Bioresource Technology 104 (2012) 616–621

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Effect of culture conditions on the competitive interaction between lactate oxidizers and fermenters in a biological sulfate reduction system

Oluwaseun O. Oyekola *, Susan T.L. Harrison, Robert P. van Hille

Centre for Bioprocess Engineering Research, Department of Chemical Engineering, University of Cape Town, Private Bag, Rondebosch 7701, South Africa

A R T I C L E I N F O

Article history:
Received 2 September 2011
Received in revised form 11 November 2011
Accepted 12 November 2011
Available online 25 November 2011

Keywords:
Acid mine drainage
Biological sulfate reduction
Fermentation
Oxidation
Kinetics
Stoichiometry

A B S T R A C T

Kinetic constants (μ_{max} and K_s) describing the predominance of lactate oxidation and fermentation were determined in chemostat cultures. The kinetics of sulfate reduction and lactate utilization were determined from 0.5 to 5 d residence times at feed sulfate concentrations of 1.0–10.0 g l^{-1}. The kinetics of lactate fermentation in the absence of sulfate were investigated at residence times of 0.5–5 d. The lactate oxidizers (LO) were characterized by a μ_{max} of 0.2 h^{-1} and K_s value of 0.6 g l^{-1} compared with a μ_{max} of 0.3 h^{-1} and K_s of 3.3 g l^{-1} for the lactate fermenters (LF). Using mathematical models, it was shown that LO competed more effectively for lactate at low lactate concentrations ($\leqslant$5 g l^{-1}) and high sulfide concentrations (0.5 g l^{-1}). Lactate fermenters outcompeted the oxidizers under conditions of excess lactate (>5 g l^{-1}) and low sulfide (0.014–0.088 g l^{-1}).

1. Introduction

Acid mine drainage (AMD) poses a threat to ecosystems. Apart from the acute toxicity of the heavy metals, the high sulfate content of untreated AMD increases the salinity of receiving water bodies which reduces the availability of readily usable water and increases the cost of water purification. Biological sulfate reduction (BSR) has potential for the treatment of AMD. The biological sulfate reduction process is mediated by sulfate reducing bacteria (SRB), which convert sulfate to sulfide (Bijmans et al., 2010). The sulfide produced can either be used to precipitate dissolved metals in AMD or removed via partial oxidation to sulfur (Mojarrad Moghanloo et al., 2010). Lactate is a potential carbon source and electron donor, offering advantages in the BSR process. However, it has been suggested that lactate-fed SRB are prone to competition from other microorganisms present in mixed cultures (Laanbroek et al., 1982). Under biological sulfate reduction conditions, lactate may be metabolized via fermentation or oxidation, or both, by a wide range of microorganisms, increasing the potential for competition (Purdy et al., 1997; Habicht et al., 2005). Competition between different microbial groups is dependent on the kinetic properties of the interacting microorganisms, as defined by the maximum specific growth rate (μ_{max}) and substrate affinity (K_s) (Bailey and Ollis, 1986). In the context of biological sulfate reduction, utilizing lactate as an electron donor, lactate fermentation is the anaerobic degradation of lactate, independent of sulfate reduction (Eq. (1)). On the other hand during lactate oxidation, there is a concurrent reduction of sulfate (Eq. (2)):

$$\text{lactate} \rightarrow \text{acetate} + 2\ \text{propionate} + HCO_3^- + H^+ \tag{1}$$

$$2\ \text{lactate} + SO_4^{2-} \rightarrow 2\ \text{acetate} + 2\ HCO_3^- + HS^- + H^+ \tag{2}$$

Oyekola et al. (2009, 2010) reported that lactate fermentation was the predominant route of lactate utilization at high lactate concentrations (4.0–8.9 ± 0.4 g l^{-1}) and high dilution rates (0.021–0.042 h^{-1}), which resulted in low sulfate conversion. At feed sulfate concentrations of 1.0 and 10.0 g l^{-1}, results showed that the volumetric sulfate reduction rate (VSRR) increased linearly with increasing volumetric sulfate loading rate (VSLR) to a maximum which was maintained with further increase in VSLR (Oyekola et al., 2009). Reactors fed with sulfate concentrations of 2.5 and 5.0 g l^{-1} showed different trends, with the highest VSRR at the dilution rates of 0.014 and 0.021 h^{-1}, respectively, followed by a decline with increasing lactate loading rate. At high dilution rates, in the reactors receiving sulfate concentrations of 2.5 and 5.0 g l^{-1}, low dissolved sulfide concentrations (0.014–0.088 g l^{-1}) were detected. The results of the reactor receiving 10.0 g l^{-1} feed sulfate suggested that at high sulfide concentrations (0.3–0.6 g l^{-1}) lactate fermenters were inhibited, resulting in the predominance of lactate oxidation. The low sulfate conversion, associated with high lactate and low sulfide concentrations was attributed to out-competition of the

* Corresponding author. Present address: Department of Chemical Engineering, Cape Peninsula University of Technology, P.O. Box 1906, Bellville 7535, Cape Town South Africa. Tel.: +27 21 9596799; fax: +27 21 9596323.
E-mail address: oyekolas@cput.ac.za (O.O. Oyekola).

0960-8524/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2011.11.052

lactate oxidizer(s) by the lactate fermenter(s) in reactors fed with sulfate concentrations of 2.5 and 5.0 g l^{-1}. The effect of feed sulfate concentration and dilution rate on shifts in the lactate metabolic pathway was described by Oyekola et al. (2009). Deviations from the trends of the kinetics of BSR, exhibited by other carbon sources such as acetate and ethanol, were reported in these studies (Oyekola et al., 2009, 2010). The aim of the current study was to validate the hypotheses proposed in these previous studies in which competition between lactate oxidizers and fermenters was proposed to depend on the operation conditions of BSR (Oyekola et al., 2009, 2010). The current study investigated the importance of an understanding of the impact of the physicochemical conditions on the metabolic dominance in optimizing biological sulfate reduction (BSR) system.

The kinetic data described by Oyekola (2008) and Oyekola et al. (2010) were used to determine the microbial growth parameters (μ_s and K_s). These parameters were used to predict the outcome of the competition between lactate oxidation and lactate fermentation under conditions of BSR. The bacterial growth rates determined using the mathematical models were compared with the experimental data to provide validation. This approach was used to elucidate the observation in the previous reports (Oyekola et al., 2009, 2010) and confirm the conditions under which lactate oxidation is dominant. The latter is key in ensuring efficient use of electron donor in BSR applications. Selection of electron donor is influenced by cost and potential formation of secondary pollutants. Owing to possible generation of secondary pollutants resulting from partial oxidation of complex electron donors and the precise chemical composition of simple substrates, simple electron donors are often preferred (van Houten et al., 1996).

2. Methods

2.1. Microorganisms and growth medium

A mixed SRB culture inoculum, isolated from the anaerobic pit of a facultative pond treating sewage and adapted to growth on lactate in the laboratory of John Duncan (Rhodes University, South Africa) was used. The culture was grown in laboratory scale chemostats (1 l, 35 °C, pH 8 ± 0.2) in modified Postgate B medium at residence times between 0.5 and 5.0 d and at sulfate concentrations between 1.0 and 10.0 g l^{-1}. Steady-state was achieved at each residence time. Steady-state data were used to estimate the kinetics of sulfate reduction, lactate fermentation and bacterial growth. Steady-state conditions were assumed to be established when both the residual sulfate and bacterial concentrations varied by <10% after a period of operation equal to three residence times since system perturbation (Oyekola et al., 2009).

Lactate fermentation studies were carried out in the absence of sulfate in the feed stream using two separate experimental runs. Magnesium sulfate was replaced with magnesium chloride to completely eliminate sulfate. In order to investigate the kinetics of lactate fermentation, the same standard experimental conditions described above were used, with the temperature and pH maintained at 35 °C and pH 8.0 ± 0.2, respectively. The feed media contained the same lactate concentration (5.6 g l^{-1}) as experiments at the feed sulfate concentration of 2.5 g l^{-1}. Other media components remained the same. The bioreactor was inoculated with culture taken from the continuous culture treating a 2.5 g l^{-1} feed sulfate concentration. Residence times were varied in the range 0.5–3.0 d (operating periods where residual lactate was detectable). The steady-state at residence time 3 d was maintained for four retention times before reducing the residence time to 2 d to allow a complete washout of sulfate and sulfide. To investigate the effect of sulfide concentration on lactate fermentation, a fermentative experiment was set-up using the inoculum from the 5.0 g l^{-1} sulfate-fed reactor. The feed media used for this experiment contained the same lactate concentration (11.1 g l^{-1}) as was utilized in the 5.0 g l^{-1} sulfate-fed reactor. Other standard experimental conditions, described above for the study of lactate fermentation kinetics, were maintained. The chemostat culture was maintained at a constant residence time of 3 d. After the steady-state was achieved, the reactor was perturbed by the addition of sulfide to the feed media, to a concentration of 0.5 g l^{-1}, using sodium sulfide. The effect of sulfide on the bacterial growth was then monitored. The pH of the media was maintained at pH 8 by the addition of HCl (32%).

2.2. Analytical methods

Total dissolved sulfide was determined spectrophotometrically at 670 nm (Cline, 1969). Acetate, propionate and lactate concentrations were determined by HPLC (Moosa et al., 2002). The sulfate concentration was measured turbidimetrically as barium sulfate (APHA, 1975). The bacterial concentration was determined as dry mass (Moosa et al., 2002).

2.3. Determination of kinetic constants and mathematical modeling

In the current study, the inverse plots of the Monod, Chen and Hashimoto and Contois (Table 1) were used to determine the microbial growth constants (μ_{max} and K_s) using the kinetic data from Oyekola et al. (2010). The approach has been previously employed by other investigators (Moosa et al., 2002; Gadekar et al., 2006). Lactate was assumed to be the dominant limiting substrate. The steady-state data were analyzed using the Chen and Hashimoto (Eq. (3)) (Chen and Hashimoto, 1980), Contois (Eq. (4)) (Contois, 1959) and Monod models (Eq. (5)) (Monod, 1949) and the kinetic constants were determined:

$$\mu = \frac{\mu_{max} S}{K_s S_o + (1 - K_s) S} \tag{3}$$

$$\mu = \frac{\mu_{max} S}{K_s'' X + S} \tag{4}$$

$$\mu = \frac{\mu_{max} S}{K_s + S} \tag{5}$$

For a continuous culture at steady-state, where cell death is negligible and the feed is sterile, $\mu = D$, where D = residence time.

Data from the experiment using 1.0 g l^{-1} feed sulfate were used to describe lactate utilization under conditions where biological sulfate reduction was dominant, in accordance with the stoichiometric analysis presented by Oyekola et al. (2009). Data obtained from the investigation of lactate fermentation kinetics, in the absence of sulfate, were used to describe the kinetics of lactate utilization under conditions where lactate fermentation was the dominant metabolic pathway (Oyekola, 2008). The model description was based on the relationship between the kinetics of bacterial growth and the lactate utilization rate (LUR) (r_L) as described

Table 1
Summary of plots used to determine the kinetic constants.

Model	Plot	Slope	Intercept
Monod	$\frac{1}{\mu}$ versus $\frac{1}{S}$	$\frac{K_s}{\mu_{max}}$	$\frac{1}{\mu_{max}}$
Chen and Hashimoto	$\frac{1}{\mu}$ versus $\frac{S_o}{S}$	$\frac{K_s}{\mu_{max}}$	$\frac{1-K_s}{\mu_{max}}$
Contois	$\frac{1}{\mu}$ versus $\frac{X}{S}$	$\frac{K_s}{\mu_{max}}$	$\frac{1}{\mu_{max}}$