Biotechnol Lett (2008) 30:467–473
DOI 10.1007/s10529-007-9566-4

ORIGINAL RESEARCH PAPER

Gas phase H_2S product recovery in a packed bed bioreactor with immobilized sulfate-reducing bacteria

Matthew J. L. McMahon · Andrew J. Daugulis

Received: 19 September 2007 / Revised: 4 October 2007 / Accepted: 8 October 2007 / Published online: 31 October 2007

Abstract An N_2 strip gas was used in a packed bed sulfate-reducing bioreactor to recover the dissolved sulfide product and improve sulfate conversion. The highest volumetric productivity obtained was 261 mol H_2S m^{-3} d^{-1}. Lowering the initial pH of the medium from 7 to 6 increased the H_2S content of the strip gas from 3.6 to 5.8 mol%. The ratio of strip gas to liquid flow rates (*G/L*) was found be to a suitable basis for scaling the process. Calculations indicated that modest *G/L* values ($<10^2$) were required to recover the residual dissolved sulfide in a downstream stripping column.

Keywords Bioreactor · *Desulfovibrio desulfuricans* · Immobilized · Hydrogen sulphide stripping · Sulfate-reducing bacteria

Introduction

Sulfate-reducing bioreactor technology is an active area of research with applications in the treatment of sulfate-laden wastewaters and bioremediation of acid mine drainage (Colleran et al. 1995; Hulshoff et al. 2001). With the recent development of H_2S splitting technologies (Ohashi et al. 1998; Ni et al. 2006), H_2S also has the potential to serve as an H_2 fuel precursor molecule in a manner analogous to the conversion of CH_4 to H_2 by steam reforming.

In the treatment of acid mine drainage, dissolved heavy metals react with biogenic sulfide and are recovered as a metal-sulfide precipitate. Although this can be accomplished simultaneously in the bioreactor, it is advantageous to recover the sulfide product as gas phase H_2S and perform the precipitation reaction in a separate vessel to prevent mixing of the metal-sulfide product with residual COD and biomass.

Production of H_2 also requires the H_2S feedstock to be in the gas phase. Unfortunately, H_2S has a high aqueous solubility and biogenic sulfide is overwhelmingly present as $H_2S_{(aq)}$ and $HS^-_{(aq)}$ (Kolmert and Johnson 2001; Baskaran and Nemati 2006; Alvarez et al. 2006). Immobilized cell, packed bed bioreactors are a preferred configuration for sulfate reducing bioprocesses but previous investigations of biogenic H_2S stripping have been limited in scope and have occurred in separate abiotic columns rather than within the bioreactor itself (Stucki et al. 1993; Selvaraj et al. 1997). In addition to being a method for product recovery, H_2S stripping has been shown to reduce sulfide inhibition and improve SRB kinetics in stirred tank, batch bioreactors (Konishi et al. 1996). It remains to be seen, however, if in situ stripping can be successfully implemented in a high-rate, continuous flow, immobilized cell bioreactor.

M. J. L. McMahon · A. J. Daugulis (✉)
Department of Chemical Engineering, Queen's University, Kingston, ON, Canada K7L 3N6
e-mail: Daugulis@chee.queensu.ca

This study addresses the aforementioned deficiency in the literature and demonstrates that H_2S can be stripped directly from a packed bed bioreactor with concomitant increases in sulfate conversion and stable bioreactor performance. Models based on fundamental equilibrium relationships are applied to predict pH and temperature conditions for enhanced sulfide recovery. Finally, calculations are performed that demonstrate the feasibility of reusing the bioreactor strip gas to recover residual dissolved sulfide in a downstream abiotic stripping column.

Materials and methods

Microorganisms, media, and culture conditions

Inoculum consisted of a mixture of *Desulfovibrio desulfuricans* subsp. *Desulfuricans* (ATCC 7757) and a sulfate-reducing bacteria (SRB) consortium enriched from municipal landfill leachate. The mixed inoculum consistently utilized sulfate and lactate at the stoichiometric ratio of 0.47 mol sulfate mol^{-1} lactate (data not shown) indicating that incompletely oxidizing SRB were the dominant population. Stock cultures were maintained at 4°C in Postgate B medium and revived at 30°C in Postgate C medium. Unless otherwise specified the medium formulations were prepared as described by Postgate (1984). Postgate C medium was supplemented with 0.1 g sodium thioglycollate/l as a reducing agent and 5 g sodium citrate dihydrate/l as a chelating agent. pH was adjusted to 6.5 using 3 M NaOH and 1 M HCl.

Packed bed bioreactor setup

Packed bed SRB fermentations were carried out in 615 ml glass columns filled with Celite R-635 diatomaceous earth pellets (6.35 mm diam × 12.7 mm ht). The bioreactors were equipped with sampling ports along the length of the column at the inlet, midpoint, and outlet. The bioreactors were operated in an upflow mode with co-current N_2 stripping and maintained at 38 ± 1°C. Packed bioreactors were autoclaved at 121°C and then inoculated by circulating 2 l stationary phase SRB culture through the bioreactor for 48 h at 200 ml h^{-1}. During bioreactor operation Postgate C medium was fed at flow rates between 40 and 200 ml h^{-1}. N_2 strip gas flow rates were between 0 and 2610 ml h^{-1} and are reported as the volumetric ratio of gas to liquid flows (*G/L*).

Three sets of PBR fermentations were conducted to investigate the effects of stripping and buffering the medium pH to initial values of 6 and 7. The first fermentation was fed with Postgate C medium prepared (unbuffered) as previously described. The second fermentation was fed with Postgate C medium containing twice the normal concentration of nutrients and was buffered to pH 6 with 8 g K_2HPO_4 l^{-1} and 14.6 g KH_2PO_4 l^{-1}. The third fermentation was conducted with 2× concentration Postgate C medium and buffered to pH 7 using 28.7 g K_2HPO_4 $l^{-1}$and 6.3 g KH_2PO_4 l^{-1}.

Analytical methods

Dissolved sulfide [$H_2S_{(aq)}$, $HS^-_{(aq)}$] was measured spectrophotometrically at 480 nm according to the CuS precipitation reaction described by Cord-Ruwisch (1985). The H_2S content of the strip gas was measured by gas chromatography equipped with a pulsed flame photo detector. Gas samples were diluted with N_2 as required using a 250 µl gas-tight syringe. Dissolved sulfate was measured at 420 nm according to the $BaSO_4$ precipitation reaction described by Kolmert et al. (2000). Lactate was measured using by HPLC equipped with an Atlantis dC_{18} column. Suspended biomass was measured by its OD_{600} and correlated to the cell dry weight (CDW). Suspended biomass concentration was used as an indicator of the relative amount of immobilized biomass present based on the assumption that all suspended cells were due to steady-state sloughing of immobilized cells at washout conditions (i.e., the dilution rate exceeded the specific growth rate).

Model development

Sulfide and carbonate equilibria

An equilibrium closed system model was formulated to estimate the effect of temperature and pH on strip gas composition by taking into account dissolved phase ionization and vapour–liquid equilibria. A model was necessary to enhance conditions for product recovery

and reduce the experimental workload. Knowledge of possible gas compositions is required to optimize downstream processes such as heavy metal precipitation and conversion of H_2S to H_2.

The closed system consisted of fixed volumes (100 ml each) of liquid and gas (N_2, 1 atm). It was assumed that N_2 was insoluble in solution and that pH did not directly affect gas solubility. 20 mM of total sulfide species was present with the stoichiometric amount of total carbonate species (40 mM) based on the incomplete oxidation of lactate [Eq. 1]:

$$2CH_3CHOHCOO^- + SO_4^{2-} \rightarrow 2CH_3COO^- + 2HCO_3^- + H_2S \quad (1)$$

The closed system model with fixed gas and liquid volumes was considered an approximation of behaviour within an open bioreactor system. Equations (2–5b, 6–9, and 10) described the vapour–liquid and dissolved ionization equilibria within the range of typical operating conditions (T = 10–50°C, pH = 6–8) and were solved simultaneously to determine the distribution of sulfide (P_{H_2S}, n_{H_2S}, n_d, n_{HS}) and carbonate species (P_{CO_2}, n_{CO_2}, $n_{H_2CO_3}$, n_{HCO_3}, n_{CO_3}). Equations and nomenclature are described below:

Sulfide equilibrium relationships

Henry's law:

$$P_{H_2S} - \frac{H_{H_2S}}{n_w} n_d = 0 \quad (2)$$

where P_{H_2S} (atm) = $H_2S_{(g)}$ partial pressure; H_{H_2S} (atm) = Henry's constant from reference data (Metcalf and Eddy 2003); n_w (5.6 mol) = moles of water in 0.100 l water; n_d (mol) = moles of $H_2S_{(aq)}$.

Ideal gas law:

$$P_{H_2S} - n_g \frac{RT}{V_G} = 0 \quad (3)$$

where n_g (mol) = moles of $H_2S_{(g)}$; R (0.082 l atm mol^{-1} K^{-1}) = universal gas constant; T (K) = temperature; V_G (0.100 l) = gas headspace volume.

Total sulfide mass balance:

$$n_g + n_d + n_{HS} = n_t \quad (4)$$

where n_{HS} (mol) = moles of $HS^-_{(aq)}$; n_t (0.020 mol) = total moles of sulfide species in the system.

Dissolved sulfide ionization equilibrium:

$$\alpha = \frac{n_d}{n_d + n_{HS}} = \left(\frac{[H_2S]}{[H_2S] + [HS^-]}\right)\left(\frac{[H_2S]^{-1}}{[H_2S]^{-1}}\right) = \frac{1}{1 + K_a/[H^+]} \quad (5a)$$

$$n_d \frac{(1-\alpha)}{\alpha} - n_{HS} = 0 \quad (5b)$$

where α = fraction of dissolved sulfides present as $H_2S_{(aq)}$; $[H_2S]$ (mol l^{-1}) = concentration of $H_2S_{(aq)}$; $[HS^-]$ (mol l^{-1}) = concentration of $HS^-_{(aq)}$; K_a (mol l^{-1}) = H_2S dissociation constant from reference data (Metcalf and Eddy 2003); $[H^+]$ (mol l^{-1}) = free proton concentration based on pH.

Carbonate equilibrium relationships

Henry's law:

$$P_{CO_2} - \frac{H_{CO_2}}{n_w} n_{H_2CO_3} = 0 \quad (6)$$

where P_{CO_2}(atm) = $CO_{2(g)}$ partial pressure; H_{CO_2} (atm) = Henry's constant from reference data (Metcalf and Eddy 2003); n_w (5.6 mol) = mol water in 0.1 l water; $n_{H_2CO_3}$(mol) = mol $H_2CO_{3(aq)}/CO_{2(aq)}$.

Ideal gas law:

$$P_{CO_2} - n_{CO_2} \frac{RT}{V_G} = 0 \quad (7)$$

where n_g (mol) = moles of $CO_{2(g)}$.

First carbonate ionization equilibrium:

$$-\frac{(n_{H^+})}{V_L^2}(n_{HCO_3^-}) + \frac{(K_{a1,H_2CO_3})}{V_L}(n_{H_2CO_3}) = 0 \quad (8)$$

where n_H (mol) = number of free protons based on pH; V_L (0.100 l) = liquid volume; n_{HCO_3} (mol) = mol $HCO^-_{3\,(aq)}$; K_{a1,H_2CO_3}(mol l^{-1}) = first H_2CO_3 dissociation constant from reference data (Metcalf and Eddy 2003); $n_{H_2CO_3}$ (mol) = mol $H_2CO_{3(aq)}$.

Second carbonate ionization equilibrium:

$$\frac{(K_{a2,H_2CO_3})}{V_L}(n_{HCO_3^-}) - \frac{(n_{H^+})}{V_L^2}(n_{CO_3^{2-}}) = 0 \quad (9)$$

where K_{a2,H_2CO_3}(mol l^{-1}) = second H_2CO_3 dissociation constant from reference data (Metcalf and Eddy 2003); n_{CO_3}(mol) = mol $CO_3^{2-}{}_{(aq)}$.

Total carbonate mass balance:

$$n_{CO_2} + n_{HCO_3^-} + n_{CO_3^{2-}} + n_{H_2CO_3} = n_{tc} \quad (10)$$

Where n_{tc} (mol) = total mol carbonate species in the system.

Abiotic counter-current stripping column equilibria

Due to the nature of co-current stripping in the bioreactor and the high solubility of H_2S, the effluent is expected to retain a significant concentration of the dissolved sulfide product. Acidifying the effluent to below pH 5 will permit the residual sulfide to be completely recovered in a downstream abiotic stripping column. An equilibrium, overall mass balance [Eq. 11] for a counter-current stripping column was performed to estimate the required *G/L* values at the following conditions: The liquid feed stream contained 20 mM $H_2S_{(aq)}$ and stripping was assumed to have negligible effects on pH. Effects of CO_2 were not evaluated and the total gas pressure was 1 atm.

$$\frac{G}{L} = \frac{(C_e - C_0)}{(y_0 - y_e)} \quad (11)$$

where C_0 (0.00036 mol mol^{-1}) = initial $H_2S_{(aq)}$ concentration; C (0 mol mol^{-1}) = exiting $H_2S_{(aq)}$ concentration; y_0 = initial mol fraction of H_2S in strip gas; y_e = exiting mol fraction of H_2S in the strip gas. *G/L* values were calculated at a column operating at 20°C and 37°C. Initial H_2S partial pressures in the strip gas were evaluated from 0 to 0.25 atm to assess the feasibility of using the bioreactor gas in lieu of fresh N_2.

Results and discussion

The results of the first PBR fermentation with a constant feed rate (L = 80 ml h^{-1}, unbuffered initial pH 6.5) indicated that a direct relationship existed between the stripping ratio (*G/L*) and effluent pH (Fig. 1a). Even in the absence of stripping, the pH rose from an initial value of 6.5–6.86 due to biogenic bicarbonate alkalinity. Gas stripping lead to further pH increases due to improved sulfate conversion (Fig. 1b) and the removal of H_2S and CO_2 according to Eqs. 12 and 13.

$$HCO_3^- + H^+ \leftrightarrow H_2CO_3 \leftrightarrow H_2O + CO_2 \quad (12)$$

$$HS^- + H^+ \leftrightarrow H_2S \quad (13)$$

The dissolved sulfide concentration remained relatively constant at 14 mM (Fig. 1b), close to the inhibitory range reported for *Desulfovibrio* spp. (Okabe et al. 1992; Reis et al. 1991; Reis et al. 1992). Despite the constant dissolved sulfide concentration, sulfate reduction increased from 15 to 28 mM as a linear function of *G/L*. Analysis of the strip gas confirmed that the balance of reduced sulfate was being removed as $H_2S_{(g)}$. The increased conversion (i.e., metabolic activity) was accomplished in part by a proportional increase in the immobilized biomass inventory (Fig. 1a). Direct removal of the sulfide product from the bioreactor was an efficient means of alleviating sulfide inhibition and effectively doubled the sulfate conversion from the original inhibition imposed limit of 14 mM.

Doubling the substrate concentrations allowed higher stripping ratios to be evaluated and the direct relationship with sulfate conversion was extended to *G/L* values of 14 without any detrimental effects (e.g., gas hold-up) from the aggressive sparging (Fig. 2). By operating at similar *G/L* values with different absolute liquid flow rates *G/L* was confirmed to be a suitable dimensionless ratio for scaling the process. Decreasing the initial medium pH from 7 to 6 increased the average H_2S content of the strip gas from 3.6 to 5.8 mol% but the effect on sulfate conversion was less pronounced. This was attributed in part to the phosphate buffer mitigating, but not eliminating the pH shifts.

In fermentations with the buffered 2× concentration medium, the steady-state dissolved sulfide concentration was 11 mM compared to 14 mM as observed with the unmodified Postgate C medium. The concentrated medium contained in excess of 200 mM each of K^+ and Na^+ and may have placed salt stress on the SRB as described by Mukhopadhyay et al. (2006) with the net result of decreasing the tolerable sulfide concentration. Even with possible salt stress, a volumetric productivity as high as

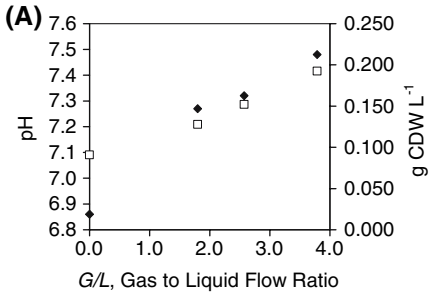

Fig. 1 (**a**) (♦) pH and (□) suspended biomass concentration (g CDW l^{-1}) as a function of *G/L* in a packed bed bioreactor ($L = 80$ ml h^{-1}). (**b**) (♦) Dissolved sulfide (mM) and (□) sulfate reduced (mM) as a function of *G/L* in a packed bed bioreactor. Samples were taken from the bioreactor's 3rd (outlet) port. Suspended biomass concentration was assumed to be proportional to immobilized biomass concentration. Postgate C medium (32 mM sulfate, 54 mM lactate) with an unbuffered initial pH of 6.5 was used at all *G/L* values

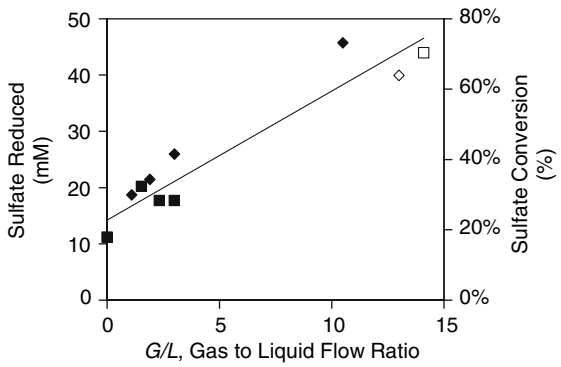

Fig. 2 Sulfate reduced (mM) and sulfate conversion (%) as a function of *G/L* in a packed bed bioreactor. Symbols indicate the initial medium pH and liquid feed rate conditions: (♦) pH = 6, $L = 100$ ml h^{-1}. (■) pH = 7, $L = 100$ ml h^{-1}. (◇) pH = 6, $L = 200$ ml h^{-1}. (□) pH = 6, $L = 50$ ml h^{-1}. Samples were taken from the bioreactor's 3rd (outlet) port. Postgate C medium with 2× substrate concentrations was used at all pH and flow conditions (63 mM sulfate, 108 mM lactate)

261 mol H_2S m^{-3} d^{-1} (residence time = 2.4 h, *G/L* = 13) was obtained based on the entire bioreactor volume. This value compares favorably with recent literature; Baskaran and Nemati (2006) employed a similar bioreactor system without stripping and reported a maximum volumetric productivity of 57 mol m^{-3} d^{-1} (residence time = 0.5 h).

Figure 3 shows the results of the closed system, gas composition model. Over the biologically relevant pH range of 6–8, the H_2S content of the head space changed dramatically from an average value of 8.3 mol% H_2S at pH 6–1.4 mol% H_2S at pH 8. Temperature shifts from 10 to 50°C resulted in marginal differences in gas phase composition. The most efficient means of partitioning the product to the gas phase was to shift the pH below 6. The carbonate system exhibited similar trends, although CO_2 is approximately three times less soluble than H_2S. Thus changes in temperature or pH to increase the H_2S mol% resulted in a proportionally greater increase in CO_2 mol%.

The results of the counter-current stripping column analysis are shown in Fig. 4. As the H_2S partial pressure in the entering strip gas increased, greater *G/L* values were required to recover fully the dissolved sulfide. When $y_0 = y_e$, *G/L* requirements rose to infinity due to the absence of a stripping driving force. Operation of the stripping column at elevated temperatures increased the equilibrium H_2S partial pressure and reduced the *G/L* requirements to recover a given quantity of sulfide. Overall *G/L* requirements for sulfide stripping remained modest ($<10^2$) compared to other common stripping processes (e.g. NH_3 removal from wastewater) which require *G/L* values in the range of 10^3 (Metcalf and Eddy 2003).

In summary, an N_2 strip gas was used in a sulfate reducing packed bed bioreactor for in situ removal of the dissolved sulfide product. Removal of the inhibitory sulfide promoted biomass growth and improved sulfate conversion. Trends with respect to *G/L* values were found to be consistent within a range of flowrates and suggested that *G/L* was a suitable design parameter for scaling the process. Manipulation of pH was found to be the most effective method of increasing the H_2S content of the strip gas while increasing the temperature in the downstream column was predicted to increase stripping efficiency

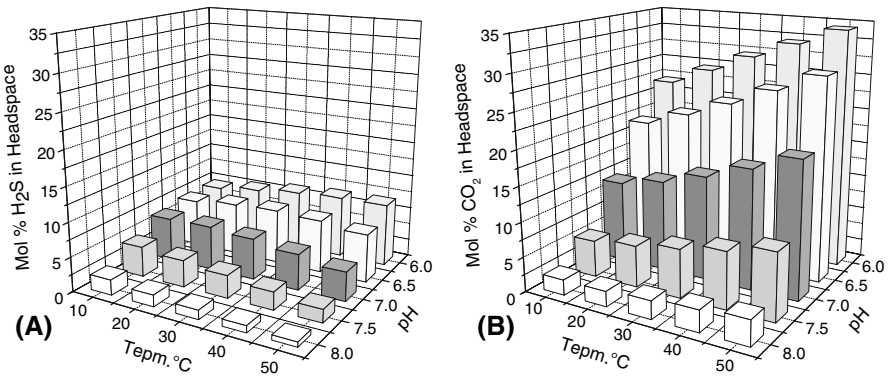

Fig. 3 Effect of temperature and pH on the mol% of H_2S (**a**) and mol% of CO_2 (**b**) in a closed system at equilibrium. The system was assumed to have a liquid volume of 100 ml, headspace volume of 100 ml (with N_2 at 1 atm), and 20 mM of total sulfide ($H_2S_{(g)}$, $H_2S_{(aq)}$, $HS^-_{(aq)}$) and 40 mM total carbonate ($CO_{2(g)}$, $H_2CO_{3(aq)}$, $HCO^-_{3(aq)}$, $CO^{2-}_{3(aq)}$)

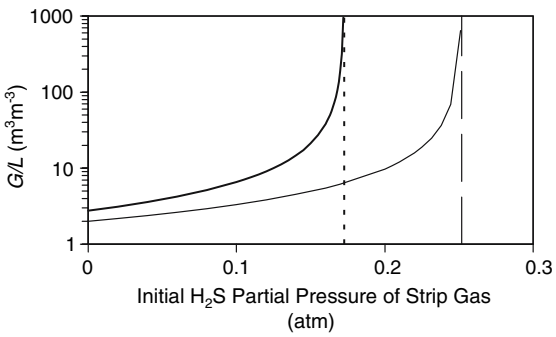

Fig. 4 *G/L* values required to remove all dissolved sulfide from a 20 mM liquid stream using an N_2 strip gas of varying initial H_2S partial pressures (atm). *G/L* values were evaluated at 20°C (*thick curve*) and 37°C (*thin curve*). The equilibrium H_2S partial pressure in the exiting gas was 0.17 atm at 20°C (*thick vertical dashed line*) and 0.25 atm at 37°C (*thin vertical dashed line*). It was assumed that all the dissolved sulfide was present as $H_2S_{(aq)}$ (i.e., pH < 5) and that temperature dependent ionization was negligible. The H_2S partial pressure of a recycled bioreactor strip gas is expected to be in the range of 0.01–0.21 atm

(i.e., decrease *G/L* requirements). These sulfide stripping methods can be applied to improve volumetric productivity in high-rate packed bed bioreactors and to recover the sulfide product in the gas phase for precipitation of heavy metals or conversion to H_2 fuel. Complete sulfide removal could also be implemented at plants treating sulfate wastewaters to significantly reduce inhibition of both SRB and syntrophic fermentative heterotrophs.

Acknowledgements The financial support of the Natural Science and Engineering Research Council of Canada, Kingston Process Metallurgy Inc., and BioCap Canada are gratefully acknowledged. Celite™ R-635 carrier materials were generously donated by World Minerals (Santa Barbara, California).

References

Alvarez MT, Pozzo T, Mattiasson B (2006) Enhancement of sulphide production in anaerobic packed bed bench-scale biofilm reactors by sulphate reducing bacteria. Biotechnol Lett 28:175–181

Baskaran V, Nemati M (2006) Anaerobic reduction of sulfate in immobilized cell bioreactors, using a microbial culture originated from an oil reservoir. Biochem Eng J 31:148–159

Colleran E, Finnegan S, Lens PE (1995) Anaerobic treatment of sulphate-containing waste streams. Anton Van Lee 67:29–46

Cord-Ruwisch R (1985) A quick method for the determination of dissolved and precipitated sulfides in cultures of sulfate-reducing bacteria. J Microbiol Meth 4:33–36

Hulshoff Pol LW (2001) New developments in reactor and process technology for sulfate reduction. Wat Sci Technol 44:67–76

Kolmert A, Johnson DB (2001) Remediation of acidic waste waters using immobilised, acidophilic, sulfate-reducing bacteria. J Chem Technol Biot 76:836–843

Kolmert A, Wikstrom P, Hallberg KB (2000) A fast and simple turbidimetric method for the determination of sulfate in sulfate-reducing bacterial cultures. J Microbiol Meth 41:179–184

Konishi Y, Yoshida N, Asai S (1996) Desorption of hydrogen sulfide during batch growth of the sulfate-reducing bacterium *Desulfovibrio desulfuricans*. Biotechnol Prog 12:322–330

Metcalf and Eddy Inc. (2003) Wastewater engineering: treatment and reuse. McGraw Hill, New York

Mukhopadhyay A, He Z, Alm EJ et al (2006) Salt stress in *Desulfovibrio vulgaris* Hildenborough: an integrated genomics approach. J Bacteriol 188:4068–4078

Ni M, Leung MKH, Sumathy K et al (2006) Potential of renewable hydrogen production for energy supply in Hong Kong. Int J Hydrogen Energ 31:1401–1412

Ohashi H, Ohya H, Aihara H et al (1998) Hydrogen production from hydrogen sulfide using membrane reactor integrated with porous membrane having thermal and corrosion resistance. J Membrane Sci 146:39–42

Okabe S, Neilson PH, Characklis WG (1992) Factors affecting microbial sulfate reduction by *Desulfovibrio desulfuricans* in continuous culture: limiting nutrients and sulfide concentration. Biotechnol Bioeng 40:725–734

Postgate JR (1984) The sulphate-reducing bacteria, 2nd edn. Cambridge University Press, London

Reis MAM, Almeida JS, Lemos PC et al (1992) Effect of hydrogen sulfide on growth of sulfate reducing bacteria. Biotechnol Bioeng 40:593–600

Reis MAM, Lemos PC, Almeida JS et al (1991) Evidence for the intrinsic toxicity of H_2S to sulphate-reducing bacteria. Appl Microl Biot 36:145–147

Selvaraj PT, Little MH, Kaufman EN (1997) Biodesulfurization of flue gases and other sulfate/sulfite waste streams using immobilized mixed sulfate-reducing bacteria. Biotechnol Progr 13:583–589

Stucki G, Hanselmann KW, Hurzeler RA (1993) Biological sulfuric acid transformation: reactor design and process optimization. Biotechnol Bioeng 41:303–315