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Biodegradability of dissolved organic matter in Boom Clay pore water under nitrate-reducing conditions: Effect of additional C and P sources

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ABSTRACT

In Belgium, Boom Clay is considered a potential host rock for the deep geological disposal of nuclear waste such as Eurobitum, an intermediate-level nitrate-containing radioactive waste form. The presence of dissolved organic matter (DOM) in the Boom Clay may play an important role in the mobility of radionuclides due to complexation and/or reduction of redox-sensitive radionuclides. The biodegradation of this DOM could therefore affect the barrier function of the Boom Clay. Due to the presence of nitrate leaching from Eurobitum into the clay formation, the biodegradation of DOM by a nitrate-reducing population cannot be ruled out in the Boom Clay surrounding a Eurobitum repository. In this paper, the biodegradability of DOM in Boom Clay pore water (BCPW) under nitrate-reducing conditions was investigated in the presence or absence of additional nutrients (*i.e.*, acetate and phosphate) expected to be present in the clay surrounding a Eurobitum repository. The results of these experiments indicate that microbial nitrate reduction and the related degradation of DOM are performed by a syntrophic microbial community but are characterised by very slow kinetics. Nitrate-reducing microorganisms seem to be (at least partially) dependent on fermentation products such as acetate provided by fermenting microorganisms. The observed slow biodegradation of DOM appears to be linked to the characteristics of Boom Clay DOM, rather than to a limitation of nutrients necessary for microbial growth. The addition of phosphate only boosts the microbial growth of certain microbial species but not the biodegradation of DOM. On the other hand, acetate significantly enhances the microbial degradation of DOM. This result can be attributed to the use of acetate as an efficient C source for certain DOM-degrading microbial species and/or as a co-substrate to stimulate DOM degradation.

1. Introduction

Boom Clay is a 30-million-year-old marine sediment in which organic matter (OM) is ubiquitous (total organic carbon or TOC content of 1–5 wt.%) and present in high concentrations in both the solid and the dissolved phases (De Craen et al., 2004; Bruggeman and De Craen, 2012; Durce et al., 2015). Boom Clay solid OM consists predominantly of organic compounds with a rather low thermal maturity, *i.e.,* showing little effect of alteration after its deposition during the Rupelian (Laenen, 1997; Van Geet, 2004; Bruggeman and De Craen, 2012). The Boom Clay pore water contains relatively high levels of dissolved organic matter (115 ± 15 mg C/l on average; De Craen et al., 2004) and is rich in both humic and fulvic acids (in approximately equal amounts) with a large variability in molecular size (Bruggeman and De Craen, 2012; Durce et al., 2015).

This Boom Clay formation is currently studied as a potential host rock for the deep geological disposal of long-lived radioactive waste, such as Eurobitum, an important Belgian bituminised intermediate-level long-lived radioactive waste form. This waste contains 20 to 30 wt % $NaNO_3$ homogeneously dispersed in a Mexphalt R85/40 bitumen matrix (Valcke et al., 2000b). After disposal of the waste in a water-saturated sedimentary formation such as Boom Clay, the uptake of water by Eurobitum due to the presence of the dehydrated and hygroscopic salts will lead to the dissolution of the $NaNO_3$ crystals and subsequently to a diffusion-controlled release of $NaNO_3$ into the Boom Clay (Valcke et al., 2000b, 2010). In addition, certain water-soluble organics are expected to leach from radioactive bituminised waste during hydration with cement-equilibrated clay water. The most important such organic compounds are acetate and formate, which are produced during both chemical and radiolytic degradation of Mexphalt R85/40 bitumen (Valcke et al., 2000a).

A possible consequence of the $NaNO_3$ release is a microbially mediated reduction of nitrate, which can occur in two distinct dissimilatory pathways, leading to the production of nitrogenous gases (denitrification) or ammonium (dissimilatory nitrate reduction to ammonium or DNRA). In both pathways, nitrite is an intermediate product,

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but depending on the availability of the electron donor, nitrate can be reduced to nitrite without subsequent production of gases or ammonium (*i.e.,* dissimilatory nitrate reduction to nitrite or DNRN), resulting in the accumulation of nitrite (*e.g.*, Almeida et al., 1995b; Oh and Silverstein, 1999).

This microbial nitrate reduction in Boom Clay would lead to the oxidation of its redox-active components, such as dissolved organic matter (DOM). This process would therefore lower the overall reducing capacity of Boom Clay towards redox-sensitive radionuclides, thereby possibly enhancing their mobility through the clay. Furthermore, biodegradation of DOM could have an impact on its chemical composition, and thus on colloid formation and/or its complexation capacity with radionuclides (Lenhart et al., 2000; Maes et al., 2006; Schmeide et al., 2012).

Previous studies have shown that microbes are able to reduce nitrate in Boom Clay pore water using DOM as an electron donor, but at a very slow rate (0.2–0.3 μM NO_3^- day^{-1}) (Mariën et al., 2011; Bleyen et al., 2016a). This rate is considerably lower than those observed in other sediments and/or natural waters, *e.g.*, ~20 μM NO_3^- day^{-1} in an anoxic borehole in the Opalinus Clay (Bleyen et al., 2017), ~40 μM NO_3^- day^{-1} in buried riverbed sediments (Pfenning and McMahon, 1997), and up to 34 μM NO_3^- day^{-1} in the anoxic layers of fresh or marine waters (Knowles, 1982). The reason for the slow nitrate reduction rates observed in Boom Clay pore water is, however, not yet clear. This property is either inherent to the biodegradation potential of DOM or to the microbial population present in the Boom Clay pore water. On the other hand, the observed microbial nitrate reduction and related DOM degradation rates in Boom Clay pore water might be underestimated due to a lack of certain nutrients that are necessary for microbial growth or activity. Some of these nutrients can be provided by the Boom Clay or by the waste itself, and could impact the degradation of DOM in the clay surrounding a repository for Eurobitum. Indeed, chemical and radiolytic degradation of bitumen yields products such as acetate that leach from the waste (Valcke et al., 2000a) and could affect certain microbially mediated processes. Furthermore, phosphor species, which are necessary for microbial growth, are limited in Boom Clay pore water (phosphate concentrations in general below detection limit of 0.5 mg L^{-1}). In Boom Clay, however, phosphor is bioavailable in the mineral fraction, such as apatite (De Craen et al., 2004), and could therefore have an impact on the microbial reaction rates.

The present paper aims to evaluate the biodegradability of DOM in Boom Clay pore water under nitrate-reducing conditions, as expected in the clay surrounding a Eurobitum repository. Batch tests were performed with Boom Clay pore water retrieved from a piezometer installed in the Boom Clay layer in Mol, to which nitrate was added. Boom clay pore water was used as is, thereby maintaining the *in situ* microbial population. The biodegradation of DOM was assessed both indirectly by studying the microbial nitrate reduction processes and directly by monitoring changes in TOC, reducing capacity and molecular size distribution of DOM. Furthermore, the effect of phosphate and acetate on the biodegradation of DOM was investigated, to gain insight into the factors that control the *in situ* biodegradation of DOM in Boom Clay surrounding a Eurobitum repository.

2. Materials and methods

2.1. Materials

All experiments were performed in an anaerobic glove box (Ar atmosphere, $pO_2 < 5 \times 10^{-4}$ kPa). All chemicals were of the highest available chemical grade. The equipment and chemicals were equilibrated with Ar ($pO_2 < 5 \times 10^{-4}$ kPa) prior to the experiments by putting them inside the glove box at least one day in advance to remove all traces of oxygen gas.

2.1.1. Boom Clay pore water

The Boom Clay pore water (BCPW) used in the batch tests was sampled from the S-1D piezometer underneath shaft 1 in the downward direction in the HADES underground research laboratory at SCK•CEN (Mol, Belgium). This piezometer was formerly known as the EG/BS (Extension Gallery Bottom Shaft) piezometer (De Craen et al., 2004; Durce et al., 2015). Care was taken to avoid any oxygen contamination during sampling by repeatedly placing the sampling equipment under vacuum and flushing it with N_2 prior to connection to the piezometer.

The S-1D piezometer contains one stainless steel cylindrical filter screen, 60 mm in length with pores ranging from 7 to 16 μm. This filter is centralised in a 13-m long coarse sand column, which enhances the water-draining capability of the piezometer and encompasses the silty Double Band of the Boom Clay. The chemical composition of the BCPW is described in detail by De Craen et al. (2004). Briefly, the pore water contains the following redox-active components: 78–160 mg C L^{-1} DOC (dissolved organic carbon), 143–871 mg C L^{-1} TIC (total inorganic carbon), 728–4425 mg L^{-1} HCO_3^-, 0.3–1.5 mg L^{-1} SO_4^{2-} and 0.5–4.6 mg L^{-1} Fe (De Craen et al., 2004).

Previous studies have demonstrated the presence of nitrate-reducing microbes (NRP or nitrate-reducing prokaryotes) in BCPW collected from the S-1D piezometer (Mariën et al., 2011; Bleyen et al., 2016a). Therefore, inoculation of the BCPW with such microorganisms was not necessary.

2.1.2. Synthetic clay water

Synthetic Boom Clay water or SBCW was used to dilute BCPW during, *e.g.*, determination of the reducing capacity of DOM, to ensure that no precipitation would occur. The chemical composition has been described by Bleyen et al. (2016a) and is representative of the major components of BCPW, with the exception of the presence of DOM and a slightly higher sulfate concentration.

2.2. Setup batch tests

Nitrate reactivity coupled to DOM degradation in BCPW was studied by adding $NaNO_3$ (Sigma Aldrich) to 1 L of BCPW at a concentration of 0.1 M $NaNO_3$ (Table 1), which corresponds to the concentration expected in the Boom Clay at a distance of 5 m from the Eurobitum disposal gallery liner (Weetjens et al., 2006). To assess the effect of acetate on DOM degradation during microbial nitrate reduction, 0.1 M $NaNO_3$ (Sigma Aldrich) and either 0.035 M or 0.07 M CH_3COONa (Sigma Aldrich) was added to 1 L of BCPW (Table 1). These two acetate

Table 1
Experimental conditions and objectives of the batch tests studying microbial nitrate reduction in BCPW with or without additional C and P sources.

Code	Test objective	Nitrate (M)	Acetate (M)	Phosphate (M) (added after 223 days)
BCPW_NO3	Nitrate reactivity in BCPW	0.1	–	–
BCPW_NO3_P	Nitrate reactivity in BCPW with added phosphate	0.1	–	0.0001
BCPW_NO3_35Ac	Nitrate reactivity in BCPW in the presence of acetate	0.1	0.035	–
BCPW_NO3_35Ac_P	Nitrate reactivity in BCPW in the presence of acetate and phosphate	0.1	0.035	0.0001
BCPW_NO3_70Ac	Nitrate reactivity in BCPW in the presence of acetate	0.1	0.07	–
BCPW_NO3_70Ac_P	Nitrate reactivity in BCPW in the presence of acetate and phosphate	0.1	0.07	0.0001
BCPW	Negative control	–	–	–