 60%, N_2 30%, CO_2 10%), whereas the series 3 was bubbled with nitrogen gaz (N_2 90%, CO_2 10%) to maintain anaerobic conditions. A solution of OM (1 mmol), consisting of a mixture of small organic acids (fumarate, acetate, lactate, formate, propionate) such as those present in pore water of argillaceous formations [7], was added in series 2 and 3. Bacterial inoculum was prepared from a bacterial culture, which was washed by centrifugation (4000 rpm, 20 min). Batches were incubated at 60°C. The initial cell density of all cultures (10^7 bacteria.ml^{-1}) was estimated by direct cell counts. Control experiments without bacteria were carried out under the same conditions.

2.2. Bacteria species and culture medium

A sulfate reducing, thermophilic (growth optimum 65°C) and anaerobic bacteria, *Thermodesulfovibrio hydrogeniphilus,* was used in the present experiments. This bacteria is able to use hydrogen, acetate, malate, formate as electron donors [9]. The culture medium was prepared by dissolving high purity chemicals in deionized water and sterilized after preparation by heating (120°C). Its chemical composition was close to the Tournemire synthetic pore water solution used in the percolation cell [8].

2.3. Chemical analyses and bacterial population measurement

Dissolved organic carbon (DOC) and sulfates in the liquid phase were periodically monitored by using total organic carbon analyzer and ion chromatography. Likewise hydrogen partial pressure was monitored by gas chromatography. The microbial activity was monitored by counting cell bacteria using a direct counting epifluorescence method (backlight kit).

3. Results

Figure 1 presents the evolution of hydrogen, sulfate and bacterial development during 49 days of experiment in series 1 and 2. The amount of hydrogen decreases in the series 1 in the biotic experiments: 4 mmol are consumed after 49 days of experiment. Such a decrease is also observed in the series 2. Regarding the bacterial population of each biotic experiment, it fluctuates around a mean value of $1.1\ 10^7$ bacteria per ml. A doubling of the population is observed in the series 2 after one month of experiment in relation to the sulfate concentration decrease (1.7 to 0.6 mmol). The sulfate reduction yield is about 83% with OM (series 2), but only 20% without OM (series 1). However, after 49 days of experiment, the OM concentration of the series 2 presents no evolution and is equal to the initial concentration (1 mmol, data not shown). Several works have shown than SRB of the genus *Desulfovibrio* required the presence of acetate in addition to CO_2 for cell synthesis (carbon source) [10], which explains the low sulfate reduction yield in the series 1.

The series 3 (not shown) reveal a decrease of the bacteria population, while no significant evolution of sulfate and OM concentration is observed. This may be an indication for a slower metabolic development in presence of a low amount of OM as electron donor (1 mmol) than in presence of hydrogen.

4. Conclusion

The batch experiments performed in the present work, which involves SRB with hydrogen and/or OM in a clay pore water, have shown that hydrogen in presence of organic matter enhances the bacterial development, while hydrogen or organic matter alone shows low or no effect. This illustrates the importance that hydrogen may have as energetic substrate for bacterial development and indicates that hydrogen may enhance bacterial development in HLW disposal conditions, even with small amounts of organic matter released in the pore water of argillaceous formations. However, the influence of parameters such as OM and hydrogen concentration or the duration of experiments should be further investigated in order to confirm these results.

Acknowledgements

We thank A. Martin-Garin and S. Bassot from IRSN and their colleagues for their technical support.

References

1. Andra, Dossier argile 2005 : phenomenological evolution a geological repository. Rapport Andra, 2005.
2. L. Urios, F. Marsal, D. Pellegrini and M. Magot, Microbial diversity of the 180 million-year-old Toarcian argillite from Tournemire, France. Applied Geochemistry, 2012. 27(7): p. 1442-1450.
3. S. Poulain, Caractérisation microbiologique de l'argile à Opalinus du Mont Terri et de l'argilite du Callovo-Oxfordien de Meuse/Haute-Marne in Thèse doctorat : Sciences chimiques. CNAB : Bordeaux 1. 2006.

4. K. Pedersen, Exploration of deep intraterrestrial microbial life: current perspectives. Fems Microbiology Letters, 2000. 185(1): p. 9-16.
5. C. Beaucaire, J.L. Michelot, S. Savoye and J. Cabrera, Groundwater characterisation and modelling of water-rock interaction in an argillaceous formation (Tournemire, France). Applied Geochemistry, 2008. 23(8): p. 2182-2197.
6. A. Courdouan, I. Christl, S. Meylan, P. Wersin and R. Kretzschmar, Isolation and characterization of dissolved organic matter from the Callovo-Oxfordian formation. Applied Geochemistry, 2007. 22(7): p. 1537-1548.
7. M. Libert, O. Bildstein, L. Esnault, M. Jullien and R. Sellier, Molecular hydrogen: An abundant energy source for bacterial activity in nuclear waste repositories. Physics and Chemistry of the Earth, Parts A/B/C, 2011. 36(17–18): p. 1616-1623.
8. C. Chautard, J.-E. Lartigue, M. Libert, F. Marsal and L. De Windt, An integrated experiment coupling iron/clay interactions with bacterial activity. Procedia Chemistry, in press.
9. O. Haouari, M.L. Fardeau, J.L. Cayol, G. Fauque, C. Casiot, F. Elbaz-Poulichet, M. Hamdi and B. Ollivier, Thermodesulfovibrio hydrogeniphilus sp nov., a new thermophilic sulphate-reducing bacterium isolated from a Tunisian hot spring. Systematic and Applied Microbiology, 2008. 31(1): p. 38-42.
10. F. Widdel and T.A. Hansen, Dissimilatory Sulfate- and Sulfur-Reducing Prokaryotes, in The prokaryotes: a handbook on the biology of bacteria: ecophysiology, isolation, identification, applications, A.T. Balows, H.G. ; Dworkin, M. ; Harder, W. ; Schleifer, K.H., Editor. 1992, Springer-Verlag. p. 582-624

Table 1: Batch experiment conditions.

	Series 1	Series 2	Series 3
Gas and dissolved hydrogen (mmol)	9	9	0
Dissolved OM (mmol)	0	1	1

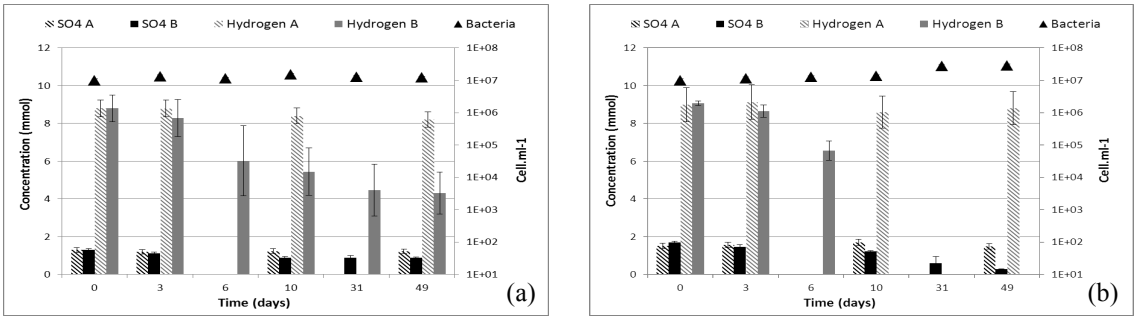

Figure 1. Evolution of sulfate, hydrogen and bacteria in series 1 (a) and in series 2 (b), average of three replicates, in both abiotic (A) and biotic (B) conditions. No bacterial development and no chemical evolution were observed in the control experiments. No measurement of hydrogen (A) and sulfate (A and B) were done at 6 days in each series. No measurement of hydrogen (A) and sulfate (A) were done at 31 days. Regarding series 2 (b), the absence of hydrogen (Hydrogen A) measured after 6 days of experiment is due to the low gas pressure of the batches because of liquid sampling in the same batches.