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Study of anaerobic lactate metabolism under biosulfidogenic conditions

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ABSTRACT

Biological sulfate reduction (BSR) has been reported to have potential for the treatment of acid mine drainage (AMD). The provision of a suitable carbon source and electron donor for this process remains a challenge. Lactate offers potential advantages as carbon source and electron donor in the biological sulfate reduction process. As this substrate is utilized by both fermentative bacteria and oxidative sulfate-reducing bacteria (SRB), the effect of feed sulfate concentration on the lactate pathways utilized under biosulfidogenic conditions was investigated. Studies were carried out in chemostat bioreactors across a range of residence times, using an enriched culture of SRB. The stoichiometry of biological sulfate reduction was affected by feed sulfate concentration and dilution rate. Incomplete oxidation of lactate was dominant at low feed sulfate concentration (1.0 g/L), while the yield of propionate from lactate metabolism increased at feed sulfate concentrations of 2.5–10.0 g/L, indicating the occurrence of lactate fermentation. Furthermore, at each sulfate feed concentration, in the range 2.5–10.0 g/L, the ratio in which lactate was metabolized by the oxidative and fermentative pathways varied with varying dilution rates. Lactate oxidation was higher at a feed sulfate concentration of 10.0 g/L relative to 2.5 and 5.0 g/L. The volumetric lactate utilization rate was enhanced by increasing the feed sulfate concentration. However, the proportion of total lactate consumed that was channelled into providing electrons for other activities apart from sulfate reduction also increased over the range of increasing sulfate concentrations studied and appeared to be a function of residual lactate and sulfide concentrations.

1. Introduction

Acid mine drainage (AMD) arises from the oxidation of sulfide minerals such as pyrite, contained in waste rocks, closed mining sites and mine tailings, on exposure to atmospheric oxygen and moisture. The characteristic composition of AMD (low pH, high heavy metal concentration, low nutrients) is detrimental to the environment and its resident organisms (Ledin and Pedersen, 1996). Sulfate is a major pollutant in the wastewaters emanating from South African mining activities and remains in solution at elevated levels following conventional treatment. Development and implementation of AMD treatment technology in South Africa seeks to reduce the sulfate concentration to acceptable levels. Anaerobic biological sulfate reduction in the treatment of acid mine drainage and other metal- and sulfate-laden wastewaters has attracted intensive research in recent years due to the advantages it offers over the other treatment technologies (Drury, 1999;

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Doye and Duchesne, 2003; Baskaran and Nemati, 2006). Sulfide and bicarbonate are produced by sulfate-reducing bacteria (SRB) as sulfate is reduced in the presence of a suitable electron donor and carbon source. The bicarbonate alkalinity neutralizes acidity while dissolved metals are precipitated by the sulfide (Reactions (1–3)) (Drury, 1999):

$$\text{Electron donor} + SO_4^{2-} \rightarrow HS^- + HCO_3^- \quad (1)$$

$$Me^{2+} + HS^- \rightarrow MeS\downarrow + H^+ \quad (2)$$

$$HCO_3^- + H^+ \rightarrow H_2O + CO_2 \quad (3)$$

where Me^{2+} = metal, such as Zn^{2+}, Cu^{2+}, Pb^{2+} and Ni^{2+}

In spite of the rigorous investigations aimed at improving the overall performance of biological sulfate reduction (BSR) (Reis et al., 1992; van Houten et al., 1996; Chang et al., 2000), there is still a dearth of literature to inform the improvement of this technology based on the kinetics of electron donor utilization. Lactate may be metabolized via fermentation or oxidation or both by a wide range of microorganisms (Reactions (4–7), Table 1) (Zellner et al., 1994). When lactate is used as an electron donor and carbon source by sulfate reducers, sulfate reduction occurs concurrently with lactate oxidation. Hence, optimization of lactate utilization via the oxidative pathway in preference to fermentation aids in maximizing the efficiency of biological sulfate reduction. Lactate is oxidized either incompletely (Reaction (4), Table 1) or completely (Reaction (5), Table 1) in the presence of sulfate by a diverse range of SRB strains (Okabe et al., 1995; Kaksonen et al., 2003). Thus, its utilization is expected to encourage high microbial diversity and consequently resilience of the system to environmental challenges (Oyekola et al., 2007).

Sulfate reducers such as *Desulfobulbus propionicus* are reported to ferment lactate in the absence of sulfate, resulting in the production of propionate and acetate (Reaction (6), Table 1) (Heimann et al., 2005). Bryant et al. (1977) showed that *Desulfovibrio* sp. co-cultured with hydrogen-utilizing methanogens, in the absence of sulfate, converted lactate via a thermodynamically unfavourable fermentative pathway to acetate (Reaction (7), Table 1). This reaction was made thermodynamically feasible by inter-species hydrogen transfer. The fermentative growth rate of *Desulfovibrio* strains on lactate is slower and produces lower growth yields compared to lactate oxidation coupled to sulfate reduction (Bryant et al., 1977).

It has been suggested that lactate-fed SRB are prone to competition from other microorganisms when present in mixed cultures since lactate is a "high-energy" substrate, which supports the growth of several groups of microorganisms (Laanbroek et al., 1982). The coexistence between non-SRB lactate fermenters and SRB in the presence of both lactate and sulfate has been reported. This occurs both in the natural environment (Laanbroek and Pfennig, 1981; Purdy et al., 1997) and anaerobic digesters (Zellner et al., 1994). Kinetic properties are key factors in determining the preferred lactate metabolic pathway. Due to their kinetic properties, high levels of lactate encourage the growth of non-SRB fermentative bacteria. In contrast, lactate oxidation becomes dominant under conditions of lactate limitation and excess sulfate (Laanbroek and Pfennig, 1981; Zellner et al., 1994). In an investigation based on a full-scale anaerobic digester by Zellner et al. (1994), *Desulfovibrio* sp. (a lactate oxidizer) was shown to have lower K_s and μ_{max} values than *Clostridium* sp. (a lactate fermenter). In a non-sterile continuous system, composed of a mixed culture of microbes, characterized by low lactate concentration, lactate degradation by *Desulfovibrio* sp. was thus the preferred metabolic pathway (Zellner et al., 1994).

In this present study, we describe the effects of sulfate concentration, lactate concentration and volumetric loading rate on the kinetics of lactate utilization and the stoichiometry of biological sulfate reduction under biosulfidogenic conditions over a wide range of feed sulfate concentrations (1.0–10.0 g/L). As sulfate was the desired limiting substrate, lactate was added to a 20% excess of the stoichiometric requirement for incomplete oxidation (Reaction (4), Table 1).

2. Materials and methods

2.1. Microorganisms and growth medium

A mixed culture of SRB, adapted to growth on lactate, was obtained from the laboratory of Prof. John Duncan (Rhodes University, South Africa). Modified Postgate B medium, in which lactate formed the sole carbon source and electron donor, was used as the growth medium (Postgate, 1984). Bromo-ethane-sulphonic-acid (BESA) (3.2 g/L) was added to the culture at the enrichment stage, prior to the culturing of the continuous reactors, to inhibit methanogenic activity (Visser, 1995). The medium containing 1.0 g/L of sulfate ions had the following composition in 1 L deionized water: 0.5 g KH_2PO_4; 1.0 g NH_4Cl; 2.0 g $MgSO_4.7H_2O$; 1.0 g Na_2SO_4; 1.0 g

Table 1 – Reactions and free-energy changes for reactions involving anaerobic metabolism of lactate.

ΔG_0 (kJ/reaction)	Reaction	
−160.1	$2\ \text{lactate} + SO_4^{2-} \rightarrow 2\ \text{acetate} + 2\ HCO_3^- + HS^- + H^+$	(4)
−225.3	$2\ \text{lactate} + 3\ SO_4^{2-} \rightarrow H^+ + 6\ HCO_3^- + 3\ HS^-$	(5)
−169.7	$3\ \text{lactate} \rightarrow \text{acetate} + 2\ \text{propionate} + HCO_3^- + H^+$	(6)
−7.98	$2\ \text{lactate} + 4\ H_2O \rightarrow 2\ \text{acetate} + 2\ HCO_3^- + 4\ H_2$	(7)