Technical Report
TR-16-05
June 2016

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SVENSK KÄRNBRÄNSLEHANTERING

ISSN 1404-0344
SKB TR-16-05
ID 1534678
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Keywords: Sulfate reduction, Monod equation, Geochemical modelling.

Abstract

Previously published experimental data from laboratory studies of sulfate reduction with either H_2 or lactate as the sole energy sources (Hallbeck 2014) are modelled in this work using Monod-type rate laws. The kinetic equations have been implemented in the widely-used PHREEQC geochemical modelling code. The rate parameters have been optimized to fit the experimental laboratory data, and they are compared with literature values. However, because the experimental conditions for the laboratory experiments (Hallbeck 2014) are quite different from those in natural environments, the rate parameters derived in this work should not be applied directly in models of sulfate reduction in groundwaters. Nevertheless, this type of numerical models will be useful in future evaluations of sulfide-related processes in nuclear waste repositories.

Sammanfattning

Denna rapport presenterar en modellering av tidigare publicerade laboratoriedata (Hallbeck 2014) av sulfatreduktion med antingen H_2 eller laktat som enda energikällor. Modelleringen har använd Monod-typ ekvationer som har lösts med den välkända geokemiska modelleringsprogramet PHREEQC. De kinetiska parametrarna har optimerats för att passa de experimentella laboratoriedata, och en jämförelse har gjorts med andra litteraturstudier. Då de experimentella betingelserna för de laboratorie-experimenten (Hallbeck 2014) skiljer sig helt från de i naturliga miljöer bör de erhållna kinetiska parametrar, som har härletts från laboratorieresultaten, inte appliceras direkt i modeller för sulfat-reduktion i grundvatten. Denna typ av numeriska modeller kan ändå vara till nytta i framtida utvärderingar av sulfidrelaterade processer i kärnavfallsförvar.

Contents

1 Introduction

In Sweden and Finland the KBS-3 concept has been adopted for the final disposal of spent nuclear fuel. In this concept, spent nuclear fuel is encapsulated in copper canisters and deposited at about 500 m depth in granitic rocks, using bentonite clay as buffer and backfill (Posiva 2012, SKB 2011). For this type of repository the sulfide present in groundwater is the main corroding agent for the copper canisters, and therefore, sulfide has a large impact on the long-term safety evaluation. Several investigations have been carried out by SKB in Sweden and by Posiva in Finland to determine the concentrations of sulfide in deep groundwater and to model the fate of sulfide in the near field of spent nuclear fuel repositories (Tullborg et al. 2010, Wersin et al. 2014).

Sources of sulfide to groundwater are limited to the dissolution of solid sulfide minerals and to the reduction of sulfate. In the Swedish and Finnish granitic rocks groundwaters have a near-neutral pH and based on their Fe(II) or sulfide contents they may be classified as anoxic or sulfidic. Under these conditions the most stable sulfide mineral is pyrite (FeS_2), which has very low solubility (King 2013). Therefore, the main source of sulfide in deep groundwater is sulfate reduction. At low temperatures (in the KBS-3 concept, the temperatures in the repository near field are always below 100 °C) this process is only carried out by sulfate-reducing bacteria (SRB) which may use a large number of reductants, that is, electron donors (Hansen 1993). In the deep Fennoscandian granitic groundwaters the source of reductants is medium- to high-molecular weight organic matter, either in dissolved or particulate form. In a spent nuclear fuel repository, organic materials are present and might enhance microbial processes, and the corrosion of metal components (rock reinforcements, etc.) will produce H_2 which may be used by SRB (Hallbeck 2010).

Sulfide concentrations in granitic groundwaters are limited by the precipitation of sulfide minerals, for example FeS (Tullborg et al. 2010). In the low temperature anoxic environment of the deep repository, the slow groundwater flow transports organic matter from the soil layers to deeper levels, and these organics are used by SRB to produce sulfide. The dissolution of iron(II) minerals in the rock matrix, and the diffusion of the resulting Fe(II) into the groundwater flowing in the fractures results in the fast precipitation of amorphous iron sulfide, which is then slowly transformed into pyrite. The concentrations of sulfide and Fe(II) in the granitic groundwaters reflect the balance between sulfide production and precipitation, processes that are affected by local hydrological and mineralogical constraints.

In 2006 a joint project was started between the companies Swedish Nuclear Fuel and Waste Management Co. (SKB) in Sweden and Electric Power Development Co., Ltd. (J-Power) in Japan to study the sulfate reduction process. Within this collaborative project laboratory experiments were conducted using the bacterium *Desulfovibrio aespoeensis* with lactate and H_2 as energy sources (Hallbeck 2014). An initial modelling of the experimental data was performed using the BIO-CORE software (Samper et al. 2006), but the agreement with the experimental data was not fully satisfactory, and the modelling results were not reported.

The aim of this study is to develop numerical models using the PHREEQC software (Parkhurst and Appelo 2013) to reproduce the results from the laboratory study (Hallbeck 2014). The final goal is to be able to use this modelling know-how in future developments of biogeochemical conceptual models which will be needed in future assessments of the long-term safety of spent nuclear fuel repositories.

In the initial 2006 joint project it was decided to perform experiments with lactate and H_2 as energy sources. As explained in the discussion, the concentration of lactate in granitic groundwaters is expected to be very low, and therefore sulfate reduction using lactate has little relevance when considering the long-term safety of spent nuclear fuel repositories. Although H_2 concentrations are also low in granitic groundwaters, there are potential deep sources of H_2 that make it a possibly important factor in the turnover of sulfide in granitic groundwaters. In addition, the possible generation of H_2 within the repository by metal corrosion indicates that it is important to be able to model H_2-induced sulfate reduction in the assessment of deep nuclear repositories.

2 Sulfate reduction

The reaction of sulfate to sulfide requires eight electrons

$SO_4^{2-} + 9\ H^+ + 8\ e^- \rightarrow HS^- + 4\ H_2O$

When hydrogen is used as electron donor, the overall reaction for sulfate reduction is

$4\ H_2 + H^+ + SO_4^{2-} \rightarrow HS^- + 4\ H_2O$ (2-1)

Lactate is one of the preferred organic substrates by the genus *Desulfovibrio* (Widdel 1988), the overall oxidation reaction is

$CH_3CH(OH)COO^- + 2\ H_2O \rightarrow CH_3COO^- + HCO_3^- + 5\ H^+ + 4\ e^-$

whereby acetate is produced. *Desulfovibrio* species are not able to oxidize acetate further (Hansen 1993, Widdel 1988). In combination with the sulfate-reduction reaction, the overall reaction becomes

$2\ CH_3CH(OH)COO^- + SO_4^{2-} \rightarrow 2\ CH_3COO^- + 2\ HCO_3^- + HS^- + H^+$ (2-2)

2.1 Biomass growth

It is to be expected that the rate of any microbial process (sulfate reduction in this case) will depend on the number of microbes (cells) performing the process, as well as on several environmental factors such as temperature, nutrient and substrate concentrations, etc. Any model of the rate of sulfate reduction will therefore be coupled to a quantification of cell growth. SRB use reactions such as (2-1) and (2-2) to obtain energy both for growth and for all other energy consuming metabolic activities. An average chemical formula for biomass is needed in order to be able to model the stoichiometry of microbial growth, and the frequently-used formula $C_5H_7O_2N$ (Criddle et al. 1991, Jin and Bethke 2007, Rittmann and McCarty 2000, VanBriesen and Rittmann 2000) will be used in this work. This formula has a molecular mass of 113.12 g/mol.

Obviously bacterial growth requires a source of carbon, and several SRB can use lactate both as a reductant for sulfate and as a source of carbon for growth (Postgate and Campbell 1966), in which case the overall reaction for cell growth may be expressed as:

$^5/_3\ CH_3CH(OH)COO^- + NH_4^+ + {}^2/_3\ H^+ \rightarrow C_5H_7O_2N + 3\ H_2O$ (2-3)

The actual ratio between lactate consumption and sulfide production must therefore be larger than the stoichiometric value of 2:1 given by Reaction (2-2), because bacterial growth requires an additional consumption of lactate by Reaction (2-3).

When H_2 is the energy source SRB also require a source of carbon. For many SRB, such as *Desulfovibrio aespoensiis* the source of carbon can be acetate in combination with inorganic carbon (i.e., CO_2). The proportion of acetate carbon incorporated into biomass for *Desulfovibrio* and *Desulfobacter* species is reported to be about 60 to 70 % (Badziong et al. 1978, Brandis and Thauer 1981, Gebhardt et al. 1983, Sorokin 1966). The overall reaction for biomass synthesis when H_2 is the energy source may therefore be written as

$^5/_3\ CH_3COO^- + {}^5/_3\ CO_2 + {}^{10}/_3\ H_2 + {}^2/_3\ H^+ + NH_4^+ \rightarrow C_5H_7O_2N + {}^{14}/_3\ H_2O$ (2-4)

However, the laboratory results in Hallbeck (2014) suggest a much lower assimilation of acetate carbon. Although acetate-related processes are certainly important in the context of nuclear waste disposal, the focus of this study is limited to simulate the sulfate reduction experiments in Hallbeck (2014), and the proportion of biomass carbon originating from acetate is of no consequence. If the proportion is 20 % instead of 67 %, then the overall reaction for biomass synthesis becomes:

$½\ CH_3COO^- + CO_3^{2-} + 3\ CO_2 + 8\ H_2 + {}^3/_2\ H^+ + NH_4^+ \rightarrow C_5H_7O_2N + 8\ H_2O$ (2-5)

equivalent to

$½\ CH_3COO^- + 4HCO_3^- + 8\ H_2 + {}^9/_2\ H^+ + NH_3 \rightarrow C_5H_7O_2N + 11\ H_2O$

2.2 Inhibition effects

Sulfide may have a toxic effect on SRB. In addition, sulfide might inhibit bacterial growth by precipitating trace metals, such as iron, needed for metabolism (Gupta et al. 1994, Hauser and Holder 1986, Khosrovi and Miller 1975, Maillacheruvu and Parkin 1996, Vosjan 1975). According to Reis et al. (1992) the un-dissociated species $H_2S(aq)$ is responsible for the observed toxicity. Almost no information is found in the literature about the toxic effects of H_2S for SRB in systems where H_2 is the substrate and CO_2 and acetate are the carbon sources. Maillacheruvu and Parkin (1996) used SRB developed from "digested sludge seed" and detected a 50 % reduction in the reaction rate at a sulfide concentration of approximately 5 mM. It was found in this work that in order to fit the sulfate reduction rate data in the H2RateAc experiments (see Section 3.1.3) it was necessary to introduce a degree of sulfide inhibition, see also Sections 4.3 and 6.1. The inhibition effect was much lower for the lactate experiments (Section 3.1.1).

Several studies have however been reported on the inhibitory effects of sulfide in systems where lactate is both the substrate and carbon source (Hilton and Oleszkiewicz 1988, Maillacheruvu et al. 1993, McCartney and Oleszkiewicz 1991, Okabe et al. 1992, 1995, Reis et al. 1992). Only three studies were performed on *Desulfovibrio* strains (Okabe et al. 1992, 1995, Reis et al. 1992), the results are however somewhat contradictory: a 50 % reduction in the growth rate of *Desulfovibrio desulfuricans* at 15 mM total sulfide is indicated by Okabe et al. (1992), while complete inhibition at a H_2S concentration of about 16 mM is reported in (Reis et al. 1992). Nevertheless, the toxic effect of sulfide should be of minor consequence for the sulfate reduction experiments conducted by Hallbeck (2014) using lactate as a substrate at pH 7, because the calculated maximum concentrations of H_2S in the aqueous phase are below 2 mM, and in addition NTA was used to prevent metal precipitation in the trace element solution described in Hallbeck and Pedersen (2008).

It has been found experimentally that acetic acid may inhibit SRB. In Nagpal et al. (2000) 50 % inhibition at 120 mM is reported for a mixed culture of SRB growing on ethanol, while 1 mM un-dissociated acetic acid is reported to inhibit SRB growth by 50 % when lactate is the substrate (Reis et al. 1990). In the sulfate reduction experiments conducted by (Hallbeck 2014) with batch experiments using H_2 as substrate at pH adjusted to 7, the optimal acetate concentration was found to be 2 mM.

When lactate was used as a substrate at pH adjusted to 7, the acetate concentrations increased up to 13 mM in the experiments by Hallbeck (2014). Chemical equilibrium calculations show that under these experimental conditions, the concentration of un-dissociated acetic acid is less than 0.1 mM, and it may therefore be concluded that the inhibition effect would be negligible.

3 The laboratory experiments

Hallbeck (2014) performed three types of experiments using the bacterium *Desulfovibrio aespoeensis*:

- **LacRate:** experiments using lactate both as a nutrient and reductant (electron-donor)
- **AcRateH2:** experiments with an excess of H_2 (electron donor substrate) and CO_2 (carbon source) while varying the concentration of acetate. This series of experiments were performed to obtain the optimal acetate concentration. As explained by Hallbeck (2014), *Desulfovibrio* species are not able to use acetate as electron donor, see also Widdel (1988, Table 10.1), however, they can use acetate in combination with CO_2 as carbon sources when either H_2 or formate is the electron-donor substrate (Hansen 1993, p. 38).
- **H2RateAc:** experiments where H_2 was varied while the concentrations of nutrients (acetate and CO_2) were kept constant at optimal levels.

These experiments are further described in the following subsections.

It must be noted that the conditions for the laboratory experiments presented in Hallbeck (2014) deviate significantly from the expected deep repository environment. In particular, an excess of nutrients was available for the bacteria, and a large concentration of electron donor-rich substrates were used. Therefore, the rates of sulfate reduction observed in these experiments will deviate substantially from those expected in the groundwaters surrounding a deep repository.

Hallbeck (2014) analyzed total sulfide with the methylene blue method (SIS 1976). According to Standard Methods Committee (2011) the methylene blue method includes dissolved H_2S, HS^- and "acid-volatile metallic sulfides" present as particulate matter. The concentration of S^{2-} is negligible at all pH values. "Acid-volatile sulfides" include amorphous iron monosulfide, mackinawite (FeS), greigitite (Fe_3S_4), and pyrrhotite (FeS). Pyrite (FeS_2) is not included in the acid-volatile sulfides.

3.1 LacRate

Sulfate reduction was studied using lactate as the energy and carbon source. Lactate is one of the preferred substrates of *Desulfovibrio*, which perform an incomplete oxidation to acetate (Widdel 1988). Yeast extract and vitamins were also added in Hallbeck (2014) to the growth medium. Five lactate concentrations, from 0.0113 to 10^{-6} M were used, while the concentration of sulfate was kept constant. Each lactate concentration was studied in triplicate (series A, B and C) for a maximum total period of 15 days. The experiments were performed in 120 mL septum bottles, and series A and B were sampled and analyzed at several intervals, while series C was only analyzed at the beginning and at the end of the experimental period. Sulfide production was detected in the two highest concentrations of lactate (11.3 and 1.13 mM). At a lactate concentration of 0.11 mM some sulfate reduction could be detected at the end of the experiment, but at lower lactate concentrations the sulfide concentrations were below the detection limit of the analyses, i.e. < 0.01 mg/L.

3.2 AcRateH2

Sulfate reduction was studied using H_2 as the energy source and CO_2 and acetate as carbon sources. Yeast extract and vitamins were also added in Hallbeck (2014) to the growth medium. The acetate concentration was varied from 53 to 0.08 mM in duplicate series while keeping the partial pressure of H_2 at about 2 bar. The optimal acetate concentration was found to be in the order of 2 mM.

3.3 H2RateAc

Sulfate reduction was studied using H_2 as the energy source and CO_2 and acetate as carbon sources. Yeast extract and vitamins were also added in Hallbeck (2014) to the growth medium. Four initial partial pressures of H_2, from about 50 to 3 % were used (total pressure 2 bar obtained with a N_2/CO_2 mixture 80/20 %), while the initial acetate concentration was kept constant at 2 mM. Each partial pressure of H_2 was studied in triplicate (series A, B and C), a separate 26 mL tube was used for each sampling point. The maximum total duration was 15 days. The initial sulfate concentration varied between 11 and 17 mM for the experiments with the highest H_2 in the gas phase (50 %), between 17 and 13.5 mM for the experiments at 25 % H_2, and kept constant at 13.5 mM for the three experiments with the lowest H_2 partial pressures.

4 Rate equations

Monod (1949) proposed a rate law for the exponential growth phase limited by the availability of substrate, an equation that has been widely used (Panikov 2011, Robinson and Tiedje 1983). In this work the Monod-type rate expressions that include thermodynamic constraints (Bethke et al. 2008, Jin et al. 2013, Jin and Bethke 2002, 2003, 2005, 2007, 2009) were tested to solve the kinetic Reactions (2-1) and (2-5) when H_2 is the energy source and (2-2) and (2-3) when it is lactate. These expressions are ordinary differential equations that may be solved using a set of initial conditions (the initial concentrations of biomass, sulfate, etc). As explained below, it turned out that in the laboratory experiments (Hallbeck 2014) the excess of nutrients and electron-donor substrates was such that the term including the thermodynamic constraint was not needed.

4.1 H_2-Acetate-CO_2

Taking into account the inhibiting effect of sulfide, the rate of sulfate reduction in experiments where H_2 is the reductant may be expressed as an extended dual Monod expression:

$$\frac{d[\mathrm{SO_4^{2-}}]}{dt} = -\frac{d[\mathrm{HS^-}]}{dt} = -[X]\cdot k_{max}\frac{[\mathrm{H_2}]}{K_{\mathrm{H2}}+[\mathrm{H_2}]}\frac{[\mathrm{SO_4^{2-}}]_{Tot}}{K_{\mathrm{SO_4}}+[\mathrm{SO_4^{2-}}]_{Tot}}\,f_{\mathrm{H2S}}\,F_T \qquad (4\text{-}1)$$

where square parentheses indicate the concentrations in the aqueous phase (mol/L), "X" is biomass, k_{max} is the maximum growth rate constant, K_{H2} and K_{SO4} are the half-saturation Monod parameters, f_{H2S} is a factor for the inhibition by sulfide discussed below (Section 4.3), and F_T is a factor expressing the thermodynamic driving force, i.e. how far the system is from equilibrium, see Bethke et al. (2008), Jin et al. (2013) and Jin and Bethke (2002, 2003, 2005, 2007, 2009). During microbial respiration sulfate reduction is coupled with ATP synthesis, and the thermodynamic driving force is given by

$$F_T = 1-\exp\left(\frac{\Delta G_{\mathrm{redox}}+m\Delta G_{\mathrm{P}}}{\chi RT}\right)$$

where m is the number of ATP molecules synthesized during the respiration reaction, χ is its average stoichiometric number, ΔG_{P} is the free energy change in synthesizing ATP from ADP, ≈ 45 kJ per mol. As in this case the redox reaction is

$$\mathrm{H_2(aq) + 0.25\ H^+ + 0.25\ SO_4^{2-} \rightarrow H_2O + 0.25\ HS^-}$$

the Gibbs free energy change becomes

$$\Delta G_{\mathrm{redox}} = \Delta G^{\mathrm{o}}_{\mathrm{redox}} + RT\ln\frac{(a_{\mathrm{HS^-}})^{0.25}}{a_{\mathrm{H_2(aq)}}(a_{\mathrm{H^+}})^{0.25}\left(a_{\mathrm{SO_4^{2-}}}\right)^{0.25}}$$

where $\Delta G^{\circ}_{\mathrm{redox}} = -65.7$ kJ·mol^{-1}. Jin and Bethke (2005) give $\chi = 2$ and $m = 1/3$ for sulfate reduction with H_2 by *Desulfovibrio vulgaris*. The calculated values of F_T are close to 1 when the driving force is large (i.e. when the concentrations of substrates are large when compared to those of the products) and it becomes zero when the substrates are exhausted.

Note that total sulfate concentrations are used in the Monod expression to make the model independent of speciation, i.e. of assumptions on the formation of sulfate complexes such as $NaSO_4^-$, $MgSO_4$(aq), etc.

The rate of H_2 consumption from Reaction (2-1) is given by

$$\frac{d[\mathrm{H_2}]}{dt} = 4\frac{d[\mathrm{SO_4^{2-}}]}{dt}$$

Because the experiments are performed by adding H_2 to the gas in the headspace above the liquid phase where the sulfate reduction occurs, the equilibrium H_2(aq) ⇌ H_2(g) must be included in the model by introducing a gas phase in equilibrium with the aqueous solution.

The rate of biomass synthesis, that is, Reaction (2-5) above, is related to the rate of sulfate reduction by a biomass growth yield coefficient, Y_{SO4}, and a biomass decay coefficient, D:

$$\frac{d[X]}{dt} = -Y_{SO4}\frac{d[SO_4^{2-}]}{dt} - D[X] \quad (4\text{-}2)$$

The rate of acetate consumption may be obtained from Reaction (2-5) and Equation (4-2):

$$\frac{d[ac]}{dt} = -\frac{1}{2}\frac{d[X]}{dt} = \frac{Y_{SO4}}{2}\frac{d[SO_4^{2-}]}{dt} + \frac{D}{2}[X] \quad (4\text{-}3)$$

Because this study proposes to model data obtained from laboratory experiments (Hallbeck 2014) conducted under conditions favorable for bacterial growth, calculations show that the thermodynamic factor F_T is always equal to one, and although D is not equal to zero, in this work it was found that this term had a negligible contribution. For models of natural systems both F_T and D should be included, as described for example in Bethke et al. (2008).

To calculate the concentration of the gaseous components CO_2 and H_2S, the following acid-base equilibria must be taken into account:

$CO_2(aq) + H_2O \rightleftharpoons HCO_3^- + H^+ \rightleftharpoons CO_3^{2-} + 2H^+$

$H_2S(aq) \rightleftharpoons HS^- + H^+$

as well as the two-phase equilibria

$H_2S(g) \rightleftharpoons H_2S(aq)$

$CO_2(g) \rightleftharpoons CO_2(aq)$

Aqueous acid-base equilibria are known to be very fast in comparison with the bacterial processes considered here. The rate of gas-liquid equilibration will depend on factors such as the relative volumes and the interphase area, but they are expected to be fast (in the order of minutes) in the laboratory experiments that will be simulated here, and therefore, these processes are modelled under the assumption of thermodynamic local equilibrium.

4.2 Lactate

When lactate is the reductant, the rate of sulfate reduction and sulfide production may be expressed as the following extended dual Monod expression

$$\frac{d[SO_4^{2-}]}{dt} = -\frac{d[HS^-]}{dt} = -[X]\cdot k_{max}\frac{[lac]}{K_{lac}+[lac]}\frac{[SO_4^{2-}]}{K_{SO_4}+[SO_4^{2-}]}f_{H2S}\,F_T \quad (4\text{-}4)$$

where square parentheses indicate the concentrations in mol/L, "*lac*" represents lactate, "*X*" is biomass, k_{max} is the maximum growth rate constant, K_{lac} and K_{SO4} are the half-saturation Monod parameters, f_{H2S} is a factor for the inhibition by sulfide discussed below (Section 4.3), and F_T is a factor expressing the thermodynamic driving force, as explained above in Section 4.1. In this case the overall redox reaction is given by Reaction (2-2) above, and the Gibbs free energy change is therefore defined as

$$\Delta G_{redox} = \Delta G^{o}_{redox} + RT\ln\frac{a_{HS^-}\,a_{H^+}\left(a_{HCO_3^-}\,a_{CH_3COO^-}\right)^2}{a_{SO_4^{2-}}\left(a_{CH_3CHOHCOO^-}\right)^2}$$

where $\Delta G^{\circ}_{redox} = -120.6$ kJ·mol^{-1}. Because values for χ and m for sulfate reduction with lactate were not found in the literature, the values of $\chi = 6$ and $m = 1$ proposed in Jin et al. (2013, Table 3) for sulfate reduction with acetate are used here. The calculated of F_T values are close to 1 when the driving force is large (i.e. when the concentrations of substrates are large when compared to those of the products) and it becomes zero when the substrates are exhausted.

Note that total lactate and sulfate concentrations are used in the Monod expression in this work to make the model independent of speciation, i.e., independent of assumptions on the formation of complexes such as $NaSO_4^-$, $MgSO_4$(aq), etc.

The rate of biomass synthesis, Reaction (2-3) above, is related to the rate of sulfate reduction by a biomass growth yield coefficient, Y_{SO4}, and a biomass decay coefficient, D, with an equation equivalent to (4-2) but with different parameter values.

$$\frac{d[X]}{dt} = -Y_{SO4}\frac{d[SO_4^{2-}]}{dt} - D[X] \quad (4\text{-}5)$$

The rate of lactate consumption, from Reactions (2-2) and (2-3), may be expressed as:

$$\frac{d[lac]}{dt} = 2\frac{d[SO_4^{2-}]}{dt} - \frac{5}{3}\frac{d[X]}{dt} \quad (4\text{-}6)$$

The rates of acetate and HCO_3^- production, cf. Reaction (2-2), are given by

$$\frac{d[ac]}{dt} = \frac{d[HCO_3^-]}{dt} = -2\frac{d[SO_4^{2-}]}{dt} \quad (4\text{-}7)$$

It is of interest to compare the magnitudes for the yield coefficients for sulfate and lactate. Neglecting the decay of biomass (the term $-D[X]$ in Equation (4-5)) the yield coefficient for lactate may be expressed as

$$\frac{d[X]}{dt} = -Y_{lac}\frac{d[lac]}{dt} \quad (4\text{-}8)$$

From Equations (4-5), (4-6) and (4-8) one obtains

$$Y_{lac} = \frac{3Y_{SO4}}{6 + 5Y_{SO4}} \quad (4\text{-}9)$$

$$Y_{SO4} = \frac{6Y_{lac}}{3 - 5Y_{lac}} \quad (4\text{-}10)$$

that is, for small yield values ($Y_{SO4} < 0.01$) $Y_{SO4} \approx 2\ Y_{lac}$.

Because this study proposes to model data obtained from laboratory experiments (Hallbeck 2014) conducted under conditions favorable for bacterial growth, the thermodynamic factor F_T is equal to one, and although D is not zero, in this work it was found that this term had a negligible contribution. For models of natural systems both F_T and D should be included, as described for example in Bethke et al. (2008) and Jin et al. (2013).

4.3 Inhibition functions

It was found during the fitting of the H2RateAc experiments (Section 3.1.3) reported in Hallbeck (2014) that a sulfide inhibition term was required. The inhibition effect was much lower for the lactate experiments (Section 3.1.1). Inclusion of the thermodynamic factor F_T, Equation (4-1), or the bacterial decay term D, Equation (4-2), was not necessary, as the calculated values of F_T were near one (see Chapter 6), and the fitted value of D was always such that the term "$D[X]$" had zero contribution in Equation (4-2). One might expect that in natural environments sulfide concentrations will be lower than in the laboratory experiments analyzed here, and inhibitory effects are expected to be negligible.

According to Reis et al. (1992) the un-dissociated species H_2S(aq) is responsible for the toxicity of sulfide to SRB growing on lactate and sulfate. However, the H_2S concentration can only be calculated if a precise pH value is available. In Hallbeck (2014) the experimental pH was initially adjusted to 7, and then buffered by the high HCO_3^- concentration and by the CO_2 in the gas phase (except for the experiments where the gas phase was 100 % H_2). There is however some uncertainty in the pH values, as indicated by the data in Tables 3-9 to 3-11 in Hallbeck (2014). Because of this, the total sulfide concentration (instead of [H_2S]) will be used to calculate the inhibition effects of sulfide in the experiments of Hallbeck (2014).

There are several ways of incorporating sulfide inhibition effects into the Monod equation (Han and Levenspiel 1988). Two commonly used expressions are

$$f_{\mathrm{H2S}} = \left(\frac{k_{\mathrm{H2S}}}{k_{\mathrm{H2S}} + [\mathrm{HS}^-]_{Tot}}\right) \qquad \text{Model "A"} \qquad (4\text{-}11)$$

$$f_{\mathrm{H2S}} = \left(1 - \frac{[\mathrm{HS}^-]_{Tot}}{k_{\mathrm{H2S}}}\right)^n = \left(\frac{k_{\mathrm{H2S}} - [\mathrm{HS}^-]_{Tot}}{k_{\mathrm{H2S}}}\right)^n \qquad \text{Model "B"} \qquad (4\text{-}12)$$

Models "A" and "B" correspond to the equations by Jerusalimsky and Neronova and by Levenspiel, respectively, in Table VI of Han and Levenspiel (1988). Figure 4-1 shows a comparison of both models, for k_{H2S} = 5 mM and n = ½. For moderate concentrations of H_2S, both models may provide similar inhibition effects. Model "A" is used in this report because it only requires one parameter.

4.4 Biomass

For convenience biomass is sometimes measured as cells/mL or as optical density with a spectrophotometer. To use the rate models described above, biomass must be converted to concentration units (mol/L).

The average dry cell mass is needed in order to evaluate bacterial growth if biomass is measured as cell counts. Bacterial populations will in general have heterogeneous size and cell mass distributions. In addition, different bacterial strains will most probably have differing shapes, sizes and compositions. For a given strain the average size will also depend on environmental factors such as nutrient availability, etc. Tang et al. (2007) report a cell dry mass in the order of 3×10^{-13} g/cell for *Desulfovibrio vulgaris* Hildenborough. For other bacteria, such as *E. coli*, *Shewanella putrefaciens*, etc, there are reports of cell dry mass in the range from 1×10^{-13} to 1.5×10^{-12} g/cell (Balkwill et al. 1988, Feijó Delgado et al. 2013, Ingvorsen et al. 1984, Loferer-Krössbacher et al. 1998, Robertson et al. 1998, Varia et al. 2014).

In this report a value of 5×10^{-13} g/cell is adopted; however, this value may be considered to be a scaling factor that can be easily changed. For example, given that biomass is measured in cell counts (e.g. cells/mL) and a different average dry cell mass is adopted, let us say *increased* by a factor of 2 (to 10^{-12} g/cell), then to obtain the same calculated rates and concentrations from the rate equations presented above, the value of k_{max} in Equations (4-4) and (4-1) must be *decreased* by a factor of 2, the yield coefficient Y in Equations (4-2), (4-6) and (4-3) must be *increased* by a factor of 2, and the initial biomass concentrations (in mol/L, calculated from the experimental values of cells/mL) must be *increased* by a factor of 2.

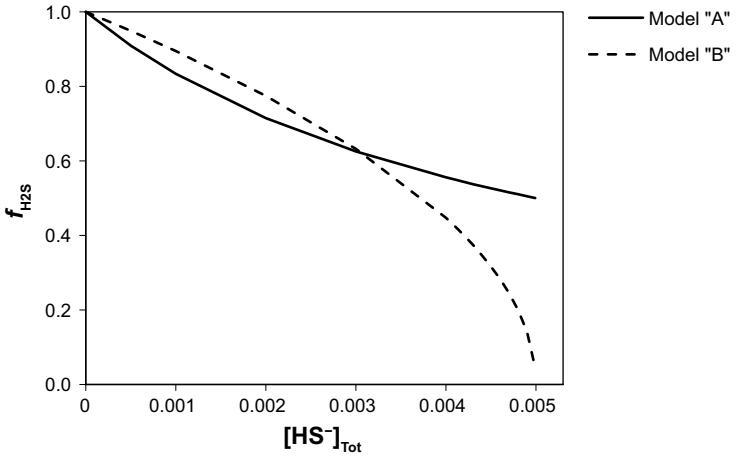

Figure 4-1. *Comparison between two H_2S inhibition models, see text for details.*

5 Numerical implementation and parameter estimation

The numerical calculations of the sulfide production rates by SRB activity were carried out by using the PHREEQC code version 3.3.3 (Parkhurst and Appelo 2013) which also includes chemical equilibrium reactions and gas-aqueous phase equilibria. This program may be downloaded from http://wwwbrr.cr.usgs.gov/projects/GWC_coupled/phreeqc/. Calibration of the Monod parameters was carried by coupling the model-independent Parameter ESTimation code PEST (Doherty 2010, 2015), an open-source, public-domain software suite that allows model-independent parameter estimation and parameter/predictive-uncertainty analysis. The PEST software, together with extensive documentation, can be downloaded from http://www.pesthomepage.org/. In this work PEST was coupled to the batch version of PHREEQC. In this methodological approach the kinetic rate variables are obtained to produce the best fit (weighted least squares minimization) to a set of experimental data (Hallbeck 2014).

Bacterial sulfate reduction occurs in systems that are not at chemical equilibrium: both an electron donor, such as H_2, and an electron acceptor, SO_4^{2-}, are present simultaneously. To simulate such a system in PHREEQC it is necessary to use a database where some redox couples have been un-coupled, as explained in Sect. 3.2.2 in Auqué et al. (2006). The same database has been used here to avoid redox equilibration between HS^-, SO_4^{2-}, H_2, etc.

Although the chemistry of Fe(II) was modelled to some extent in the calculations, it had no effect, neither on the rate of sulfate reduction nor on the sulfide concentrations, because the precipitation of Fe(II)-sulfide was not included. Nevertheless, as mentioned above in Chapter 3, the analyzed sulfide concentrations would include the small amounts of FeS(ppt) that could have precipitated during the experiments, and therefore, including a precipitation reaction in the calculations would not affect the model curves presented in Chapter 6.

6 Model simulations of the laboratory data

6.1 Experiments with varying H_2 partial pressures at constant acetate concentration

The sulfide concentrations (in mol/L) and the logarithm of the total number of cells per mL for experiment H2RateAc reported in the Appendix of Hallbeck (2014) were fitted with the optimization program PEST described above, using Equations (4-1), (4-2) and (4-11). The weight factors used in the least-squares minimization were set equal to the reciprocal of the average for each of the eight data sets (that is, the time-series for both $[HS^-]_{Tot}$ and log(cells/mL) for each of the four H_2(g) pressures investigated). A zero weight was given to the values at time zero.

The appendix provides a PHREEQC input file incorporating the optimized parameters. It must be recalled that the values of Y_{SO4} and k_{max} are linearly depend on the value adopted here for the dry mass of the cells (5×10^{-13} g/cell). Furthermore, these values will also depend, but to a lesser degree, on the several other model assumptions, such as the biomass composition ($C_5H_7O_2N$, 113.115 g/mol), the stoichiometry for the biomass growth (Reaction (2-5)), and on the inhibition function (Equation (4-11)).

The uncertainty of the initial pH was addressed by repeating the optimization using as initial pH either 7.00 or 7.20. The results of both optimizations are given in Table 6-1. Although the least-squares sum is somewhat lower for the case pH_{ini} = 7.20, and the parameter uncertainties are also somewhat lower, the investigated initial pH variation on the parameter values is found to be negligible.

The goodness of the fit between the experimental data and the model may be seen in Figure 6-1 and Figure 6-2. The predicted values of the hydrogen partial pressure (Figure 6-3) agree well with the measured data. The model fails however to reproduce the sulfate concentrations: While the initial reported experimental SO_4^{2-} values for experiment H*553 vary between 16.7 and 10.9 mM, the final values differ by less than 1 mM, see Figure 6-4. The predicted small variation of the acetate concentrations with time, Figure 6-4, is the result of adopting Reaction (2-5) for biomass synthesis, where only 20 % of the biomass carbon originates from acetate. If Reaction (2-4) had been used instead, then the model calculations would have predicted a larger consumption of the initial acetate.

The precipitation reaction for Fe(II)-sulfide was not included in the model, and Figure 6-5 shows that according to the calculations FeS(ppt) should not have precipitated during the experiments. The calculations indicate however that the solutions become oversaturated with more crystalline solid phases such as mackinawite, greigite or pyrite; however, more crystalline solid phases would not be formed unless the more soluble phase precipitates first (Stumm and Morgan 1996, p. 356).

The calculated values for the thermodynamic driving force function, F_T in Equation (4-1), are always larger than 0.94 for all experimental points (not shown in the graphs), except for the last two points of the experiments with the lowest partial pressures of hydrogen (points H2*33 in Figure 6-3, corresponding to an initial H_2 concentration of 33 ‰) where the initial amount of H_2 becomes exhausted.

Table 6-1. Parameters providing the best fit to the experimental data for experiment H2RateAc in Hallbeck (2014).

Parameter*	pH_{ini} = 7.0	pH_{ini} = 7.2
Y	0.109 ± 0.078	0.109 ± 0.076
k_{max}	$(1.48 \pm 0.97) \times 10^{-4}$ s^{-1}	$(1.48 \pm 0.95) \times 10^{-4}$ s^{-1}
K_{H2}	$(1.95 \pm 0.43) \times 10^{-5}$ M	$(2.02 \pm 0.42) \times 10^{-5}$ M
K_{SO4}	$< 1 \times 10^{-3}$ M	$< 1 \times 10^{-3}$ M
k_i	$(4.67 \pm 0.74) \times 10^{-4}$ M	$(4.69 \pm 0.72) \times 10^{-4}$ M

* Uncertainties are 95 % confidence limits.

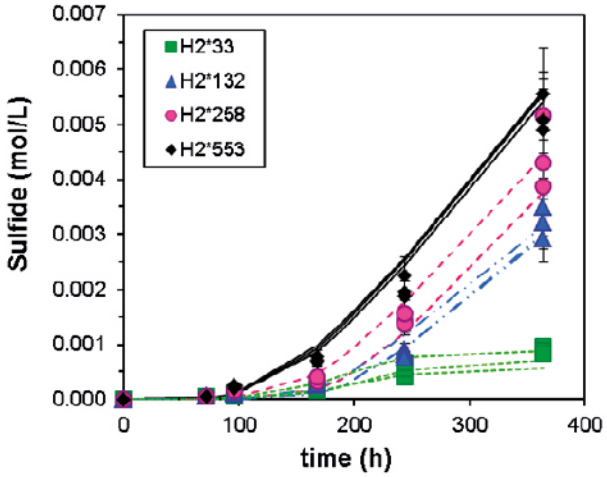

Figure 6-1. *Fitted model calculations of total aqueous sulfide concentrations compared with the experimental values reported in Hallbeck (2014) for the H2RateAc experiments. The uncertainty bars represent 15 % of the reported experimental analysis. Because each experiment series was performed in triplicate by Hallbeck (2014) with slightly different initial conditions (for example for the biomass and sulfate concentrations), three calculation are re reported for each experiment series.*

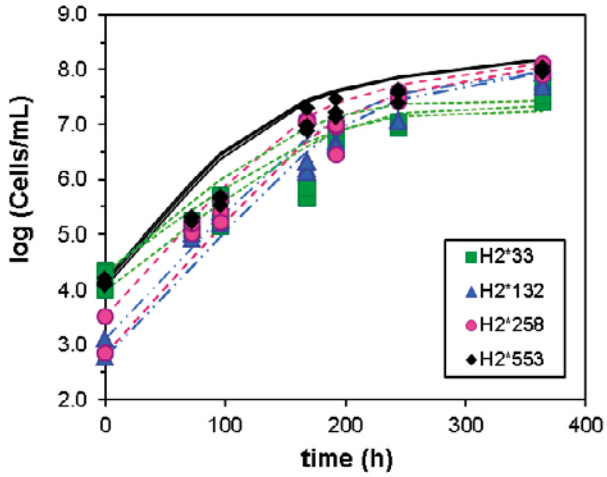

Figure 6-2. *Fitted model calculations of biomass compared with the experimental values reported in Hallbeck (2014) for the H2RateAc experiments. Because each experiment series was performed in triplicate by Hallbeck (2014) with slightly different initial conditions (for example for the biomass and sulfate concentrations), three calculation are re reported for each experiment series.*

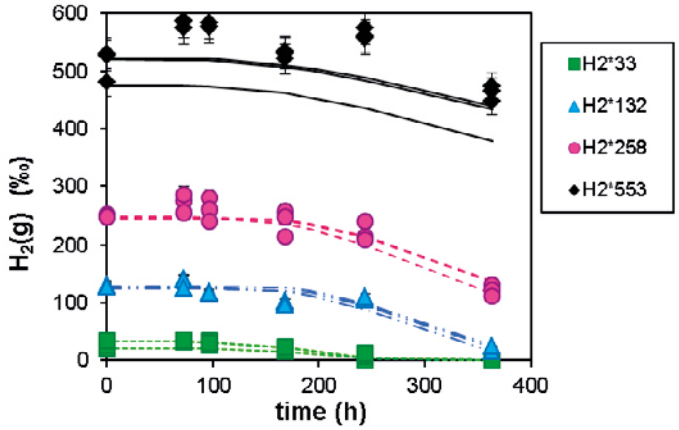

Figure 6-3. *Predictive model calculations of hydrogen partial pressures compared with the experimental values reported in Hallbeck (2014) for the H2RateAc experiments. The uncertainty bars represent 5 % of the reported experimental values. Because each experiment series was performed in triplicate by Hallbeck (2014) with slightly different initial conditions (for example for the biomass and sulfate concentrations), three calculation are re reported for each experiment series.*

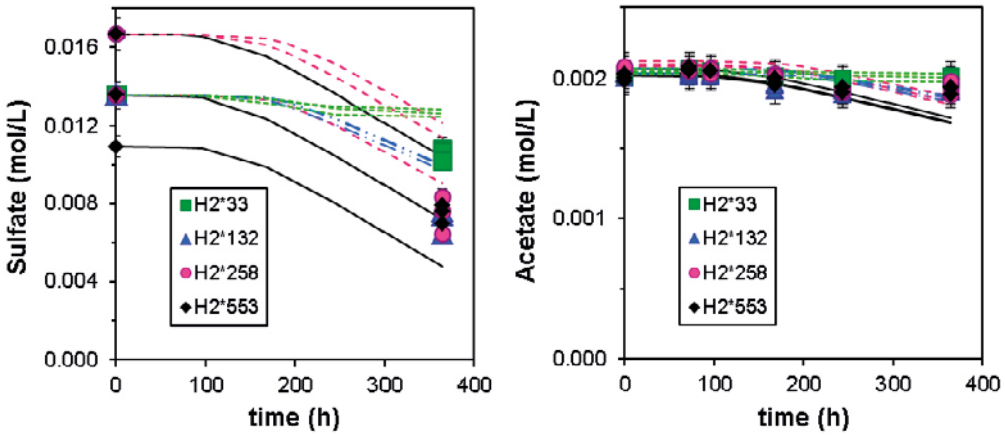

Figure 6-4. *Predictive model calculations of sulfate and acetate concentrations compared with the experimental values reported in Hallbeck (2014) for the H2RateAc experiments. The uncertainty bars represent 5 % of the reported experimental values. Because each experiment series was performed in triplicate by Hallbeck (2014) with slightly different initial conditions (for example for the biomass and sulfate concentrations), three calculation are re reported for each experiment series.*

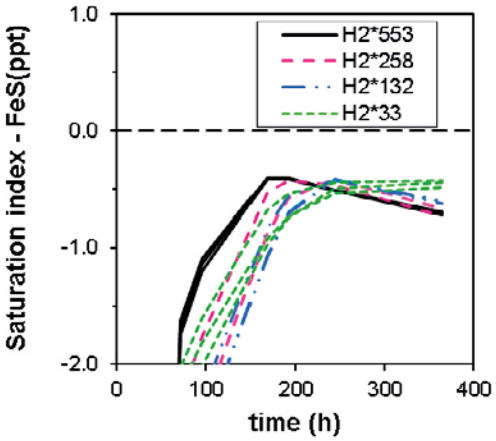

Figure 6-5. *Calculated values for the saturation index of amorphous (precipitated) Fe(II)-sulfide corresponding to the H2RateAc experiments reported in Hallbeck (2014). Some of the calculated curves for the triplicate experiments are plotted on top of each other.*

6.2 Lactate experiments

The logarithm of the sulfide concentrations (in mol/L) and the logarithm of the total number of cells (cells/mL) for experiment LacRate reported in the Appendix of Hallbeck (2014) were fitted with the optimization program PEST described above, using Equations (4-4), (4-5) and (4-11), and the resulting parameters are listed in Table 6-2. Only the experiments at initial lactate concentration of 11.3, 1.13 and 0.11 mM were used. The weight factors were set equal to the reciprocal of the average for each of the six data sets (that is, for the three initial lactate concentrations 11.3, 1.13 and 0.11 mM investigated in Hallbeck (2014), the time-series of both $\log([HS^-]_{Tot})$ and log(cells/mL)). As the initial values of $\log([HS^-]_{Tot})$ are not defined, they were not included in the averages. Also, a zero weight was given to the values at time zero.

The appendix provides a PHREEQC input file incorporating the optimized parameters. It must be recalled that the values of Y_{lac} and k_{max} are linearly dependent on the value adopted for the dry mass of the cells (5×10^{-13} g/cell). Furthermore, these values will also depend, but to a lesser degree, on the several other model assumptions, such as the biomass composition ($C_5H_7O_2N$, 113.115 g/mol) and the inhibition function (Equation (4-11)).

The goodness of the fit between the experimental data and the model may be seen in Figure 6-6 and Figure 6-7. Note that Figure 6-6 displays sulfide concentrations, although their logarithms were used in the model fitting. The fitting of the biomass data (cells/mL) is only good at the highest lactate concentration. The predicted concentrations of lactate (Figure 6-8) and sulfate (Figure 6-9) agree well with the measured data. The model predicts reasonably well the acetate concentrations at the two highest lactate initial concentrations, but for the experiment with the lowest lactate concentrations the agreement is not so good, see Figure 6-8.

The precipitation reaction for Fe(II)-sulfide was not included in the model, and Figure 6-10 shows that according to the calculations, FeS(ppt) should not have precipitated during the experiments. The calculations indicate however that the solutions are oversaturated with more crystalline solid phases such as mackinawite, greigite or pyrite; however, more crystalline solid phases would not be formed unless the more soluble phase precipitates first (Stumm and Morgan 1996, p. 356).

The calculated values for the thermodynamic driving force function, F_T in Equation (4-4), are always larger than 0.89 for all experimental points (not shown in the graphs), even when, as seen in Figure 6-8, the lactate concentration becomes exhausted.

Table 6-2. Parameters providing the best fit to the experimental data for the LacRate experiment in Hallbeck (2014) having initial lactate concentrations ≥ 0.11 mM.

Parameter*	Fitted value
Y_{lac}	0.13 ± 0.07
Y_{SO4}[a]	0.35 ± 0.24
k_{max}	$(8 \pm 7) \times 10^{-5}$ s^{-1}
K_{Lac}	$(60 \pm 170) \times 10^{-6}$ M
K_{SO4}	$(360 \pm 11000) \times 10^{-6}$ M
k_i	$(4.7 \pm 14) \times 10^{-3}$ M

* Uncertainties are 95 % confidence limits.
[a] Y_{SO4} calculated using Equation (4-10).

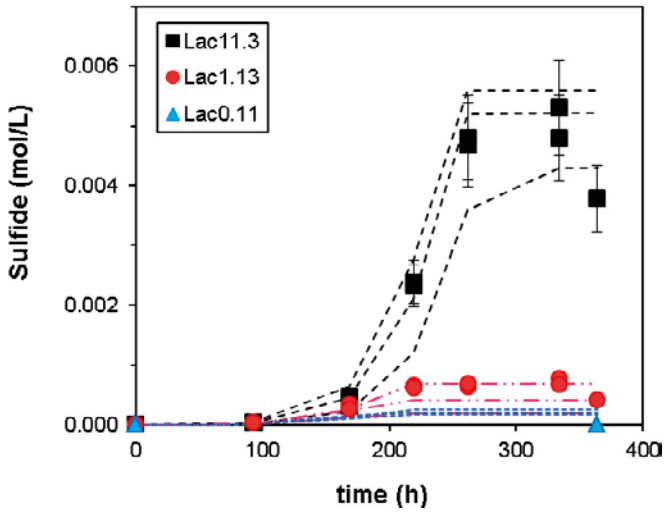

Figure 6-6. *Fitted model calculations of total aqueous sulfide concentrations compared with the experimental values reported in Hallbeck (2014) for the LacRate experiments with initial lactate concentrations ≥ 0.11 mM. The uncertainty bars represent 15 % of the reported experimental analysis. Because each experiment series was performed in triplicate by Hallbeck (2014) with slightly different initial conditions (for example for the biomass and lactate concentrations), three calculation are re reported for each experiment series.*

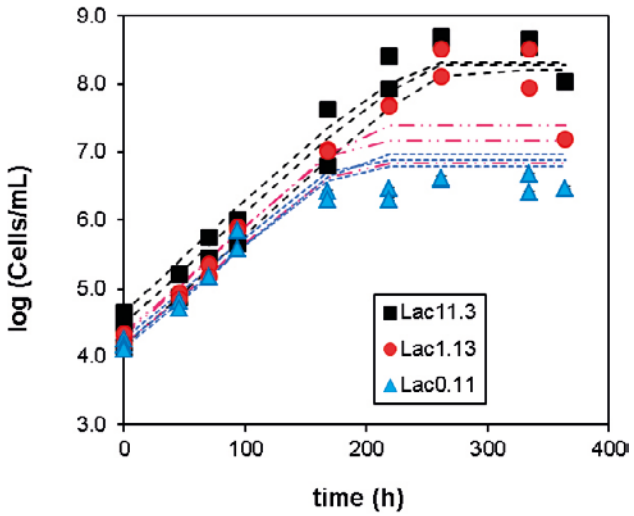

Figure 6-7. *Fitted model calculations of biomass compared with the experimental values reported in Hallbeck (2014) for the LacRate experiments with initial lactate concentrations ≥ 0.11 mM. Because each experiment series was performed in triplicate by Hallbeck (2014) with slightly different initial conditions (for example for the biomass and lactate concentrations), three calculation are re reported for each experiment series.*

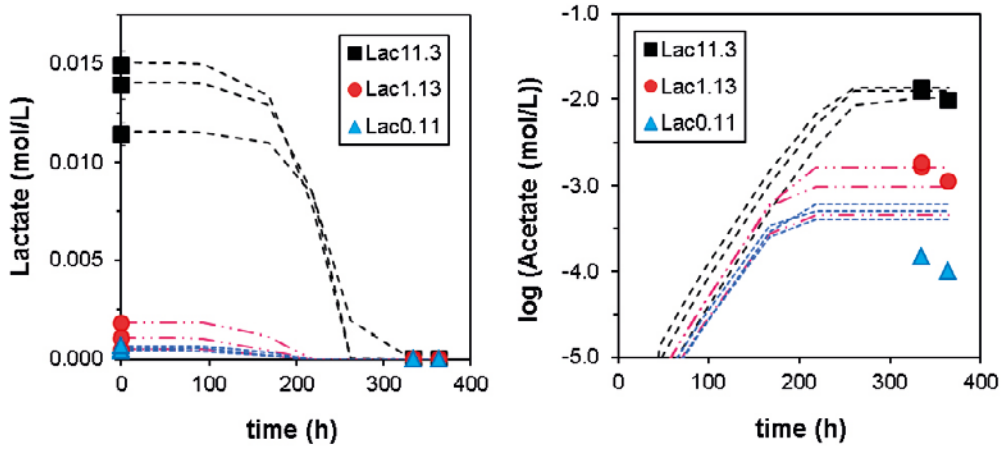

Figure 6-8. *Predictive model calculations of lactate and acetate concentrations compared with the experimental values reported in Hallbeck (2014) for the LacRate experiments with initial lactate concentrations ≥ 0.11 mM. Because each experiment series was performed in triplicate by Hallbeck (2014) with slightly different initial conditions (for example for the biomass and lactate concentrations), three calculation are re reported for each experiment series.*

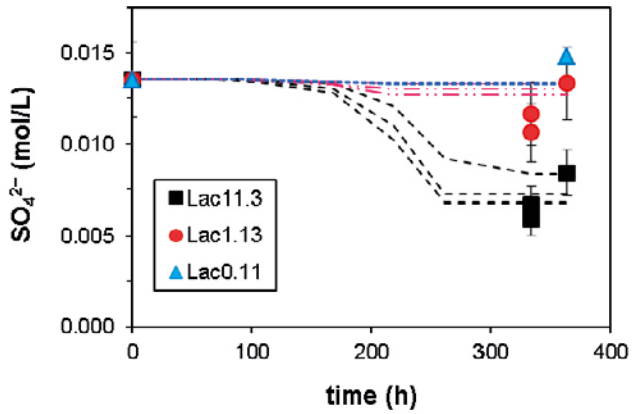

Figure 6-9. *Predictive model calculations of sulfate concentrations compared with the experimental values reported in Hallbeck (2014) for the LacRate experiments with initial lactate concentrations ≥ 0.11 mM. The uncertainty bars represent 15 % of the reported experimental analysis. Because each experiment series was performed in triplicate by Hallbeck (2014) with slightly different initial conditions (for example for the biomass and lactate concentrations), three calculation are re reported for each experiment series.*

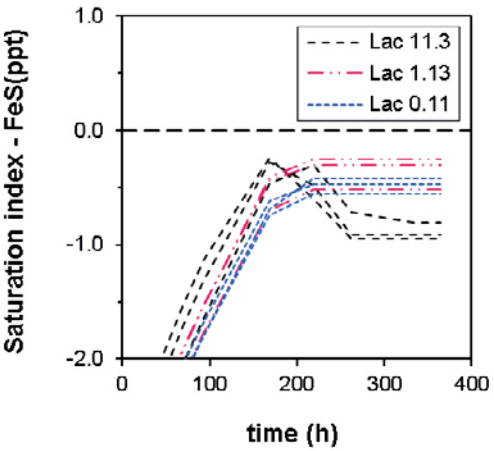

Figure 6-10. *Calculated values for the saturation index of amorphous (precipitated) Fe(II)-sulfide corresponding to the LacRate experiments reported in Hallbeck (2014). Because each experiment series was performed in triplicate by Hallbeck (2014) with slightly different initial conditions (for example for the biomass and lactate concentrations), three calculation are re reported for each experiment series.*

7 Comparison with other literature data

7.1 Lactate as substrate

Table 7-1 presents the literature values for growth yields and Monod parameters from laboratory studies of sulfate reduction by *Desulfovibrio* species using lactate as the source of energy and carbon. A comparison between Table 6-2 and Table 7-1 shows that the growth yields obtained from the data in Hallbeck (2014), namely $Y_{lac} = 0.13 \pm 0.07$, is among the span of reported values, (in the range from 0.02 to 0.68). Similarly, the value $k_{max} = (8 \pm 7) \times 10^{-5}$ s^{-1} (Table 6-2) lies between the reported values, $(0.7 \text{ to } 11) \times 10^{-5}$ s^{-1}. Table 7-1 shows that there is a large variability on the reported half-saturation constants of both lactate and sulfate, K_{lac} and K_{SO4}, from a few millimolar to a few micromolar in both cases. Furthermore, it was not possible to obtain precise values for the half-saturation constants by fitting the model to the LacRate data from Hallbeck (2014). The model fitting suggests however that K_{SO4} may be as high as a few millimolar, but the data is best fitted with K_{SO4} of about 400 µM. The fitting of the data in Hallbeck (2014) also suggest that K_{lac} should be about 60 µM.

Steger et al. (2002, Fig.2) presented experimental data on the growth of *Desulfovibrio desulfuricans* subsp. *aestuarii* on lactate during sulfate reduction. Their figure shows the formation of sulfide to a concentration up to 8×10^{-3} M in a time span of 32 hours, almost ten times faster than the sulfate reduction observed in Hallbeck (2014), see Figure 6-6 above. In Steger et al. (2002, Fig.2) the optical density at 600 nm (OD_{600}) is reported to provide relative changes in biomass as a function of time. The relationship between OD_{600} and the cell density for *Desulfovibrio* is reported to be in the range $(8 \text{ to } 43) \times 10^8$ cells · $(\text{mL} \cdot \text{OD6}_{00})^{-1}$ (Bender et al. 2007, Chen et al. 2015, Redding et al. 2006, Tang et al. 2007). Tang et al. (2007) report a biomass of 132 mg/L at $OD_{600} = 0.35$, and using a linear relationship, the biomass corresponding to the OD_{600} in the figure of Steger et al. (2002) may be estimated. PHREEQC calculations using the estimated biomass values indicate that k_{max} should be about 60×10^{-5} s^{-1} to reach the reaction rate displayed in the figure of Steger et al. (2002), a value which is much larger than the literature values represented in Table 7-1. The calculations are however qualitative, as some experimental details are unknown, such as the volume of the headspace, and therefore it has not been possible to establish the reason for the high rate displayed in the figure in Steger et al. (2002).

Noguera et al. (1998, Fig.2) performed batch experiments of growth of *Desulfovibrio vulgaris* on lactate. Their figure shows a biomass increase by approximately a factor of 20 (1.5 $\log_{10}$ units) in a time span of 48 hours, which is faster (about a factor of two) than the biomass increase observed in Hallbeck (2014), see Figure 6-7 above. PHREEQC calculations are performed here using the parameters in Table 6-2, except that the value of k_{max} is adjusted to 25×10^{-5} s^{-1}. Figure 7-1 and Figure 7-2 show the agreement, or lack thereof, between the calculated values and the experimental data reported in Noguera et al. (1998). It should be noted that the value of k_{max} used in the calculations (25×10^{-5} s^{-1}) is larger than the other values $(0.7 \text{ to } 11) \times 10^{-5}$ s^{-1} reported in the literature (Table 7-1).

Table 7-1. Literature values for growth yields and Monod parameters for *Desulfovibrio* sulfate reduction using lactate as energy and carbon source.

Parameter					Conditions and Reference
Y_{lac}*	Y_{SO4}*	k_{max} (s^{-1})	K_{lac} (mol/L)	K_{SO4} (mol/L)	
0.27	0.98[b]	10×10^{-5}	0.047×10^{-3}		*Desulfovibrio desulfuricans*, 30 °C, pH 7.4 (Cappenberg 1975)
0.25	0.86[b]	5×10^{-5}			*Desulfovibrio vulgaris*, 30 °C, pH 7.0 (Khosrovi and Miller 1975)
≈ 0.05[a]	≈ 0.10				*Desulfovibrio vulgaris* (Liu and Peck 1981)
0.06	0.13[b]				*Desulfovibrio vulgaris*, 30 °C, pH 7.2 (Traore et al. 1981)
0.016 to 0.036	(0.03 to 0.08)[b]	$(1.7 \text{ to } 2.9) \times 10^{-5}$			diverse *Desulfovibrio*, 30 °C, pH 7.2 (Traore et al. 1982)
0.07	0.16[b]	8×10^{-5}	1.65×10^{-3}		*Desulfovibrio vulgaris*, 30 °C, pH 7.0 (Traore et al. 1983)
≈ 0.05[a]	≈ 0.11			$(5 \text{ to } 77) \times 10^{-6}$	diverse *Desulfovibrio*, 30 °C, pH 7.1 (Ingvorsen and Jørgensen 1984)
0.05	0.11[b]	5×10^{-5}			*Desulfovibrio vulgaris*, 35 °C, pH 6.8 (Pankhania et al. 1986)
		6.9×10^{-5}	1.5×10^{-3}		*Desulfovibrio*, 37 °C, pH ≈ 7.1 (Zellner et al. 1994)
0.68	1.7	0.7×10^{-5}		11×10^{-3}	*Desulfovibrio desulfuricans*, 30 °C, pH 7.2 values from Table IV in Herrera et al. (1991)
0.019	0.04[b]	11×10^{-5}	$(16, 115) \times 10^{-6}$		*Desulfovibrio desulfuricans*, 25 °C, pH 7.0 (Okabe and Characklis 1992)
≈ 0.018	0.04[b]	$\approx 10 \times 10^{-5}$	25×10^{-6}	19×10^{-6}	*Desulfovibrio desulfuricans*, 35 °C, pH 7.0 (Okabe et al. 1992)
0.06[a]	0.13				*Desulfovibrio*, 37 °C, pH 6.6 (Reis et al. 1992)
			58.1×10^{-6}	244×10^{-6}	*Desulfovibrio desulfuricans*, 30 °C, pH 7.2 (Fukui and Takii 1994)
		4.8×10^{-5}			*Desulfovibrio desulfuricans*, 35 °C, pH 7 (Okabe et al. 1995)
			29×10^{-3}	210×10^{-6}	*Desulfovibrio vulgaris*, 30 °C, pH 7.2 (Noguera et al. 1998)
0.054	0.12[b]	2.8×10^{-5}	27×10^{-3}		*Desulfovibrio vulgaris* (Tang et al. 2007)

* Calculated from reported yields of biomass per mol substrate, using a biomass composition of $C_5H_7O_2N$ (113.115 g/mol).
a Y_{lac} calculated using Equation (4-9).
b Y_{SO4} calculated using Equation (4-10).

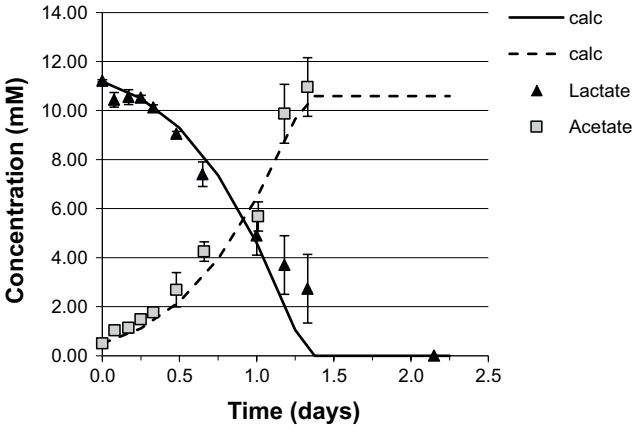

Figure 7-1. *Model calculations of lactate and acetate concentrations compared with the experimental values reported in Noguera et al. (1998). Initial conditions: lactate 11.2 mM, acetate 0.5 mM, SO_4^{2-} 10.7 mM, biomass 4.9 mg/L. See text for details of the parameters used in the calculations.*

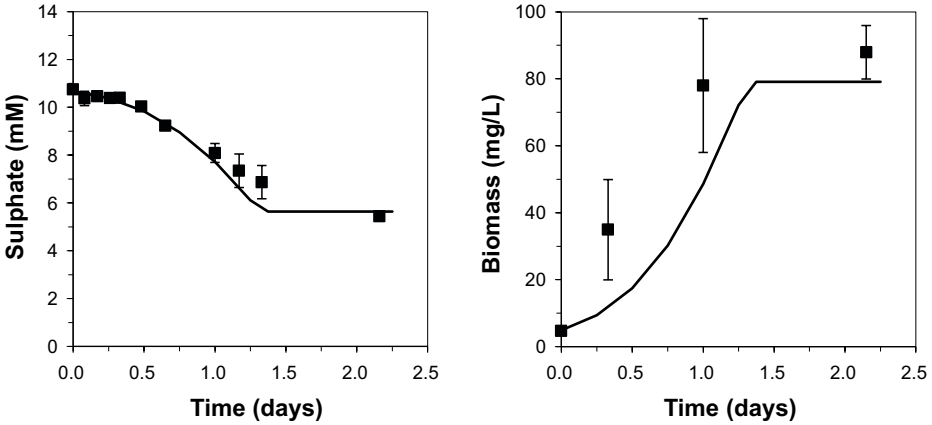

Figure 7-2. *Model calculations of sulfate and biomass concentrations compared with the experimental values reported in Noguera et al. (1998). See text for details of the parameters used in the calculations, and Figure 7-1 for initial conditions.*

7.2 H_2 as substrate

Table 7-2 provides an overview of literature values for growth yields and Monod parameters from laboratory studies of sulfate reduction by *Desulfovibrio* species using H_2 as the energy source and CO_2 and acetate as the carbon sources. The growth yield, Y, obtained from the data in Hallbeck (2014), namely 0.11 ± 0.08, Table 6-1, is at the upper limit of the reported values. The value k_{max} = $(1.5 \pm 1) \times 10^{-5}$ s^{-1} (Table 6-1) is close to the reported values, (1.6 to 6.4) $\times 10^{-5}$ s^{-1}. The fitted values for the half-saturation constants for hydrogen, K_{H2}, $(20 \pm 4) \times 10^{-6}$ M is however a power of ten higher than the reported values in the literature (Table 7-2). It was not possible to obtain a half-saturation constants for sulfate from the data in Hallbeck (2014).

Steger et al. (2002, Fig.2) presented experimental data on the growth of *Desulfovibrio desulfuricans* subsp. *aestuarii* on H_2 during sulfate reduction. Their figure shows the formation of sulfide to a concentration up to 6×10^{-3} M in a time span of 32 hours, almost ten times faster than the sulfate reduction observed in Hallbeck (2014), see Figure 6-1 above. The carbon source in appears to be solely yeast extract and CO_2 from the gas in the headspace. However, some experimental details are unknown, such as the volume of the headspace, and therefore it is not possible to use these data in any modelling.

Table 7-2. Literature values for growth yields and Monod parameters for *Desulfovibrio* sulfate reduction using H_2 as the energy source and CO_2 and acetate as the carbon sources.

Parameter				Conditions and reference
Y_{SO4}*	k_{max} (s^{-1})	K_{H2} (mol/L)	K_{SO4} (mol/L)	
0.06	2×10^{-5}			*Desulfovibrio vulgaris*, pH 6.5 Fig.2 and 3 (pH 7) in Badziong and Thauer (1978)
0.04				*Desulfovibrio*, pH 7.2 (Badziong et al. 1978)
0.06 to 0.10				Diverse *Desulfovibrio*, 37 °C, pH 6.2 (Brandis and Thauer 1981)
		1.3×10^{-6}		*Desulfovibrio vulgaris*, 35 °C, pH 6.9 (Kristjansson et al. 1982)
0.01		4×10^{-6}		*Desulfovibrio vulgaris*, 30 °C (Lupton and Zeikus 1984)
0.11	6.4×10^{-5}		$(10 \pm 5) \times 10^{-6}$	*Desulfovibrio vulgaris*, 35 °C, pH 6.8 (Nethe-Jaenchen and Thauer 1984)
0.008	1.6×10^{-5}	3.3×10^{-6}		Diverse *Desulfovibrio*, 37 °C, pH 6.7 (Robinson and Tiedje 1984)
0.086	3.4×10^{-5}		$< 100 \times 10^{-6}$	*Desulfovibrio desulfuricans*, 32 °C, pH 6.8 (Seitz and Cypionka 1986)

* From reported yields of biomass per mol of substrate, using a biomass composition of $C_5H_7O_2N$ (113.115 g/mol).

Noguera et al. (1998, Fig.4) performed batch experiments of growth of *Desulfovibrio vulgaris* on H_2, with acetate and CO_2 as the carbon sources, and reported the H_2 consumption in their figure, which shows a complete consumption of H_2 (initially 0.025 atm) in 24 hours. As in the case of the experiments by Noguera et al. (1998) discussed above that used lactate, these reaction rates are much faster than those observed in Hallbeck (2014). For example, the experiment H2*33 in Hallbeck (2014) starting with $H_2 \approx 0.066$ atm required more than 200 hours to decrease substantially the H_2 concentration, see Figure 6-3 above. To simulate the batch experiments reported by Noguera et al. (1998), PHREEQC calculations are performed here using the parameters in Table 6-1, except that the value of k_{max} is adjusted to 50×10^{-5} s^{-1}. Figure 7-3 shows the agreement between the calculated partial pressures for H_2 and the experimental data reported in Noguera et al. (1998, Fig.4). It should be noted that the value of k_{max} used in the calculations (50×10^{-5} s^{-1}) is larger than the other values $(1.6 \text{ to } 6.4) \times 10^{-5}$ s^{-1} reported in the literature (Table 7-2).

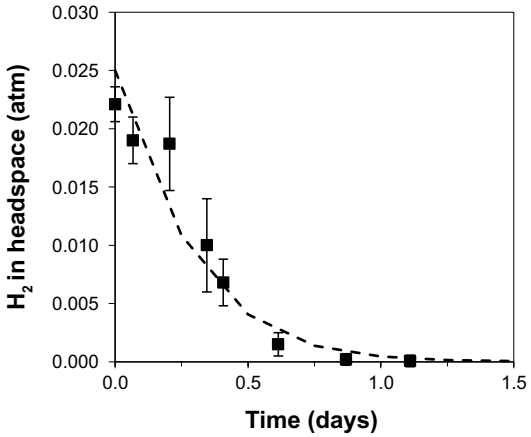

Figure 7-3. *Model calculations of the H_2 partial pressures compared with the experimental values reported in Noguera et al. (1998). Initial conditions: H_2 0.025 atm, acetate 12 mM, SO_4^{2-} 9.3 mM, biomass 9.4 mg/L. See text for details of the parameters used in the calculations.*

8 Discussion and conclusions

The Monod-type model presented is based on several premises that deserve additional attention. To simulate biomass growth when cell numbers are measured requires a conversion, first to mass, and then to moles. Here we have assumed a biomass composition of $C_5H_7O_2N$, and a dry cell mass of 5×10^{-13} g/cell. Although the bacterial biomass composition adopted here is widely used, the elemental analysis of *D. vulgaris* Hildenborough grown on lactate was approx. $C_5H_{8.2}O_{1.7}N_{1.3}$ (Traore et al. 1981). There is therefore some uncertainty concerning the chemical composition of the bacteria in the model. Using a different biomass formula would change the stoichiometric coefficients in Reactions (2-3) and (2-4) (or (2-5)), which would affect the calculated rates of consumption of lactate and acetate. Nevertheless, the parameters for the sulfate reduction rate would remain unaffected.

There are large uncertainties in the half-saturation constants for sulfate and lactate obtained in this work, Table 6-1 and Table 6-2, because the concentrations of these substrates were not sufficiently varied in the experiments by Hallbeck (2014). Even if the tables indicate that the values of k_{max} have relatively low uncertainty, as Equations (4-1) and (4-4) show, the value of k_{max} is always multiplied by the biomass concentration, and it will therefore depend on any assumption made on the concentration of SRB. For example, the dry cell mass used, 5×10^{-13} g/cell, has, as discussed previously, a direct influence on k_{max} in Equations (4-1) and (4-4) and on the yield coefficient, Y, in Equations (4-2), (4-3) and (4-6).

The laboratory experiments presented in Hallbeck (2014) were performed using an excess of nutrients and a large concentration of electron donor-rich substrates, and therefore, the experimental conditions deviated significantly from those expected in a deep repository environment. Therefore, the kinetic constants obtained here (Table 6-1 and Table 6-2) are not expected to be applicable in natural systems. There is a need to increase the knowledge on SRB activity under *in-situ* conditions, or in laboratory environments mimicking the nutrient- and substrate-poor conditions in deep granitic groundwaters.

Although lactate-utilizing SRB are found in sediment environments (Hordijk and Cappenberg 1983, Parkes et al. 1989, Sass et al. 1997), the concentration of lactate in granitic groundwaters is expected to be very low, and therefore sulfate reduction using lactate has very little relevance when considering the long-term safety of spent nuclear fuel repositories. Hydrogen concentrations are also low in granitic groundwaters (Hallbeck and Pedersen 2008, Table 8), but there are potential deep sources of H_2 (Sherwood Lollar et al. 2014) that make H_2 a possibly important factor in the turnover of sulfide in granitic groundwaters. In addition, the possible generation of H_2 within the repository by the corrosion of metal rock reinforcements means that it is important to be able to model H_2-induced sulfate reduction in the assessment of deep nuclear repositories.

The overview of literature values presented in Table 7-1 and Table 7-2, all based in laboratory investigations, shows that there is a large variability in the Monod parameters. This may be due to different data analysis procedures, but also to differences in experimental conditions, and in bacterial species or strains. It has to be concluded that the use of literature data when modelling sulfate reduction in natural environments has to be made with caution.

With respect to the long-term safety of spent nuclear fuel repositories, the main outcome of the present work is the development and testing of models for sulfate reduction using PHREEQC. The microbial rate equations implemented within the PHREEQC software environment have been shown here to be well suited to reproduce experimental laboratory data, and the same programming technique should be valuable when modelling sulfate reduction in the context of assessing the long-term safety of spent nuclear fuel repositories.

9 Acknowledgements

This work started in 2006 as a collaboration between the Electric Power Development Co., Ltd., Japan (J-Power) and the Swedish Nuclear Waste and Management Co., Sweden (SKB). The help provided by Marcus Laaksoharju (Geopoint AB, Sweden) as organiser of that collaborative project is kindly acknowledged. Several persons within J-Power were very helpful, in particular we would like to thank Mr. Ajima and Mr. Todaka for their efforts in this project.

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Appendix 1

PHREEQC input file for LacRate

```
TITLE -------------------
Sulfate reduction rate project (J-Power + SKB)
Lactate experiments.
Model "with" chemistry. H2S exists in the gas phase.
****  Initial pH 7.0  ****  Cell-dry-weight 5x10^-13  ****
-------------------

SOLUTION_MASTER_SPECIES
#element   species   alk   gfw_or_formula  element_gfw
Lac        Lac-      0.0   C3H5O3          89.07     # Lactate
Ace        Ace-      0.0   C2H3O2          59.044    # Acetate
Nta        Nta-3     1.0   188.117         188.117
Z_bio      Z_bio     0.0   113.115         113.115   # biomass C5H7O2N


SOLUTION_SPECIES
    Lac- = Lac-                # Lactate
      log_k 0
    Ace- = Ace-                # Acetate
      log_k 0
    Nta-3 = Nta-3
      log_k 0
    Z_bio = Z_bio              # Biomass
      log_k 0

    H+ + Lac- = HLac
      log_k   3.86
      delta_h +0.08 kcal
    Mg+2 + Lac- = MgLac+
      log_k   1.37
    Mg+2 + 2 Lac- = Mg(Lac)2
      log_k   2.2
    Ca+2 + Lac- = CaLac+
      log_k   1.48
    Ca+2 + 2 Lac- = Ca(Lac)2
      log_k   2.6
    Fe+2 + Lac- = FeLac+
      log_k   1.37
    H+ + Ace- = HAce
      log_k   4.757
      delta_h +0.41 kJ
    Mg+2 + Ace- = MgAce+
      log_k   1.26
    Ca+2 + Ace- = CaAce+
      log_k   1.18
      delta_h +1. kcal
    Fe+2 + Ace- = FeAce+
      log_k   1.4
    Fe+3 + Ace- = Fe(Ace)+2
      log_k   4.023
    Fe+3 + 2Ace- = Fe(Ace)2+
      log_k   7.572
    Fe+3 + 3Ace- = Fe(Ace)3
      log_k   9.587
    H+ + Nta-3 = H(Nta)-2
      log_k   10.278
      delta_h  -18.83 kJ
    2H+ + Nta-3 = H2(Nta)-
      log_k   13.22
      delta_h  -17.99 kJ
    3H+ + Nta-3 = H3(Nta)
      log_k   15.22
      delta_h  -16.32 kJ
    Fe+2 + Nta-3 = Fe(Nta)-
      log_k   10.19
    Fe+2 + 2Nta-3 = Fe(Nta)2-4
      log_k   12.62
    Fe+2 + Nta-3 + H+ = FeH(Nta)
      log_k   12.29
    Fe+2 + Nta-3 + H2O = FeOH(Nta)-2 + H+
      log_k   -1.06
```

```
USER_PRINT
-start
  10 PRINT "Biomass (mg/L) = ",TOT("Z_bio")*113.115*1000
  20 PRINT "Cells/mL = ",TOT("Z_bio")*113.115/(5e-13*1000)
  30 x = -99
  40 IF (TOT("Z_bio")>0) THEN x=log10(TOT("Z_bio")*113.115/(5e-13*1000))
  50 PRINT "log Cells/mL = ",x
 100 RT = 8.3145*298.15/1000 #kJ/mol
 120 X_ = 6
 130 m_ = 1
 140 DG0 = -120.6 #kJ/mol
 150 DGP = 45 #kJ/mol
 160 lnLac = -230.
 165 lnAce = -230.
 170 lnSO4 = -230.
 175 lnHCO3 = -230.
 180 lnH = -230.
 190 lnHS = -230.
 200 IF(ACT("Lac-")>0)  THEN lnLac=LOG(ACT("Lac-"))
 201 IF(ACT("SO4-2")>0) THEN lnSO4=LOG(ACT("SO4-2"))
 202 IF(ACT("HCO3-")>0) THEN lnHCO3=LOG(ACT("HCO3-"))
 203 IF(ACT("H+")>0)    THEN lnH=LOG(ACT("H+"))
 204 IF(ACT("Ace-")>0)  THEN lnAce=LOG(ACT("Ace-"))
 205 IF(ACT("HS_-")>0)  THEN lnHS=LOG(ACT("HS_-"))
 210 DGredox = DG0 + RT*(lnH+lnHS+2*(lnHCO3+lnAce)-lnSO4-2*lnLac)
 220 FT = 1 - EXP( (DGredox + m_*DGP) /(X_*RT))
 300 PRINT "lnH=",lnH," lnHCO3=",lnHCO3
 305 PRINT "lnLac=",lnLac," lnAce=",lnAce
 310 PRINT "lnHS=",lnHS," lnSO4=",lnSO4
 320 PRINT "------  DGredox = ",DGredox,"   FT = ",FT
-end

CALCULATE_VALUES
Hours
-start
  10 SAVE SIM_TIME/3600
-end
logCells
-start
  10 x = -99
  20 IF (TOT("Z_bio")>0) THEN  x=log10(TOT("Z_bio")*113.115/(5e-13*1000))
  50 SAVE x
-end
Gas_P
-start
  10 SAVE GAS_P
-end
H2ppt
-start
  10 x = -999
  20 IF (GAS_P>0) THEN  x = 1000*10^(SI("H2(g)"))/GAS_P
  50 SAVE x
-end
logSulfide
-start
  10 x = -10
  20 IF (TOT("S_")>1e-10) THEN  x=log10(TOT("S_"))
  50 SAVE x
-end
logAce
-start
  10 x = -10
  20 IF (TOT("Ace")>1e-10) THEN   x=log10(TOT("Ace"))
  50 SAVE x
-end
logLac
-start
  10 x = -10
  20 IF (TOT("Lac")>-10) THEN  x=log10(TOT("Lac"))
  50 SAVE x
-end
```

```
F_T
-start
 100 RT = 8.3145*298.15/1000 #kJ/mol
 120 X_ = 6
 130 m_ = 1
 140 DG0 = -120.6 #kJ/mol
 150 DGP = 45 #kJ/mol
 160 lnLac = -230.
 165 lnAce = -230.
 170 lnSO4 = -230.
 175 lnHCO3 = -230.
 180 lnH = -999.
 190 lnHS = -999.
 200 IF(ACT("Lac-")>0)  THEN lnLac=LOG(ACT("Lac-"))
 201 IF(ACT("SO4-2")>0) THEN lnSO4=LOG(ACT("SO4-2"))
 202 IF(ACT("HCO3-")>0) THEN lnHCO3=LOG(ACT("HCO3-"))
 203 IF(ACT("H+")>0)    THEN lnH=LOG(ACT("H+"))
 204 IF(ACT("Ace-")>0)  THEN lnAce=LOG(ACT("Ace-"))
 205 IF(ACT("HS_-")>0)  THEN lnHS=LOG(ACT("HS_-"))
 210 DGredox = DG0 + RT*(lnH+lnHS+2*(lnHCO3+lnAce)-lnSO4-2*lnLac)
 220 FT = 1 - EXP( (DGredox + m_*DGP) /(X_*RT))
 300 SAVE FT
-end

RATES

aSO4_red
-start
    1 moles = 0
    2 rate = 0  # (mol /(L·second))
    5 IF(TOT("S(6)") <= 1E-10 OR TOT("Lac") <= 1E-10 OR TOT("Z_bio") <= 1E-15) THEN GOTO 200
   10 KLac =           5.8150000E-05         # half sat. for Lactate (mol of Lac/L)
   20   Ks =           3.6270000E-04         # half sat. for SO4 (mol of SO4/L)
   30 Kmax =           8.1110000E-05         # max. rate of SO4 reduction by SRB (1/second))
   40   Ki =           4.6760000E-03         # half sat. inhibition for H2S (mol of H2S/L)
   50 f1 = 1
   51 IF(TOT("Lac") > 0 AND KLac > 0) THEN f1 = TOT("Lac")/(KLac + TOT("Lac"))
   60 f2 = 1
   61 IF(TOT("S(6)") > 0 AND KS > 0) THEN f2 = TOT("S(6)")/(Ks + TOT("S(6)"))
   70 f3 = 1   # term for inhibition by HS-
   71 IF(TOT("S_") > 0 AND Ki > 0) THEN f3 = Ki/(Ki + TOT("S_"))
  100 IF(TOT("Z_bio") > 0 AND Kmax > 0) THEN rate = -Kmax * f1 * f2 * f3 * (TOT("Z_bio"))
  110 moles = rate * TIME
  120 REM # note: rate of SO4 change must be <= 0 (sulfate disappears)
  150 IF(moles < 0 AND (TOT("S(6)") + moles) >1e-10 AND (TOT("Lac") + 2*moles) > 1e-10) THEN GOTO
200
  160 REM PRINT "Obs? KH=",KH," Ks=",Ks," Kmax=",Kmax," Ki=",Ki," Lac=",TOT("Lac"),"
S(6)=",TOT("S(6)")," TOT(S_)=",TOT("S_")," Bio=",TOT("Z_bio")," rate_SO4=",rate,"
moles_SO4=",moles
  170 moles = 0
  180 rate = 0
  200 PUT(rate, 1)
  210 SAVE moles
-end

Biomass
-start
   1001 moles = 0
   1005 if (TOT("S(6)") <= 1E-10 OR TOT("Lac") <= 1E-10 OR TOT("Z_bio") <= 1E-15) then GOTO 1200
   1006 IF(TOT("Lac") <= 1E-10 OR TOT("N(-3)") <= 1E-10) THEN GOTO 1200
   1010 Y =            1.3398776E-01     # specific yield of SRB (mol of Bio/mol of Lac)
   1020 D =            1.0000000E-15     # Decay coefficient (second-1)
   1030 IF(Y < 0) THEN Y = 0
   1040 R = GET(1)                       # uses rate calculated in rate SO4_red
   1050 IF(R >0) THEN R = 0   # note: rate of SO4 change must be <= 0 (sulfate disappears)
   1060 rate = -Y * R - D * TOT("Z_bio")
   1070 REM # note: rate of biomass change should be >= 0 (unless R=0 and D>0)
   1080 REM #       moles must be <0 here because there seems to be a bug in PreeqC:
   1090 REM #       when moles > 0 and the stoichiometry for biomass in KINETICS is >0
   1100 REM #       then the kinetic calculation fails
   1150 moles = - rate * TIME
   1160 IF((TOT("Z_bio") + moles) > 1E-15) THEN GOTO 1200
   1170 REM PRINT "Obs! Y=",Y," Lac=",TOT("Lac")," S(6)=",TOT("S(6)")," TOT(S_)=",TOT("S_")","
Bio=",TOT("Z_bio")," rate_bio=",rate," moles_bio=",moles
   1180 moles = 0
   1200 SAVE moles
-end
```

```
PRINT
  -reset true
  -user_print true

SELECTED_OUTPUT 1
    -file       LacRate.prn
    -reset                                  false
    -pH                                     true
    -calculate_values          Gas_P Hours logCells logSulfide logAce logLac F_T
    -totals     Lac  Ace  S(6)  S_  Z_bio  C(4)
    -kinetic_reactants         aSO4_red  Biomass
    -saturation_indices        H2S_(g) FeS(ppt) Mackinawite

#-------------------------------------------------------------------------------

SOLUTION 1 Hallbeck (2014), SKB-TR-14-14 -- Lac11.3A
    temp      22
    pH        9.5 # adjusted to get pH=7 after N2/CO2 equil.
    pe        -8
    redox     pe
    units     mol/kgw
    density   1
    C         0.059995
    Ca        0.006802
    Cl        0.165988
    Fe        3.06e-005
    K         0.010089
    Mg        0.015992
    N(-3)     0.018756
    P         0.001102 as H2PO4
    N_        1e-20
    S_        1e-20
    Ace       1e-20
    Na        0.194816 charge
    S(6)      0.013533
    Lac       0.015044
    Nta       7.85e-5
    Z_bio     19.892e-8
    -water    1 # kg
EQUILIBRIUM_PHASES 1
    CO2(g)    -0.3979          1
    N_2(g)    0.2041           1
SAVE solution 1
END

USE solution 1
GAS_PHASE 1
    -fixed_volume
    -equilibrium with solution 1
    -volume 1.4
    -temperature 22
    CO2(g)
    N_2(g)
    H2S_(g)
    H2O(g)

KINETICS 1

aSO4_red #dS/dt
    -formula  Lac 2 SO4 1 Ace -2 HCO3 -2 HS_ -1 H -1
    -m0   0
    -tol  1e-012

Biomass #dX/dt
    -formula  Lac 1.6667 NH4 1 H 0.6667 Z_bio -1 H2O -3
    -m0   0
    -tol  1e-012

-steps       0  45 25 23 75 51 43 72 30 hours
-step_divide 100000
-cvode true
-cvode_order 5
-cvode_steps 10000

INCREMENTAL_REACTIONS true
END
```

(Note: the other solutions are not included for brevity)

Appendix 2

PHREEQC input file for H2RateAc

```
TITLE -------------------
Sulfate reduction rate project (J-Power + SKB)
Variable H2 added in the gas phase. Constant acetate concentrations
Model "with" chemistry. H2S exists in the gas phase.
****  Initial pH 7.0  ****  Cell-dry-weight 5x10^-13  ****
-------------------

SOLUTION_MASTER_SPECIES
#element  species alk gfw_or_formula  element_gfw
Ace   Ace-    0.0 C2H3O2  59.044    # Acetate
Nta        Nta-3     1.0   188.117         188.117
Z_bio Z_bio   0.0 113.115 113.115   # biomass C5H7O2N

SOLUTION_SPECIES
  Ace- = Ace-   # Acetate
    log_k 0
  Nta-3 = Nta-3
    log_k 0
  Z_bio = Z_bio # Biomass
    log_k 0

  H+ + Ace- = HAce
    log_k           4.757
    delta_h +0.10 kcal
  Mg+2 + Ace- = MgAce+
    log_k           1.26
  Ca+2 + Ace- = CaAce+
    log_k           1.18
    delta_h +1. kcal
  Fe+2 + Ace- = FeAce+
    log_k           1.
  H+ + Nta-3 = H(Nta)-2
    log_k   10.278
    delta_h  -18.83 kJ
  2H+ + Nta-3 = H2(Nta)-
    log_k   13.22
    delta_h  -17.99 kJ
  3H+ + Nta-3 = H3(Nta)
    log_k   15.22
    delta_h  -16.32 kJ
  Fe+2 + Nta-3 = Fe(Nta)-
    log_k   10.19
  Fe+2 + 2Nta-3 = Fe(Nta)2-4
    log_k   12.62
  Fe+2 + Nta-3 + H+ = FeH(Nta)
    log_k   12.29
  Fe+2 + Nta-3 + H2O = FeOH(Nta)-2 + H+
    log_k   -1.06

USER_PRINT
-start
  10 PRINT "Biomass (mg/L) = ",TOT("Z_bio")*113.115*1000
  20 PRINT "Cells/mL = ",TOT("Z_bio")*113.115/(5e-13*1000)
  30 x = -99
  40 IF (TOT("Z_bio")>0) THEN x=log10(TOT("Z_bio")*113.115/(5e-13*1000))
  50 PRINT "log Cells/mL = ",x
 100 RT = 8.3145*298.15/1000 #kJ/mol
 120 X_ = 2
 130 m_ = 1/3
 140 DG0 = -65.7 #kJ/mol
 150 DGP = 45 #kJ/mol
 160 lnH2 = -230.
 170 lnSO4 = -230.
 180 lnH = -230.
 190 lnHS = -230.
 200 IF(ACT("H2")>0)    THEN lnH2=LOG(ACT("H2"))
 201 IF(ACT("SO4-2")>0) THEN lnSO4=LOG(ACT("SO4-2"))
 202 IF(ACT("H+")>0)    THEN lnH=LOG(ACT("H+"))
 203 IF(ACT("HS_-")>0)  THEN lnHS=LOG(ACT("HS_-"))
 210 DGredox = DG0 + RT*(0.25*(lnHS-lnSO4-lnH)-lnH2)
 220 FT = 1 - EXP( (DGredox + m_*DGP) /(X_*RT))
 300 PRINT "lnH=",lnH," lnH2=",lnH2
 305 PRINT "lnHS=",lnHS," lnSO4=",lnSO4
 320 PRINT "------  DGredox = ",DGredox,"   FT = ",FT
-end
```

```
CALCULATE_VALUES
Hours
-start
  10 SAVE SIM_TIME/3600
-end
logCells
-start
  10 x = -99
  20 IF (TOT("Z_bio")>0) THEN  x=log10(TOT("Z_bio")*113.115/(5e-13*1000))
  50 SAVE x
-end
Gas_P
-start
  10 SAVE GAS_P
-end
H2ppt
-start
  10 x = -999
  20 IF (GAS_P>0) THEN  x = 1000*10^(SI("H2(g)"))/GAS_P
  50 SAVE x
-end
logSulfide
-start
  10 x = -10
  20 IF (TOT("S_")>1e-10) THEN  x=log10(TOT("S_"))
  50 SAVE x
-end
logAce
-start
  10 x = -10
  20 IF (TOT("Ace")>1e-10) THEN   x=log10(TOT("Ace"))
  50 SAVE x
-end
F_T
-start
 100 RT = 8.3145*298.15/1000 #kJ/mol
 120 X_ = 2
 130 m_ = 1/3
 140 DG0 = -65.7 #kJ/mol
 150 DGP = 45 #kJ/mol
 160 lnH2 = -230.
 170 lnSO4 = -230.
 180 lnH = -230.
 190 lnHS = -230.
 200 IF(ACT("H2")>0)     THEN lnH2=LOG(ACT("H2"))
 201 IF(ACT("SO4-2")>0) THEN lnSO4=LOG(ACT("SO4-2"))
 202 IF(ACT("H+")>0)     THEN lnH=LOG(ACT("H+"))
 203 IF(ACT("HS_-")>0)   THEN lnHS=LOG(ACT("HS_-"))
 210 DGredox = DG0 + RT*(0.25*(lnHS-lnSO4-lnH)-lnH2)
 220 FT = 1 - EXP( (DGredox + m_*DGP) /(X_*RT))
 300 SAVE FT
-end
```

```
RATES

aSO4_red
-start
    1 moles = 0
    2 rate = 0  # (mol /(L·second))
    5 IF(TOT("S(6)")<=1E-10 OR TOT("H(0)")<=1E-10 OR TOT("Z_bio")<=1E-15) THEN GOTO 200
   10   KH =           1.9500000E-05         # half sat. for H2 (mol of H2/L)
   20   Ks =           1.0000000E-06         # half sat. for SO4 (mol of SO4/L)
   30 Kmax =           1.4830000E-04         # max. rate of SO4 reduction by SRB (1/second))
   40   Ki =           4.6700000E-04         # half sat. inhibition for H2S (mol of H2S/L)
   50 f1 = 1
   51 IF(TOT("H(0)") > 0 AND KH > 0) THEN f1 = 0.5*TOT("H(0)")/(KH + (0.5*TOT("H(0)")))
   60 f2 = 1
   61 IF(TOT("S(6)") > 0 AND KS > 0) THEN f2 = TOT("S(6)")/(Ks + TOT("S(6)"))
   70 f3 = 1 # term for inhibition by HS-
   71 IF(TOT("S_") > 0 AND Ki > 0) THEN f3 = Ki/(Ki + TOT("S_"))
  100 IF(TOT("Z_bio") > 0 AND Kmax > 0) THEN rate = -Kmax * f1 * f2 * f3 * (TOT("Z_bio"))
  110 moles = rate * TIME
  120 REM # note: rate of SO4 change must be <= 0 (sulfate disappears)
  150 IF(moles<0 AND (TOT("S(6)")+moles)>1e-10 AND (TOT("H(0)")+2*moles)>1e-10) THEN GOTO 200
  170 moles = 0
  180 rate = 0
  200 PUT(rate, 1)
  210 SAVE moles
-end

Biomass
-start
   1001 moles = 0
   1005 IF(TOT("S(6)")<=1E-10 OR TOT("H(0)")<=1E-10 OR TOT("Z_bio")<=1E-15) THEN GOTO 1200
   1006 IF(TOT("Ace")<=1E-10 OR TOT("C(4)")<=1E-10 OR TOT("N(-3)")<=1E-10) THEN GOTO 1200
   1010  Y =            1.0941000E-01     # specific yield of SRB (mol of Biomass/mol of H2)
   1020  D = 1E-15                        # Decay coefficient (second-1)
   1030 IF(Y < 0) THEN Y = 0
   1040 R = GET(1)                        # uses rate calculated in rate SO4_red
   1050 IF(R >0) THEN R = 0 # note: rate of SO4 change must be <= 0 (sulfate disappears)
   1060 rate = -Y * R - D * TOT("Z_bio")
   1070 REM # note: rate of biomass change should be >= 0 (unless R=0 and D>0)
   1080 REM #       moles must be <0 here because there seems to be a bug in PreeqC:
   1090 REM #       when moles > 0 and the stoichiometry for biomass in KINETICS is >0
   1100 REM #       then the kinetic calculation fails
   1150 moles = - rate * TIME
   1160 IF((TOT("Z_bio") + moles) > 1E-15) THEN GOTO 1200
   1180 moles = 0
   1200 SAVE moles
-end

PRINT
  -reset true
  -user_print true

SELECTED_OUTPUT 1
    -file     H2Rate.prn
    -reset      false
    -pH     true
    -calculate_values H2ppt Gas_P Hours logCells logSulfide logAce F_T
    -totals     Ace  S(6)  S_  Z_bio  H(0) C(4)
    -kinetic_reactants  aSO4_red  Biomass
    -saturation_indices H2S_(g) FeS(ppt) Mackinawite
```

```
#------------------------------------------------------------------------------

SOLUTION 1 Hallbeck (2014), SKB-TR-14-14 -- H2*533 Repl.A
    temp      22
    pH        6.85    # adjusted to get pH=7 after N2/CO2 equil.
    pe        -7
    redox     pe
    units     mol/kgw
    density   1
    C         0.059995
    Ca        0.006802
    Cl        0.165988
    Fe        3.06e-005
    K         0.010089
    N(-3)     0.018756
    P         0.001102 as H2PO4
    N_        1e-20
    S_        1e-20
    Ace       0.0020154
    Na        0.181787 charge
    Mg        0.013390
    S(6)      0.010930
    Nta       7.85e-5
    Z_bio     5.3044e-8
    -water    1 # kg
EQUILIBRIUM_PHASES 1
    CO2(g)   -0.68849   1
    N_2(g)   -0.08643   1
    H2(g)    -0.02254   1
SAVE solution 1
END

USE solution 1
GAS_PHASE 1
    -fixed_volume
    -equilibrium with solution 1
    -volume 1.6
    -temperature 22
    CO2(g)
    N_2(g)
    H2(g)
    H2S_(g)
    H2O(g)

KINETICS 1

aSO4_red #dS/dt must be <= 0 and "moles" <= 0, i.e., SO4 will be removed
    -formula  H2 4 H 1 SO4 1 H2O -4 HS_ -1
    -m0       0
    -tol      1e-012

Biomass #dX/dt "moles" will be <= 0, i.e., biomass produced
    -formula  Ace 0.5 CO3 1 NH4 1 H 1.5 CO2 3 Z_bio -1 H2O -6
    -m0       0
    -tol      1e-012

-steps       0 72 24 72 24 52 120 hours
-step_divide 100000
-cvode true
-cvode_order 5
-cvode_steps 10000

INCREMENTAL_REACTIONS true
END
```

(Note: the other solutions are not included for brevity)

Arkitektkopia AB, Bromma, 2016

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