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Experimental and numerical modeling of bacterially induced pH increase and calcite precipitation in saline aquifers

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ABSTRACT

As a part of an effort to investigate potential implications of microbial activity upon CO_2 geological sequestration, both the alkalinization of a urea-containing artificial ground water and the subsequent calcium carbonate precipitation, induced by *Bacillus pasteurii*, have been studied in batch experiments. Four reproducible stages of this microbial process were identified and numerically modeled: (1) a rapid rise of pH values caused by bacterial ureolysis, (2) a pH plateau due to a dynamic equilibrium between CO_2 transfer through the liquid/gas interface and the ureolysis process, (3) a decrease in pH due to $CaCO_3$ precipitation (4) a slow long term evolution of pH depending on the presence of viable microorganisms which have survived to carbonate precipitation. Correlations between the durations and pH values of these four steps were also evidenced. To interpret quantitatively the observed trends, the geochemical code CHESS© was adapted for taking into account the enzymatically catalyzed ureolysis reaction as well as the kinetics of gas/solution exchanges and the rate of calcium carbonate precipitation. Finally, new original aspects of *B. pasteurii* biomineralization were evidenced, namely a cellular calcium phosphate precipitation preceding the formation of calcite and a negative impact of phosphate on ureolysis and calcite precipitation.

1. Introduction

Microbiological activities that occur in subsurface environments are largely unknown, although numerous studies have documented their impact on various geological processes (e.g. Edwards et al., 2005; Severmann et al., 2006; Tuck et al., 2006). Their roles in carbonate precipitation, mineral alteration, oil and gas souring and petroleum maturation have strong economic and environmental implications (e.g. Premuzic and Lin, 1999; Wolfgang, 2003). Recently, intensive research on CO_2 geological storage has been undertaken to tackle climate changes by mitigating the environmental consequences of energy production from hydrocarbon fuels. As a result, large amounts of this gas may need to be stored underground as part of a future global atmospheric stabilization strategy. In particular, it might be sequestrated into depleted oil and gas reservoirs as well as in deep saline aquifers, deep-seated coal beds or fractured (ultra)basic rocks, which altogether offer very important storage capacities around the world (e.g. White et al., 2003; Bachu et al., 2007). This approach will require the ability to model the injection of carbon dioxide into deep geological reservoirs and to predict its fate for thousands of years after disposal. In accordance, identification and proper knowledge of the critical controlling processes are strongly required. The deep biosphere could have, in that perspective, far reaching implications for CO_2 geological sequestration. However, at present, little is known about the involved biogeochemical processes. The development of conceptual and numerical models that would incorporate biotic processes both at the pore and reservoir scales has been hindered by a lack of understanding of the major involved parameters. To define relevant parameters needed for kinetic and thermodynamic computational models focused on microbe/mineral/pore fluid processes, improved understanding of interactions between subsurface microorganisms and CO_2 enriched environment is mandatory. In particular, the ability of a large variety of aerobic and anaerobic microorganisms to initiate and enhance carbonate precipitation (e.g. Boquet et al., 1973; Visscher and Stolz, 2005) is of noticeable interest as it may be employed to convert the injected CO_2 into mineral, which is seen as one of the safest CO_2 trapping mechanisms (Bachu, 2008). By considering that this process could constitute a potential mean of sealing pores and fractures in rocks, microbially mediated mineralization could also have adverse effects by decreasing porosity, hence injectivity, close to the well and thus should also be studied in this respect. Among the biological processes leading to CO_2 mineralization into solid carbonate phases, the bioalkalinization, i.e. pH increase of the local environment related to metabolic activities of microorganisms, constitutes a well-documented mechanism. The pH increase

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could indeed act against the acidification yielded by the CO_2 injection, which usually prevents precipitation of solid carbonates. In addition, biologically induced precipitation of calcium carbonates by alkalinizing bacteria appears to be an ubiquitous phenomenon related to various metabolisms including photosynthesis, sulphate-reduction, methanogenesis, oxidation of organic carbon or organic nitrogen and ureolysis (e.g. Boquet et al., 1973; Knorre and Krumbein, 2000; Braissant et al., 2004; Baumgartner et al., 2006).

To quantitatively evaluate the alkalinizing potential of microorganisms and its consequences in terms of biomineralization, we developed a combined experimental and numerical approach based on *Bacillus pasteurii*, a well-known model of carbonate precipitating microorganism (e.g. Ferris et al., 2003; Fujita et al., 2004), which was recently reclassified as *Sporosarcina pasteurii* (Yoon et al., 2001). This microorganism is not directly relevant to deep subsurface applications but is commonly used for modeling underground biomineralization. This ureolytic bacterium uses urea hydrolysis for both energy generation by ionic gradients (Mobley and Hausinger, 1989; Smith et al., 1993; Jahns, 1996) and nitrogen assimilation (Nielsen et al., 1998; Swensen and Bakken, 1998). As summarized in Eqs. (1) and (2), urea hydrolysis generates an increase in pH which can induce calcium carbonate precipitation by saturation of the calcite system (Ferris et al., 1996; Stocks-Fisher et al., 1999).

$$(H_2N)_2{-}CO + 2H_2O \rightarrow 2NH_4^+ + CO_3^{2-} \quad (1)$$

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3 \quad (2)$$

The kinetics of calcium carbonate precipitation in response to the hydrolysis of urea by *B. pasteurii* in an artificial ground water (AGW) has been extensively investigated, in particular by Ferris et al. (2003) and Mitchell and Ferris (2005). Some specific aspects, however, were not considered in these previous studies, namely the details of the pH-time history and the explicit calculation of the gas/solution exchanges allowing to predict exact pH values at the different stages of the process. The main purpose of the present study is thus to measure accurately and calculate quantitatively the pH variations upon alkalinization and subsequent carbonate precipitation induced by *B. pasteurii* inoculated in an AGW taking into account exchanges between the aqueous and gaseous phases. For this purpose, a set of experiments was first conducted for providing a full range of pH measurements by varying the main parameters of the ureolysis reaction and resulting precipitation, namely inoculum size, urea concentration, atmospheric contact and presence of phosphate, which is a known carbonate crystallization inhibitor (e.g. Stumm and Morgan, 1996). The advanced geochemical speciation modeling tool CHESS© (van der Lee, 1998) was adapted and used in order to analyze the involved biogeochemical process and gas/solution exchanges and interpret the evolution of this complex system.

2. Materials and methods

2.1. Artificial ground water (AGW)

The AGW medium used during these experiments was prepared in Ultra Pure Water (UPW, resistivity = 18 MΩ), based on the aqueous chemistry of the Dogger aquifer (Paris Basin, France), a limestone assemblage (200–300 m thick) of Middle Jurassic age presenting a maximum burial depth of 1900 m below sea level. This stratigraphic level constitutes a possible pilot site for CO_2 sequestration in France. The saline solutions from the aquifer are essentially of the Na–Cl type with relatively low Ca concentrations (average of 20 mM). An average composition of the 67 °C Dogger level coming from analyses of water samples from 17 wells and described by Azaroual et al. (1997) was considered. AGW included Na^+ (42 mM), K^+ (2 mM), Mg^{2+} (8 mM), Ca^{2+} (20 mM), SO_4^{2-} (8 mM), and Cl^- (84 mM), with a final ionic strength of 0.135 M. The solution was complemented with urea (2 or 0.2%), with or without phosphoric acid (4 mg.l^{-1}), and equilibrated at room temperature with the atmosphere at a pH value of 6.00 by acidification with hydrochloric acid. Once sterilized by Millipore filtration (0.2 µm Isopore™), the solutions were kept at 4 °C in sealed bottles before the start of the experiments. At this time, they were equilibrated to the atmosphere at 30 °C with agitation (200 rpm) half an hour before inoculation time (t_0). No significant pH change was observed during this stage.

2.2. Microbial inoculation

B. pasteurii was grown in Brain Heart broth (MERCK) complemented with 20 g.l^{-1} of urea. Pilot cultures were freshly inoculated with the strain ATCC11859 (as referenced by the American Type Culture Collection) from the Deutsche Sammlung von Mikroorganismen und Zellkulturen, DSMZ (N° 33) and kept overnight at 30 °C with agitation (200 rpm). The cultures that were sustaining an exponential growth were washed two times by centrifugation (8500 *g*, 10 min) in UPW and used afterward as inoculation material for the experiments. For this purpose, cells were suspended in sterile UPW and then mixed at t_0 in equal parts with amended double strength AGW at a final optical density at 600 nm (OD_{600}) of 0.05 or 0.15. All the solutions were thermally equilibrated with the experimental temperature (30 °C) before use. No crystal seeds were added to the solutions.

2.3. Microbial carbonate precipitation experiments

To evaluate the effects of gas diffusion between atmosphere and solution, experiments were conducted either in 50 ml Falcon® tubes or in 100 ml Erlenmeyer flasks with a total solution volume of 30 ml and 60 ml, respectively. In both experiments, the container was maintained at 30 °C with agitation (200 rpm) but while the Falcon® tubes were loosely covered to allow direct contact with air, the Erlenmeyer flasks were kept closed by cotton caps between the samplings to hinder gas exchanges. The pH and OD_{600} measurements were accomplished regularly throughout all the experiments. The OD_{600} measurements were done by retrieving respectively in the Erlenmeyer and Falcon® experiments, 1 ml and 0.5 ml of medium that were immediately acidified by an equal volume of HCl 1 M. In this process, the solid carbonates were dissolved but the bacterial OD_{600} was preserved as it was checked in separate tests without precipitates (data not shown). Samples of 1 ml were collected for ammonium and calcium analyses. In those aliquots, solid carbonates and cells were immediately separated by centrifugation (8500 *g*, 10 min), and then the pellets and supernatants were both kept at −20 °C. The sampling was limited to a maximal total volume loss of 1/5. At the end of the experiments (24 h), the precipitates were collected by scratching the inner recipient wall. The collected powders were then dried at 40 °C and weighted. Additional pH measurements were also conducted with 40 ml of solution in open beakers maintained in the same experimental conditions. All the experiments were triplicated.

2.4. Chemical and mineralogical analysis

pH, dissolved inorganic nitrogen (DIN), and calcium were measured from samples taken at different experimental steps. Total dissolved inorganic nitrogen issued from ureolysis (i.e. ammonium and ammonia) and calcium were determined using a Shimadzu spectrophotometer (UV-1650 PC) with the Spectroquant® kits 1.14752.0001 ammonium test and 1.14815.0001 calcium test at respectively 690 nm and 550 nm. pH measurements were accomplished with a combined pH glass electrode HANNA HI 1131 (single junction). The calibration of the electrode (two points procedure, HANNA Instruments PH210, precision = ±0.01 pH unit) was realised with certified pH buffer solutions (Fisher Chemical, precision = ±0.01 pH unit) and without adjustments for the ionic strength. To avoid any trouble linked to the deposition of precipitates on the electrode surface, this one was placed in an acidic