Biohydrometallurgy: Biotech key to unlock mineral resources value

Proceedings of the 19th International Biohydrometallurgy Symposium

( IBS 2011 )

18 – 22 September 2011

School of Minerals Processing and Bioengineering, Central South University
Key Laboratory of Biohydrometallurgy, Ministry of Education of China
Changsha, Hunan Province, China

Edited by

Guanzhou Qiu¹,², Tao Jiang¹,², Wenqing Qin¹,², Xueduan Liu¹,², Yu Yang¹,², Haidong Wang³

¹ School of Minerals Processing and Bioengineering, Central South University, Changsha, China
² Key Laboratory of Biohydrometallurgy, Ministry of Education of China, Changsha, China
³ Central South University Press, Changsha, China
Sulphate-reducing fixed-bed bioreactors fed with different organic substrates for metal precipitation

Pavlina Kousi*, Emmanouella Remoundakib, Artin Hatzikioseyianc, Marios Tsezosd

National Technical University of Athens, School of Mining and Metallurgical Engineering, Laboratory of Environmental Science and Engineering
Heroon Polytechniou 9, 15780, Athens, Greece
pkousi@metal.ntua.gr, *remoundaki@metal.ntua.gr, *artin@metal.ntua.gr, *tsezos@metal.ntua.gr

Keywords: SRB; Desulfbacter postgatei; sulphate reduction; acetate oxidation; ethanol oxidation; lactate oxidation; metal precipitation

Abstract: Batch upflow fixed-bed sulphate-reducing bioreactors have been set up and monitored for the treatment of synthetic, acidic (pH 3.5) solutions containing divalent iron (100 mg/L), zinc (100 mg/L), copper (100 mg/L), nickel (100 mg/L) and sulphate (1800 mg/L), using acetate, ethanol and lactate as sole electron donors at 60% excess over stoichiometry. The reactors were inoculated with an ethanol-grown microbial community, which was dominated by Desulfbacter postgatei, an acetate-utilizing species. This paper comparatively discusses the results obtained from the batch runs of the reactors in terms of sulphate reduction and organic substrate utilization, metal precipitation capacity as well as solids composition and formation mechanisms. Quantitative precipitation of the soluble metal ions has been achieved. XRD and SEM-EDS analyses showed poorly crystalline phases of marcasite, covellite and wurtzite as well as several mixed metal sulphides. The reduction of sulphate was approximately 95% for the ethanol-and lactate-fed reactor whereas it was limited to about 80% in the acetate-fed reactor after 30 h of operation. TOC degradation and residual acetate content (< 100 mg/L for the ethanol-and lactate-fed reactor) showed that the original microbial community sustained its acetate-oxidizing capacity. Acetate, ethanol and lactate are completely degraded and this is important for the overall efficiency of the process.

1 Introduction

Wastewater originating from mining and metallurgical industries is often acidic and typically characterized by a significant content of sulphate and soluble metals, such as Fe, Zn, Cu, Ni, Pb and Cd. Biological treatment of such wastewater, based on Sulphate-Reducing Bacteria (SRB) [1, 2], is a viable option due to lower cost and better sludge qualities compared to conventional chemical treatment [3]. In such processes, SRB obtain energy for cell synthesis and growth by coupling the oxidation of organic substrates or molecular hydrogen (H2), under anaerobic conditions, to the reduction of sulphate (SO42-) to sulphide (H2S, HS-) [2]. Sulphide react with divalent metal ions which are then sequestered from wastewater as insoluble metal sulphides in the form of various mineral phases [4]. Sulphide and bicarbonate ions, which are formed during sulphate reduction and carbon source oxidation, equilibrate into a mixture of H2S, HS-, S2-, CO2, HCO3- and CO32-, which buffers the solution pH around neutral to slightly alkaline values [5].

However, the dissolved organic carbon content of metal-containing wastewater is very low, usually < 10 mg/L [6]. Therefore, addition of a suitable carbon source and electron donor for sulphate reduction is necessary to promote biogenic H2S production. The preferred carbon sources for SRB are low molecular-weight compounds such as organic acids (e.g. lactate, pyruvate, formate and malate), fatty acids (e.g. acetate) and alcohols (e.g. ethanol, propanol, methanol and butanol) [7, 8]. Nevertheless, several other materials have been examined as cost-effective electron donors for the SRB-based sulphate reduction, such as hydrogen (coupled with CO or CO2 as carbon source) [8 - 10] and various organic materials [8, 11], such as molasses [5, 12 - 15] or wastes [16, 17]. The carbon/energy substrate is clearly an influential variable because of its effect on growth rate and culture composition as well as a potential effect on the economics of a full-scale industrial process.

The oxidation of lactate, which is a relatively common substrate, is a two-stage process as it proceeds via the oxidation of the intermediates-produce of acetate; thus, depending on the SRB species, lactate can be incompletely oxidized to acetate (reaction i) or completely oxidized to CO2 (reaction ii) via reaction [2].

\[
\begin{align*}
2\text{CH}_3\text{CHOHCOO}^- + \text{SO}_4^{2-} & \rightarrow 2\text{CH}_3\text{COO}^- + 2\text{HCO}_3^- + \text{H}_2\text{S} \\
2\text{CH}_3\text{CHOHCOO}^- + 3\text{SO}_4^{2-} & \rightarrow 6\text{HCO}_3^- + 3\text{H}_2\text{S} \\
\text{CH}_3\text{COO}^- + \text{SO}_4^{2-} & \rightarrow 2\text{HCO}_3^- + \text{HS}^{-}
\end{align*}
\]


Ethanol is proposed as carbon source/electron donor for sulphate-reducing bacteria for several reasons, including ease of availability and low cost. Moreover, White & Gadd demonstrated that ethanol was more effective in stimulating sulphide production than lactate which, however, produced the greatest biomass [18]. Ethanol, like lactate, can be incompletely oxidized to acetate (reaction) or completely oxidized to CO₂ (reaction), depending on the presence of acetate-utilizing SRB in the microbial consortium; has been proven the critical step, as it controls the generation of alkalinity and the residual organic content of the effluent. Acetate may also inhibit sulphate reduction at high concentration and low pH [19], being highly toxic to SRB in undissociated forms [20]. Thus, in order to avoid acetate accumulation, efforts have been made towards engineering biosulfidogenic systems by enriching cultures with acetate-utilizing bacteria [21] or by developing syntrophic sulfidogenic microbial consortia [20] or by even supplying alternative electron acceptors, such as oxygen or nitrate [22]. Therefore, acetate oxidation is considered a key factor for the optimization of the entire process, since it determines the neutralization potential of the acidic feeding solution and the effluent organic carbon content.

The present work reports experimental results from the batch runs of reactors inoculated with a known acetate-utilizing culture, already grown on ethanol, when fed on acetate, ethanol and lactate. Experimental set-up and methodology included liquid phase monitoring for the determination of the residual total organic carbon, metals, sulphate, ethanol and acetate in solution. On the base of the experimental results, main operating conditions and efficiency of the reactors are comparatively discussed.

2 Materials and methods

2.1 Sulphate-reducing fixed-bed reactors

In order to study the acclimation potential of an ethanol-grown culture to acetate and lactate, the final microbial degradation of the substrate and the intermediated produced acetate as well as the efficiency of the reactors, batch-fed set-up was selected.

Three identical reactors were set-up and operated using three different feeding media. The sulphate-reducing fixed-bed reactors, operating in upflow mode, were PVC tubes (length: 51 cm; I. D.: 6.7 cm) which were filled with porous, sintered-glass pipes (length: 1.5 cm; wall thickness: 5 mm; specific surface: 1,200 m²/L-SintoMec®, JBL Germany), resulting in a bed height of 43 cm and reactor effective volume of 1,590 mL. The reactors were inoculated by transferring sufficient support material with already grown bacterial biomass from an ethanol-fed bioreactor [23]. This mixed sulphate-reducing bacterial culture was largely dominated (83% of the clones) by dsrAB sequences closely related (92% - 94% similarities) to that of Desulfbacter postgatei [23].

The reactors operated continuously for 6 months at constant room temperature (25°C) in batch-fed mode. They were fed from a 2 L bottle via a peristaltic pump (recirculation rate: 720 mL/h). The feeding solution was replaced upon depletion, to ensure zero residual sulphate and acetate content in the reactor liquor at the end of each feeding cycle without emptying the reactor, to avoid any oxidation effects due to air inflow.

The feeding solutions were a modified variation of Postgate’s medium (DSMZ GmbH, Desulfovibrio medium no. 63), using lactate (reactor [L]), ethanol (reactor [E]) and acetate (reactor [A]), respectively, as sole carbon/electron sources. Lactate was substituted with ethanol and acetate for the ethanol-and acetate-fed reactors maintaining the organic surplus of the original Postgate’s medium (i.e. 60% over stoichiometry) to ensure non substrate limiting conditions. The feeding solutions also contained divalent iron (100 mg/L, added as FeSO₄·7H₂O), zinc (100 mg/L, added as ZnCl₂), copper (100 mg/L, added as CuCl₂·2H₂O), nickel (100 mg/L, added as NiSO₄·6H₂O) and sulphate (1,800 mg/L, added as Na₂SO₄, MgSO₄·7H₂O, FeSO₄·7H₂O and NiSO₄·6H₂O). The pH of the feeding solutions was adjusted to 3.5 by addition of HCl (Merck, analytical grade).

2.2 Liquid phase monitoring

The liquid phase parameters were monitored systematically after the reactors microbial community reached a stable condition. The experimental cycles presented herein were conducted simultaneously for the three reactors running in parallel. Each experimental cycle was repeated three times and the duration of each consecutive experimental cycle was 30 hours.

During each experimental cycle, sampling was performed at the bottle each time the solution was renewed as well as at the reactor outlet every 2 hours, since the reactor liquor was fully recirculated after 2.2 h.

Solution pH was determined on unfiltered samples, which were then vacuum filtered through 0.45 μm sterile membranes (Whatman) before any determination. Sulphate concentration was determined by turbidimetry at 450 nm after formation of BaSO₄ (Hach DR/2000, Method 8051). Total organic carbon (TOC) content was determined by colorimetry after persulphate oxidation of carbon to carbon dioxide.
and colour change of a pH indicator (Hach DR/2500, Method 10129). Copper, nickel, zinc and iron concentrations were determined by inductively coupled plasma spectrometry ICP (Leeman Labs, Direct Reading Echelle, detection limit for Fe, Zn, Cu, Ni < 0.2 mg/L). The concentrations of ethanol and acetate were determined by gas chromatography (HPLC 5890 II) equipped with a flame ionization detector and a 30 m x 0.32 mm (ID) capillary column coated with a 0.25 mm film of polyethylene glycol (Agilent DB-FFAP) which is recommended for the analysis of aquatic VFA solutions [24 - 26]. The temperatures of the injector and detector were 250°C and 300°C, respectively. The initial temperature of the oven was set at 70°C for 2 min followed by a ramp of 20°C/min up to the final temperature of 170°C which was kept steady for 1 min.

Nitrogen was used as a carrier gas at a flow rate of 2.5 mL/min, which remained constant throughout the thermal programme of the analysis. Propanol and propionic acid were used as internal standards for ethanol and acetate, respectively. The lactate content of the samples collected from the lactate-fed reactor was calculated by subtracting the TOC equivalent of the analytically determined acetate concentration from the total TOC concentration of the respective samples. The result was converted into the equivalent lactate concentration in mg/L.

All analytical determinations were performed induplicate. Based on calibration and results repeatability, the precision of the sequentially repeated determinations was estimated as: (a) pH: 1%; (b) sulphate: 5%; (c) TOC: 10%; (d) ethanol, acetate: 5%. The reproducibility of the results is given as standard deviation of the three mean values calculated for each run.

3 Results and discussion

3.1 pH variation

Figure 1 (a) presents the pH profile during the studied experimental cycles. The initial pH of the fresh feeding solution was 3.5, whereas pH at the end of each cycle always reached a value of 6.80 in reactor [A] and 7.10 in reactors [E] and [L] as a result of the alkalinity generated during the SRB metabolism.

Based on the calculation of the molar [organic substrate] : [generated alkalinity] ratio from the incomplete-complete oxidation reactions for the three different substrates tested (L: (1) – (2); E: (4) – (5); A: (3)); greater alkalinity would be expected in reactor [L]. However, the experimental results show that solution pH is neutralized faster in reactor [E] reaching its maximum value after 12 h, due to the higher oxidation rate of ethanol (Figure 1 (e)) which is due to the greater affinity of D. postgatei for ethanol as demonstrated by Laanbroek et al. [27]. Moreover, the generation of alkalinity was not as high as theoretically expected for reactor [L] as part of the utilized lactate may have been involved in processes other than sulphate reduction (section 3.2 and [28]).

This pH increase proves that the culture adapted successfully to the change of the electron donor in reactors [L] and [A]. It also indicates that the SRB metabolic process was not adversely affected nor inhibited by the initial low pH. This was observed for the three organic substrates tested. Sulfidogenesis is, therefore, possible at low initial pH values, thus confirming the viability and the efficiency of the process when the mixed SRB community is in direct contact with acidic wastewater [20, 29 - 31].

3.2 Organic carbon utilization and sulphate reduction

The data depicted in Figure 1 (b) present that sulphate were reduced by 97% in reactors [E] and [L] and by 80% in reactor [A]. This finding is in agreement with the results regarding the utilization of the total organic carbon available in the feeding media (Figure 1 (c)). It is shown that organic content was oxidized by 95% in reactors [E] and [L] and by 82% in reactor [A]. Moreover, Figure 1 (d) and Figure 1 (f), presenting ethanol and lactate oxidation respectively, show that the intermediated produced acetate was oxidized to less than 100 mg/L (equivalent TOC < 40 mg/L) in both reactors [E] and [L] after 30 hours of operation. This strongly confirms that the established biofilm maintained its capacity to oxidize the intermediated produced acetate. The substrate change, therefore, did not significantly alter the original sulphate-reducing community.

Initial sulphate reduction rates were calculated based on the reduction of the available sulphate content within 2 h after the commencement of the experiments (Figure 1 (b)). These calculated sulphate reduction rates are in agreement with the findings reported for a previously run lactate-fed reactor [32], operating continuously at HRT = 9 h, where the microbial community was more diverse [33]. Table 1 summarizes the key process parameters as they were determined for the three sulphate-reducing reactors examined in the present study.

<table>
<thead>
<tr>
<th>REACTOR</th>
<th>Sulphate reduction (%)</th>
<th>TOC degradation (%)</th>
<th>Initial sulphate reduction rate (g/L - d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>80</td>
<td>82</td>
<td>5.3</td>
</tr>
<tr>
<td>E</td>
<td>97</td>
<td>95</td>
<td>7.2</td>
</tr>
<tr>
<td>L</td>
<td>97</td>
<td>95</td>
<td>7.4</td>
</tr>
</tbody>
</table>
Figure 1  (a) pH, (b) sulphate reduction, (c) total organic carbon degradation, (d) acetate oxidation in the acetate-fed reactor, (e) ethanol/acetate oxidation in the ethanol-fed reactor and (f) lactate/acetate oxidation in the lactate-fed reactor during the studied experimental cycles. (Data points are the mean values acquired from three replicate experimental runs and duplicate analytical determinations of each sample. Error bars represent the standard deviation of the three sequentially run experiments.)

Regarding the experimental molar substrate: sulphate ratio reported in Table 2, all ratios are higher than the theoretical values by 60% – 75%. In a previous work, it has been shown that the molar ethanol/sulphate ratio should be 0.75 to attain complete ethanol oxidation and sulphate reduction, i.e. 12.5% higher than the theoretical stoichiometric ratio of 0.67 [23]. The molar ethanol:sulphate ratio computed from the data of the present study is 40% higher than this value (Table 2) due to the high initial concentration of ethanol which is, however, consumed. This is probably due to the fact that, in these carbon-rich conditions, ethanol and acetate may have also served as carbon source for other non sulphate-reducing, anaerobic bacterial strains (e.g. methane-producing bacteria [34 – 36]). This is also seen in the case of reactor [L] where lactate degradation is utilized only by 56.6% in sulphate reduction (Table 2) since lactate, at high initial concentrations, has been reported to support the growth of non sulphate-reducing microorganisms preferring different metabolic pathways than the ones discussed in the present study [28, 37].
Table 2 Utilization of organic substrate for sulphate reduction

<table>
<thead>
<tr>
<th>Reactor</th>
<th>Substrate degraded (mM)</th>
<th>Sulphate reduced (mM)</th>
<th>Stoichiometric molar substrate:Sulphate ratio</th>
<th>Substrate utilized in sulphate reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>21.9</td>
<td>18.6</td>
<td>1.18 (reaction)</td>
<td>56.6</td>
</tr>
<tr>
<td>A</td>
<td>27.1</td>
<td>15.6</td>
<td>1.74 (reaction)</td>
<td>57.6</td>
</tr>
<tr>
<td>E</td>
<td>20.4</td>
<td>19.2</td>
<td>1.06 (reaction)</td>
<td>62.7</td>
</tr>
</tbody>
</table>

3.3 Metal removal and sulphide precipitation

Iron, zinc, copper and nickel, at initial concentration 100 mg/L each, precipitated as sulphides within the first 2 h of each experimental cycle in the reactors (residual concentrations < 0.2 mg/L). This result points out that the metal-precipitating capacity of the columns was maintained high despite the substrate change and the presence of a less diverse sulphate-reducing community.

SEM-EDS analysis revealed aggregates of microcrystalline iron-zinc-copper-nickel sulphides (Figure 2). The biogenic formation of microcrystalline sulphides has already been discussed in terms of formation pathways at ambient temperature, alkaline pH and reducing conditions [33]. XRD analysis of the precipitates demonstrated the presence of marcasite as the main iron sulphide, covellite as the main copper sulphide, wurtzite as the main zinc sulphide and heazlewoodite as the main nickel sulphide [23].

4 Conclusions

The following conclusions can be drawn from this study:

(1) From the monitoring of pH, it has been evident that the culture adapted successfully to the change of the electron donor in reactors [L] and [A]. The SRB metabolic process was not adversely affected nor inhibited by the initial low pH for the three organic substrates tested. Sulfidogenesis is, therefore, possible at low initial pH values when the mixed SRB community is in direct contact with acidic wastewater.

(2) The experimental results have shown that sulphate is reduced by 97% in reactors [E] and [L] and by 80% in reactor [A] and organic content oxidized by 95% in reactors [E] and [L] and by 82% in reactor [A].

(3) All metals precipitated quantitatively within the first 2 h of each experimental cycle. The precipitated metal sulphides were mainly microcrystalline.

(4) The intermediately produced acetate was oxidized to less than 100 mg/L (equivalent TOC < 40 mg/L) in both reactors [E] and [L]; meaning that the established biofilm maintained its capacity to oxidize the intermediately produced acetate and that the substrate change did not significantly alter the original sulphate-reducing community.

(5) From the comparative study of the three substrates, it is concluded that acetate, ethanol and lactate are completely degraded and this is important for the overall efficiency of the process. Based on the theoretical stoichiometry of the relative reactions, 58% - 63% of the substrates is utilised in the respective sulphate-reducing processes because part of the substrate is involved in processes other than sulphate reduction.

Acknowledgements

The authors acknowledge the financial support granted to the Laboratory of Environmental Science and Engineering, NTUA, Greece by the Greek Government in the frame of supporting research activities.
References


