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O_2 depletion in granitic media

The REX project

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ABSTRACT

The redox conditions are of consequence for the performance of nuclear repositories. The presence of molecular oxygen (O_2) would affect the corrosion of canisters and the migration of radionuclides eventually released from a damaged canister. The rate of disappearance of O_2 left in voids at repository closure is therefore an important information when evaluating repository designs.

The REX project (Redox Experiment in Detailed Scale) has been carried out within the frame of SKB Äspö Hard Rock Laboratory in Sweden. The aim was to determine how O_2 trapped in the closed repository would react with the rock minerals in the tunnel and deposition holes and in the water conducting fractures. The REX project consisted of the following sections:

- *Field investigations at Äspö*:
  - Microbial O_2 consumption at several sites in the Äspö tunnel
  - The *in situ* experiment: Injection of oxygen and monitoring of O_2 uptake in a confined fracture surface.
- *The Replica Experiment*: a laboratory study using the other half of the fracture surface used for the *in situ* experiment.
- *Laboratory experiments*: needed to support the interpretation of the field and replica experiments. Conducted with groundwaters, bacteria, and mineral samples from Äspö.

The results from the *in-situ* experiment were confirmed by those of the replica experiment performed in the CEA laboratory in France. Both were concordant in showing time scales for O_2 uptake in the order of days. The agreement was remarkable when taking into account the differences in experimental conditions. For example, different microbial processes took place in the two experiments.

Laboratory studies with rock samples demonstrated that microbial activity induced increased chemical weathering, with the formation of clay minerals. Rates of O_2 uptake by fracture filling minerals were determined under laboratory conditions. These rates were faster than those reported in literature studies of pure mineral systems, and supported the fast O_2 uptake rates obtained in the field and replica experiments.

The main conclusions from the REX project were:

- O_2 uptake by the geologic medium was demonstrated. For performance assessment the rate of O_2 uptake may be considered to be instantaneous.
- Microbial activity contributed substantially to the O_2 reduction.
- A substantial reducing capacity should be assigned to CH_4 and H_2, which diffuse from deep geological sources. These compounds may be used by microbes as a redox buffer against the intrusion of O_2-rich waters, independently of surface climatic conditions.

SAMMANFATTNING

Funktionen av ett förvar för utbränt kärnbränsle påverkas av de rådande redoxförhållandena. Till exempel kan molekylärt syre (O_2) påverka korrosionen av metallkapslar och radionuklidernas migration från en defekt kapsel ut i geosfären. Det är därför viktigt att beakta kapaciteten för syrekonsumtion på förvarsnivå när man planerar ett förvar.

REX projektet (”Redox Experiment in Detailed Scale”) utfördes inom ramen för SKBs berglaboratorium på Äspö. Målet var att bestämma hur O_2 som lämnas kvar vid stängningen av ett förvar kan reagera med mineral och grundvatten i berget i tunneln, deponeringshålen eller längs de vattenförande sprickorna. REX projektet bestod av följande delar:

- *Fältförsök på Äspö*:
  - Studier av mikrobiellt upptag av O_2 på flera platser längs Äspötunneln.
  - Injektering av O_2 och uppföljning av syreupptaget vid en isolerad sprickyta.
- *Replica experiment*: Ett laboratorieförsök med den andra hälften av sprickytan som användes vid *in situ* experimentet.
- *Laboratorieexperiment*: Dessa utfördes med grundvatten, bakterie- och mineralprov från Äspö i syfte att stödja tolkningen av fält- och replica-försöken.

Resultaten från in-situ försöket bekräftades av replicaexperimentet utfört i CEA-laboratoriet i Frankrike. Båda undersökningarna visade att O_2 hade konsumerats helt efter några dagar. Överensstämmelsen var påfallande god med tanke på de olikheter som fanns beträffande experimentförhållanden. Till exempel förekom olika mikrobiella processer i de två experimenten.

Laboratorieförsök med bergfragment visade att mikrobiell aktivitet orsakade en ökad bildning av lermineral. Hastigheten för O_2 upptag på sprickfyllnadsmaterial (huvudsakligen hydrotermalt omvandlad Äspödiorit med klorit, kalcit och lermineral) bestämdes under laboratorieförhållanden. Dessa hastigheter var snabbare än de som finns rapporterade i litteraturen för rena mineralsystem, och stödde det snabba O_2 upptag som uppmättes i såväl fält- som replicaexperiment.

De huvudsakliga slutsatserna från REX projektet är:

- En påtaglig och snabb O_2-förbrukning påvisades för geosfären. I säkerhetsutvärderingen kan man anta att hastigheten för O_2 konsumtionen är ögonblicklig.
- Mikrobiell aktivitet bidrog väsentligt till O_2-konsumtionen.
- Metan och vätgas som diffunderar upp genom jordskorpan förväntas bidra med betydande reduktionskapacitet. Dessa ämnen kan användas av mikrober som redox buffert mot intrång av O_2-rika vatten, oberoende av klimatiska förhållanden.

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1 INTRODUCTION

This report describes the results and conclusions from the REX project, which had as primary aim to confirm previous experimental models for the uptake of O_2 by geologic media, in conditions similar to those of a deep nuclear waste repository.

In addition to this experimental confirmation, new findings have established the importance of microbial activities for keeping reducing conditions around nuclear repositories. Oxidation of CH_4 and H_2 has been found to be important processes for O_2 reduction that had not been considered in previous models of nuclear waste repository performance (SKB, 1999; SKBF/KBS, 1983)

The SKB concept for disposal of high level nuclear waste is based on isolation of spent fuel in copper canisters buried approximately 500 meters deep in granitic bedrock. A critical safety aspect is the mechanical and chemical stability of engineered barriers: canisters, compacted bentonite in the deposition holes between canister and rock, and sand/bentonite mixtures as backfill in tunnels and shafts. Performance assessment must evaluate the long-term stability and consequences of the eventual failure of these barriers. If radionuclides are released from the engineered barriers, the exposure of the biosphere to long-lived radionuclides will depend on groundwater flow and the capacity of the geologic media to confine radionuclides through adsorption, precipitation and diffusion into rock matrix.

Redox chemistry issues that concern repository performance assessment are:

1. The presence and fate of molecular oxygen, O_2, in the deep environment at the time of repository closure. Oxygen affects the corrosion of copper metal and the redox state of several long-lived radionuclides, such as Np, Pu, Tc, and U. Those are much more soluble and mobile under oxygenated conditions.
2. Microbes may affect the underground redox conditions by "catalysing" chemical processes, in particular the oxidation of dissolved organic material but also H_2 and CH_4 formed in the deep geosphere. These respiration activities result in decreasing O_2 levels.
3. Microbial production of hydrogen sulphide, HS^-, from sulphate reduction in the deep environment after the closure of the repository. The presence of sulphide would negatively affect the corrosion properties of the copper canister.

The reduction of dissolved O_2 has been addressed in former performance assessments by model calculations based on data obtained under laboratory conditions (SKB, 1999; SKBF/KBS, 1983). These laboratory studies are also supported by general field observations. However, the rate of reduction

of dissolved O_2 under realistic field conditions has not been determined previously.

The analysis of the possible effects of dissolved O_2 on the performance of a repository is best described by considering two different time periods:

- During a period of perhaps some days or weeks after closure, O_2 will remain in the repository:
  - In the air occupying the porosity voids of the back fill and in the bentonite buffer.
  - In dissolved form due to diffusion into the tunnel walls, or as dissolved O_2 that is transported by groundwater circulating in fractures intersecting the tunnel.

  This O_2 may create locally oxidising environments.

  The redox processes taking place during this period are indicated in Figure 1.

- The second time period takes place during a glaciation. Glacial recharge melt waters are likely to contain dissolved O_2 in larger quantities than rainwater. Because of large hydrostatic gradients under an ice sheet, they might reach repository levels and result in oxidising environments. Under these climatic conditions the input of organic matter from the surface biosphere is negligible. Hence, the usual reduction of oxygen by dissolved organic carbon from recharge surface waters will not take place (see the Redox Experiment in Block Scale, Section 2.2.2).

  One of the main results of the REX project is the demonstration that microbial activities are activated when intruding O_2-rich surface waters are mixed with deeper groundwaters, which contain reductants such as CH_4 that emanates from deeper sources. These processes, depicted in Figure 2 and described in Section 2.1, will favour a reducing environment in the near- and far-fields surrounding a nuclear repository.

The REX project was started with the main objective of investigating O_2 reduction by creating a controlled oxidising perturbation in the deep rock environment at the Äspö Hard Rock Laboratory. An important requirement was that the experiment should be performed in a fracture zone representative of a deep repository environment. The purpose of the REX project was to:

1. Assess experimentally the capacity of the host rock system to reduce an intrusion of oxygen.
2. Determine the kinetics (half-life) of the O_2 reduction under *in-situ* conditions.
3. Develop quantitative descriptions of the O_2 reduction processes that can be used to assess the repository redox status during the post-closure phase.

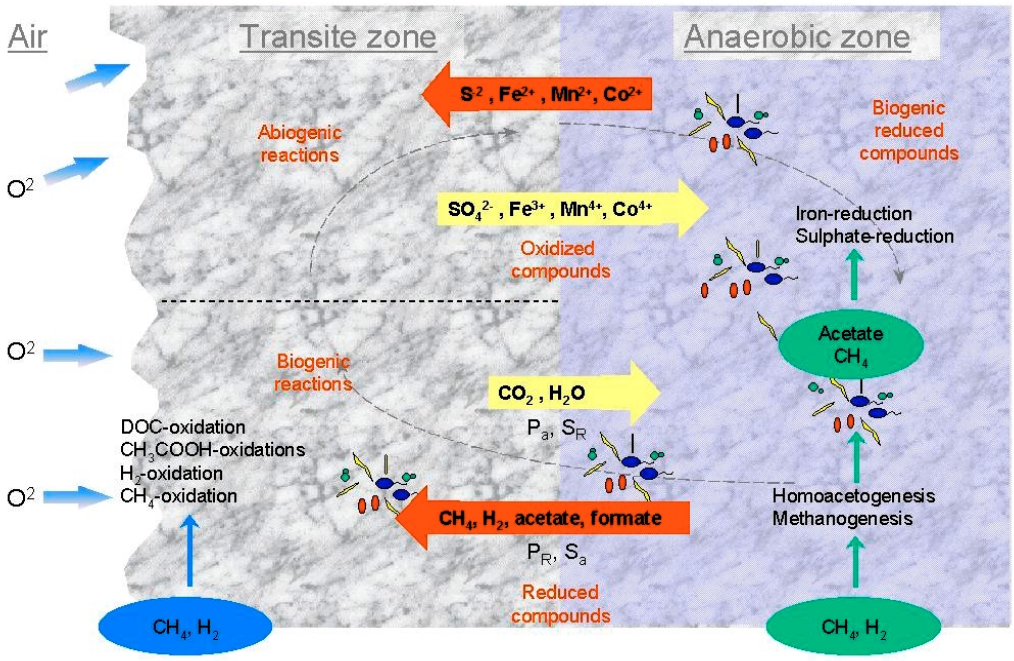


Figure 1. Redox processes at the interface between a reducing rock/groundwater system and an aerated tunnel. Several of these processes are microbially mediated. From (Kotelnikova and Pedersen, 1999).

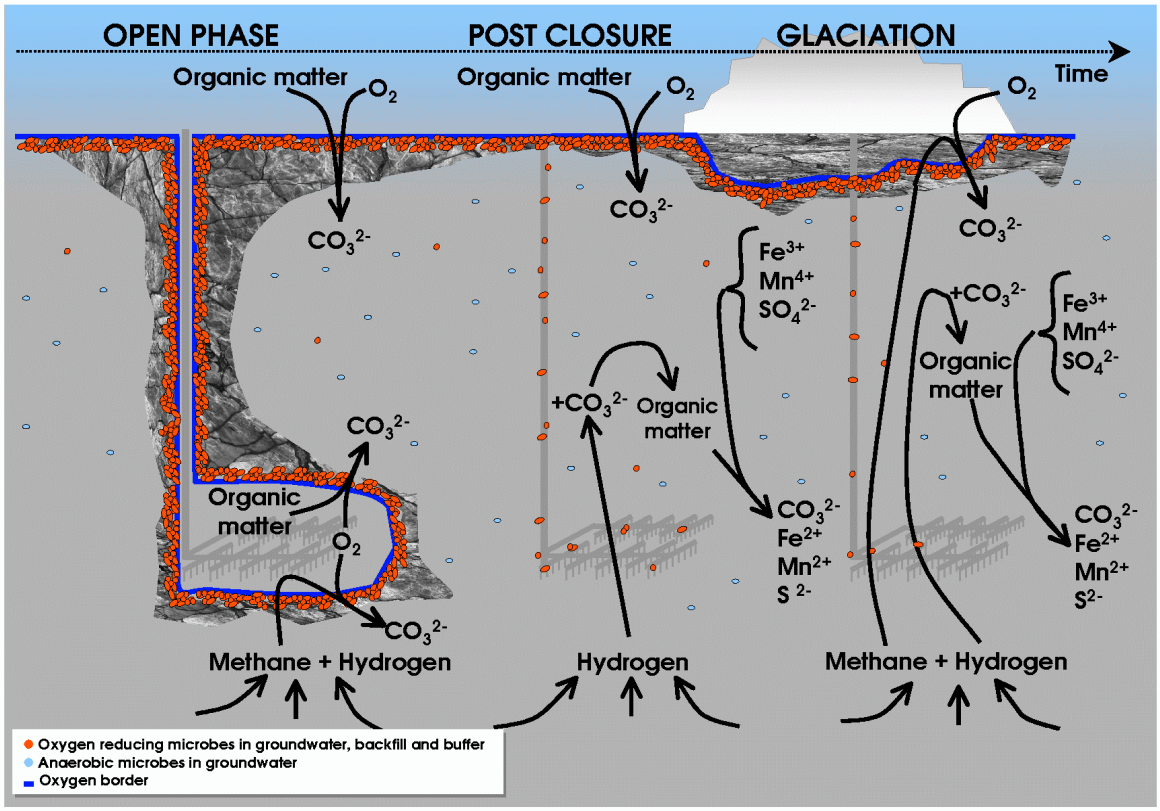


Figure 2. Schematic model of how microbes in the geosphere hinder O_2 from reaching a nuclear repository and keep the groundwater redox potential at low levels. From (Pedersen, 2000).

2 CONCEPTUAL MODELS

The REX project, as well as the earlier Redox Project in Block Scale (Section 2.2.2), were made to obtain understanding of the reduction of O_2 in a repository and the redox buffer capacity of rock and groundwater. The design and planning of the REX project (Puigdomenech et al., 2000b) was based on previously well-established models and data on reactions between O_2 and inorganic reductants, with or without the mediation of microbes.

It is well known that the main reductants in the rock matrix and in fracture filling minerals are aluminosilicates and sulphides containing iron(II). This is also the case for the bentonite buffer and other engineered barriers in a nuclear repository. In addition it is known that microbes consume O_2, which is their preferred electron acceptor. The major reductants that microbes may use to consume O_2 in the deep groundwaters at Äspö are CH_4 and organic carbon. Dissolved H_2, HS^- and Fe(II) may also contribute to the O_2 reduction, but these species generally have a much lower concentrations than do CH_4 and organic carbon. Locally, however, they may have a significant effect.

Lately evidence has been gathered that show that there is a deep biosphere driven by H_2, CH_4, and CO_2 generated in deep geological sources.

2.1 THE UNDERGROUND BIOSPHERE

Recent microbiological data suggest that H_2 is an important electron and energy source, and that CO_2 is an important carbon source in the subsurface biosphere (Pedersen, 2000). The consumption of H_2, CH_4 and CO_2 generated in deep geological sources is important to sustain the biological activity as described schematically in Figure 3.

These recent data, partly from the REX project and other SKB projects demonstrate that microorganisms may be able to buffer against oxidising disturbances in deep repository environments independently of climatic and hydrological conditions on the surface. The presence of an active and diversified microbiota at repository depths is well documented (Pedersen, 2000), as is the capacity of microorganisms in "catalysing" processes that eventually result in reducing conditions.

Microbial decomposition and the production of organic material depend on the sources of energy and on the electron acceptors present. Organic carbon, H_2, CH_4 and reduced inorganic molecules are possible energy sources in subterranean environments. The solubility of O_2 in water is low, despite this oxygen is the preferred electron acceptor for many microbes, because they obtain more energy per organic molecule than with other electron acceptors.

Therefore, the microbes use electron acceptors in the following order: first O_2, followed by NO_3^-, Mn(IV), Fe(III), sulphate, sulphur, and CO_2. Simultaneously, fermentation processes supply the respiring microbes with H_2 and simple organic acids. This is illustrated in Figure 4.

How does the underground biosphere affect the performance of a nuclear repository? Figure 2 illustrates three repository scenarios: the open repository, the post-closure situation, and a glaciation period.

During the repository operation period O_2 will be supplied by recharging groundwater into the basement rock O_2 will also diffuse from the tunnel air into the rock matrix. The recharging groundwater will also contain organic matter, and microbes will continuously use this to reduce O_2.

Anaerobic microbes in hard rock aquifers are known to use organic carbon to reduce ferric iron, manganese(IV) and sulphate to ferrous iron, manganese(II) and sulphide. These species will be transported by groundwater that will react with O_2 when it reaches an open repository tunnel. Layers of other types of microbes then grow on the tunnel walls where groundwater seeps out and they produce organic carbon with the energy derived from these groundwater components. Other microbes can later use the organic matter for additional O_2 reduction.

Glaciation presents a special case (Figure 2). During such a period the input of organic carbon with recharging groundwaters will be very low because there is no photosynthetic production of organic carbon. The REX project has demonstrated a significant activity of CH_4-oxidising bacteria. Methane is produced in deep rocks and migrates upwards (Apps and van de Kamp, 1993). The continuous flow of CH_4 from deep mantle rocks will not depend on glaciation events, and therefore there is a mechanism for O_2 consumption that is independent on climatic conditions. Hydrogen is an even better oxygen reducer for microbes than methane, but it has lower concentration in the groundwaters examined (Kotelnikova and Pedersen, 1999). The upward diffusion flow of CH_4 and H_2 is therefore an important parameter that needs to be determined in order to determine the reducing capacity of nuclear waste repositories. Work is ongoing to assess this flow.

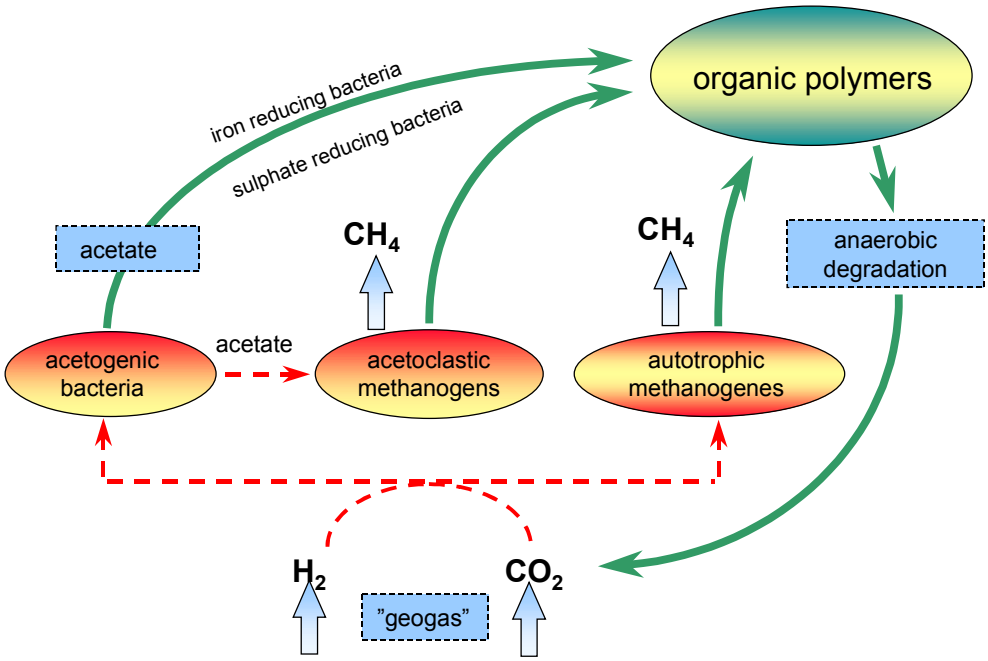


Figure 3. The deep hydrogen-driven biosphere hypothesis illustrated by the carbon cycle. Under repository conditions subterranean microorganisms are theoretically capable of a life cycle independent of sun-driven ecosystems. Gases from the deep crust of the Earth (H_2 and CO_2) can be used as energy and carbon sources. From (Pedersen, 2000).

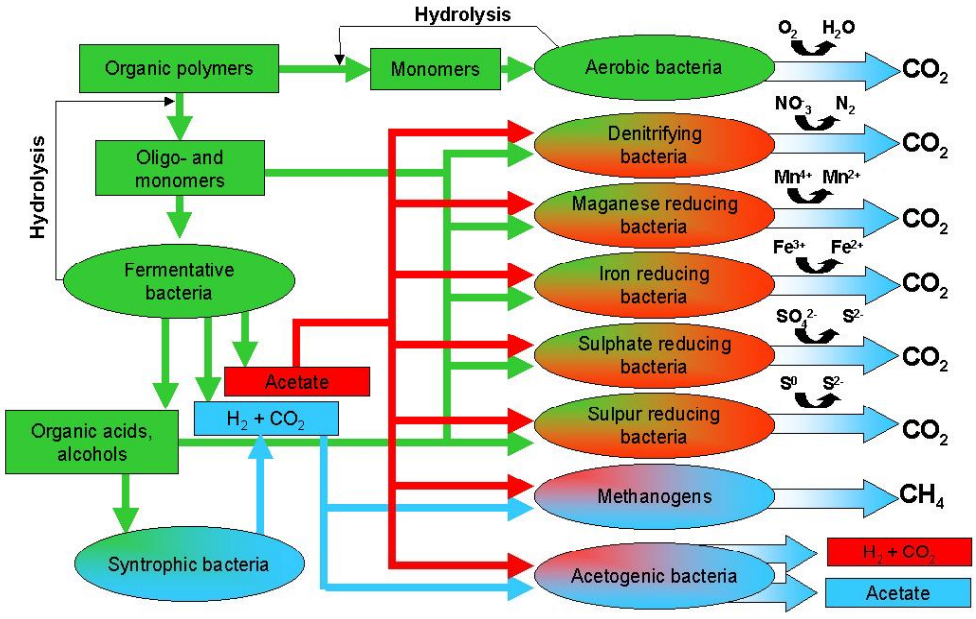


Figure 4. The different degradation processes of organic carbon are characterised by the principal electron acceptor. Of importance for nuclear waste disposal is the production of HS^-, which is a potential corrodant of canister materials, and the turnover of gases such as CO_2, H_2 and CH_4. From (Pedersen, 2000).

2.2 PREVIOUS STUDIES ON O_2 UPTAKE

2.2.1 Previous Laboratory Studies

A large body of knowledge has been accumulated on the chemical reactions between O_2 and groundwater components and geological materials. Most of these data have been obtained in well-controlled laboratory experiments. It must be noted however that microbial analyses and controls have in general *not* been performed in these studies. Therefore some of the results concerning "abiotic" O_2-uptake reactions might have been affected by microbial activities.

In the absence of organic matter (methane, acetate, *etc*), the most important reductants in groundwaters are Fe^{2+} and sulphide. They react with oxygen according to:

$$Fe^{2+} + 0.25\ O_2 + 2.5\ H_2O \rightarrow Fe(OH)_3(s) + 2\ H^+ \qquad (1)$$

$$HS^- + 2\ O_2 \rightarrow SO_4^{2-} + H^+$$

It has been found in laboratory studies that these reactions are relatively fast at the pH ranges prevailing in natural waters. The reaction between oxygen and Fe(II) has a half-life shorter than 1 h at pH > 7, and the half-life for sulphide is shorter than one month (Eary and Schramke, 1990). These reactions may therefore be considered instantaneous when compared to the time scales used in performance assessment calculations for nuclear repositories.

Minerals that release reductants like iron(II) and sulphide will result in oxygen removal at the same rate as the release. For the sites studied by SKB, ferrous silicates, and to a minor extent sulphide minerals, are the major sources of reduction capacity.

The production of hydrogen sulphide, HS^-, in the deep repository environment could be detrimental for the corrosion of canister materials; it was addressed in the Äspö Sulphate Reduction Project (Laaksoharju et al., 1995).

Literature data on the reaction between dissolved O_2 and pyrite (FeS_2), Fe(II)-aluminosilicate minerals and rock samples were reviewed in (Puigdomenech et al., 1999). Based on these data, estimated time scales for reduction of dissolved O_2 in a typical fracture in granitic rock were estimated to be between 60 and 350 years, see Table 1. Similarly, calculations for the backfill and bentonite clay buffer showed time scales for O_2 depletion between 7 and 290 years (Wersin et al., 1994).

2.2.2 The Redox Experiment in Block Scale

The Äspö Redox Experiment in Block Scale (Banwart et al., 1994; Banwart et al., 1995; Banwart et al., 1996) focused on surface water inflow to vertical fractures during construction and operation of a deep repository. The ex-

periment was carried out in a fracture zone at 70 m depth in the entrance tunnel to Äspö. In spite of massive surface water input, the fracture zone remained persistently anoxic. The main conclusion from this study was that the increased inflow of relatively organic-rich shallow groundwater instead of adding dissolved oxygen, resulted in reducing conditions in the deeper parts of the fracture zone as a result of microbiological oxidation of the added organic material. These conclusions are specific to this particular fracture zone, experimental conditions, and the time scale (3 years) of the experiment, but are probably also relevant for other conductive fracture zones.

2.2.3 Other Field Experiments

Measurements of dissolved O_2 in the Kamaishi Test Site in Japan (Sasamoto et al., 1999), showed that the O_2 concentration decreased from ≈8 ppm at the tunnel wall to 0.3 and < 0.01 ppm at a distance of 1.8 and 20 m, respectively, from the drift wall. A model was proposed for the diffusion of atmospheric O_2 into the excavation disturbed zone (EDZ) of the granodioritic rock, and reaction with Fe^{2+} ions in the groundwater.

The rate of oxygen uptake in granite in the absence of dissolved organic carbon was determined during a field experiment at the Underground Research Laboratory of AECL in Manitoba, Canada (Gascoyne, 1997). In this experiment O_2 was monitored in water circulating a borehole section in "unfractured" granite. From the results, the minimum O_2 consumption rate was estimated to 1.3×10^{-5} mol·m^{-2}·day^{-1}. Assuming that the oxidation of Fe(II) in biotite is the cause of O_2 reduction, the minimum reaction rate is 2.6×10^{-4} mol·m^{-2}·day^{-1}. This is larger than the laboratory results 1.2×10^{-8} mol-O_2·m^{-2}·day^{-1} reported in (Malmström and Banwart, 1997). The discrepancy might be due the uncertainties associated with the field experiment: microbial activities, unknown surface area, etc.

3 EXPERIMENTAL RESULTS

The REX project has been based on several simultaneous lines of research (Puigdomenech et al., 1999; Puigdomenech et al., 1996; Puigdomenech et al., 2000b):

1. *Laboratory studies* using minerals, rock samples, groundwater, and microbes from the Äspö site. These experiments were conducted under well-controlled laboratory conditions, and they included both batch and flow-through column experiments.

2. *Underground field experiments* at Äspö. Because of uncertainties in hydraulic parameters, the *in-situ* experiment focused in injection and monitoring of O_2 into a borehole reaching a hydraulically isolated fracture surface. This concept excluded the hydraulic complications and costs associated with experiments involving several boreholes.

3. *A replica experiment*, performed under well defined laboratory conditions. This experiment was performed with the other half of the fracture surface used in the *in-situ* experiment, which was obtained when the drillcore was extracted.

The Appendix describes with some degree of detail the results obtained from the different parts of the REX project. Additional sources of information may be found in reports cited in the corresponding parts of the Appendix and in (Puigdomenech et al., 1999; Puigdomenech et al., 2000b). This section presents only a short summary of the main results.

3.1 LABORATORY EXPERIMENTS

3.1.1 The Effects of Microbes on Water/Rock Interactions

The effects of iron and sulphate reducing bacteria on rock/groundwater interactions were determined using flow columns and mixed-flow reactors, as described in Section A2.2 of the Appendix. The experiments showed that bacterial processes may mediate clay formation, even during the low-temperature alteration of granitic rocks. This is important for repository performance because the mobilisation of fine-grained material, aided by biological processes, may have a significant impact on groundwater flow-paths, and on the capacity for radionuclide sorption.

3.1.2 O_2 Uptake by Fracture Filling Minerals from Äspö

O_2 uptake by fracture-filling minerals from Äspö was determined using batch and a recirculating batch reactor experiments. The mineral samples were collected both from fractures intersecting the tunnel wall, and from a specially drilled borehole. They were subsequently sieved and analysed. The results are described in Section A2.3 of the Appendix. The observed rate constants varied approximately between 0.0001 and 3 $L\cdot(g\cdot day)^{-1}$. With one exception, the fastest reaction rates were observed with the finest particle size fraction. The observed O_2 uptake rates were larger than what could be expected from previous laboratory experiments using pure minerals. Although there is no direct evidence that microbial processes were completely excluded in this study (microbial analysis were not performed), the experimental method avoided microbial contamination, and a test in the presence of a microbial inhibitor showed no decrease in the O_2 uptake rate.

3.1.3 The *Replica* Experiment

This study was done with the other half of the fracture surface used in the *in-situ* experiment, which was recovered at the end of the drilling of the REX borehole (KA2861A). As in the case of the *in-situ* experiment, a fracture surface (≈0.03 m^2) was isolated and set into contact with ≈1 L of groundwater to which different amounts of O_2 had been added. Because most of the reporting for this study was in French, a quite detailed description is given in Section A2.1 of the Appendix. The experimental conditions were similar for both the *replica* and *in-situ* experiments. For example, the groundwater was sampled at the REX site in the Äspö tunnel and sent to the CEA laboratory in Cadarache. Nevertheless, some differences existed in the materials of the set-up, as well as in temperature, pressure, and gas content.

The *replica* study demonstrated that O_2-uptake took place in time scales similar to those of the *in-situ* experiments. It was also demonstrated that microbial activities had a principal role in the decrease of O_2 concentrations. The *replica* experiment was found useful in complementing and confirming the conclusions from the *in-situ* study.

3.2 FIELD EXPERIMENTS

3.2.1 The *In-situ* Experiment

In this experiment a fracture surface (≈0.03 m^2) was isolated and set into contact with ≈1 L of groundwater to which different amounts of O_2 had been added. Details of the set-up and results are described in Section A1.1 of the Appendix. The experiments were performed under *in-situ* conditions (temperature, pressure, *etc*). The experimental site has some of the most saline groundwaters at Äspö ($[Cl^-]$≈0.4 M) and isotopic data show that these waters are perhaps the oldest at this site (Mahara et al., 1998), although this is not significant for the results of the experiment.

Several parameters were monitored continuously, like O_2-concentration and pH, while samples for chemical and microbial analysis could only be taken after some of the O_2-uptake tests.

The *in-situ* experiments demonstrated that O_2-uptake took place in time scales ranging from a couple of days to one or two weeks. Although the mechanism for O_2-uptake could not be established unequivocally, it was demonstrated that microbial activities had a principal role.

3.2.2 Microbe REX

Microbial O_2 consumption experiments using microbes sampled in different parts of the Äspö tunnel, including borehole groundwaters, tunnel-walls and ponds. These experiments, described in Section A1.2 of the Appendix, show that the indigenous microbial populations are well capable of O_2 consump-

tion. As expected, at temperatures of 30 and 60°C an adaptation period of ≈2 weeks was required for the microbial O_2-uptake. The rates observed varied between 0.31 and 4.5 µM/day. These rates are however substantially slower than those observed in the *in-situ* and *replica* experiments. Methane oxidising bacteria were found to have a substantial contribution to the total O_2 consumption.

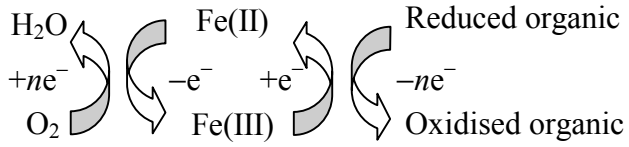
The main conclusions from this study were:

a) microbes showed O_2 consumption capacities independently of where they were sampled in the different parts of the Äspö tunnel; and

b) CH_4 oxidation is a mechanism for microbial O_2 uptake that is not dependent on the input of dissolved organic carbon from a surface biosphere, and thus, it is independent of future climatic conditions. This constitutes a new safeguard in the performance nuclear waste repositories.

4 DISCUSSION AND CONCLUSIONS

Results using experimentally independent methods, both from the REX project and from the Redox project in Block Scale (Section 2.2.2), confirm that microbes in hard rock aquifers and tunnels are capable of O_2 uptake. Microbially mediated processes will also accelerate the decrease of O_2. For example, iron reducing and oxidising bacteria might participate in the Fe(III)-Fe(II) system, which may act as an electron carrier, as indicated in the following diagram:



The results from the REX project indicate that geosphere microbes are capable of using available reductants to protect the host rock and repository from O_2, and to produce groundwater components that lower the redox potential. This represents a large benefit for repository performance.

In addition some evidence has been gathered that microbial activity may accelerate rock weathering and induce the formation of clay minerals. This was demonstrated in the column experiments described in Section A2.2 of the Appendix. The formation of clays and Fe(III) oxy-hydroxides would favour the retention of radionuclides through sorption. The results obtained are however too preliminary to be included in performance assessments of nuclear waste repositories.

Another important result from the REX project has been to emphasise the importance of clay-type fracture-filling minerals in O_2-uptake processes. Previous borehole drillings were inadequate in preserving the clay fraction of fracture fillings. Triple-tube drilling procedures may be used to recover this small size fraction, and they are essential to a proper characterisation of a candidate site for a future nuclear waste repository.

4.1 ESTIMATED TIME SCALES FOR O_2 DEPLETION

Models of O_2-uptake for performance assessment calculations must include both rates and reducing capacity considerations. Time scales for the depletion of dissolved O_2 with reducing minerals may be estimated from the results of the REX project. These time scales for oxygen consumption do not necessarily represent how long O_2 will last in the repository environment after closure, but do represent time scales for chemical reaction that can be compared with rates of diffusion and groundwater flow. Comparison of these time scales provides a structured basis for developing the proper mathematical description for coupled reaction and transport.

4.1.1 Time Scales for O_2 Uptake in Conductive Fracture Zones

The time scale for depletion of dissolved O_2 by reactions with rock, or by reaction with dissolved Fe(II) released from rock, can be estimated for the case of O_2 in a conductive fracture at the time of repository closure.

Equation (2) calculates the time scale (τ) to deplete a closed reservoir of dissolved molecular oxygen from the initial concentration, $[O_2]_o$, and the rate of oxygen consumption, R. This equation is related to the zero-order reaction for the slow release of Fe(II) into solution followed by rapid reaction with dissolved O_2. The half-life for O_2 cannot be calculated because the overall rate of O_2 depletion is only dependent on the very slow dissolution reaction, and not on the concentration of O_2 in solution.

$$\tau = \frac{[O_2]_o}{R} \tag{2}$$

$$R = \frac{R_o f_m P_{Fe} A_w}{4} \tag{3}$$

Equation (3) calculates the rate of O_2 uptake where A_w ($m^2 \cdot L^{-1}$) is the area of rock surface in contact with the groundwater in a fracture zone; f_m is the volume fraction of the Fe(II)-bearing mineral or rock type in a fracture; and P_{Fe} is the mole fraction of total iron as Fe(II) in the reactive mineral or rock type. Each mole of O_2 reacts with 4 moles of Fe(II), *cf.* Eq.(1). R_o is the rate of oxygen uptake by: 1) reaction in solution with Fe(II) released from the mineral by dissolution, or 2) reaction directly at Fe(II) sites on the mineral surface.

In the case of direct O_2 uptake at mineral surfaces, the half-life ($t_{½}$) for oxygen can be calculated assuming first-order decay for oxygen concentration in solution, *cf.* Eq. (4).

$$\frac{d[O_2]}{dt} = -k[O_2] \tag{4}$$

Other processes, perhaps microbially mediated, might also follow apparent first-order kinetics for O_2 uptake. The half-life is in this case calculated by integrating Eq. (4) between $t = 0$ and t, and setting the concentration $C(t_{½}) = ½\ C(t=0)$. In the experiments of (White and Yee, 1985), the oxygen concentration was approximately 0.25 $mmol \cdot L^{-1}$ when they determined R_o for slow oxygen uptake by mineral surfaces. Because the rate of uptake was slow, $[O_2]$ did not change dramatically during the rate determination. The first-order rate constant k is thus approximated by τ^{-1}, *cf.* Eq. (5) and Table 1. Values of τ may be obtained from the measured rate of oxygen uptake at $[O_2] = 0.25\ mmol \cdot L^{-1}$. Equation (6) gives the half-life for dissolved oxygen in those experiments.

$$-k = \frac{1}{[O_2]}\frac{d[O_2]}{dt} = \frac{R}{[O_2]} = \frac{1}{\tau} \quad (5)$$

$$t_{½} = \tau \ln(0.5) \quad (6)$$

Values of τ for various mineral or rock types are reported in Table 1. The relative abundance of the reactive mineral or rock type is based on the amount of biotite present in fresh granite sampled during the Block Scale Redox Experiment (Banwart et al., 1992, p.36). The other minerals or rock types are calculated assuming the same relative abundance. Samples of biotite and chlorite from the Block Scale Redox Experiment (Banwart et al., 1994) show iron(II) content of 76-84% and 56-67% of total Fe respectively. Similar results were found in fracture filling mineral samples collected for the REX project (Table 9 in Section A2.3). For simplicity, and given the very approximate nature of these calculations, we assign a value of $P_{Fe} = 1$ for all rock types and minerals.

The surface area of rock in contact with the flow path (10 $m^2 \cdot L^{-1}$) is based on results from tracer studies carried out in a fracture at Stripa (Andersson et al., 1989). Recent results from the TRUE experiment have resulted in values in the range 1.9 to 33 $m^2 \cdot L^{-1}$ (Winberg et al., 2000, Sections 8.7, 8.8.7, 8.8.12, and 11.6).

Table 1. Characteristic time scales for uptake of dissolved O_2 by Fe(II) in fracture minerals or dissolved in groundwater. The initial dissolved O_2 concentration was 8 $mg \cdot L^{-1}$ (0.25 $mmol \cdot L^{-1}$) in all cases. See text for details.

Solid Phase	Process	R_o ($mol \cdot m^{-2} \cdot day^{-1}$)	R ($mol \cdot L^{-1} \cdot day^{-1}$)	τ (years)
Minerals [a]				
Biotite	dissolution	45×10^{-9}	11×10^{-9}	60
Biotite	O_2 uptake	8×10^{-9}	2×10^{-9}	330
Hornblende	O_2 uptake	19×10^{-9}	5×10^{-9}	150
Augite	O_2 uptake	90×10^{-9}	22×10^{-9}	30
Granite in URL (Canada) [b]	O_2 uptake?	1.3×10^{-5}	1.3×10^{-4}	2 *days*
Fracture minerals from Äspö [c]	O_2 uptake		5×10^{-5} to 1.3	(6 to <0.1) *days*
REX *in-situ* and *replica* [d]	O_2 uptake?		9×10^{-3} to 0.09	<0.1 *days*

a: Eq.(3) has been used with: $f_m = 0.1$, $P_{Fe} = 1$, and $A_w = 10$ $m^2 \cdot L^{-1}$.
c: *R* calculated with $A_w = 10$ $m^2 \cdot L^{-1}$.
c: *R* calculated with a rock-groundwater ratio of 1.8 $kg \cdot L^{-1}$.
d: *R* calculated with $A_w = 0.024$ $m^2 \cdot L^{-1}$ in the REX experiment and $A_w = 10$ $m^2 \cdot L^{-1}$ in the fracture.

Table 1 tabulates time scales for O_2 depletion as calculated by Eqs. (2) to (5). Iron(II) dissolution rates and O_2 uptake rates measured by (Malmström and Banwart, 1997; Malmström et al., 1996) and (White and Yee, 1985), respectively, can be used as measures of R_o.

Table 1 also reports the time scale for O_2 depletion obtained from the experiments performed in the URL by (Gascoyne, 1997). This time scale is shorter than that obtained from laboratory experiments. The mechanisms for O2 uptake in these experiments can not be ascertained because not enough details were reported in the original publication. A contribution from microbial activities can not be excluded.

The time scales given in Table 1 represent the relative rate of the chemical reaction to be compared with rates of other processes such as diffusion, aerobic respiration of any organic carbon, groundwater flow.

Recent triple tube sampling of borehole cores shows that the amount of gouge material was under-represented when drilling with the usual method. The sampling of borehole KA3065, performed under REX, gave 110 $mg \cdot cm^{-2}$ (Puigdomenech et al., 1999). Using an estimated fracture aperture of 1 mm, the ratio of gouge material to groundwater volume is calculated to be 1800 $gm \cdot L^{-1}$, when correction is made for the volume taken up by the gouge material. Table 1 also reports estimated time scales for O_2 depletion when the results from the REX laboratory experiments (Section A2.3) are combined with this mineral to water ratio.

The time scale to develop anoxic conditions, based on laboratory data using pure mineral samples, is between 60 and 350 years, with the longest time based on chlorite dissolution rate. The time scale to reach typical dissolved iron(II) concentrations in the groundwater, after anoxic conditions are established, is estimated to be on the order of one year. This is a consequence of solubility limitations for Fe(II) to build up in solution. Dissolved iron(II) therefore provides only a small reservoir of reducing capacity compared to the amount that is present in the rock.

Time scales to develop anoxic conditions based on gouge material from the Äspö tunnel are however much shorter: a few days.

4.1.2 Rate Models for Microbial Processes

Simple microbial growth kinetics is usually found to follow the Monod equation:

$$\frac{d[\mathrm{O_2}]}{dt} = -\frac{r_{\max}[\mathrm{O_2}]}{K_\mathrm{S} + [\mathrm{O_2}]} \tag{7}$$

It must be noted that in this equation $r_{\max}$ (the maximum rate) depends on the amount of biomass in the system, while K_S is the substrate concentration at which the rate is ½ of the maximum value. Integration of Eq.(7) gives:

$$[\mathrm{O_2}] + K_\mathrm{S} \ln[\mathrm{O_2}] = \left([\mathrm{O_2}]_0 + K_\mathrm{S} \ln[\mathrm{O_2}]_0\right) - r_{\max}\, t$$

This expression may be used to calculate the half-life ($t_{½}$) to consume O_2 in a closed repository, which is found to be dependent on the initial oxygen concentration:

$$t_{½} = \frac{0.5[\mathrm{O_2}]_0 + K_S \ln(0.5)}{r_{\max}}$$

In the *in-situ* REX experiment the observed rate of dissolved O_2 consumption follows the Monod expression with $r_{\max} \approx$ (5 to 90) μM·day^{-1} and $K_\mathrm{S} \approx$ (200 to 300) μM (Kotelnikova and Pedersen, 2000). The values $r_{\max}$ = 35 μM·day^{-1} and K_S = 200 μM give a calculated half-life in the REX *in-situ* experiment of $t_{½}$ = 7.5 days for $[O_2]_o$ = 8 mg·L^{-1}.

It has been observed in the Äspö subterranean environment, that multiply substrate utilisation by microorganisms is the rule, owing to the low nutrient levels found in the ground water. Multi-substrate Monod kinetics can be used to describe the influence of many substrates. For two substrates, namely oxygen and a carbon source, this takes the Double Monod form:

$$\frac{d[\mathrm{O_2}]}{dt} = -\frac{r_{\max}[\mathrm{O_2}]}{K_{\mathrm{O2}} + [\mathrm{O_2}]} \frac{[\mathrm{S}]}{K_\mathrm{S} + [\mathrm{S}]}$$

where S represents the carbon source. In general several groups of microbes will consume oxygen in parallel, and knowledge of the microorganisms inhabiting the environment can provide information about the rate of specific substrate transformations. For example, methanotrophs have K_S = (0.097 to 15) μM, and hydrogen consuming bacteria have K_S = (10 to 50) nM (Kotelnikova and Pedersen, 2000).

Before these values are used in estimating O_2 depletion times in rock fractures, the value of $r_{\max}$ should however be corrected for the number of microbes present, taking into account that most microbes are attached to mineral surfaces, see for example Table 8-18 in (Kotelnikova and Pedersen, 2000). In the REX *in-situ* experiment the surface to volume ratio was 0.6 m^2·L^{-1}, while for a typical fracture the expected ratio is $A_\mathrm{w} \approx$10 m^2·L^{-1}. The

maximum Monod rate, r_{max}, must therefore be increased for the increased bacterial population attached to the larger surface area of the fracture. Taking as corrected values[†] $r_{max} \approx 2500\ \mu M \cdot day^{-1}$ and $K_S \approx 200\ \mu M$, the following half-life times are obtained for a fracture, depending on the initial O_2 concentration:

$[O_2]_0$ / mg·L^{-1}	Calculated $t_{½}$ / days
1	0.06
8 (air saturation)	0.16
30 (glacial meltwater)	0.25

4.2 CAPACITIES FOR O_2 DEPLETION

4.2.1 Abiotic Inorganic Reactions

The total amount of reductants in an average conductive fracture may be estimated assuming that only Fe(II) contributes to the reducing capacity:

$$Fe^{2+} + ¼\ O_2 + {}^5/_2\ H_2O \rightarrow Fe(OH)_3(s) + 2\ H^+ \qquad (1)$$

Other reductants, such as sulphide and Mn(II), will also contribute to the total reducing capacity, although they are usually present in lesser amounts.

4.2.1.1 Fracture Characteristics and Amount of Gouge Material

As an example, a fracture with an average aperture of 1 mm will be considered. The amount of gouge material is set equal to 110 mg·cm^{-2}, as found in the triple-tube drilling of KA3065A (Puigdomenech et al., 1999), with an expected density of 2.6 g·cm^{-3}. This gives:

Gouge volume ≈ 0.4 L·m^{-2}
Groundwater volume ≈ 0.6 L·m^{-2}
Mineral / groundwater ratio ≈ 1.9 g·L^{-1}

The groundwater volume corresponds to the amount of water per square meter of fracture, and it does *not* correspond to the total mineral surface area in contact with the circulating groundwater ("wetted" surface area), which is naturally much larger.

4.2.1.2 Iron(II) Contents and O_2 Depletion Capacity

Samples of biotite and chlorite from the Block Scale Redox Experiment (Banwart et al., 1994) showed iron(II) content of 76-84% and 56-67% of total Fe respectively, in agreement with the data obtained in the REX project (Table 9 in Section A2.3).

[†] The internal surface of PEEK tubing in the REX *in-situ* experiments (0,46 m^2) was neglected, as the tubing had the lowest number of attached microorganisms, *cf.* Table 8-18 in (Kotelnikova and Pedersen, 2000).

A total Fe content of only 3%, with 70% Fe(II), will be considered here. The amount of iron(II) in the gouge material for an average fracture then becomes ≈ 0.4 mol·m^{-2}, which is equivalent to ≈ 0.7 mol of Fe(II) per litre of groundwater.

The capacity for O_2 uptake in the gouge material is therefore calculated to be 0.17 mol-O_2 per litre of groundwater flowing in the fracture, which is equivalent to reducing capacity of 0.1 mol-O_2 per m^2 of fracture. As a comparison, it could be noted that air-saturated water contains 2.5×10^{-4} mol-O_2·L^{-1}.

4.2.2 Capacity for O_2 Depletion Through Microbially Mediated Processes

The reducing capacity due to microbial processes is equivalent to the amount of reductants available for the microbes. As mentioned in Section 2, the main reductants available in the deep groundwaters at Äspö that microbes are able to use for O_2 consumption are CH_4 and organic carbon:

$$CH_2O + O_2 \rightarrow CO_2 + H_2O$$

$$CH_4 + 2\ O_2 \rightarrow CO_2 + 2\ H_2O$$

Although dissolved H_2, HS^- and Fe(II) could also contribute to O_2 uptake, they generally have much lower concentrations than CH_4 and organic carbon.

Total organic carbon levels in the deep groundwaters of Äspö are usually a few mg·L^{-1}. Methane levels varied between 0.2 and 1000 μM, as shown in Figure 5. These values lead to the conclusion that the calculated reducing capacity due to dissolved organic carbon is limited: *e.g.* 10 mg-C·L^{-1} correspond to a capacity to reduce 0.8 mol-O_2·L^{-1}. However, the capacity for O_2 consumption due to CH_4 may be quite large, especially when taking into account a constant diffusive flow of CH_4(g) from deeper geological layers. One of the consequences of the REX project has been to start efforts in order to quantify the flows of H_2 and CH_4 at repository levels in Swedish sites.

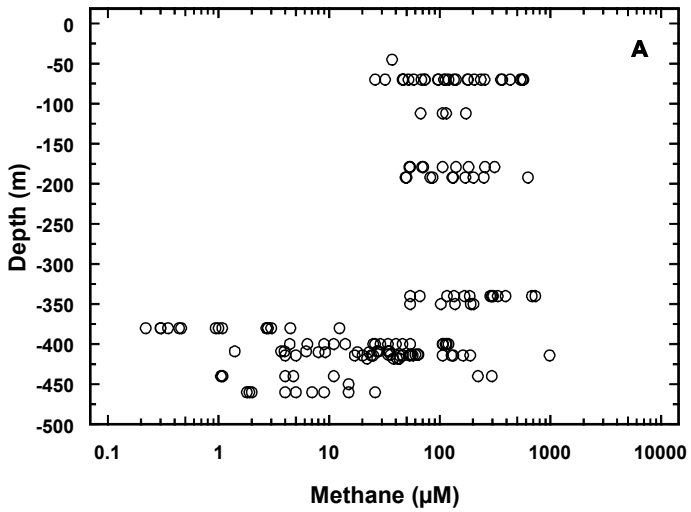


Figure 5. CH_4 concentrations in Äspö groundwaters measured as gas in the head space of sample vessels and re-calculated to dissolved gas. From (Kotelnikova and Pedersen, 1999).

4.3 CONCLUSIONS

- O_2 uptake by geologic media has been demonstrated.
- Microbes play a substantial role in O_2 consumption, and microbially mediated processes implicate added reducing capacity in a repository environment.
- Lab experiments with rock samples demonstrated that microbial activities induced increased chemical weathering, with the formation of clay minerals.
- When surface water containing O_2 encounters the "stationary" groundwater system, there is an important increase in the microbiological activities, resulting in O_2 depletion, transformation of organic material, formation of biofilm, and increased chemical weathering.
- The hydrodynamic effects of microbial formation of biofilms, clays and iron oxy-hydroxides are unknown at this moment. There is no information either on the increased sorption capacity for radionuclides due to these phenomena.
- Similar time scales for O_2 uptake were obtained in the *in-situ* and replica experiments. Nevertheless different microbial processes were taking place in the two experiments. This was reflected e.g. in the different pH-evolutions.
- Replica experiments have been found to be useful complements that may confirm the results from field experiments.
- Rates of O_2 reduction by fracture filling minerals were determined under laboratory conditions. These rates were faster than those reported in literature studies of pure mineral systems.
- The rate of microbial O_2 consumption was at least as fast as the corresponding inorganic processes.
- The time scale for O_2 reduction in typical fractures is estimated to be in the order of a few days.
- Microbes may reduce O_2 using H_2 and CH_4, which are common components of the gases dissolved in groundwaters. The reduction capacity of a repository site must therefore include the presence of organic materials, and the fluxes of H_2 and CH_4 from the deeper parts of the geosphere. This implicates added reducing capacity in a repository environment that is independent of surface climate conditions.
- Site characterisation for nuclear repository emplacements should therefore include
    - indigenous microbial populations
    - dissolved gases: H_2, CH_4, CO_2
    - fracture filling minerals carefully sampled with the triple tube drilling techniques
    - dissolved organic carbon

5 APPENDIX

A1 THE FIELD EXPERIMENTS

A1.1 THE REX IN SITU EXPERIMENT

The design and results from the REX in situ experiment have been reported in detail elsewhere (Kotelnikova and Pedersen, 2000; Puigdomenech et al., 1999; Puigdomenech et al., 2000a). A concise summary is given in this section.

A1.1.1 Description of the Experiment

The aim of the *in situ* experiment was to isolate a single fracture surface and to monitor the uptake of O_2 as a function of time. A borehole (KA2861A, $\Phi \approx 200$ mm) was drilled at 380 m depth in the tunnel of the Äspö Hard Rock Laboratory. The drillcore was sent to CEA (Cadarache, France) where a replica of the field experiment was completed as described in Section A2.1. The REX *in situ* experiment was conducted in a single fracture at 8.81 m from the tunnel wall. A detailed description of the set-up for the REX *in situ* experiment has been reported (Puigdomenech et al., 2000a), and it is summarised in Figure 8.

Because the O_2 probe requires a constant flow of the fluid, the experiment was performed in a closed re-circulating system. The circulation loop had a total volume of about one litre, and before each O_2 pulse it was filled with fresh O_2-free groundwater from the adjacent borehole, KA2862A. The O_2 injection pulse started by replacing part of the volume by a groundwater sample that had been previously saturated with either air or O_2(g). Because of the limited volume, the injection was performed with simultaneous withdrawal of the same volume of groundwater from the circulation loop. The procedure was performed under the system hydrostatic pressure of ≈35 bar.

The reaction chamber was made of gold-plated stainless steel. Tubing connections and valves were stainless steel. The tubing had 6.35 / 3.15 mm as outer / inner diameters, and the material was PEEK (poly ether ether ketone). A gear pump was used to circulate the groundwater from the reaction chamber to the measuring electrodes. The pump head was a Micropump®, type 1802R.125, with 316 stainless steel pump body and Ryton® (polyphenylene sulfide) gears. The pump motor speed control unit allowed circulation flow rates within 0 – 200 cm^3/min. Three o-rings ensured hydraulic confinement of the investigated fluid by fitting the reaction cap to the borehole

core. These o-rings were made of polyurethane because this material may be manufactured at many different degrees of flexibility.

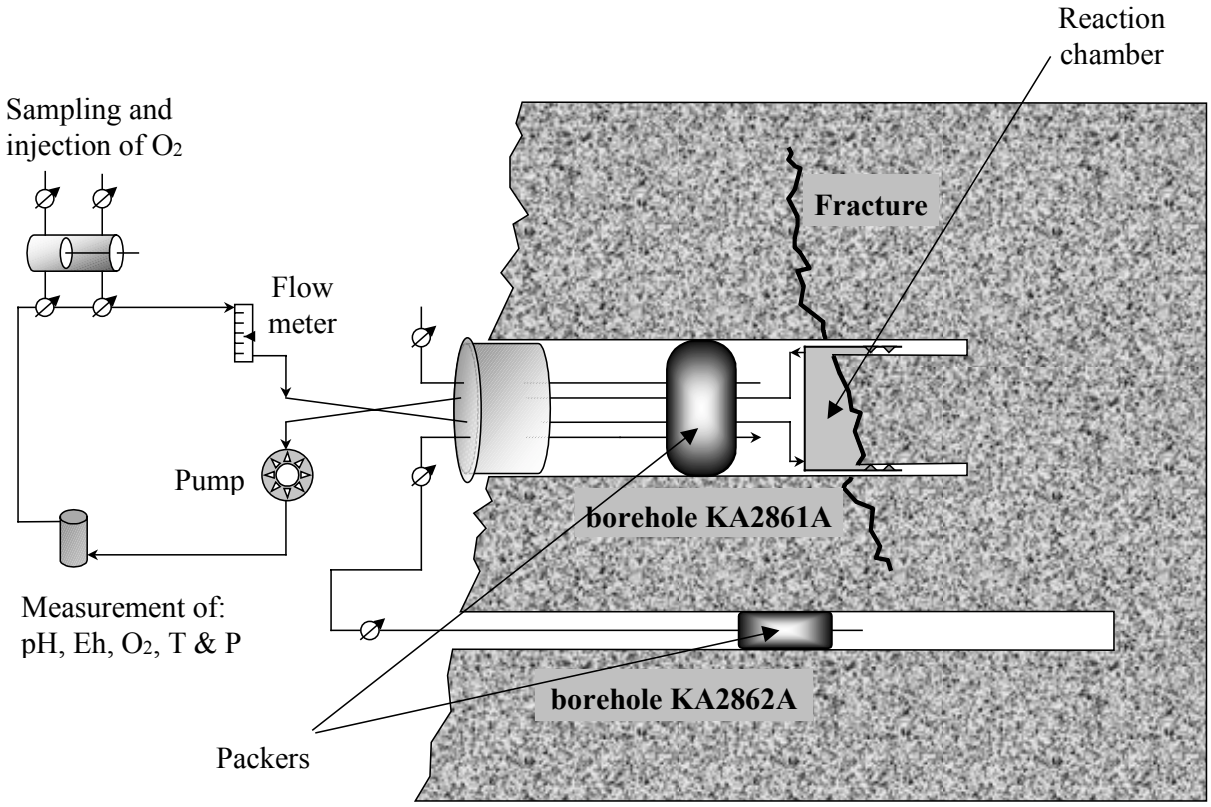


Figure 6. Set up for the REX *in situ* experiment.

Tracer tests with uranine were conducted to ascertain hydraulic confinement. It was found however that a substantial amount of tracer was lost, probably through the o-rings of the steel cap, to the surrounding borehole section. For periods larger than 10 days, almost 50% of the fluid in the reaction loop was interchanged with groundwater from the surrounding section of KA2861A (Puigdomenech et al., 2000a). The results of the tracer tests were interpreted as first order kinetics,

$$C = C_0 \, \mathrm{e}^{-k_{\mathrm{leak}} \, t}$$

where C is the tracer concentration, C_0 is the initial concentration of tracer, k_{leak} is the leakage rate constant, and t is the elapsed time. The results gave a total volume of 0.960 L for the test loop, and a leakage rate constant of 0.041 day^{-1}. In the data analysis of the O_2 uptake experiments a correction was introduced for this leakage.

Atmospheric leakage tests were also performed (Puigdomenech et al., 2000a). The data showed that atmospheric O_2(g) contaminated the groundwater in the reaction loop at a rate that amounted to ≈1 mg/L in a one-week period. This introduced an uncertainty in the results of the field experiment. The atmospheric leakage that was observed implies that the O_2-uptake rates

obtained in the field experiment were conservative, that is, the reported O_2-uptake rates are smaller than the real values.

A1.1.2 Results from the REX Field Experiment

O_2 uptake rates were studied in the REX *in situ* experiment by performing a series of O_2 injection pulses. Initial O_2 concentrations were in the range 0.5 - 26 $mg \cdot L^{-1}$, as specified in (Puigdomenech et al., 2000a).

A1.1.2.1 O_2 Uptake in the *In Situ* Experiment

The injected amounts of O_2 were consumed in the experiments within 5 to 10 days. Data for one of the O_2-pulses is shown in Figure 7.

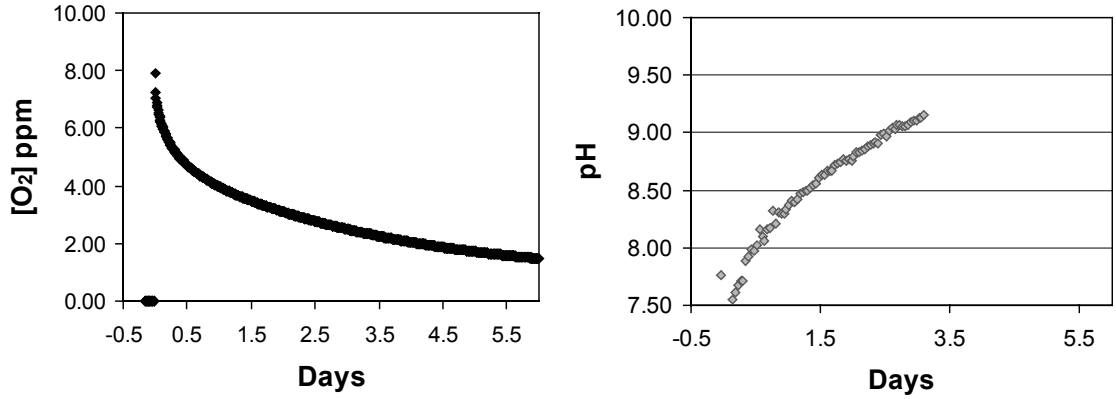


Figure 7. Results from one of the O_2-pulses in the REX field experiment.

A detailed presentation of the experimental data is given in (Puigdomenech et al., 2000a). All data could be described with a first order rate equation. Further refinements of the kinetic model were not possible owing to the limitations of the field experiment, described above, mainly: hydraulic leakage through microfractures and o-rings, and diffusion of atmospheric O_2 into the reaction loop. The rate constants found when fitting the experimental data varied between –0.9 and –0.1 day^{-1}.

A1.1.2.2 Chemical, Microbial and Mineralogical Characterizations in the *In Situ* Experiment

Microbial data from the field experiment have been reported in detail (Kotelnikova and Pedersen, 2000). Analyses of groundwater samples from the circulation loop showed O_2-induced aerobic microbial respiration and a succession of microbial groups in the groundwater and on mineral surfaces. Quite unexpectedly the number of iron-reducing organisms increased during the O_2 pulses in some cases. Similar effects were observed in the replica experiment. It appears that iron-reducing bacteria developed because of the increased Fe(III) concentrations that followed the O_2 pulses.

Chemical analyses for the REX field experiment were reported in (Puigdomenech et al., 2000a). The carbon balance is of importance because it is governed by, and it affects microbial activities. Both the inorganic and organic carbon content of the groundwater in the REX chamber increased during the O_2 pulses. Thus the initial inorganic and organic carbon content are 115 μM (alkalinity 7 mg/L) and (17 to 290) μM (DOC = 0.2 to 3.5 mg/L), respectively, giving a total initial carbon content in the range 130 to 400 μM.

At the end of an O_2 pulse the final inorganic and organic carbon content are 280 to 640 μM (alkalinity 17 to 39 mg/L) and (416 to 3250) μM (DOC = 5 to 11 mg/L), respectively, giving a total final carbon content in the range 700 to 3900 μM.

A possible explanation for these carbon increases could be leaching and/or bacterial degradation of plastic materials such as electrode components, o-rings, and PEEK tubing. No evidence for such degradations were however observed, as expected taking into account the small amounts of dissolved organic carbon observed.

The fracture surface was extracted from the REX borehole and characterised after completion of the field experiment (Puigdomenech et al., 2000a). SEM examination of the fracture surface showed that:

- The calcite patches contained well-crystallised calcite. Dissolution textures of the calcite were not observed.
- Pyrite grains were observed with sizes from 100 μm to < 1 μm. There was no indication of dissolution or oxidation of the pyrite.
- Flakes of chlorite and Fe-containing clay minerals are present on the surface.
- The fracture coating was not continuous, and during the experiment the water was in contact with the altered wall rock minerals: albite, epidote, chlorite, quartz, K-feldspar, fluorite and micro-grains of hematite.

Microbial characterisation of the fracture surface and PEEK tubing showed that 91% of the whole microbial population was located on the interior tubing walls, 8% in the gold-plated cap and fracture surface, while only 1% was suspended in the circulating groundwater (Kotelnikova and Pedersen, 2000).

A1.1.3 Main Conclusions from the *In Situ* Experiment

A quantitative interpretation of the data obtained from the field REX experiment is difficult due to the inherent uncertainties in the experimental set-up: insufficient hydraulic confinement, and atmospheric oxygen leakage. The results lead to the following qualitative conclusions:

- O_2 uptake takes place in periods of one to two weeks for the present experimental conditions.
- Microbial processes consuming O_2 give a substantial contribution to the observed uptake rate.

- The mechanisms for O2 uptake in the field experiment and in the replica experiment were maybe different, because of the different pH-variation during the O_2-pulse experiments. Two main differences exist between the field and replica experiments that may contribute to these differences: the materials for the reaction chamber (plastic versus gold-plated stainless steel), and the dissolved gases in the groundwater.

A1.2 MICROBIAL O_2 CONSUMPTION IN ÄSPÖ TUNNEL ENVIRONMENTS: THE MICROBE-REX PROJECT

Investigations of *in situ* microbial consumption of dissolved O_2 at Äspö have been performed by Göteborg's University, Sweden. This work was labelled as the "MicrobeREX" project (Kotelnikova and Pedersen, 1999). The main results and conclusions from these activities are summarised in this section. Further details may be obtained from the progress and final reports of this investigation (Kotelnikova and Pedersen, 1998; Kotelnikova and Pedersen, 1999).

A1.2.1 Introduction

Theoretically predicted respiration potentials for organic carbon, H_2 and CH_4 dissolved in the groundwater preclude that significant contribution to the process of rock reduction may be expected by heterotrophic aerobes, H_2 and CH_4 oxidising bacteria. It was hypothesised that microbial oxidation of organic carbon, H_2 and CH_4 are important processes that will reduce O_2 in and around a future HLW repository, and a field study (MicrobeREX) was undertaken to demonstrate this.

To test the hypothesis the following objectives were studied:

- Investigation of microbial O_2 reduction in Äspö HRL environments
- Qualification of dominating electron donors and microbial activities
- Isolation of CH_4-utilising microorganisms from the groundwater and study of their kinetic parameters
- Development of models for microbial O_2 uptake which may be used for predicting the time needed for the repository to return to anoxic conditions. The model should be based on available electron donors and microbial 16S rRNA gene diversity.

Our experiments showed that microbial O_2 reduction does occur in deep groundwater. Carbon dioxide was produced concurrently with O_2 reduction confirming the biogenic nature of the reduction.

A1.2.2 Results from the MicrobeREX Project

A1.2.2.1 Microbial O_2 Respiration in Deep Granitic Groundwaters

The data on microbial O_2 reduction activities by microorganisms were obtained with different techniques: Winkler method, gas chromatography, most probable numbering, enrichment technique, inhibitor analysis and radiotracer measurements. Details on these techniques are given in (Kotelnikova and Pedersen, 1999).

The samples were collected from boreholes and open tunnel ponds at Äspö tunnel at depths of 200-460 m below surface from January 1996 to May, 1998. The evaluation of the microbial activities in open ponds may be used to predict the future microbial activities after the O_2 intrusion around the future repository.

The investigated populations developed O_2 reduction rates and capacity that depended on the initial concentration of dissolved O_2. After approximately two weeks the unattached microbial population *in vitro* was able to consume O_2 both at 30°C and at 60°C. No delay in O_2 consumption was observed at 16°C. The results demonstrated that methanotrophs survive in deep groundwaters and that they were induced by O_2.

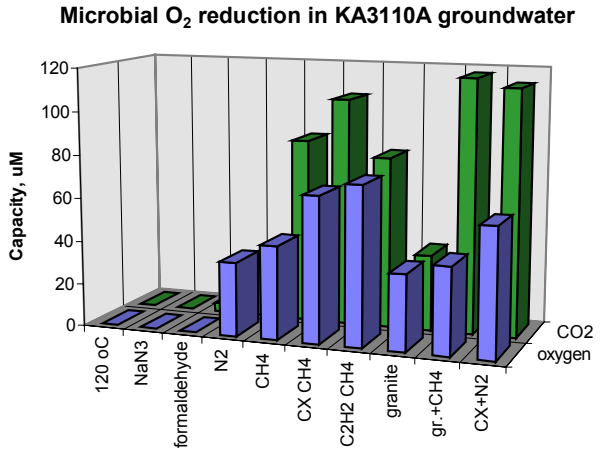


Figure 8. Effect of heating and different additions on microbial O_2 reduction in KA3110A groundwater at 16°C. *NaN_3*: sodium azide (1%); *granite*: mineral:water ratio 20:80; *CH_4*: 1% CH_4 in head space; *CX*: cycloheximide (1%); *N_2*: no additions, air in head space; *C_2H_2*: acetylene 5%; *formaldehyde*: 2%; *120°C*: the samples were heat autoclaved at 120°C for 20 min; *gr.+ CH_4*: mineral:water ratio 20:80 and 1% CH_4 in head space.

Rates of O_2 consumption ranged from 0.31 to 4.5 μM/day and decreased in the presence of well-known microbial inhibitors. Carbon dioxide was produced concurrently with O_2 consumption confirming the biogenic nature of the O_2 disappearance. O_2 consumption rates and specific cell respiration were reproducible in different ground water and with time.

Depending on temperature and the type of groundwater the approximate time needed for consumption of 500 μM of dissolved O_2 by unattached microbial population *in vitro* ranged from 9 days to 3.99 years. The O_2 consumption decreased exponentially with time

Diverse microbial populations capable of O_2 consumption were present both in oxic and anoxic ground water. There was no significant difference in O_2 consumption rates, capacities or efficiencies between the anoxic and oxic ground water.

The O_2 consumption capacity of the tested groundwater reached 700 μM. The O_2 consumption efficiency ranged between 40 and 100%, depending on O_2 concentration and duration of the tests.

At ambient temperature (15-16°C) the O_2 consumption was observed both in oxic and anoxic ground water without induction period. The microbial population *in vitro* was able to start active reduction at 30°C and 60°C after an induction period of 2 weeks. Thermophilic organisms were present in the groundwater. The adaptation period is expected to be longer at temperatures that differ from the ambient rock temperatures.

Different groups of microbes take a part in the consumption of O_2 in the flux ground water and on rock surfaces. Specific inhibitor of Eukaryota, cycloheximide, reduced O_2 consumption on 45-65%, indicating that eukaryotic microorganisms took part in O_2 consumption. Different microbial catalysis impact O_2 respiration in accordance with available nutrients, observed microbial activities and viable competent organisms. Such substrates as CH_4, H_2, acetate and formate were actively used in the presence O_2 (Table 1).

Table 2. Uptake and mineralisation of carbon substrates by microorganisms in ^{14}C radiotracer experiments with the KA2862A groundwater injected in REX chamber 980707 and circulating there until 980724. Values in the table are the average of four replicates. The activities in killed controls were subtracted from the activities in the non-inhibited samples.

Microbial processes	Rate of ^{14}C-substrate incorporation	
	Product, $\mu M \cdot day^{-1}$	Biomass, $\mu M \cdot day^{-1}$
Aerobic processes		
Glucose oxidation	0.05	642
Acetate oxidation	12030	9470
Formate oxidation	0	8880
Aerobic CH_4 oxidation	2.07	40
H_2 oxidation	*n.d.*	30
Anaerobic processes		
Glucose fermentation	1135	740
Homoacetogenesis	0.64	*n.d.*
Methane production	0.03	*n.d.*
Anaerobic CH_4 oxidation	0	0.5

A1.2.2.2 Methane Oxidation in the Groundwater

Methanotrophs were found to survive in deep groundwater at low numbers and they were active. Specific MO (methane oxidation) rates ranged between 3.08 and 220 nmol CH_4 per litre per day. The percentage of methanotrophs in the total cell counts in groundwater ranged from 0.15 to 30%. Methanotrophs could be induced by oxidation of the groundwater. The attached populations appeared to be more active than the free-living.

25 pure cultures actively oxidising methane were isolated from Äspö, affiliated to *Methylomonas, Methylosinus, Methylococcus, Methylobacterium* and *Methylocystis*. Half of the isolates grew without copper. Isolate 3500 developed at low temperature (6-36°C) and high salinity (1 M). A new species *Methylomonas scandinavica* SR5 was isolated and characterised.

Acetylene, which is a specific inhibitor of CH_4 oxidation, reduced O_2 reduction by 16-70%. Methane oxidation was responsible at least for 2.7-6.67% of the O_2 reduction in the deep groundwater and for 5.1-9.1% on preoxidised deep rock surface. The gas-chromatography tests showed that methane oxidation is responsible for 0.32-6.7% of total O_2 reduction in the deep groundwater and 9.08-57% in oxidised pond water. Efficiency of methane oxidation ranged between 38 and 80%.

Some bacteria use H_2 or CH_4 as electron donor instead of organic matter, which means that microbial O_2 reduction may occur also in deep groundwater where the availability of organic carbon is limited. Specific CH_4 oxidation rates ranged between 3 and 220 nM CH_4 per litre per day. Comparison of the total O_2 reducing activities by gas chromatography and radiotracer tests showed that CH_4 oxidation was responsible for 6.7% of the total O_2 re-

duction in the groundwater, for 9.1% of the total O_2 reduction on stones and 57% of total O_2 reduction in pond water. Methane oxidising organisms constituted 0.15% and 35% of the total cell population in the groundwater and pond water, respectively. Methane oxidation is proposed as one of the dominating microbial mechanisms for O_2 reduction.

Photographs of CH_4 oxidising bacteria are shown in Figure 9.

A1.2.3 Modeling of Microbial Respiration

The structure of the mixed microbial population depends on the energy sources available. Chemical analysis of groundwater and gases may predict the dominating microbial groups, which will be activated by the addition of dissolved O_2.

The respiration process may be approximated by the Double Monod form. A model for microbial O_2 consumption was proposed in (Kotelnikova and Pedersen, 1999). Multisubstrate Monod kinetics was found to describe the influence of the microbial energy sources available in groundwater on respiration capacity.

The stoichiometric and weight coefficients for each physiological group of microorganisms participating in the respiration were included in the model. Metabolic characteristics of indigenous organisms for the Monod rate equation provided the necessary data for testing the capacity model. Some of the results of the model are shown in Figure 10. Full details may be found in (Kotelnikova and Pedersen, 1999).

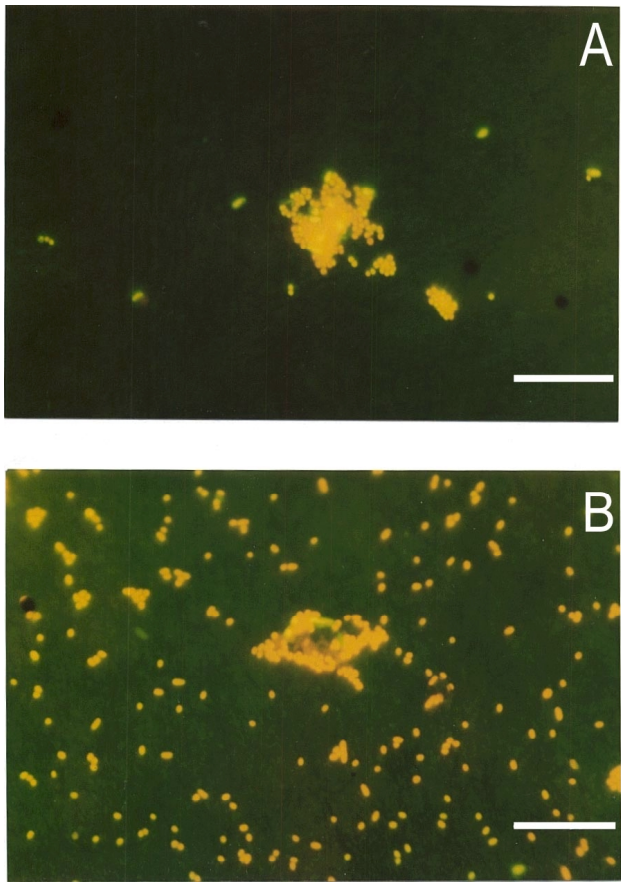


Figure 9. Phase-contrast micrographs of methane-utilising bacteria isolated in pure cultures from KA3105A (A) and KA3110A (B) groundwaters, grown in NMS medium (A) and sterile groundwater from KA3110A (B) with CH_4 and O_2. Bars: 10 µm.

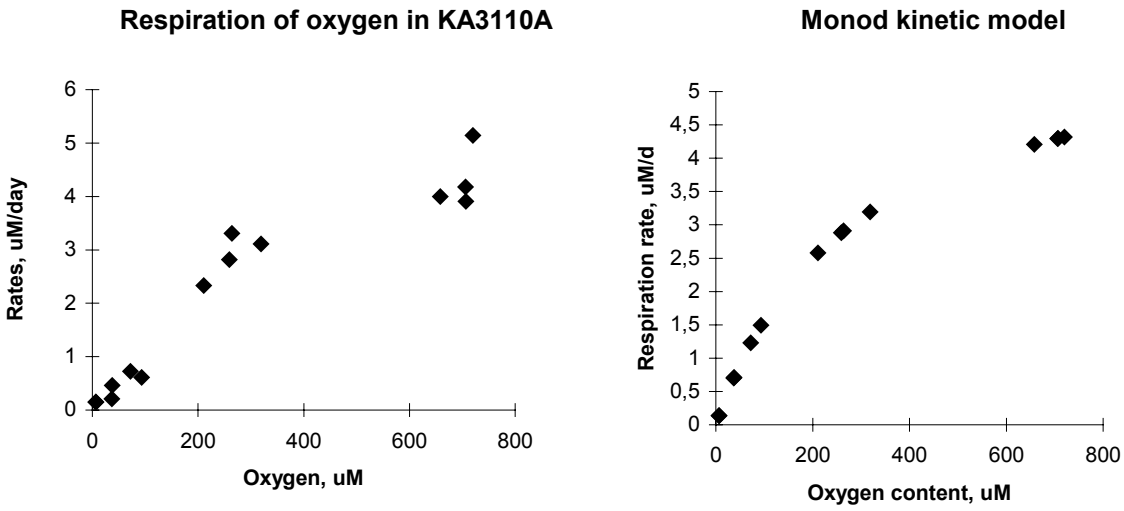


Figure 10. Microbial reduction of O_2 in KA3110A groundwater and the Monod-modeled reduction rate.

A1.2.4 Conclusions from the MicrobeREX Experiments

The experimental results obtained indicate that microbial activity will greatly contribute to O_2 reduction in groundwater in a HLW repository.

O_2 reduction is affected by different microbial groups depending on available electron donors and viable competent organisms. Substrates such as CH_4, H_2, acetate and formate will be actively oxidised in the presence O_2. Gases dissolved in a groundwater (CH_4 and H_2) will add to the reducing capacity. This process will be mediated by microorganisms. Methane oxidation will be one of the dominant processes for microbial O_2 reduction.

The biochemical O_2 reduction depends on the microbial diversity in the groundwater. The structure of mixed population depends on the electron donors available. Therefore, the *qualitative chemical analysis of groundwater and gases* may predict dominating microbial groups, which will be activated by O_2. While *quantitative chemical analysis of groundwater and gases* may predict the extent of these microbial processes. *Specific Monod kinetic parameters for microorganisms* may be used to obtain an estimate of the ability of different biota to use electron donors and reduce O_2. The model proposed in this report is based on the above listed characteristics. The model may be used to estimate the O_2 reduction capacities and rates of microorganisms in the groundwater.

A2 LABORATORY INVESTIGATIONS

A2.1 THE REPLICA EXPERIMENT

A2.1.1 Introduction

This section describes results obtained since February 1998 on the REPLICA experiment, which is part of the REX project. The results presented here in condensed format are described in greater detail in other reports (Trotignon et al., 1998; Trotignon et al., 1999). The present summary however includes recently acquired data, gathered after the end of the experiment in May 1999.

The concept, use and objectives of the REPLICA experiment proposed by ANDRA and CEA, within the framework of the REX project were presented in (Puigdomenech et al., 1999). The use and purposes of such an experiment is to simulate closely the *in situ* REX experiment by:

- comparing lab results with *in situ* testing: such a comparison is a powerful tool to test the importance of parameters often considered as irreproducible in the laboratory, i. e. water composition, microbial populations, dissolved gases, etc
- preparing and dimensioning the *in situ* experiment: lab experiments allow to test the sensitivity of the system by testing the dissolved O_2 uptake in blanks and by rock samples. It is also possible to test some sensitive parameters such as temperature, water composition, materials to be used and sensors.

The principle of the REPLICA experiment was to realise a laboratory study in which the second half of the fracture surface used in the *in situ* experiment would be submitted to the same succession of O_2 injections as in the field experiment.

After an initial period, during which materials, sensors and protocols were tested and the experimental set-up was built (Puigdomenech et al., 1999), final blanks and leakage tests were conducted. The REPLICA experiment was launched on June 1998. After an initial period of about three weeks during which the core in contact with water was isolated, the first O_2 pulses started. The last oxygen pulse ended in February 1999. The set-up was then left isolated in anoxic conditions for a period of about 100 days during which the evolutions of pH and Eh were recorded. The experiment was then stopped in May 1999.

A2.1.2 Experimental Procedure and First Observations

A2.1.2.1 Core Sampling and Characterisation

The diorite core (176 mm in diameter) was drilled on Oct. 28th 1996 (borehole KA2861A). The end section of the core was sent to the CEA at Cada-

rache, France. The fracture surface does not show strong signs of present day alteration or water circulation. The core was sawed in three parts: *i*) a basal section used to prepare thin sections and measure the uptake of dissolved oxygen by small diorite fragments, *ii*) an intermediate section of about 100 mm thickness, called "ghost core", used to simulate the real core in tests of the set-up, *iii*) the top section of the core, of about 120 mm thickness, carrying the target section. In addition to mineralogical investigations on thin sections (Lartigue et al., 1997b; Puigdomenech et al., 1999), the target section was observed under the binocular prior to the REPLICA. This investigation showed that the fracture surface had a number of asperities, open mineral joints and cavities (Trotignon et al., 1998). Mineral fragments are also found on the surface. This means that the effective surface area of the core surface will be significantly larger than the section area of the core. Test performed on the ghost core also showed that water could slowly permeate through it by using fractures filled with white alteration products (prehnite, calcite, clays).

A2.1.2.2 Groundwater Sampling

To be as close as possible to *in situ* conditions, special care was taken to minimise contamination of the water. After various tests and investigations (Lartigue et al., 1997a; Lartigue et al., 1997b; Puigdomenech et al., 1999), it was decided to use the water from the KA2862A borehole (inner section), located at 1 meter from the REX site with chemical characteristics close to those of the REX borehole. Groundwater used for the REPLICA was sampled in sterile, teflon coated, steel bottles, kept at site pressure, in order to minimise chemical and microbiological perturbations before injection in the experimental set-up in Cadarache. Upon arrival at Cadarache, the bottles were stored in dark at 5°C (site temperature is 15°C). Chemical analysis of this water may be found in Section A2.1.4 of this report. It is a Ca-Na-Cl rich solution (ionic strength about 0.4 molar). For leakage tests, which may consume large volumes of water, sampling in 5 litre sterile pockets was realised at atmospheric pressure.

A2.1.2.3 Eperimental Set-Up

The experimental set-up is described in detail in (Lartigue et al., 1997a; Puigdomenech et al., 1999; Trotignon et al., 1998). Figure 11 gives a schematic representation of this set-up and Figure 12 is a photographic view of it. The main parts of the set-up are the core unit (where the core is placed, with an O-ring fitted around and pressed by the cap), the measurement unit including a dissolved oxygen sensor (Orbisphere polarographic cell) , an Eh sensor (Pt combined) and a pH electrode. The Eh and pH sensors are Mettler-Ingold Xerolithe gel electrodes with Ag-AgCl references. Forced circulation of water by the pump (120 mL/min) is required for normal operation of the O_2 sensor. The volume of water used for filling the set-up is 1178±25 mL. The set-up works at atmospheric pressure and is placed in an incubator at 15±1°C. The water equilibration unit is used to pre-equilibrate the Äspö water with a gas mixture containing a known concentration of oxygen. Two equilibration procedures are used, respectively for pulses with low or high

concentration of dissolved oxygen (Puigdomenech et al., 1999). The homogenisation time for the O_2 concentration ranges from 20 to 60 minutes.

A2.1.2.4 Preliminary Experiments

The first investigations were made of O_2 uptake by the materials used in the experimental set-up. These investigations showed that the rate of reduction of dissolved oxygen by stainless steel 316L at 15°C in the saline Äspö groundwater was of the same order of magnitude as that of small diorite cubes under the same conditions. Therefore, stainless steel fittings and valves were avoided as much as possible. In addition, the cap and measurement unit were made in PETP (polyethylene terephthalate), which is a chemically inert and dense plastic material (PEEK, poly-ether-ether-ketone, could not be used because it was not possible to find cylinders large enough for the cap). As an alternative and in order to try comparable materials to those selected for the *in situ* experiment, a stainless steel cap and measurement unit plated with gold were also manufactured and tested (see Section A2.1.2.6).

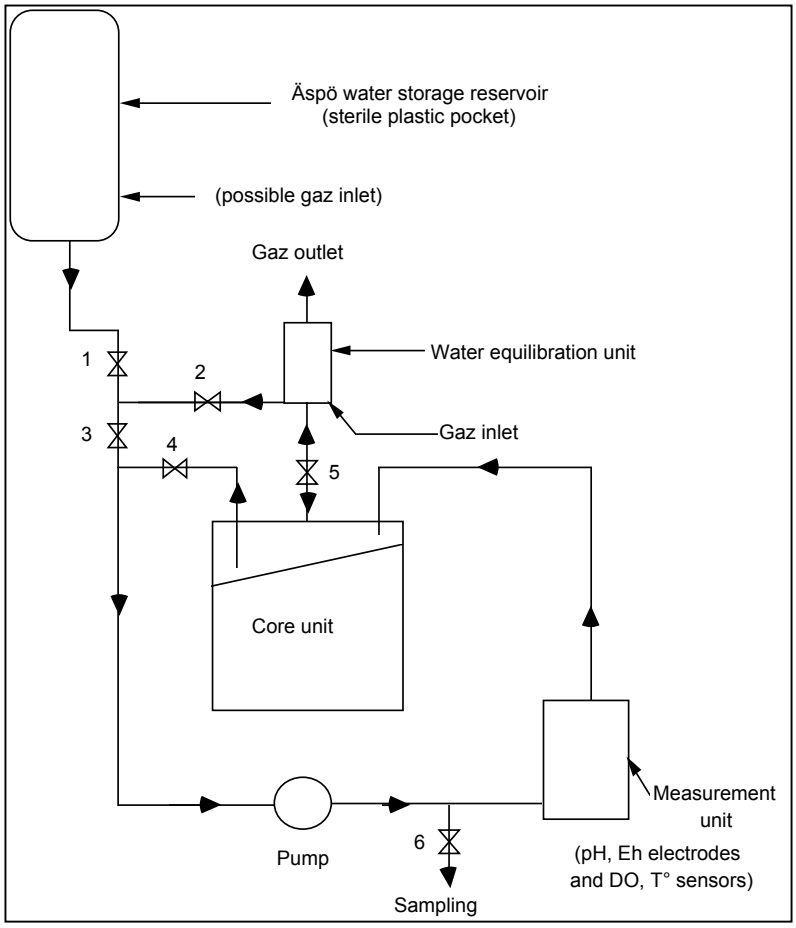


Figure 11. Scheme of the REPLICA set-up.

In order to test the reproducibility and stability of dissolved O_2 measurements, many blank tests were performed in a PETP vessel. Progressively increasing O_2 uptake observed in these blanks was shown to be due to microbial activity (Lartigue et al., 1997b; Puigdomenech et al., 1999). Tests were then continued with the ghost core. A series of O_2 pulse injections showed that:

- the replica set-up worked in a stable and reproducible way;
- the time needed to return to a zero O_2 concentration was of the order of magnitude of 2 weeks for a 10 mg/L initial O_2 concentration, much shorter than anticipated from literature data (Puigdomenech et al., 1999);
- the approx. linear shape of the O_2 evolution curves was unexpected and required an explanation;
- sulphate reducing bacteria sampled in the groundwater were able to colonise the experimental set-up;
- aerobic (or facultative) microorganisms developed in the set-up.

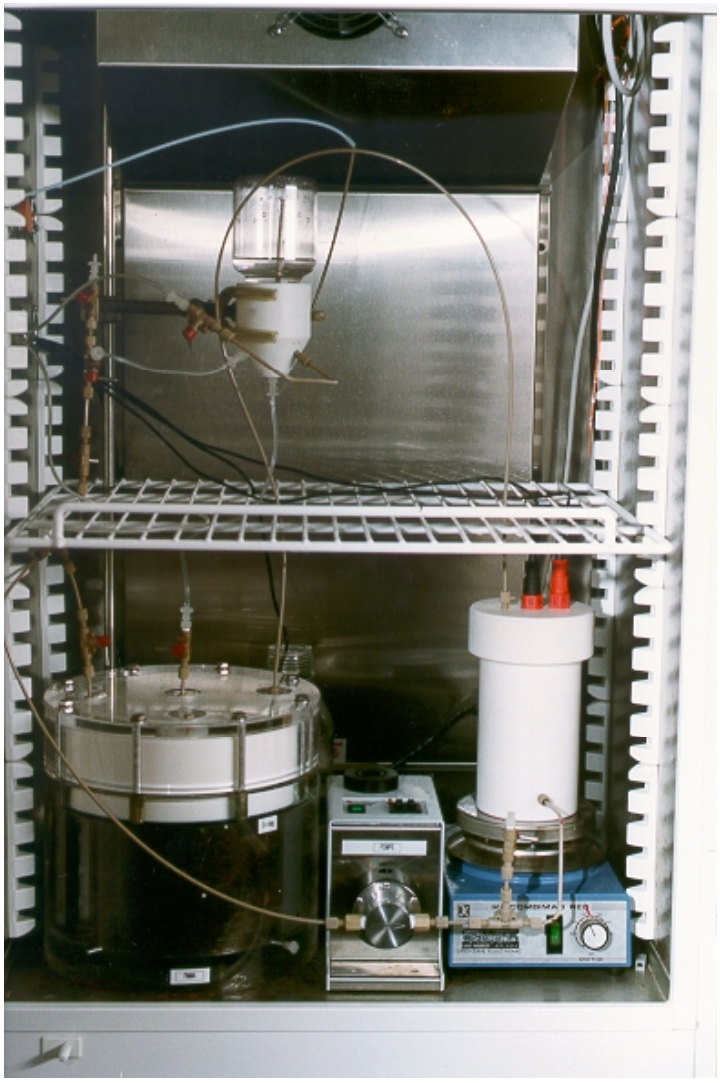


Figure 12. General view of the REPLICA set-up.

A2.1.2.5 The Experimental Protocol

Based on previous results, a protocol for the injection of O_2 pulses was proposed (Puigdomenech et al., 1999). This was based on the injection of O_2 pulses of increasing intensity in order to explore the dependence of the kinetic rate law on the O_2 concentration. Another advantage of this procedure was that it began with small perturbations. The injection of several pulses of identical intensity enabled to test the reproducibility. Rest periods between series of pulses made it possible to study the kinetics of return from anoxic to reducing conditions. The solution was sampled after the injection of each pulse. A final period of high O_2 concentration was used in order to evaluate the buffering capacity of the system. The protocol as described in (Puigdomenech et al., 1999) was followed with some adaptations:

- the initial period, preceding the injection of the first pulse, was limited to 3 weeks although a slow drift of pH and Eh (in anoxic conditions) was observed;
- the O_2 concentration for the first series of pulses was 0.8 mg/L;

- the time lag between the first series of pulses and the second one was extended to 2 months in order to explore the evolution towards reducing conditions;
- the O_2 concentration for the 2nd series of pulses was 4.5 mg/L; the membrane of the O_2 sensor had to be changed during the 1st pulse of the series;
- the time lag between the second and third series of pulses was limited to 15 days;
- the O_2 concentration of the two first pulses of the third series is limited to 7.5 mg/L in order to gradually perturb the system; the third pulse was about 11 mg/L in O_2 concentration; a replacement of O_2 sensor membrane is also realised during this series of pulses;
- at this stage, two small O_2 pulses (1 mg/L) have been added in order to confirm results obtained at the beginning with integration of the history of the system (microbial growth). The pH electrode was changed after these pulses;
- high O_2 concentration maintained during several weeks, by the successive injection of strong oxygen concentrations;
- after the O_2 concentration returned to zero, the system was left isolated during 15 weeks with continuous monitoring of pH and Eh. During this period, several solution samplings were realised without perturbing the reducing conditions.

A2.1.2.6 Preliminary Tests and Blanks

A2.1.2.6.1 The REX00 run

The REX00 run was an experiment conducted on the ghost core in order to ensure that the set-up was working correctly over long time periods, with continuous monitoring of Eh, pH and O_2. This experiment, run at the beginning of 1998, had a total duration of 48 days. The experimental configuration was very close to that of the REPLICA, except that the water used was collected at atmospheric pressure in a large PE vial. Details are given in (Trotignon et al., 1998). The set-up was carefully cleaned with ethanol. Before loading the set-up with the groundwater from the KA2862 borehole, this had been equilibrated with a CO_2 (100 ppm) – H_2 (2000 ppm) – N_2 (qsp) mixture under anoxic conditions. A O_2 pulse was then injected.

After an initial period were O_2 concentration increased up to 2 mg/L (probably due to a re-equilibration with trapped air bubbles), it rapidly decreased with a constant rate (21 µmol/(L day)) and reached zero concentration after about 4 days. The Eh potential showed an initial increase, followed by a plateau and then a rapid decrease as O_2 vanished. The pH decreased continuously as long as O_2 was present. After O_2 exhaustion, pH increased slowly and Eh went on decreasing. After 35 days, a sudden drop of Eh was observed, with simultaneously pH fluctuations. Solution analyses before and after the run, showed a marked increase of dissolved Fe. Dissolved sulphides were detected at a low level in the reduced solution.

In summary, the REX00 run showed that the set-up worked correctly over time periods of at least two months. The evolution of O_2 concentration was

consistent with previous runs performed on small batch systems (Puigdomenech et al., 1999). The pH drop, in oxic conditions, was perhaps linked to carbonic acid production by microbes. Marked reducing conditions (E_{NHE} = −150 mV for pH = 6.8) were obtained after about 5 weeks in anoxic conditions. Unfortunately, no microorganism analyses were planned for this run.

A2.1.2.6.2 Blanks and Leaching Test

Before starting the REPLICA experiment, blanks were performed on the set-up equipped with the gold-plated cap and measurement unit. A PETP core was placed in the set-up as a replacement of the diorite core. A series of blanks was realised with water from the KA2862A borehole with or without sterilisation (using NaN_3, although a slight interaction transiently exists between O_2 and the azide anion). The O_2 uptake rate in non-sterile conditions was higher than in sterile conditions. However, a contribution to O_2 uptake remained in sterile conditions; the corresponding data points set on a straight line through the origin, consistently with a first order rate law (k_2 about 0.1 day^{-1}). At the end of blanks, the set-up was opened and the probable cause of this O_2 uptake was identified: swellings and blisters and corrosion evidence was seen on the gold plating. As it was not possible to guaranty that a new plating of the cap and measurement unit would be efficient, it was decided, in agreement with ANDRA, to start the REPLICA by using the PETP version of the equipment.

A2.1.3 O_2 Uptake in the REPLICA Experiment

Before starting the REPLICA experiment, the set-up was sterilised with ethanol and then loaded with the real core section, that had been kept under nitrogen in the lab. To avoid spilling of pressurised water and in order not to contaminate the set-up, the leakage test was made with water stored in 5 L sterile pockets. The set-up was flushed by nitrogen before introducing the water. After only few minutes of water circulation, leaks were seen on the cylindrical surface of the core, corresponding to a fracture network. No leakage was noticed at the o-ring; water can be transported under low pressure through the fracture network mainly as a result of capillary forces. The leakage test was stopped and the cylindrical surface and base of the core were covered with a resin (Struers Epofix 40200029). A second leakage test was then performed and gave good results on a 12 days period. The pressurised bottles arrived then at the lab in Cadarache and the REPLICA could start immediately.

Table 3 gives the general chronology of the REPLICA experiment, from the beginning to the end in May 1999.

Table 3. Chronology of the DO (dissolved O_2) pulses performed in REPLICA experiment.

Date	Time (d)	Note		Reference	Chemistry	Biology
10/06/98	0	Start		REP01	yes	yes
		Initial anoxic state				
02/07/98	22	First small DO pulse	P1	REP02	yes	no
06/07/98	26	Second small DO pulse	P2	REP03	yes	no
09/07/98	29	Third small DO pulse	P3	REP04	yes	yes
15/09/98	97	First medium DO pulse	P4			
		after long anoxic period		REP05	yes	yes
23/09/98	105	Second medium DO pulse	P5	REP08	yes	yes
05/10/98	126	Third medium DO pulse	P6	REP09	yes	no
26/10/98	138	First large DO pulse	P7			
		after anoxic state		REP10	yes	yes
09/11/98	152	Second large DO pulse	P8	REP11	yes	no
23/11/98	166	Third large DO pulse	P9	REP12	yes	yes
14/12/98	187	Small DO control pulse	P10	REP13	yes	no
17/12/98	190	Small DO control pulse	P11	REP14	yes	no
18/12/98	191	High DO (28 ppm)	P12a		no	no
21/12/98	194	DO increases to 38 ppm	P12b		no	no
7/1/99	211	Sampling at 17.5 ppm	P12c	REP15	yes	yes
21/1/99	225	Sampling at 7 ppm	P12d	REP16	yes	yes
4/2/99	239	DO reaches 0 ppm				
4/3/99	267	Sampling in anoxic conditions		REP17	yes	yes
14/4/99	308	Sampling in anoxic conditions		REP18	yes	yes
11/5/99	335	Sampling in anoxic conditions and stop		REP19	yes	yes

A2.1.3.1 Initial Period

Water sampled in pressurised bottles on May 12th in Äspö arrived in Cadarache on May 29th. One of the bottles was set aside for chemical and microbiological analyses. The transfer from the bottles to the set-up (which works at atmospheric pressure) was done in two stages: *i*) transfer from the bottle to a sterile pocket to avoid degassing in the set-up, *ii*) transfer from the sterile pocket to the set-up after 3 days degassing at 5°C in the dark and keeping the gas in the pocket. Prior to loading, the set-up was flushed by a CO_2-H_2-CH_4-N_2 gas mixture, to obtain the mean gas concentrations of the field groundwater. After loading, a O_2 concentration of 0.16 mg/L, a pH of 7.09 and a Eh of –100mV (measured against $Ag/AgCl_2$). The set-up was then left isolated during three weeks. Dissolved O_2 introduced in the water was consumed in about 10 hours. Eh decreased rapidly as soon as O_2 was exhausted; pH decreased during about 1 hour and then started again to increase. After about 4 days, Eh began to increase again while pH started to decrease again. At this stage of the experiment, it was observed by switching on and off the O_2-meter that a slight (and reversible) interaction existed between the O_2 sensor and the Eh and pH sensors, causing a shift of about 0.2 pH units and 10 mV in Eh. It was nevertheless decided to go on with the injection of oxygen pulses, without modifying the set-up.

A2.1.3.2 First Series of O_2 Pulses (P1, P2 and P3)

The first series of experiments were made from July 2nd 1998 to July 10th 1998. They were started by withdrawing with a sterile syringe 120 mL of water through the sampling port (Figure 11), while the same volume of solution was introduced from the equilibration module, bubbled with an adequate N_2-O_2 mixture. The evolutions of dissolved O_2, Eh and pH are given in Figure 13 to Figure 15.

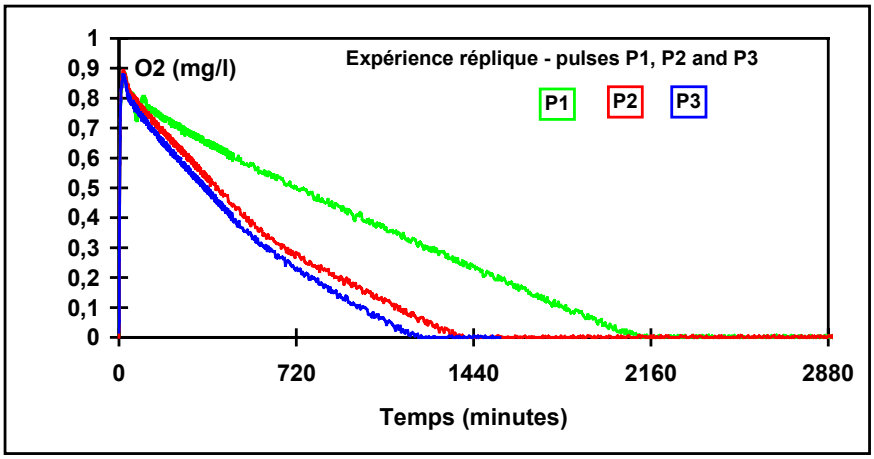


Figure 13. Time evolution of dissolved O_2 for pulses P1, P2 and P3.

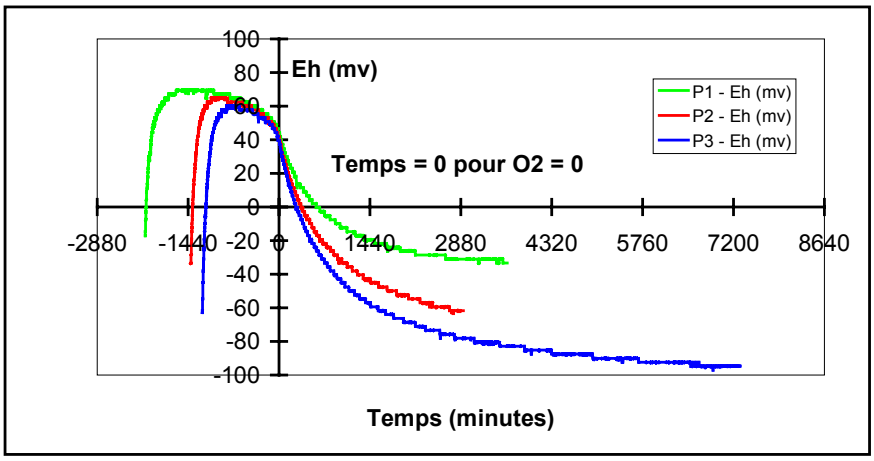


Figure 14. Time evolution of Eh (against Ag/AgCl) for pulses P1, P2 and P3. The time origin is set at the moment when O_2 is exhausted.

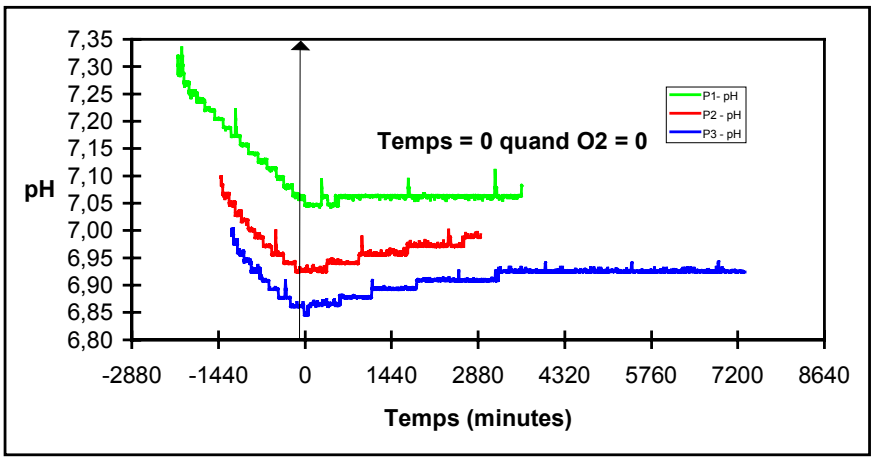


Figure 15. Time evolution of pH (15°C) for pulses P1, P2 and P3. The time origin is set at the moment when O_2 is exhausted.

For pulse P1, a linear O_2 uptake was observed, while for P2 and P3 a succession of two faster linear uptakes was found, *cf.* Figure 13. The Eh potential followed very similar evolutions during the three pulses: a rapid increase at O_2 injection, followed by a plateau and then a rapid decrease at O_2 exhaustion. Temporal evolutions of pH were also similar from pulse P1 to P3: a decrease during the O_2 uptake phase followed by a slow increase in anoxic conditions. The injection of O_2 thus leads to a progressive acidification of the medium.

A2.1.3.3 Anoxic Time Lag

After the first series of O_2 pulses, it was decided to maintain the set-up in isolated anoxic conditions for a long period (2 months) in order to evaluate the time necessary to reach stable reducing conditions. Eh and pH evolutions are given in Figure 16 and Figure 17. Their coupled evolution will be commented in Section A2.1.7 with respect to solution analyses. At the end of

this period, the solution reached a reducing state (Eh(ENH)= –50 mV for pH = 6.7) that was slightly drifting towards lower potentials and pH.

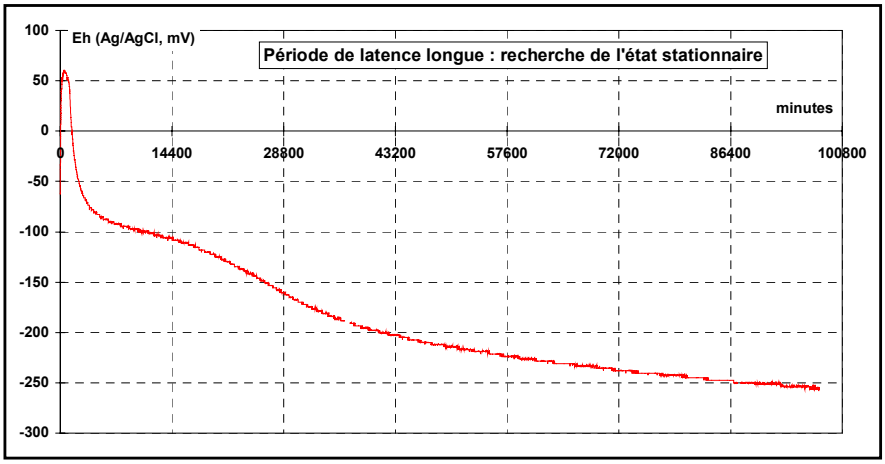


Figure 16. Anoxic period: Time evolution of Eh (against Ag/AgCl) during 3 months. The potential against the normal hydrogen electrode may be calculated according to: E_{NHE} = Eh(Ag/AgCl) + 207 mV.

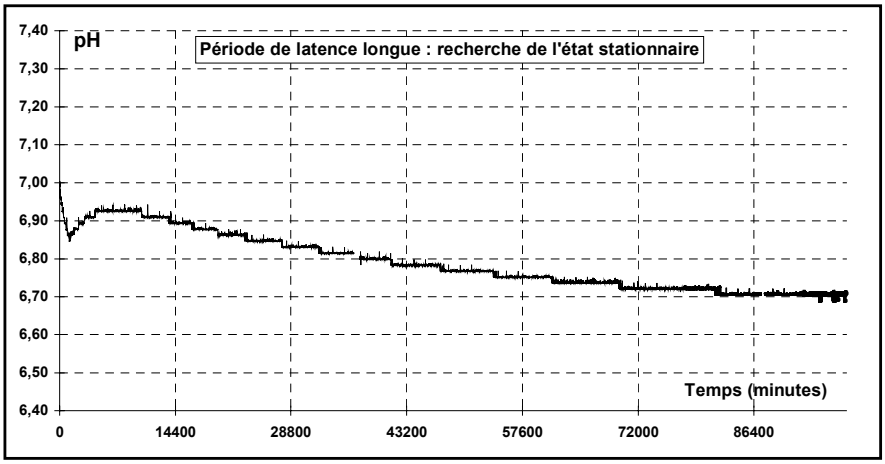


Figure 17. Anoxic period: Time evolution of pH (15°C) during 3 months..

A2.1.3.4 Second Series of O_2 Pulses (P4, P5 and P6)

As for the first series of pulses, described in previous section, the 2nd series of O_2 pulses were conducted by a combined injection/drawing-off operation of 120 mL of solution. The injected solution was bubbled with pure oxygen prior to injection. During pulse P4, a replacement of the membrane of the oxygen sensor was done because the O_2 concentration signal was becoming noisy. This operation is detailed in (Trotignon et al., 1998). For pulses P4 to P6, a stronger dependence of the uptake rate versus the O_2 concentration was observed (Figure 18). The mean O_2 uptake rate diminished from pulse P4 to P6. The evolutions of pH and Eh (Figure 19 and Figure 20) were similar to those obtained from pulses P1 to P3.

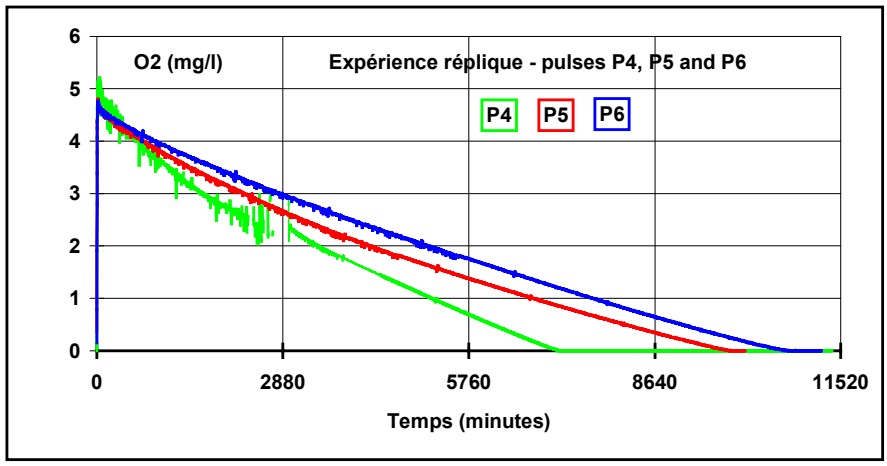


Figure 18. Time evolution of dissolved O_2 for pulses P4, P5 and P6.

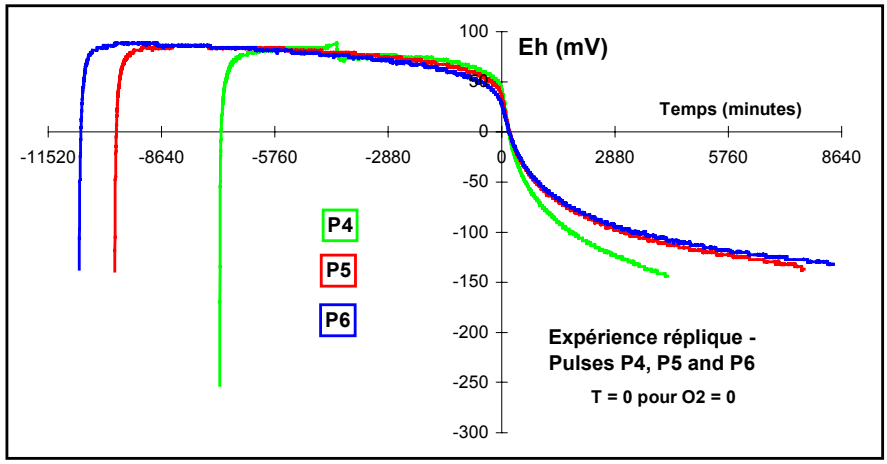


Figure 19. Time evolution of Eh (against Ag/AgCl) for pulses P4, P5 and P6. The time origin is set at the moment when O_2 is exhausted.

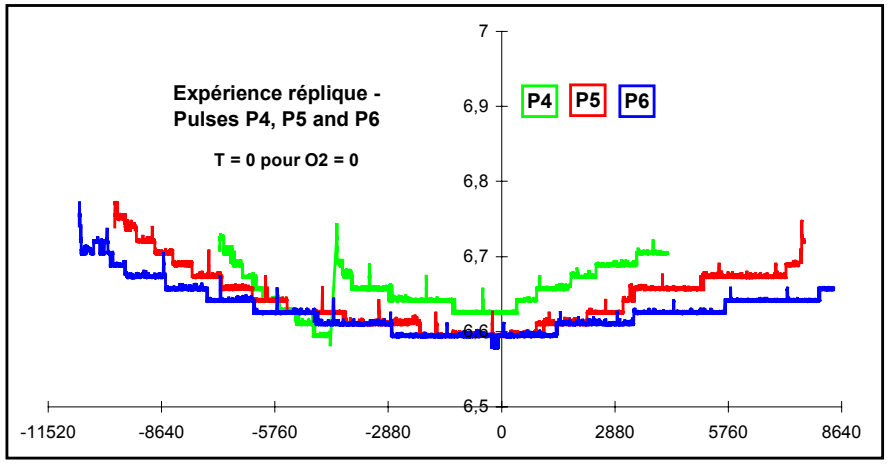


Figure 20. Time evolution of pH (15°C) for pulses P4, P5 and P6. The time origin is set at the moment when O_2 is exhausted.

A2.1.3.5 Third Series of O_2 Pulses (P7, P8 and P9)

The time lag between the second and the third series of pulses was restricted to 2 weeks. During this period pH increased to 6.6 while Eh(ENH) decreased to about +50 mV. A second replacement of the membrane in the O_2-sensor was done after P8. The O_2 concentration injected for P9 (11 mg/L) was higher than for P7 and P8, due to an unexpected overpressure during bubbling in the equilibration module. As for pulses P4 to P6, a dependence of O_2 uptake with respect to O_2 concentration was noted (Figure 21).

The temporal pH and Eh evolutions were consistent with other pulses, although the pH seemed to get buffered around 6.5. The pH electrode started to give a noisy signal during pulse P9 and was changed in December 1998 (Figure 22 and Figure 23).

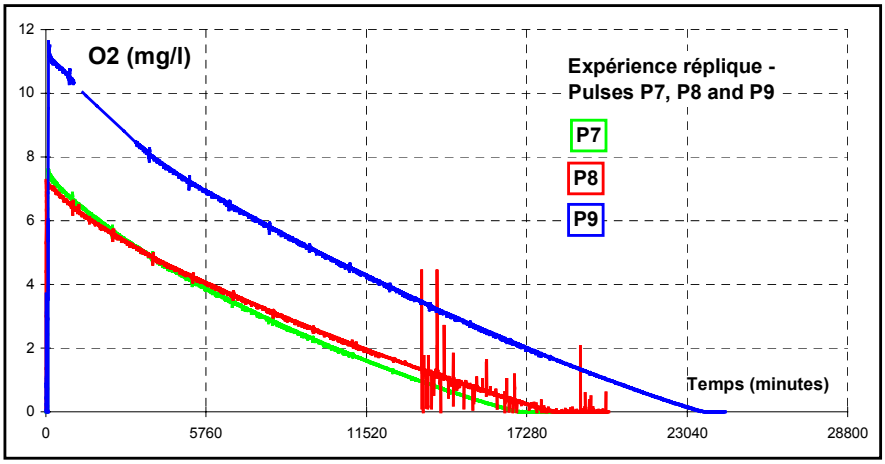


Figure 21. Time evolution of dissolved O_2 for pulses P7, P8 and P9.

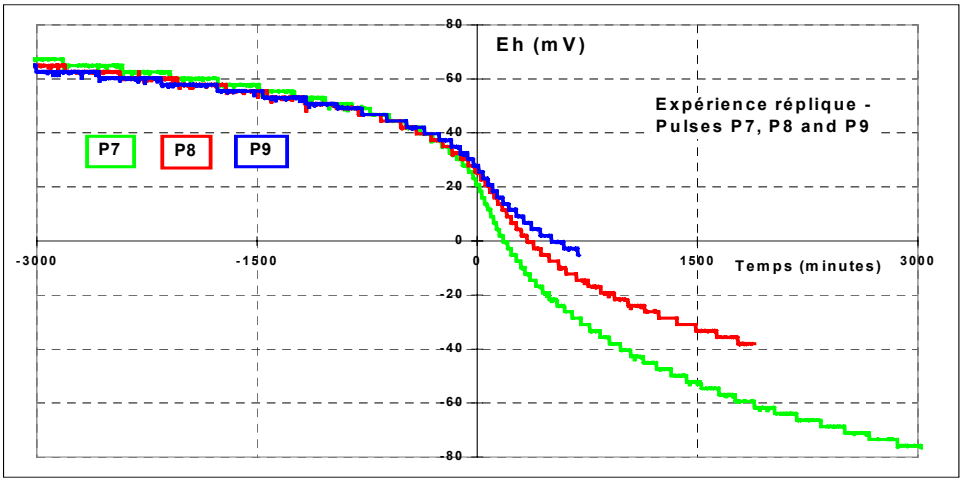


Figure 22. Time evolution of Eh (against Ag/AgCl) for pulses P7, P8 and P9. The time origin is set at the moment when O_2 is exhausted.

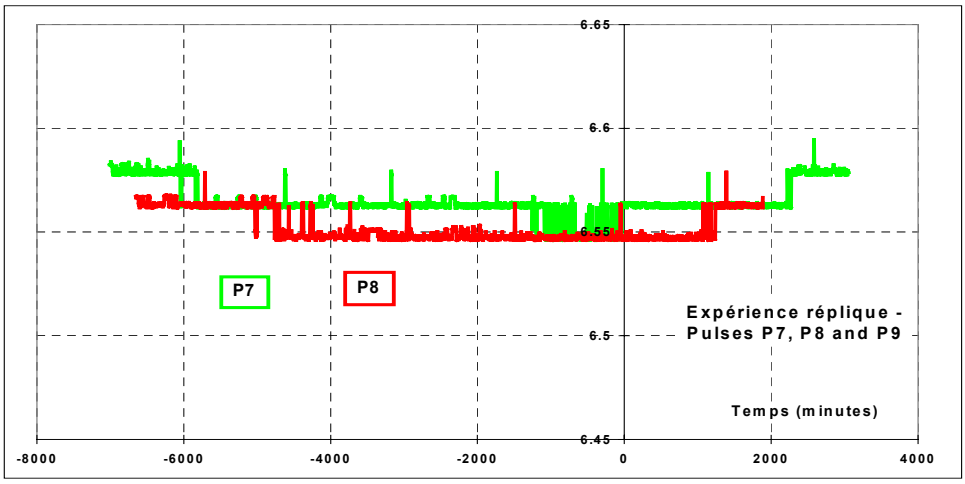


Figure 23. Time evolution of pH (15°C) for pulses P7 and P8. The time origin is set at the moment when O_2 is exhausted. The pH signal for P9 is not shown because of electrode failure.

A2.1.3.6 Supplementary Small O_2 Pulses (P10 and P11)

It was decided, before launching the last sequence of the experimental protocol, to try to reproduce one or two pulses of small intensity. This decision was motivated by the fact that, although microbial populations had strongly evolved (see below), the O_2 uptake rates stayed in the same order of magnitude. Pulse P10 of intensity 1 mg/L compared perfectly well with pulse P1 (Figure 24); this fact suggested that there was a rate limiting factor for O_2 uptake by microbes. The following pulse (P12) was performed in order to test whether this rate-limiting factor was linked to convection of the solution, i.e. to the pumping flow rate in the set-up loop. During pulse P12, the pumping rate was changed from 80 mL/min to 160 mL/min without any noticeable effect on O_2 uptake rate. The rate-limiting factor for O_2 uptake by microbes is thus not convective flow.

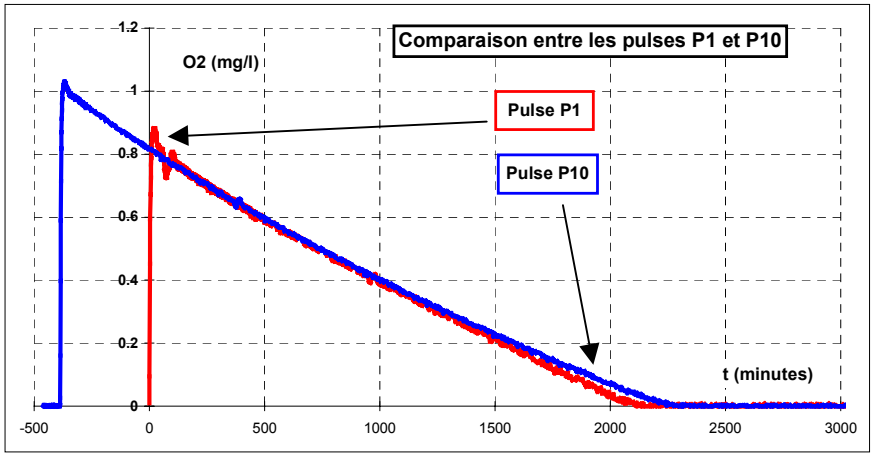


Figure 24. Comparison of pulses P1 and P10.

A2.1.3.7 Intense and Long Oxygenation Period (P12a to P12d)

This long oxygenation period was included in the protocol to be the highest perturbation undergone by the fracture surface. The first injection of O_2 was obtained by recirculating the whole water volume in the equilibration module through which pure oxygen bubbled. At different periods, the oxygen concentration was renewed (Figure 25) and simultaneously a solution sample was extracted. Some problems with the O_2 sensor (noisy signal probably due to deposits on the membrane) were encountered and led us to shut the sensor off during some periods. The Eh potential remained stable at about +300 mV (*versus* NHE) during the oxygenated period (Figure 26); a short transient was noted at each O_2 new injection. When O_2 was exhausted, Eh dropped rapidly, consistently with previous observations on other pulses. Upon each O_2 injection, pH rapidly increased (Figure 27) and then continuously decreased during O_2 uptake. After O_2 exhaustion, pH started to increase again.

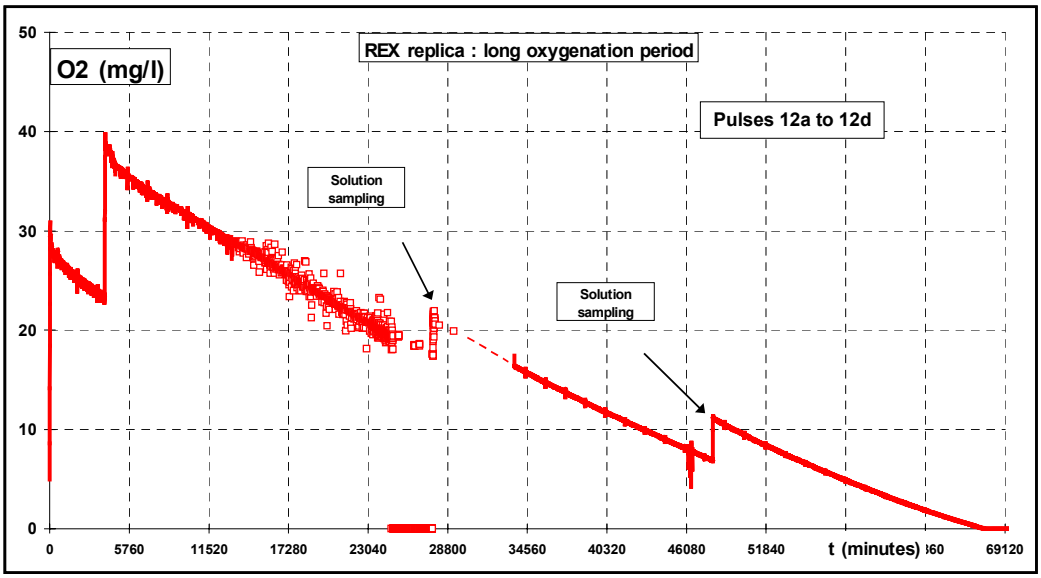


Figure 25. Evolution of dissolved O_2 concentration during the composite pulse P12.

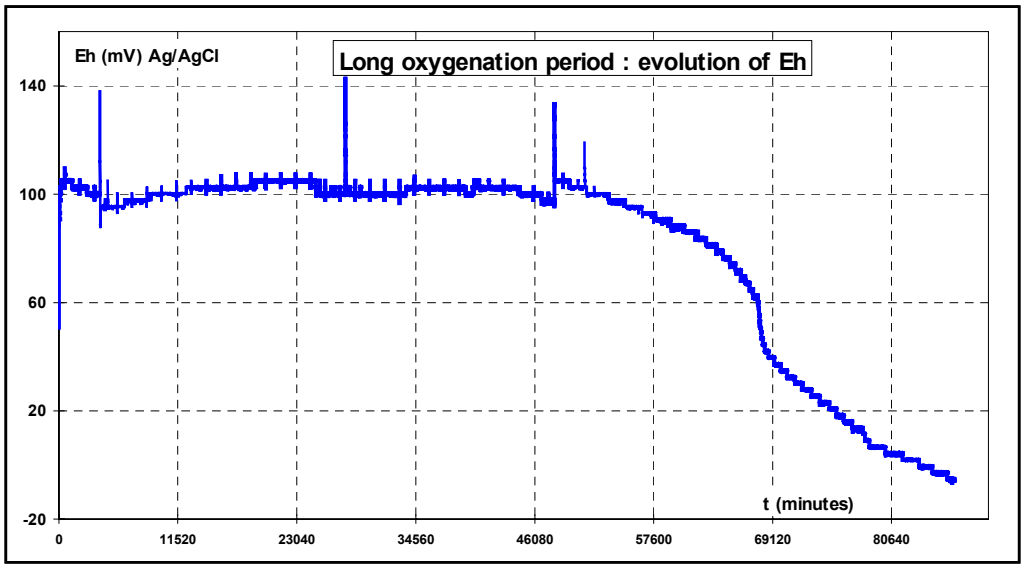


Figure 26. Time evolution of Eh (against Ag/AgCl) during and after pulse P12.

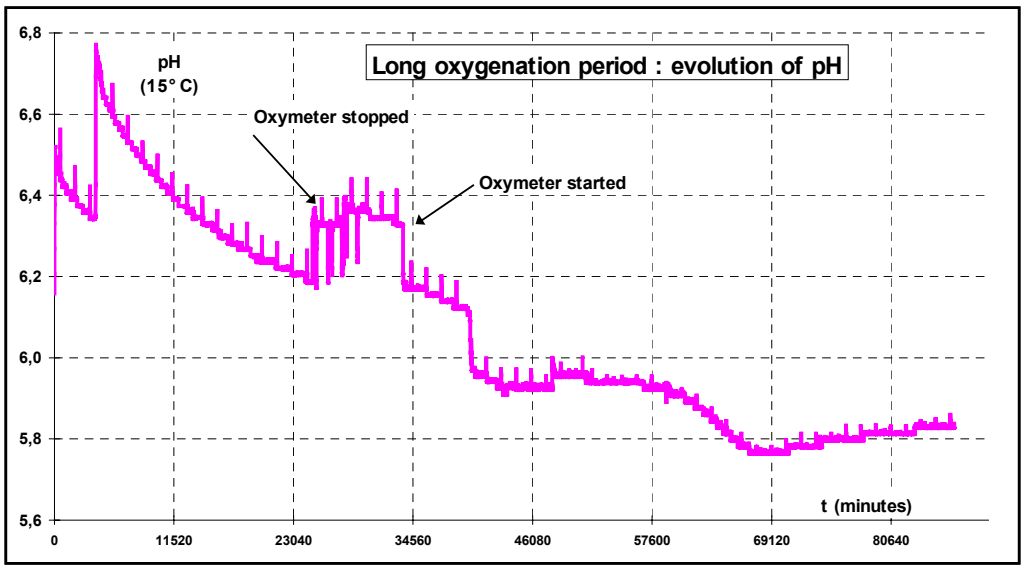


Figure 27. Time evolution of pH during and after pulse P12.

A2.1.3.8 Final Anoxic Period

After the dissolved O_2 was totally exhausted, the set-up was left in function and Eh and pH parameters evolved spontaneously. Eh decreased continuously to about –10 mV (ENH) and pH steadily increased from 5.9 to about 6.1 in three months. Three samples were collected during this period, without perturbing the reducing conditions and gave access to chemical and microbiological evolutions (see Sections A2.1.4 and A2.1.5 below). The coupled Eh-pH evolution will be commented in Section A2.1.7.

A2.1.4 Chemical Analyses

At each O_2 injection, a solution sample was collected and analysed. Chemical analyses were done for Na, K, Ca, Mg, Cl, SO_4, Fe, Mn, Al, Sr, Si. Total organic carbon (TOC) and inorganic carbon, sulphide and several organic acids were analysed on some samples. Analytical methods are given in (Trotignon et al., 1999). Table 4 summarises analytical data. A more detailed presentation of chemical data is made in (Trotignon et al., 1999).

Concentrations of main elements (Na, Ca, Cl and Mg) are stable with time. The potassium concentration increases strongly with time: this is perhaps due to the Eh and pH electrodes, which release KCl through the porous junction, or to the release by the resin (see data for TOC). The levelling of concentrations after 01/11/98 could be linked to a precipitation process. The second increase after 31/12/98 could be linked to the electrode replacement. Silicon concentrations remain fairly stable, at a level consistent with chalcedony-solution equilibrium. The Mn concentration of solution is usually around 250 µg/L, except at the end of long anoxic periods were concentrations up to 1.3 mg/L were measured. For iron, a similar evolution is observed: the first samples show a low iron concentration (30 µg/L); at the end of the first anoxic period, the iron concentration in solution is 600 µg/L. During pulses P4 to P12, the iron concentration seems to be stable, around 150 to 200 µg/L. During the last anoxic period, iron concentration progressively increases up to 1000 µg/L. Al concentrations fluctuate between values less than the detection limit (25 µg/L) up to 70 µg/L, with no clear trend.

Total organic carbon (TOC) in solution shows a very strong increase, probably due to the plastic parts of the set-up (PETP cap and measurement unit, O-ring, resin). The stabilisation of TOC in December 1998 could be linked to a direct reaction of O_2 with organic carbon, as at this period high O_2 pulses were injected. Inorganic carbon increases markedly in solution with time and this supports the idea that bacterial respiration generates CO_2 which leads to the progressive acidification of the solution. The pH of the solution stabilises around 6 at the end of the experiment. Exploratory analyses were done on simple organic acids, showing that these molecules remain at low levels, probably because they are rapidly degraded by microbes. Temporal evolution of sulphate in solution shows a trend to decrease: this is possibly to precipitation phenomena or to sulphate reducing microorganisms.

In conclusion, chemical analyses give essential information that help to constrain the interpretation of O_2 uptake kinetics. Major facts evidenced during the REPLICA experiment were:

- increase of total organic carbon (TOC) in solution: this contributes probably to bacterial growth. However, the degradation of organic carbon in small molecules is probably limited by an unidentified process. This could give an explanation to the limitation of O_2 uptake by microbes.

- continuous increase of inorganic carbon and correlative decrease of pH down to about 6;
- stabilisation of iron concentrations at a significant level during the period of strong oxygen pulses. The literature review had shown that iron(II) oxidation in solution follows a first rate law with respect to O_2. Going back to the data summarised by (Wehrli, 1990), supposing that iron concentration is maintained in solution at 200 µg/L, the kinetic coefficient of this first-order rate law was evaluated at 25°C, pH = 6 and 6.5. Calculated values are respectively 0.02 and 0.19 day^{-1}, which is the order of magnitude derived for pulses after P4. For earlier pulses, the fitted value is higher, consistently with higher pHs. The oxygen-sensor contribution to the O_2 reduction is about 0.002 day^{-1}. Oxidation of iron(II) in solution is thus a candidate mechanism to explain the first order contribution observed on O_2 signals. An explanation remains however to be given to explain why iron remains at a constant level in solution although rapidly oxidised and precipitated, and why the first order rate is nearly stationary.
- the iron and manganese concentrations increase strongly during long anoxic periods: this is consistent with the known redox properties of these elements.
- manganese concentrations remain relatively stable during periods of O_2 injection: Mn could have contribution of similar form to that of Fe to O_2 uptake kinetics. A literature review is necessary to evaluate this point.
- sulphate concentration decreases, probably due to precipitation or microbial reduction.
- Ca, Na, Mg, Cl, Si, Sr concentrations are stable or show weak fluctuations.

Table 4. Chemical analyses for water samples from the REPLICA experiment.

Sample	Date	Ca	Na	K	Mg	Al	Fe	Si	Mn	Sr++	Cl-	SO4--	HS-	TOC unfiltered
		g/L	g/L	mg/L	mg/L	µg/L	µg/L	mg/L	µg/L	mg/L	g/L	mg/L	µg/L	mg/L
REX B1	10/06/98	5,04	3,80	18	36	35	15	4,0	220	95	15,44	850		1,47
REP02	02/07/98	4,15	3,06	212	34	65	29	4,0	239	99	16,25	932		119
	02/07/98						18							
REP03	06/07/98	5,56	3,32	174	40	<25	27	4,0	252	101	14,46	875		161
	06/07/98						20							
REP04	09/07/98	5,77	3,55	172	40	<25	50	4,0	259	101	16,60	965		160
REP05	15/09/98	5,65	3,47	484	37	<25	366	4,4	286	99	16,77	940		392
	15/09/98						600		1300				50	
REP08	23/09/98	5,49	3,73	538	38	<25	133	4,8	275	107	16,39	730		290
	23/09/98					60	30		30					309
REP09	05/10/98	5,57	3,70	554	38	<25	160	5,0	288	110	16,57	747		312
REP10	26/10/98	5,79	3,72	597	37	<25	238	5,1	299	106	16,84	774		335
REP11	09/11/98	5,58	3,62	505	36	38	162	4,6	292	102	16,06	705		305
REP12	23/11/98	5,62	3,48	465	37	47	178	4,6	293	102	16,43	775		292
REP13	14/12/98	5,65	3,31	475	38	34	167	3,8	307	108	15,55	684		254
REP14	17/12/98	5,51	3,76	435	38	37	155	3,9	304	104	14,81	667		
REP15	07/01/99	5,88	3,55	430	38	44	88	3,9	295	103	14,99	687		282
REP16	21/01/99	5,86	3,26	558	38	63	108	4,0	319	101	14,57	637		325
REP17	04/03/99	4,14	3,13	735	38	48	258	3,9	331	99	14,77	674	10	
REP18	14/04/99	5,79	2,98	867	41	47	455	4,7	348	103	15,03	663		
	14/04/99						700		930				10	
REP19	11/05/99	5,54	2,87	769	39	37	606	4,9	337	99	15,67	736		
	11/05/99						1000		1080				250	

Table 4. (Continued)

Sample	Date	TOC filtered	Inorg. C unfiltered	Inorg. C filtered	HCOO- Formiates	CH3COO- Acetate	CH3CH2CO2- Propionate	Phtalate	Oxalate	Citrate	pH (15°C)
		mg/L	mg/L	mg/l	mg/L	mg/L	mg/l	mg/l	mg/l	mg/l	
REX B1	10/06/98		1,6 **								7,65
REP02	02/07/98				<1	1,25					7,23
	02/07/98										
REP03	06/07/98		2,1		<1	2,25					7,08
	06/07/98										
REP04	09/07/98		2,15		<2	1,25					6,99
REP05	15/09/98		3,4		<2	<2	<2	<2	<2	<2	6,70
	15/09/98										
REP08	23/09/98	286	5,4	4,55	<2	<2	<2	<2	<2	<2	6,72
	23/09/98										
REP09	05/10/98	311	6,1	5,96	<2	<2	<2	<2	<2	<2	6,69
REP10	26/10/98	331	7,4	8,31							6,69
REP11	09/11/98	320	11,3	11,1	<2	<2	<2				6,58
REP12	23/11/98	282	13,6	15,7							6,56
REP13	14/12/98	260	17,2	11,5	<2	4,0	<2				6,61
REP14	17/12/98										6,63
REP15	07/01/99	283	14	13,9							6,34
REP16	21/01/99	320	15,3	16,5							5,98
REP17	04/03/99	508		14,2	<2	4,0	<2				5,90
REP18	14/04/99	552									6,00 *
	14/04/99										
REP19	11/05/99	533		11,4	<2	4,0	<2				6,06 *
	11/05/99										

*** : DO sensor stopped**
**** : HRL data**

A2.1.5 Microbial Analyses

Fractions of samples collected during the experiment were used for microbiological cultures. Analysed parameters were:

- counting of total flora (epi-fluorescence)
- culture and counting of total viable aerobic bacteria
- culture and counting of total fermentary anaerobes
- culture and counting of sulfate reducing bacteria
- culture and counting of methanogenic bacteria
- culture and counting of methanotrophic bacteria
- culture and counting of iron reducing bacteria

Analytical methods are described in (Daumas, 1999). In addition to solution cultures, 5 samplings of materials surfaces (2 on the core, cap, O-ring, resin) were done at the opening of the set-up in May 1999.

Table 5 gives results obtained on solutions. Among studied bacteria, three groups developed strongly: aerobes, fermentative anaerobes and iron reducing bacteria (IRB). In addition, at the end of the experiment, a very strong increase of sulphate reducing bacteria (SRB) is observed. Figure 28 and Figure 29 display the temporal evolution of these groups of bacteria. Two groups of bacteria were not detected quantitatively: methanogens and methanotrophic bacteria.

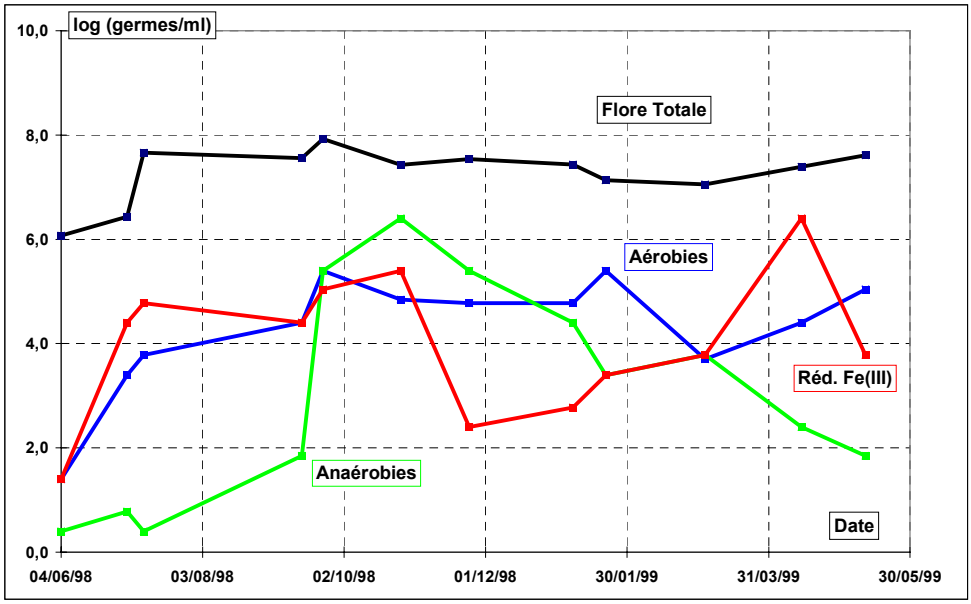


Figure 28. Time evolution of main bacteria groups in solution (log. scale).

Table 5. Microbiological data for water samples from the REPLICA experiment.

Echantillon	Date	Flore totale	Aérobies hétérotrophes	Anaérobies fermentaires acidogènes	Sulfato-réductrices	Méthanotrophes	Méthanogènes	Réductrices du fer
		germes/ml	germes/ml	germes/ml	germes/ml	germes/ml	germes/ml	germes/ml
Eau initiale	04/06/98	1,18E+06	25	2,5	25	0	0	25
REP02	02/07/98	2,70E+06	2,50E+03	6	25	0	0	2,50E+04
REP04	09/07/98	4,56E+07	6,00E+03	2,5	0	0	0	6,00E+04
REP05	14/09/98	3,63E+07	2,50E+04	70	0	0	0	2,50E+04
REP08	23/09/98	8,45E+07	2,50E+05	2,50E+05	0	0	0	1,10E+05
REP10	26/10/98	2,66E+07	7,00E+04	2,50E+06	0	0	0,6	2,50E+05
REP12	24/11/98	3,45E+07	6,00E+04	2,50E+05	2,5	0	0	2,50E+02
REP15	07/01/99	2,73E+07	6,00E+04	2,50E+04	2,5	0	0	6,00E+02
REP16	21/01/99	1,35E+07	2,50E+05	2,50E+03	0	0	0	2,50E+03
REP17	04/03/99	1,13E+07	5,00E+03	6,00E+03	2,5	0	0	6,00E+03
REP18	14/04/99	2,45E+07	2,50E+04	2,50E+02	2,50E+02	0	0	2,50E+06
REP19	11/05/99	4,12E+07	1,10E+05	70	2,50E+03	0	0	6,00E+03
Echantillon	Date	log	log	log	log	log	log	log
Eau initiale	04/06/98	6,07	1,40	0,40	1,40			1,40
REP02	02/07/98	6,43	3,40	0,78	1,40			4,40
REP04	09/07/98	7,66	3,78	0,40				4,78
REP05	14/09/98	7,56	4,40	1,85				4,40
REP08	23/09/98	7,93	5,40	5,40				5,04
REP10	26/10/98	7,42	4,85	6,40			-0,22	5,40
REP12	24/11/98	7,54	4,78	5,40	0,40			2,40
REP15	07/01/99	7,44	4,78	4,40	0,40			2,78
REP16	21/01/99	7,13	5,40	3,40				3,40
REP17	04/03/99	7,05	3,70	3,78	0,40			3,78
REP18	14/04/99	7,39	4,40	2,40	2,40			6,40
REP19	11/05/99	7,61	5,04	1,85	3,40			3,78

Total flora (Figure 28) shows a very strong increase after the first oxygen pulses, in correlation with the main groups. Total flora counting includes both living cells and a large proportion of dead cells, which explains why the sum of aerobes, anaerobes and other groups is far less than the total flora. Inspection of results shows that there certainly is an overlap between groups: part of the IRB (iron-reducing bacteria) is most probably able to develop with O_2 as energy source and are thus counted in both groups. Aerobes and IRB increase simultaneously after the injection of the first O_2 pulse. Anaerobes develop strongly after the first long anoxic period (summer 1998). They then become the dominating group and decrease strongly and continuously after the injection of the strong O_2 pulses. IRB seem to increase strongly in conditions of intermediate O_2 concentration: they decrease markedly after anoxic periods or when high O_2 pulses are injected. SRB (sulphur-reducing bacteria) show a rather interesting behaviour: during most of the experiment they remain detectable at a low level or are even not detected. At the end of the experiment, a demographic explosion is observed for this group. It is interesting to observe that SRB develop anti-correlatively to IRB. Data obtained on SRB show that, although these microorganisms remain undetectable during long periods of time, although the set-up has been strongly oxygenated, they are able to survive and grow again when conditions are more favourable.

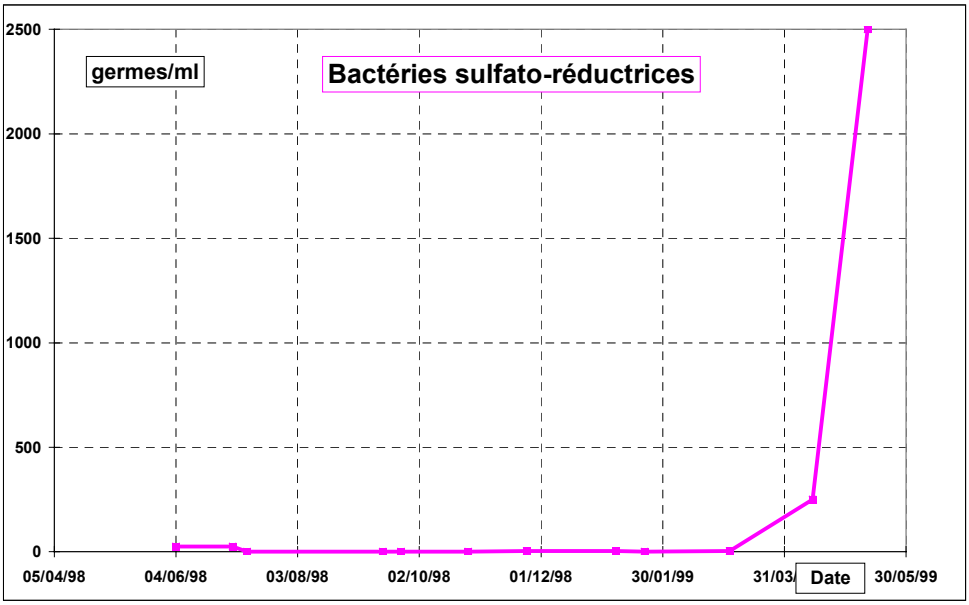


Figure 29. Temporal evolution of SRB bacteria in solution (linear scale).

Methanotrophic microorganisms were never detected, not even on the water sampled directly in pressurised bottles. This is remarkable because these microorganisms are systematically detected on site, see for example (Kotelnikova and Pedersen, 1999). The culture protocol was checked and a second culture protocol was added without any success.

Methanogens were not detected at a significant level.

Table 6 displays data obtained on surface samplings at the opening of the set-up. The contrast between core samples and plastic surfaces is evident: the core sample is the host surface for SRB. The PETP surface contains very low levels of all groups. In contrast the resin displays high concentrations of anaerobes and is thus probably the source of organic carbon in the solution. IRB and aerobes are present on most materials, significantly on the core. The microbe densities derived from culture do not support the idea that there is a continuous biofilm on the solids, especially the core. Probably, colonies are formed at discrete sites on the diorite surface.

Table 6. Microbiological data on surface samplings.

Sample	Surface area (cm2)	Flore totale (cell/cm2)	Aérobies hétérotrophes (cell/cm2)	Anaérobies fermentaires acidogènes (cell/cm2)	Sulfato-réductrices (cell/cm2)	Méthanotrophes (cell/cm2)	Méthanogènes (cell/cm2)	Réductrices du fer (cell/cm2)
Core 1	2	3,88E+08	6,00E+04	1,10E+04	1,30E+04	<0,5	<0,5	2,50E+04
Core 2	3	1,76E+08	1,66E+05	1,66E+04	1,66E+05	<0,5	<0,5	1,66E+04
Resin	5	1,40E+07	1,00E+03	1,00E+05	10	<0,5	<0,5	1,00E+04
PETP cap	6	6,70E+05	1,10E+05	1,00E+02	10	<0,5	<0,5	24
O-ring	5	8,49E+05	2,30E+05	6,60E+02	2	<0,5	<0,5	1,90E+04

In conclusion, the microbiological analyses show that:

- the main groups of bacteria that developed in the set-up are: aerobic, anaerobic fermentative and iron reducing bacteria and sulphate reducing bacteria;
- important oxygen pulse injection inhibit temporarily the SRB but these bacteria can grow again if conditions evolve favourably; when SRB begin to develop, IRB seem to be inhibited;
- iron reducing bacteria develop best in intermediate conditions of O_2 level and are thus one of the most efficient promoters of O_2 uptake because they produce iron (II) and because they probably are also able to consume O_2 directly;
- the source of organic carbon in the set-up is most probably the resin and not the PETP parts;
- the physical substrate for SRB is on the diorite; however, densities measured do not support the idea of a continuous biofilm;

A2.1.6 Modelling the Kinetics of O_2 Uptake

A2.1.6.1 The Kinetic Model

In order to model the rate of O_2 uptake, a purely chemical kinetics rate equation was sought, neglecting diffusion phenomena in the pores of the core. A literature review established the form taken by O_2 reaction kinetics due to: *i*) oxidation of reduced elements in solution (especially Fe(II), S(-II)) (Millero, 1985; Millero et al., 1987a; Millero et al., 1987b; Stumm and Lee, 1961; Wehrli, 1990; Zhang and Millero, 1991), *ii*) surface reaction of O_2 on minerals (Shoesmith et al., 1996; White and Yee, 1985; Williamson and Rimstidt, 1994), *iii*) dissolved O_2 uptake by microorganisms (Rittmann and Vanbriesen, 1996; van Cappellen and Gaillard, 1996).

Comparison of experimental facts with bibliographical data lead to a rate law for dissolved O_2 uptake that is the sum of three terms, a Monod type rate law (direct influence of microbes, enzymatic catalysis), a first order rate law (mainly iron(II) oxidation in solution) and a ½ order rate law (surface reaction of dissolved O_2 with solids):

$$\frac{dc}{dt} = -\frac{k_1 c}{K + c} - k_2 c - k_3 c^{0.5} \qquad (8)$$

The definition and dimensions of each parameter for this equation are given in the following table.

Constant	Dimension	Definition
c_0	$[MOL][L]^{-3}$	initial O_2 concentration
k_1	$[MOL][L]^{-3}[T]^{-1}$	limiting rate (Monod law)
K	$[MOL][L]^{-3}$	critical concentration (Monod law)
k_2	$[T]^{-1}$	first order rate constant
k_3	$[MOL]^{0.5}\,[L]^{-1.5}\,[T]^{-1}$	0.5 order rate constant

This rate law was tested against some of the preliminary data sets (Lartigue et al., 1997b; Puigdomenech et al., 1999) with following conclusions:

- in non sterile runs, a Monod-type contribution is necessary to reproduce correctly the evolution of O_2. This contribution (k_1) generally dominates the global rate at low concentrations ($O_2 < 60$ µmol/L) and may be up to 50 µmol/(L day).
- first order or ½ order contributions are evidenced at higher O_2 concentrations or in sterile conditions. The set of constants fitting the data set is often not unique and k_2 and k_3 are best differentiated by studying O_2 pulses of increasing intensity. Typical ranges found for k_2 and k_3 rate constants are respectively from 0 to 0.3 day^{-1} and from 0 to 3 $(\mu mol/L)^{0.5}$ day^{-1}.
- there was no evidence of diffusion control from these first data-sets.

A2.1.6.2 Fitting Procedure

All three contributions to O_2 uptake shown in Eq.(8) are multiple: the first term groups the influence of several bacterial groups and probably several catalytic mechanisms, the second one (first order kinetic) includes iron(II) oxidation in solution, sensor uptake and probably some solid-solution interactions, while the third term probably collects the influence of several reactions, mainly solid-solution reactions (e.g. pyrite oxidation, …).

The model is simplistic because transport of reactive species in cracks and open pores is neglected; in addition, the time evolution of the activity of many species is not taken into account, which may have had an important effect on kinetics. This is namely the case for: H^+ (important role on iron(II) oxidation kinetics and minerals dissolution kinetics), carbonates (coupled to microbiological metabolism), iron (used by some microorganisms as an energy source), organic carbon (used in microbiological metabolism), etc. The fact that this model can describe our data in many instances only means that a quasi-stationary state of chemical conditions, and thus O_2 uptake, is rapidly reached in our experiment.

The fitting procedure used to derive rate-law coefficients was improved (Trotignon et al., 1999) and included several steps: evaluation of the time derivative of O_2 uptake (using a software written by E. Castelier); choice of the most suitable kinetic model, including appropriate contributions (Monod, 1st order, …); optimisation by a least squares procedure of the rate law coefficients on a finite difference formulation (better suited for this purpose than the analytical formulation). The analytical solution and analytical derivative using these coefficients were then computed and compared to the data.

In most cases, it was found that a kinetic law including only 2 terms (Monod + 1st order kinetics) was sufficient to describe properly the data sets. This simpler model was considered more satisfactory from the scientific point of

view. Adding more parameters to the rate equation would probably increase the agreement with the experimental data, but it would not give additional insight on the mechanism of the process. Frequently, a transient was observed during the first hours; the system reached then a stationary state of O_2 uptake.

An exhaustive presentation of data treatment cannot be presented in the framework of this report; the interested reader should contact the authors at the CEA.

A2.1.6.3 Modeling Pulses P1, P2 and P3

The study of pulse P1 is illustrated in Figure 30 and Figure 31. An initial transient, during which a first order contribution exists and then vanishes, was evidenced. The second part of the signal corresponds well to a Monod kinetic contribution. Pulses P2 and P3 show a more complex evolution, discussed in detail in (Trotignon et al., 1999). A combination of Monod and first order kinetics seems sufficient to describe properly the data, although at least for pulse P2, the data suggests a succession of two distinct Monod kinetics superimposed with a first order rate law.

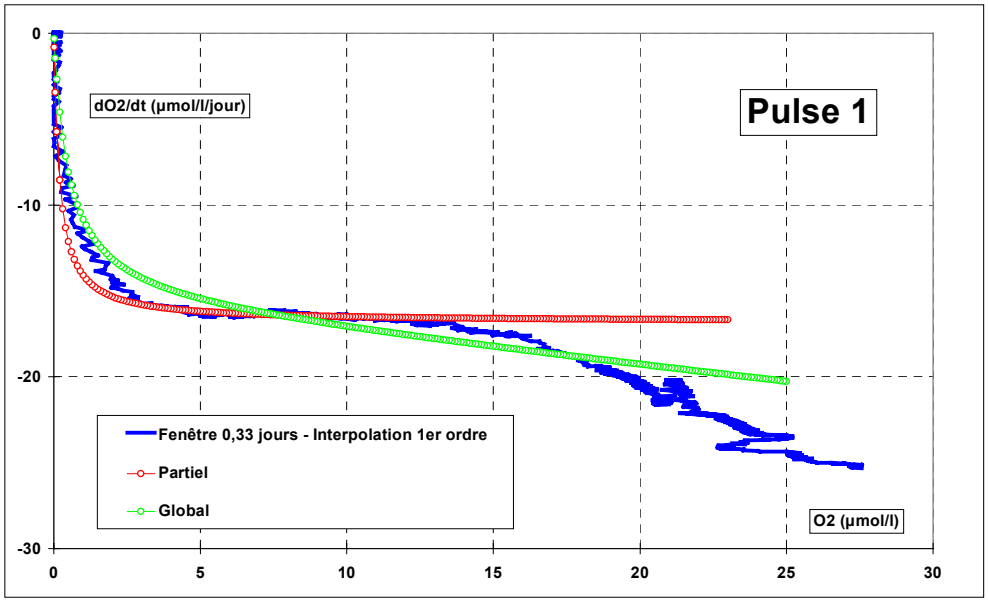


Figure 30. Pulse P1: study of the time derivative.

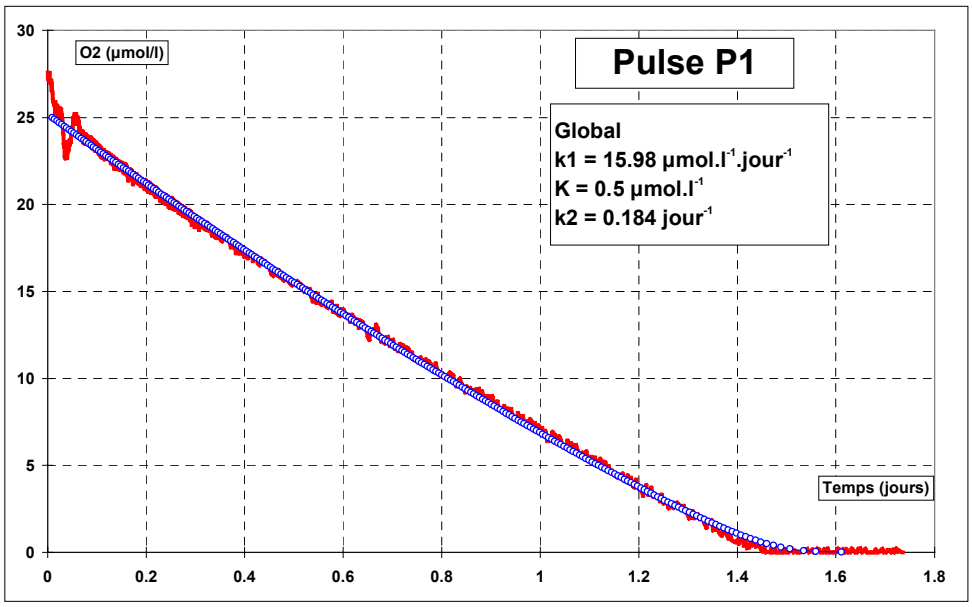


Figure 31. Pulse P1: comparison between experimental data and global fit.

A2.1.6.4 Modeling Pulses P4 to P9

For pulse P4, only the end of the signal was treated because of the membrane change. The dissolved O_2 data for these pulses shows a stabilisation of kinetic constants used in the model; Monod + first order kinetics are sufficient to describe correctly the signal.

The transient period at the beginning of pulses P4 to P6 was decomposed in two successive stages: *i*) a very rapid transient due to internal equilibration of the set-up after O_2 injection, *ii*) a longer transient (1 to 2 days) probably corresponding to the adjustment of some chemical species under the influence of microbial activity or mineral dissolution.

The same observations were made for pulses P7 to P9.

A2.1.6.5 Modeling Pulses P10 and P11

Pulse P10, realised directly after pulse P9, showed that although microbial populations had markedly evolved, the Monod contribution to O_2 uptake was rather stable. The kinetic constants derived from pulse P10 are well in the average of previously derived coefficients for pulses P1 to P3. Pulse P11 (see Section A2.1.3.6) showed that the rate limiting factor for the Monod kinetics was not convective flow. This rate limiting factor is probably of chemical origin (chemical intermediate species or process).

A2.1.6.6 Modeling Pulses P12a to P12d

Pulse 12 is a large composite pulse during which O_2 was renewed several times. Because of some noise on signal due to the dirtying of the membrane of the oxygen sensor, only the last part of the signal was treated. Once again, the combination of Monod + first order kinetics showed to be the simplest and best choice, giving a high quality fit (Figure 32 and Figure 33).

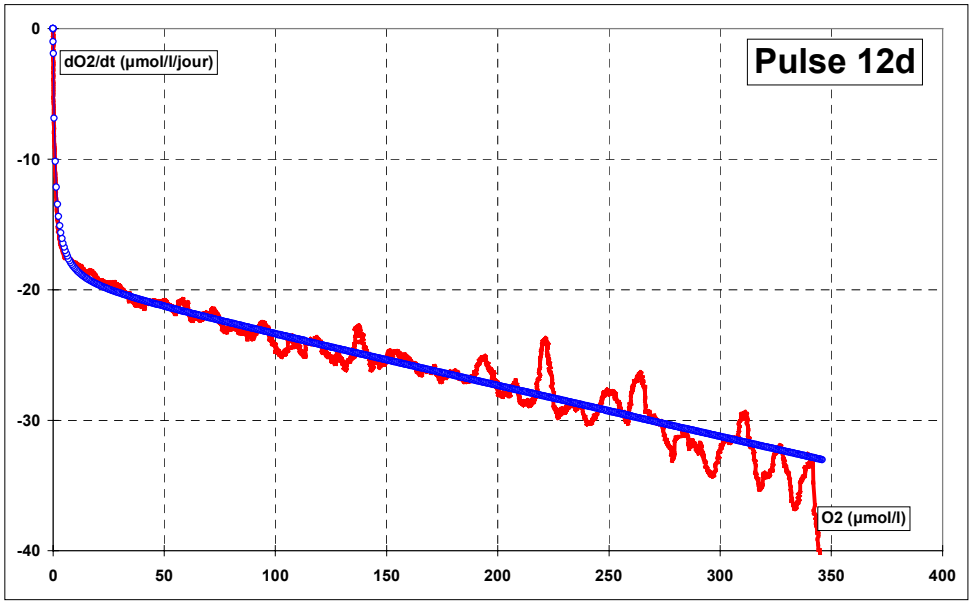


Figure 32. Pulse P12: study of the time derivative.

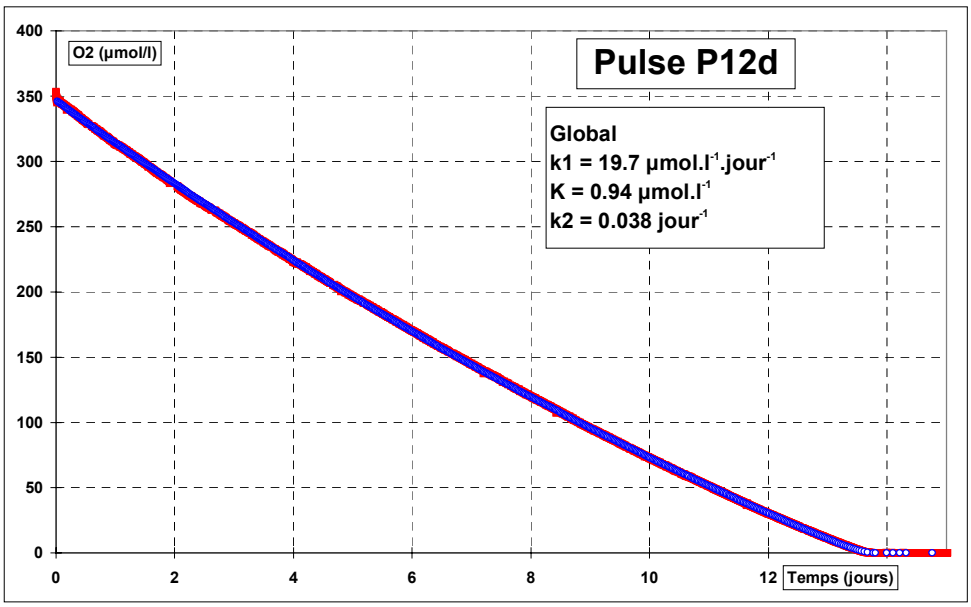


Figure 33. Pulse P12: comparison between data and partial fit.

A2.1.6.7 Conclusions from the Kinetic Modeling

The main conclusions from the kinetic modeling of the REPLICA experiment are:

- for nearly all the pulses (except P2 and perhaps P3), the sum of a Monod and a first order kinetic rate laws gives a good description of the data $[O_2] = f(t)$;

- typical values of the rate constants in Eq.(8) are: for k1, 15 µmol/(L day), for K, 0.2 µmol/L and for k2, 0.07 day^{-1}. The next tables summarise values obtained on the fittings of global signals and of the end of pulses (thus not influenced by the initial transient);
- a transient is observed during the first hours of each pulse, during which O_2 uptake is stronger;
- the evolution observed in pulses P2 and P3 suggests the temporal succession of several Monod processes;
- a reasonable chemical/microbiological model has been established that describes important redox reactions taking place in underground systems.

Table 7. Summary of fitted rate-law coefficients for the fitting procedure on global signals.

Echantillon	Date	Pulse	c0	k1	K	k2
				global	global	global
REX B1	10/06/98					
REP02	02/07/98	1	25.18	15.98	0.5	0.184
REP03	06/07/98	2	26.6	12.997	0.0123	1.39
REP04	09/07/98	3	26.08	15.825	0.016	1.457
REP05	15/09/98	4	71.51	21.66	0.5	0.107
REP08	23/09/98	5	142.11	10.96	0.016	0.1648
REP09	05/10/98	6	141.93	12.98	0.013	0.097
REP10	26/10/98	7	228.48	10.13	0.3	0.099
REP11	09/11/98	8	216.96	10.1	0.3	0.0753
REP12	23/11/98	9	313.06	13.78	0.91	0.04
REP13	14/12/98	10				
REP14	17/12/98					
REP15	07/01/99					
REP16	21/01/99	12d	346.66	19.69	0.9429	0.03869

Table 8. Summary of fitted rate-law coefficients for the fitting procedure on the end of the signals.

Echantillon	Date	Pulse	c0	k1	K	k2
				partiel	partiel	partiel
REX B1	10/06/98					
REP02	02/07/98	1	23.65	16.82	0.193	0
REP03	06/07/98	2	18.97	18.265	0.11	0.232
REP04	09/07/98	3	22.57	18.355	0.069	0.912
REP05	15/09/98	4	70.78	22.59	0.224	0.071
REP08	23/09/98	5	124.74	14.04	0.108	0.079
REP09	05/10/98	6	135.21	15.231	0.49	0.055
REP10	26/10/98	7	217.87	11.38	0.0126	0.0784
REP11	09/11/98	8	204.35	11.822	0.274	0.049
REP12	23/11/98	9				
REP13	14/12/98	10	30.91	12.9	0.1029	0.3032
REP14	17/12/98					
REP15	07/01/99					
REP16	21/01/99	12d				

A2.1.7 Return to Reducing Conditions: Reaction Pathways Through Eh-pH Diagrams

The introduction in the protocol of long anoxic periods enabled us to study the evolution of pH and Eh parameters as well as other chemical or biological parameters after dissolved O_2 had totally disappeared. These studies showed that important chemical evolutions were going on after exhaustion of O_2 leading through different pathways to a reducing state. It will be shown here that Eh and pH values derived during these periods make sense and are consistent with chemical and microbiological data.

Several anoxic periods were studied here by the use of Eh-pH diagrams. These diagrams were made with the GWB modelling tool (Bethke, 1994) by using the chemical analyses available for solutions sampled at the end of the corresponding anoxic period.

The REX00 run: This case corresponds to a run made at the beginning of 1998 on the ghost core. Figure 34 gives the trajectory of (Eh,pH) couples on the diagram. At O_2 injection, Eh increases at constant pH and then pH decreases at constant Eh during O_2 uptake. At O_2 exhaustion, Eh drops at nearly constant pH until it reaches the magnetite-solution equilibrium limit and where it follows this limit until pH = 6.4, where Eh suddenly drops. Representative points reach the pyrite-solution limit. There is also a good consistence between this diagram and HS^- concentrations measured in the solution, see (Trotignon et al., 1999).

Start of the REPLICA experiment, before injection P1: Although O_2 is exhausted since two weeks, the potential starts to increase again (see Section A2.1.3.1). The corresponding Eh-pH diagram (Figure 35) is more difficult to

interpret because at this stage of the experiment no data on solutions is available, especially for dissolved iron. Measured potentials are too low in order to involve Mn redox couples. The diagrams suggest that, if we assume a very low dissolved iron concentration ($< 10^{-8}$ mol/L), the approach towards a magnetite-solution equilibrium is a plausible hypothesis.

Summer 1998; between P3 and P4: The Eh-pH diagram (Figure 36) was drawn by taking into account total dissolved iron measured at the end of the anoxic period. After a transient period, corresponding to the injection and uptake of dissolved O_2, the points in the diagram follow a direction perpendicular to the goethite-solution, and are located on this limit at the end of the run (the computed limit on the diagram correspond effectively to solution conditions at the end of the run). This curve suggests that a goethite-solution equilibrium is attained while dissolved iron concentrations increase with time. This is consistent with iron concentration evolutions over anoxic periods.

Spring 1999; after P12: This was the longest anoxic period and several samplings were possible. The Eh-pH diagram in Figure 37 shows our interpretation. After the initial transient, representative points accumulate on the hematite-solution limit. A diagram drawn with the goethite-solution limit instead, also gives a good fit. As solution analyses show that the Fe concentrations increase in solution, a similar interpretation as for Figure 36 is proposed: the points follow a shifting solid/solution border, represented here by the hematite-Fe^{2+} equilibrium limit.

In conclusion, the study of the anoxic periods shows that:
- Eh and pH measurements realised in anoxic conditions have a thermodynamic sense with respect to the Fe-H-O-S systems;
- different reaction pathways were deduced from the Eh-pH diagrams; it is probable that initial pH plays an important role, because of the high dependence of Fe(III) hydrolysis kinetics with respect to pH;
- the evolution towards stable reducing conditions is long and may last for several months (to be compared to the short O_2 uptake period) and it is probably catalysed by bacterial activity.

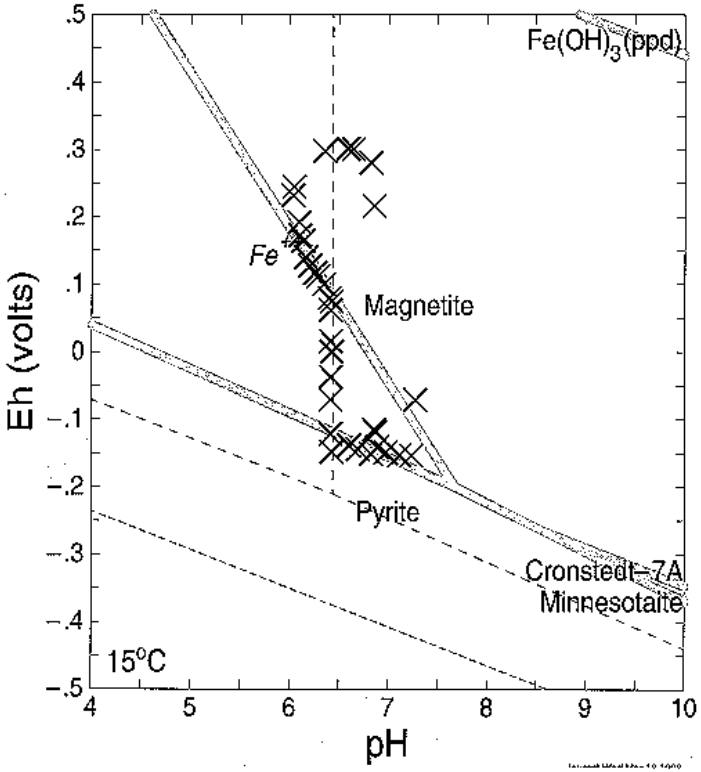


Figure 34. Eh-pH diagram corresponding to the REX00 run (Jan. 1998). Total dissolved Fe is constrained by solution analyses.

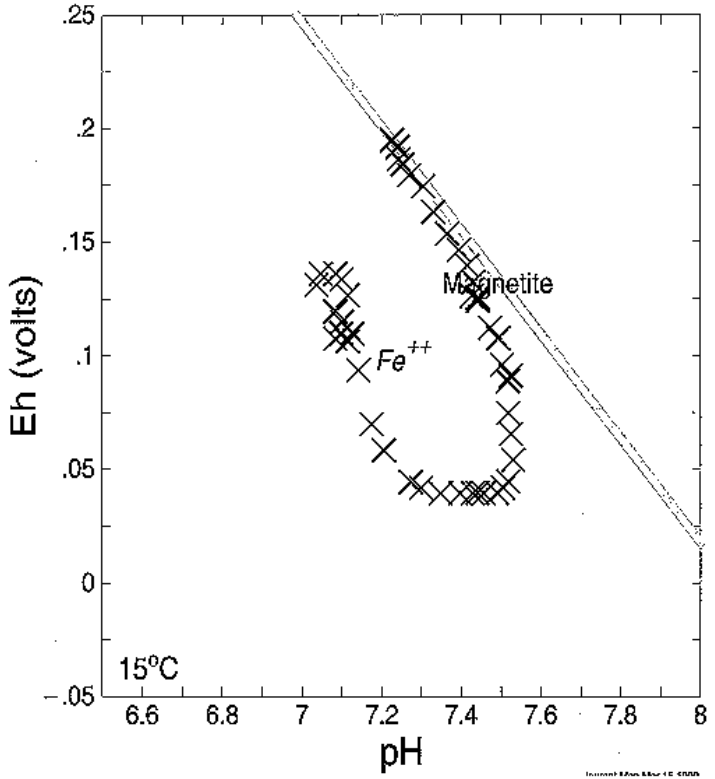


Figure 35. Eh-pH diagram corresponding to the period preceding pulse P1. Total dissolved Fe fixed at 10^{-9} mol/L.

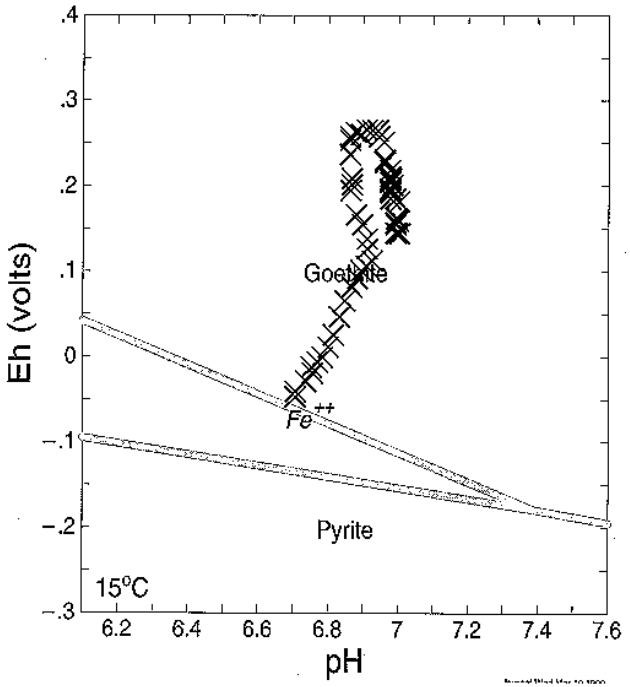


Figure 36. Eh-pH diagram for the anoxic period after pulse P3. Total dissolved Fe is constrained by solution analyses.

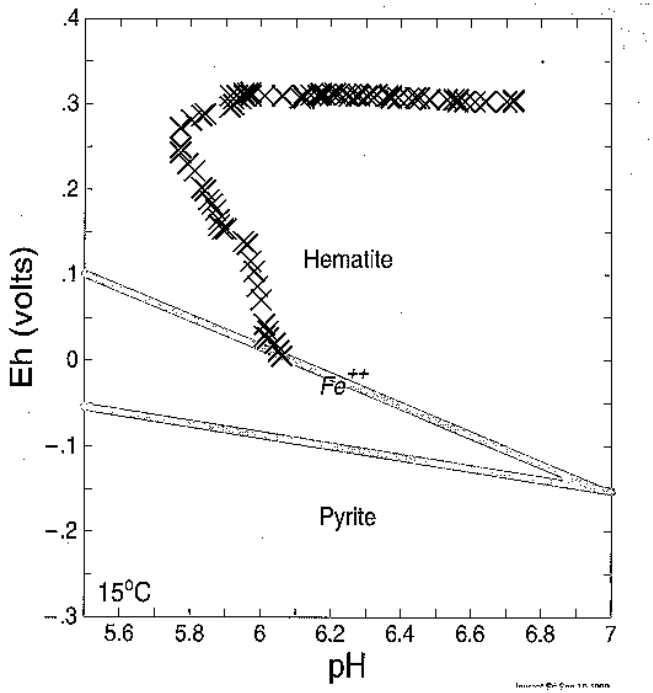


Figure 37. Eh-pH diagram for the anoxic period after pulse P12. Total dissolved Fe is constrained by solution analyses.

A2.1.8 Discussion and Conclusions from the REPLICA Experiment

A2.1.8.1 Contributions from the Methodology

One of the aims of the REPLICA was to parallel an *in situ* experiment and to help its preparation and interpretation. The nature of the REX project was a particularly adequate test for this because the corresponding *in situ* experiment involved a limited volume of rock and also complex physico-chemical phenomena, difficult to reproduce. During the course of the project, the following conclusions were drawn that could be consequently used by other teams involved in the project:

- evidence that the interaction of stainless steel 316L with O_2 in Äspö groundwater could contribute significantly to O_2 uptake;
- evidence of solution transport through the core;
- rapid acquisition of kinetic data and aid to elaborate an experimental protocol;
- acquisition of kinetics for the transformation from oxic to anoxic conditions and further to a reducing state;
- data on bacteria in the lab compared to the field;
- contribution to modelling.

A2.1.8.2 Limitations of the REPLICA Experiment

Some limitations were however identified:

- release of organic carbon by the resin used to cover the core edge; this has probably had an influence on bacterial growth, although the degradation of organic carbon seems rate limited. Improvements for this must be sought first in the lab by making more blanks and tests;
- due to the grouping of sensors in a single unit, a slight interaction between sensors probably led to contamination of the membrane of the O_2 sensor, and resulted in a slight interaction between the O_2 probe and the Eh and pH sensors. This point is easy to correct by re-organising the sensors in the loop;
- release of potassium by Eh and pH sensors; this has probably a minor effect. It could be improved by using an optode for pH;
- the water/solution ratio could probably be improved;
- the dissolved gas measurements could probably be improved by complementary technique (Membrane Inlet Mass Spectrometry, …);
- the fact that methanotrophic did not develop, even in samples collected directly in pressurised bottles, is not understood.

A2.1.8.3 Conclusions from the REPLICA Experiment

The approach used for the REPLICA seems thus useful but can probably only be applied to *in situ* experiments with a restricted spatial scale. Improvements are possible on certain aspects: choice of set-up components and organisation of sensors.

The combined study of O_2 uptake kinetics, chemical analyses in solution and evolution of microbial populations shows that:

- O_2 uptake is correctly described by a kinetic rate law combining a enzymatic catalysis term (Monod kinetics) and a first order term;
- the first-order term (k2 in Eq.(8)) is most probably linked to iron (II) oxidation in solution; its order of magnitude (0.07 day^{-1}) is consistent with literature data and chemical analyses;
- the microbial rate constant (k1 in Eq.(8)) is in the order of 10 to 15 µmol/(L day);
- O_2 uptake rates lead to total exhaustion of oxygen in periods of 1 day to a few weeks following initial concentration;
- the coupling between iron reducing bacteria (IRB) and the reaction between O_2 and dissolved Fe(II) is probably an important process that could be the basis of a simple and conservative description of the system; the IRB are especially active at intermediate O_2 concentrations and this gives them a key role;
- a rate limiting factor was evidenced for O_2 uptake by microbes;
- SRB are present and are not totally killed by O_2 pulses; they are able to develop again when anoxic conditions are back; SRB are present on the fracture surface, and not in other parts of the equipment;
- the concept of buffering capacity of the deep granitic medium is thus not only linked to reductants present in the site, but also to the availability of bacterial substrates that may be transported by groundwater;
- once anoxic conditions have been reached, the return towards reducing conditions proceeds in several months (1 to 3 in the REPLICA). Different reaction paths were identified and are probably catalysed by bacterial activity. Equilibrium of solution with magnetite, pyrite and Fe(III) oxyhydroxides are shown to control these reaction paths;

A2.2 COMPLEMENTARY LABORATORY STUDIES TO EXAMINE MICROBIAL EFFECTS ON REDOX

A2.2.1 Introduction

Detailed studies of the subsurface microbiology of Äspö (Pedersen and Karlsson, 1995) has revealed the presence of many different bacteria in the deep groundwaters including iron and sulphate reducing bacteria. A series of experiments were conducted as part of a BGS-JNC collaborative programme to study the rock-water and microbial interactions. Results from these experiments have been reported in detail elsewhere (Bateman et al., 1999; Bateman et al., 1998a; Bateman et al., 1998b; Milodowski et al., 1996; West et al., 1997; West et al., 1998; West et al., 2001; Yoshida et al., 1999).

A2.2.2 Experiments

Results from previous work (Bateman et al., 1999) using batch systems showed that microbes have complex effects on the geochemistry of the system. The experiments discussed here were designed to represent more realistic conditions, by using flowing systems. Both columns and mixed-flow reactors were used in these experiments (Figure 38 and Figure 39). The experiments were conducted within an anaerobic chamber with a controlled $N_2/H_2/CO_2$ atmosphere. A series of experiments were conducted in which crushed Äspö Diorite was reacted with Äspö groundwater flowing into the system. Half the experiments were inoculated with a mixture of sulphate reducing bacteria (SRB) and iron reducing bacteria (IRB). The fluid was continuously analysed for chemical and microbial changes during the experiments. The fluid pH and flow rate was also monitored. The reacted solid residues from the experiments were examined for mineralogical and petrographic changes at the end of the experiments.

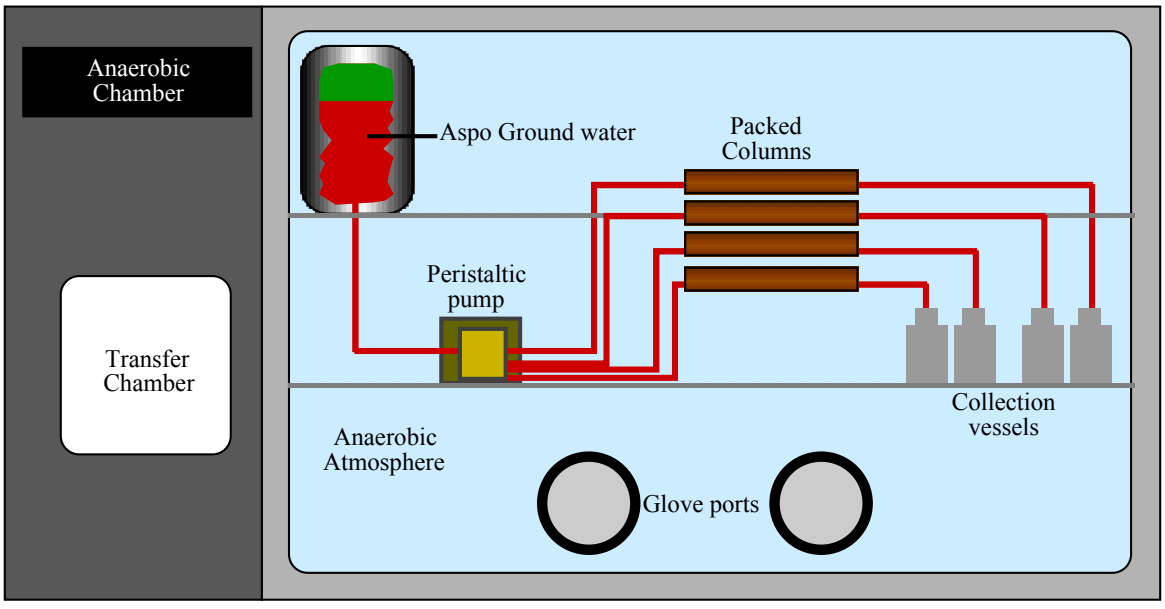


Figure 38. Schematic of column set up.

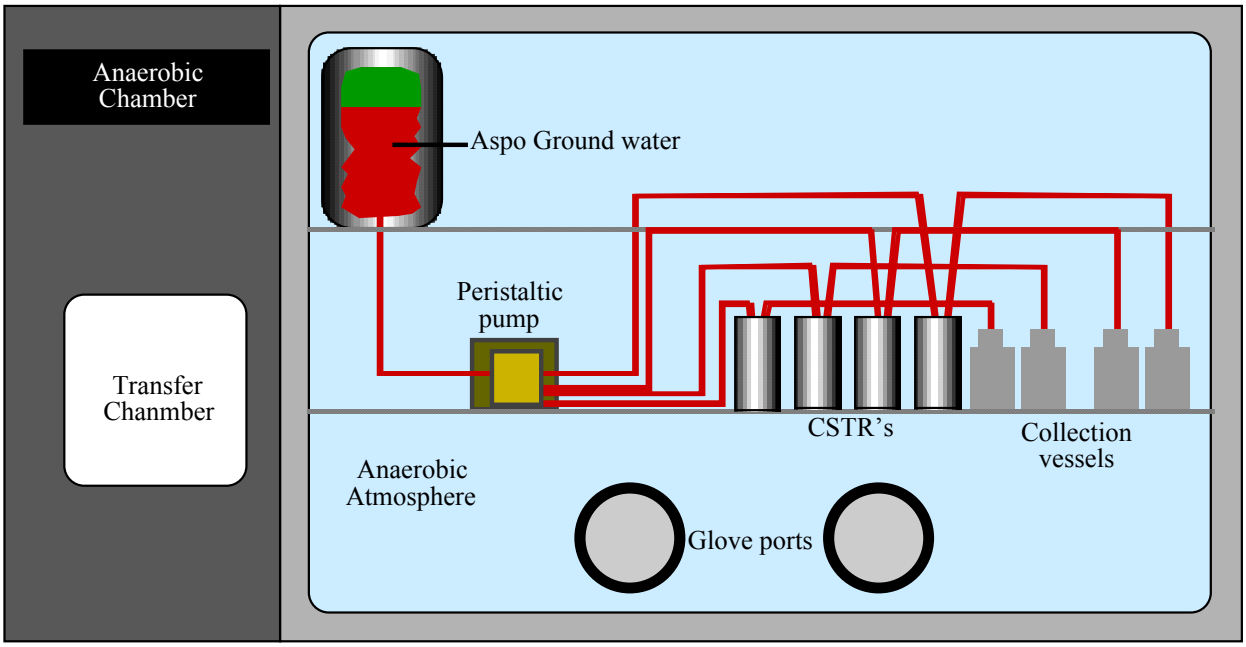


Figure 39. Schematic of CSTR (Continuously Stirred Tank Reactor) set up.

A2.2.3 Results

The column experiments were run for about two weeks before the columns with bacteria added became blocked and flow fluid ceased. The mixed-flow experiments were ran anaerobically for three months, after which two of the experiments were allowed to run aerobically for a further three months.

Microbiology Results. Bacterial populations were sustained throughout the experiments.

Mineralogy Results. Some evidence of biofilm development was observed during analysis of residues from the column experiments. However, the mixed-flow experiments showed no clear evidence (or preservation) of biofilm development.

Scanning electron microscopy (SEM) observation of the solid residues from the mixed-flow experiments showed no significant evidence for reaction when compared to the starting material. However, there was a loss of fine grained (i.e. <5µm) material, which originally adhered to grain surfaces in the starting material. There was also evidence for the formation of minor amounts of smectite on primary mineral surfaces; smectite was not present in the starting materials. X-ray diffraction analysis supported the identification of smectite in the residues. Mineralogical observations indicate that minor conversion of chlorite and chloritised biotite fines present within the crushed diorite has produced smectite and mixed-layer chlorite-smectite. The degree of alteration was greater in the experiments when bacteria were

present, but no difference in reaction was seen between the anaerobic experiments, and those that were initially anaerobic and subsequently run aerobically. This suggests that the smectite formation occurred under anaerobic conditions, with little subsequent alteration under aerobic conditions (Figure 40).

The observations are consistent with those of the column experiments. The columns with bacteria became blocked very rapidly, and minor amounts of smectite were observed in the reaction residues examined after this period. Experiments without bacteria did not block up. These results are consistent with bacterially enhanced smectite formation being responsible for the blocking of the column experiments. However, additional research is required to confirm whether or not this hypothesis is correct. If bacterial action is indeed shown to be responsible for the enhanced rate of smectite formation, the chemical mechanism needs to be established by further work.

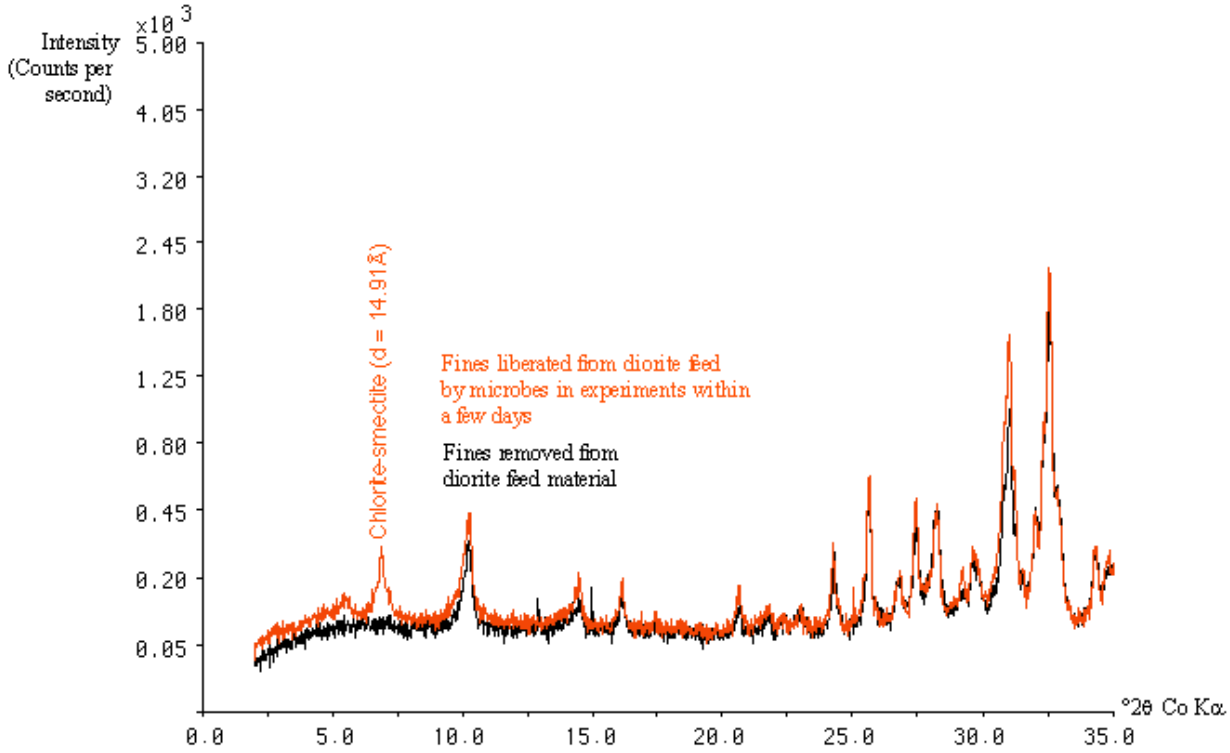


Figure 40. Comparison of the XRD traces (air-dried) obtained from the orientated fine fraction (<5 µm) material separated from the crushed Äspö Diorite starting material and the experimental residue from column experiments.

Chemistry Results. Little evidence for rock-water interactions is seen in the chemical analysis data for all the experiments. There are some slight initial increases observed for some elements (i.e. Si, Al) but this is probably the result of the re-establishment of equilibrium between the ground water and the crushed rock as no further trends are observed. Modelling of the fluids showed them to be saturated with respect to clay minerals. Possibly, the ob-

served mineralogical changes reflect alteration occurring in microbially mediated microenvironments close to mineral surfaces. Consequently, these changes may be too small to be detected in the chemical analysis of the bulk fluid.

A2.2.4 Conclusions

These experiments have demonstrated that, during the low temperature alteration of granitic rocks by groundwaters, bacterial processes may mediate clay mineral formation. In addition, they have shown that microbial activity can impact on fluid flow through porous media even in the nutrient poor conditions of a crystalline granitic bedrock.environment. The mobilisation of fine grained material, aided by biological processes, may have a significant impact on groundwater movement around a repository by blocking of constrictions in fracture flow paths and pore throats. This could be further enhanced by any supply of nutrients from the waste itself that could increase microbial activity. The formation of new clay minerals (smectite, chlorite-smectite) is potentially important for ion exchange and sorption reactions. Although these experiments used crushed diorite, the observations will be applicable to a wide variety of host rock lithologies in which quartz, K-feldspar, plagioclase, chlorite and mica are major components.

A2.3 OXYGEN UPTAKE BY FRACTURE MINERALS FROM ÄSPÖ

A2.3.1 Introduction

This section gives details of laboratory experiments carried out at the University of Bradford since September 1997 for the REX project.

Two types of experiment were carried out in order to measure O_2 consumption rates of rock samples from the Äspö Hard Rock Laboratory. These were the Recirculating Batch Reactor Experiment and the Batch Bottle Reactor Experiments. The first is a sophisticated method of observing O_2 uptake by a fracture mineral sample over a number of days or weeks in highly controlled circumstances. It allowed the sample to be isolated from the atmosphere and any leakage of oxygen into the kit during the experiment to be quantified and taken into account when calculating oxygen uptake. The second is a simple and cheap method for measuring a sample's reactivity using 'off the shelf' laboratory equipment.

The Recirculating Batch Reactor Experiment allows O_2 uptake to be observed almost continuously and pH development and solution compositions were monitored with time during these experiments.

The Batch Bottle Experiment is an ideal method for quickly and cheaply assessing the reactivity of different rock samples under various different conditions and has the potential to be developed further for use on a large scale.

Results of these experiments using samples from the Äspö HRL are summarised in this section.

A2.3.2 Methodology

Fieldwork carried out at the Äspö HRL and reported in (Rivas Perez and Banwart, 1998) allowed samples of fracture material to be collected by hand from fractures intersecting the tunnel wall. These mineral samples were dried in a vacuum desiccator and then dry sieved. Each size fraction was sonicated in ethanol to remove the clay fraction that could be adhering to mineral particles in larger fractions, in preparation for experimental work. In addition fracture mineral material recovered from a core taken from the Hard Rock Laboratory (borehole KA3065) was treated in a similar fashion. It was also dried and sieved into various size fractions, and used in a number of experiments reported in this document and its appendices.

Once sieved into size fractions the samples were characterised mineralogically using optical microscopy and XRD (X-ray diffraction) techniques. The samples were typical of cataclastic fracture material from a fault zone in an acid igneous pluton. The material was poorly sorted containing particle sizes ranging from that of boulder size blocks down to clay size. Quartz,

plagioclase and K-feldspar dominated the mineral assemblages (Puigdomenech et al., 1999). The rock samples also typically contained significant amounts of chlorite, epidote and biotite, indicating a significant percentage of Fe(II) in the samples.

The initial concentration of ferrous iron surface sites for a sample used in an experiment can be calculated if the wetted surface area, the percentage total iron and the percentage of Fe(II) of total iron are known. Analytical methods, BET nitrogen adsorption, X-ray Fluorescence (XRF) and Mössbauer analysis can quantify these values respectively and for some of the samples collected from the Äspö tunnel we have done this, *cf*. Table 9.

The observed experimental data pertaining to dissolved O_2 consumption agrees qualitatively with the conceptual model presented in (Rivas Perez, 1996). A favourable comparison can be made between the rate and manner of O_2 consumption observed for the fracture mineral samples and that predicted by scoping calculations based on the conceptual model, also reported in (Rivas Perez, 1996). The conceptual model has now been tested quantitatively using characterised rock samples and data collected from the recirculating batch reactor experiment. In addition experiments using batch bottle reactors have enabled have provided further data on the quantity and rate of oxygen uptake by fracture mineral samples.

Table 9. Initial concentration of Fe(II) in the samples

Sample Name	Experiment Name	Wetted Surface Area (m^2/g)	Total Fe in Mineral Sample (%)	% Fe(II) of Total Fe	Rock to solution ratio (g/l)
1762m <0.25mm	081098	16.7	5.14	74.50	7.24
1762m <0.25mm	081098	16.7	5.14	74.50	7.42
1762m 0.25-0.5mm	230198	10.3	4.35	73.40	17.29
1762m.25-0.5mm	230198	10.3	4.35	73.40	15.86
1762m 0.25-0.5mm	300998	10.3	4.35	73.40	5.89
1762m 0.25-0.5mm	300998	10.3	4.35	73.40	9.04
1762m 0.5-1mm	180298	11.9	4.23	76.90	25.11
1762m 0.5-1mm	180298	11.9	4.23	76.90	24.82
1762m 0.5-1mm	240398	11.9	4.23	76.90	5.4
1762m 0.5-1mm	240398	11.9	4.23	76.90	8.0
1762m 1-2mm	240398	19.2	3.77	74.90	5.74
1762m 1-2mm	240398	10.6	3.77	74.90	5.45
1762m 1-2mm	230399	19.2	3.77	74.90	5.3
1762m 1-2mm	230399	10.6	3.77	74.90	5.4

A2.3.3 Recirculating Batch Reactor Experiment

A2.3.3.1 Description

The Recirculating Batch Reactor Experiment (Figure 41 and Figure 42) consisted of a batch reactor in which the sample to be tested was housed and also incorporated a filter holder and filter in its lid. The batch reactor was connected to a pump, which was in turn connected to a flow through cell. This housed the oxygen probe. To complete the circuit the flow through cell

was connected to the batch reactor. Solutions were drawn through the batch reactor and filter into the pump and then through the flow through cell before returning to the batch reactor. The total volume of the kit was 415 mL.

Solution samples were drawn from the flow through cell and pH measurements made when the pump was stopped. Solution removed from the equipment for sampling was replaced with fresh deoxygenated solution from a reservoir.

The batch reactor, the filter holder, the flow through cell and all tubing are constructed of PEEK (Poly-Ether-Ether-Ketone) a plastic selected for its density and non-reactivity. The entire experimental kit with the exception of the pump was housed in a temperature control box.

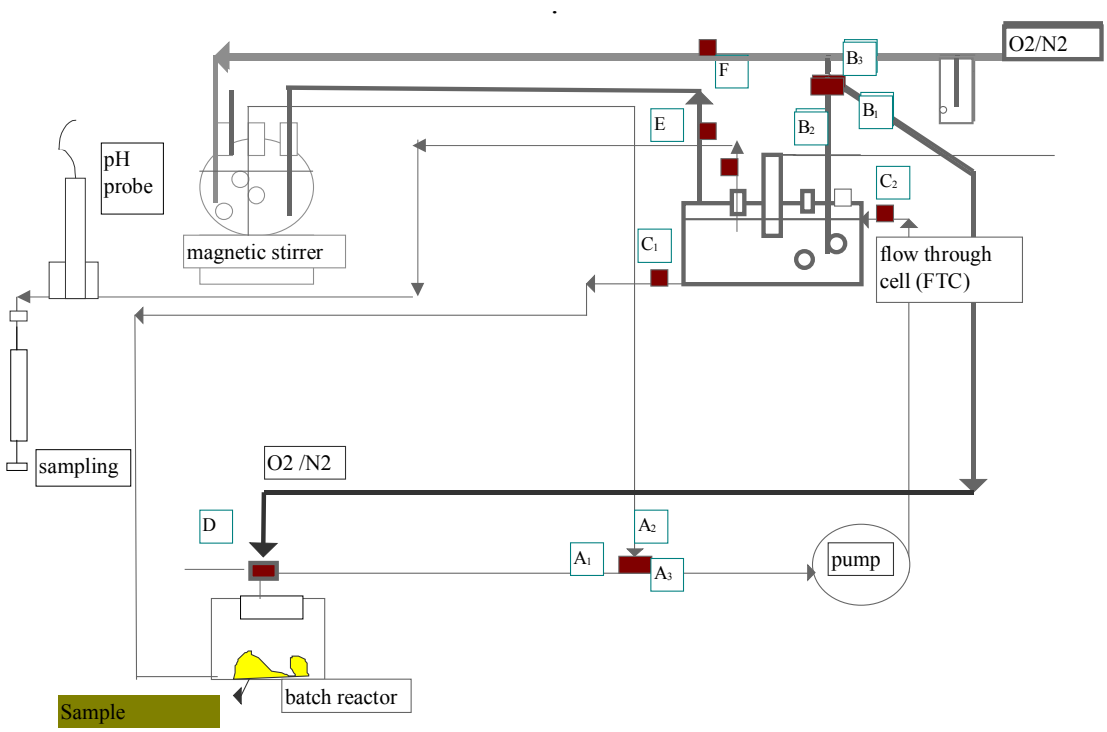


Figure 41. Scheme of the Recirculating Batch Reactor Experiment.

Figure 42. The Recirculating Batch Reactor Experiment.

The procedure for the recirculating batch reactor experiment is as follows.

First the amount of O_2 ingress into the recirculating batch reactor is measured. The kit is assembled and 3µm filter put in the filter holder and filled with deoxygenated 10^{-2} M NaCl solution and the pump is switched on. This solution is allowed to circulate around the equipment at a rate of 5ml per minute and the concentration of oxygen is measured. After several hours the rate of O_2 ingress into the equipment is calculated from the recorded dissolved oxygen data. If the rate of ingress is at an acceptable level then the experiment can proceed, if not the equipment must be taken apart and reassembled to minimise oxygen leaking into it and the ingress measured once again.

When the oxygen ingress rate is at an acceptably low level the kit is drained and fresh solution is equilibrated at a known temperature with 20 % O_2 in N_2 gas in the reservoir. A known weight of the sample to be tested is placed in the batch reactor and the equipment is filled with the oxygenated 10^{-2} M

NaCl solution. This solution is circulated around the equipment at a rate of approximately 5 mL per minute. Dissolved oxygen concentrations and temperature are recorded and the experiment is left to run for up to 14 days.

Solution samples and pH measurements are taken periodically. The pump is stopped and the sample line valve in the lid of the flow through reactor is opened to allow 8-11 mL of solution to be drawn through a pH flow through cell and collected in a syringe. It is then transferred to a labelled polythene bottle, acidified and frozen. Synchronous with the solution sample being removed, deoxygenated solution from the reservoir replaces it.

At the end of the experiment the solution is drained from the kit and some of it set aside for an alkalinity test. The mineral sample is transferred to a vacuum dessicator to dry out before being stored. The equipment is then taken apart and washed before the next experiment.

A2.3.3.2 Results

Graphs of dissolved O_2 concentration *versus* time for the Recirculating Batch Reactor Experiment are shown in Figure 43 to Figure 49.

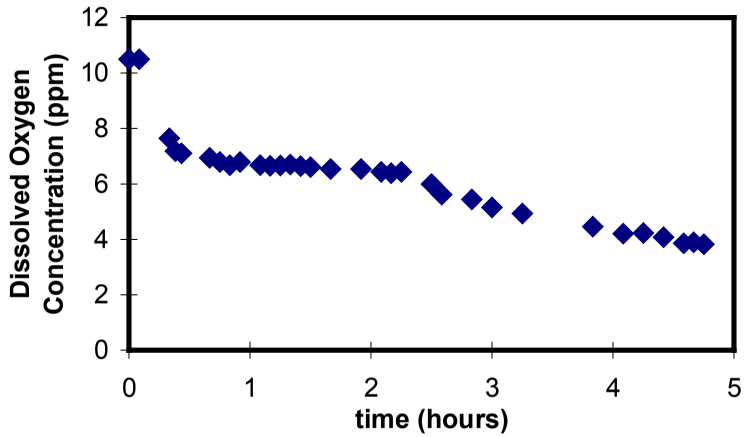


Figure 43. Plot of $[O_2]$ *versus* time for recirculating batch reactor experiment 100998 using 10.0 g of sample Äspö 1762 m, 1-2 mm size fraction in 415 mL of oxygenated 10^{-2} M NaCl solution. Data corrected for dilution and atmospheric ingress.

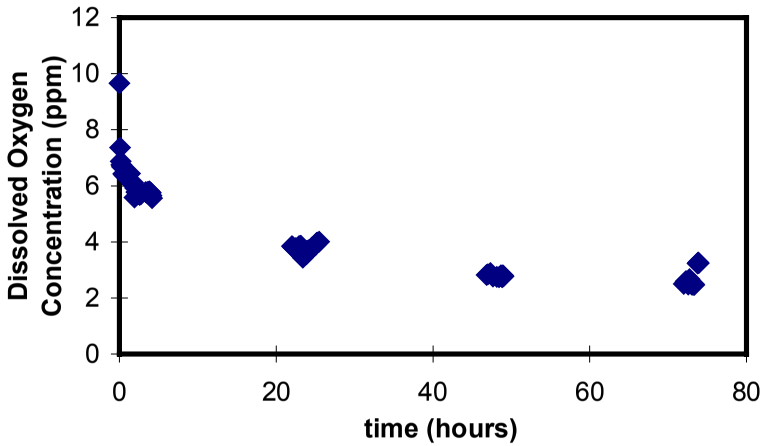


Figure 44. Plot of $[O_2]$ *versus* time for recirculating batch reactor experiment 170698 using 2.0 g of sample Äspö 1762 m, 1-2 mm size fraction in 415 mL of oxygenated 10^{-2} M NaCl solution. Data corrected for dilution and atmospheric ingress.

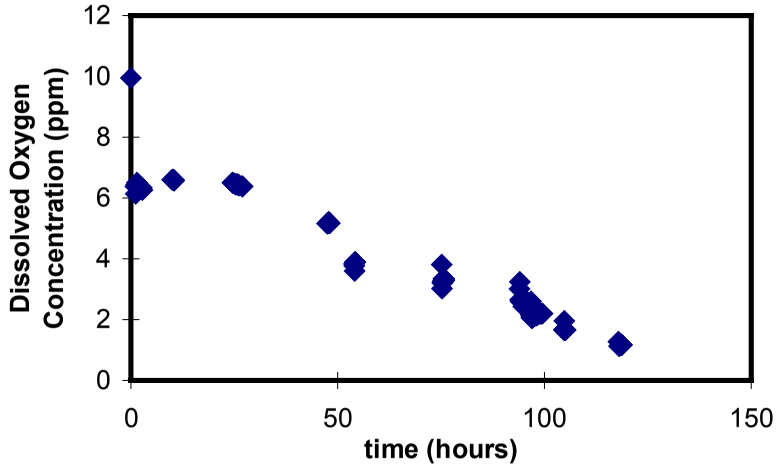


Figure 45. Plot of $[O_2]$ *versus* time for recirculating batch reactor experiment 160798 using 2.01 g of sample Äspö1762 m, 1-2 mm size fraction in 415 mL of oxygenated 10^{-2} M NaCl solution. Data corrected for dilution and atmospheric ingress.

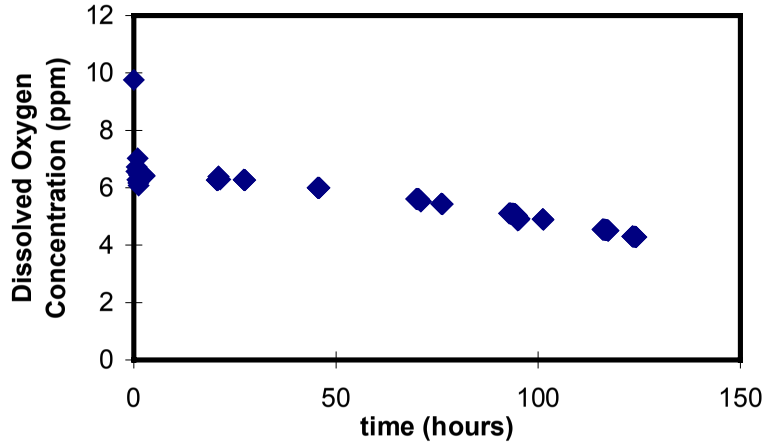


Figure 46. Plot of $[O_2]$ *versus* time for recirculating batch reactor experiment 240798 using 2.0 g of sample Äspö 1762 m, 1-2 mm size fraction in 415 mL of oxygenated 10^{-2} M NaCl solution at pH=3. Data corrected for dilution and atmospheric ingress.

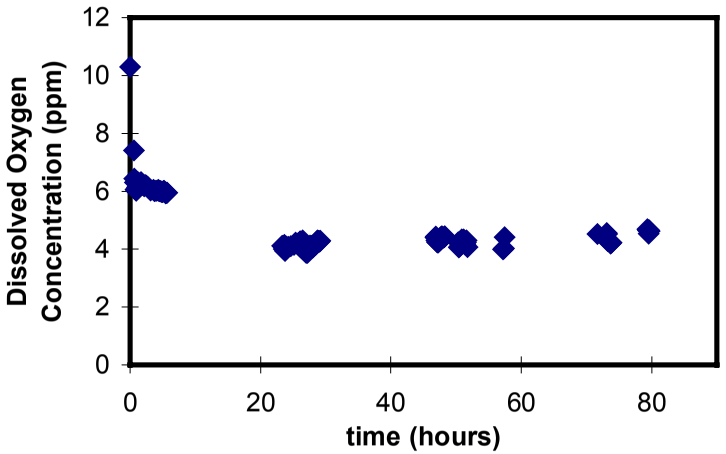


Figure 47. Plot of $[O_2]$ *versus* time for recirculating batch reactor experiment 100898 using 2.0 g of sample Äspö1762 m, 1-2 mm size fraction in 415 mL of oxygenated 10^{-2} M NaCl solution at pH=11. Data corrected for dilution and atmospheric ingress.

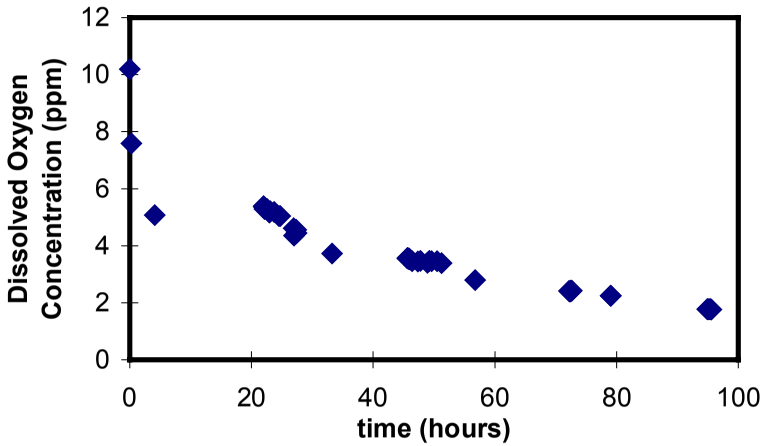


Figure 48. Plot of $[O_2]$ *versus* time for recirculating batch reactor experiment 071098 using 2.0 g of sample KA3065 0.3-0.7 m, 1-2 mm size fraction in 415 mL of oxygenated 10^{-2} M NaCl solution. Data corrected for dilution and atmospheric ingress.

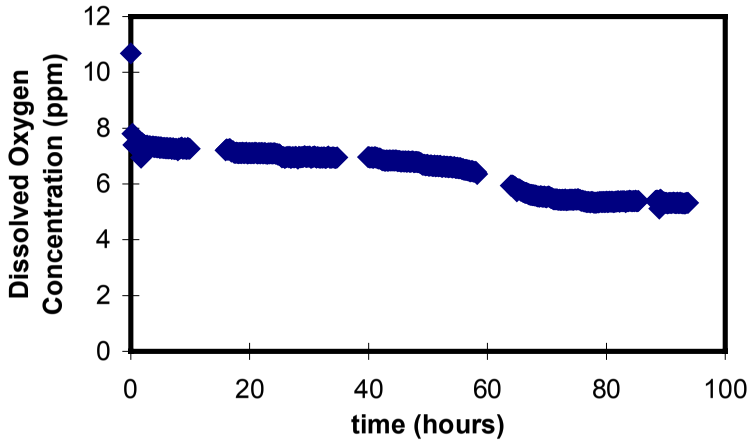


Figure 49. Plot of $[O_2]$ *versus* time for recirculating batch reactor experiment 121098 using 2.0 g of sample KA3065 0.7-0.9 m, 1-2 mm size fraction in 415 mL of oxygenated 10^{-2} M NaCl solution. Data corrected for dilution and atmospheric ingress.

A2.3.4 Batch Bottle Reactors

These experiments were developed to allow O_2 consumption data for fracture mineral samples that could not be used in the Recirculating Batch Reactor Experiment to be gathered. On average a typical Recirculating Batch Reactor experiment will take about 14 days to test one sample, therefore due to time constraints the number of samples that were used in these experiment were limited. In the same time period many more samples could be tested by the Batch Bottle Reactor method which although not as accurate or as precise as the Recirculating Batch Reactor Experiment allowed an assessment of a samples reactivity to be made.

The equipment required for the batch bottle experiment (Figure 50) consisted of

- 280 mL glass bottles with ground glass stoppers
- magnetic stirrer
- magnetic fleas
- Hanna HI 964400 Microprocessor Bench Dissolved Oxygen Meter
- Hanna portable pH meter
- filtration equipment
- Hach alkalinity testing kit

A measured quantity of rock fracture filling material from the Äspö HRL was reacted in a stoppered glass bottle with approximately 280 mL of oxygenated artificial groundwater. The bottles were kept at room temperature. Dissolved O_2 was measured using a Hanna HI 964400 microprocessor bench dissolved oxygen meter. The ground glass stopper of the bottle was removed and the probe was submerged in the solution in the bottle. The solution was stirred using a magnetic stirrer to ensure that an accurate dissolved O_2 measurement was made. After each measurement, a measured volume of deoxygenated solution was added to the bottles to replace solution displaced from the bottle by the electrode. Oxygen consumption was observed to a greater or lesser degree for practically all samples.

A control experiment consisting of a bottle filled with deoxygenated solution was used to measure the maximum oxygen ingress during the measurement procedure. A further control contained only oxygenated solution, to demonstrate that dissolved oxygen consumption was due to the presence of sample. Dissolved O_2 concentration was also measured in these bottles.

Each experiment was assumed completed when oxygen consumption has slowed significantly, due either to a decrease in the sample capacity for uptake or to the consumption of all the dissolved oxygen in the bottle. At this point the pH of the solution was measured and then the contents of the bottles are filtered. The mineral material was dried in a vacuum dessicator, a solution sample acidified and frozen and the remaining solution was used to measure final alkalinity.

The results of the Batch Bottle Experiments are given in Table 10 to Table 14.

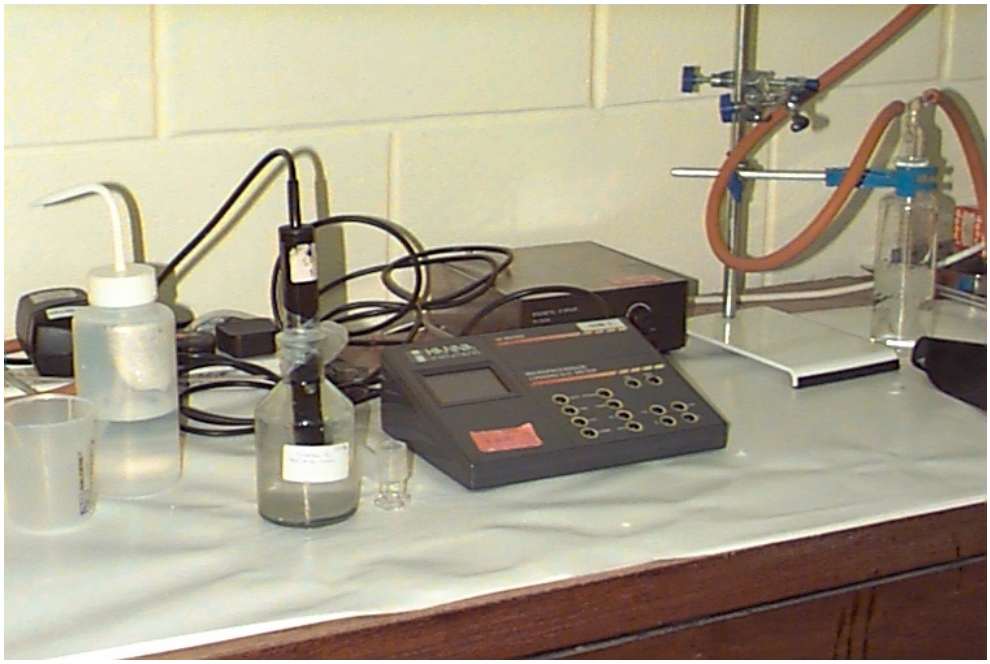


Figure 50. Measurement of O_2 in a Batch Bottle Reactor.

Table 10. Samples Äspö 1762 m. Summary of rate constants, k' ($L \cdot g^{-1} \cdot d^{-1}$).

Particle Size	k'	standard deviation
<0.25 mm	-6.05×10^{-3} -3.16×10^{-3}	1.67×10^{-3} 2.0×10^{-3}
0.25 -0.5 mm	-9.82×10^{-4} -4.47×10^{-4}	5.73×10^{-4} 2.67×10^{-4}
0.5 -1 mm	-1.16×10^{-2} -2.54×10^{-3}	1.98×10^{-3} 7.40×10^{-4}
1-2 mm	-7.21×10^{-3} -9.43×10^{-3} -8.38×10^{-3} -8.38×10^{-3} -2.23×10^{-3} -1.65×10^{-3}	7.05×10^{-3} 8.04×10^{-3} 9.43×10^{-3} 8.76×10^{-3} 1.55×10^{-3} 1.99×10^{-3}
2-10 mm	-6.46×10^{-4} -9.16×10^{-4}	4.44×10^{-4} 4.77×10^{-4}

Table 11. Samples Äspö 1955 m. Summary of rate constants, k' ($L \cdot g^{-1} \cdot d^{-1}$).

Particle Size	k'	standard deviation
<0.25 mm	-1.04×10^{-1} -1.61×10^{-1}	4.90×10^{-2} 4.61×10^{-2}
0.25 -0.5 mm	-1.35×10^{-4} -3.08×10^{-3}	8.19×10^{-5} 9.26×10^{-4}
0.5 -1 mm	-3.03×10^{-3} -2.47×10^{-3}	3.11×10^{-4} 3.29×10^{-4}
1-2 mm	-2.00×10^{-2} -4.64×10^{-2}	6.91×10^{-2} 2.98×10^{-2}
2-10 mm	-9.11×10^{-4} -1.01×10^{-4}	6.22×10^{-4} 5.70×10^{-4}

Table 12. Samples Äspö 1985 m. Summary of rate constants, k' ($L \cdot g^{-1} \cdot d^{-1}$).

Particle Size	k'	standard deviation
<0.25 mm	-3.22×10^{-1} -3.78×10^{-1}	9.56×10^{-2} 1.15×10^{-1}
0.25 -0.5 mm	-1.91×10^{-3}	2.91×10^{-3}
0.5 -1 mm	-1.05×10^{-3}	1.48×10^{-3}
1-2 mm	-1.49×10^{-3} -8.45×10^{-4}	4.77×10^{-4} 3.75×10^{-4}

Table 13. Samples Äspö 2180 m. Summary of rate constants, k' ($L \cdot g^{-1} \cdot d^{-1}$).

Particle Size	k'	standard deviation
<0.25 mm	-5.45×10^{-1} -6.03×10^{-1}	1.91×10^{-2} 2.11×10^{-1}
0.25 -0.5 mm	-2.43×10^{-3} -3.30×10^{-3}	1.00×10^{-3} 1.64×10^{-3}
0.5 -1 mm	-3.23×10^{0} -2.75×10^{0}	5.46×10^{-1} 4.95×10^{-1}
1-2 mm	-3.43×10^{-3} -3.63×10^{-4}	3.49×10^{-3} not available
2-10 mm	-8.69×10^{-4} -6.68×10^{-4}	2.63×10^{-4} 5.14×10^{-4}

Table 14. Samples Äspö borehole KA3065, 0.7-0.9m, 0.25-1 mm. Summary of rate constants, k' ($L \cdot g^{-1} \cdot d^{-1}$).

Particle Size	k'	standard deviation
sonicated in ethanol	-3.38×10^{-3}	2.37×10^{-3}

not cleaned	-4.90×10^{-3}	2.70×10^{-3}

A2.3.5 Inhibitor Experiments

An additional type of bottle experiment was conducted using microbial inhibitors to establish whether the decreasing O_2 concentrations observed were due to microbial consumption. Other bottles contained mineral sample that had been sterilised at 120°C in a N_2 atmosphere.

The results were inconclusive. The highest oxygen consumption observed occurred in bottles containing inhibitor, suggesting that the inhibitor contained either a reducing agent, or that it was poisoning the oxygen electrode, or both.

Heat sterilisation of samples in this experiment appeared to reduce oxygen consumption slightly, either by reducing microbial activity or physically changing the reactive surfaces of the sample.

A2.3.6 Conclusions

The experiments carried out at the University of Bradford successfully demonstrated that O_2 uptake by rock samples taken from the Äspö Hard Rock Laboratory can be observed and quantified.

The Batch Bottle Experiment is an ideal method for quickly assessing the reactivity of different rock samples under various different conditions. The Recirculating Batch Reactor Experiment is a reliable method of measuring oxygen uptake and data collected from these experiments can be used in the modelling of repository safety.

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