Performance of a lactate-fed UASB reactor treating sulfate containing waters

Sueli Moura Bertolino[1], Tácia Costa Veloso[1], Versiane Albis Leão[2]

[1] *Rede Temática em Engenharia de Materiais. Universidade Federal de Ouro Preto. Praça Tiradentes, 20. Centro. Ouro Preto, MG. CEP: 35400-000. suelibertolino@yahoo.com.br;* [2] *Laboratório de Bio&Hidrometalurgia. Universidade Federal de Ouro Preto. Campus Morro do Cruzeiro, Ouro Preto, MG. CEP: 35400-000, versiane@demet.em.ufop.br.*

Abstract This study aimed to improve the understanding of biological sulfate reducing in a continuous reactor. The effects of lactate concentration and recirculation on sulfate reduction in a lab scale UASB reactor were studied. The reactor performance was assessed in experiments with 1 day residence time, 2g/L sulfate concentration and organic loading increasing from 3.5 kgCOD/m^3.d to 5.9 kgCOD/m^3.d. In the absence of recirculation, the best outcome was noticed with the lowest organic loading where a SRB population of 9.5x10^9 SRB cells/mL oxidized 68% of the lactate and reduced 60% of the sulfate present in the influent solution. Propionate concentration increased with the lactate content, suggesting fermentation. When recirculation was applied, sulfate reduction yields increased to 89%, corresponding to a sulfate removal rate of 1.94 kgSO_4^{2-}/m^3.d. Incomplete lactate oxidation to acetate predominated as a result of presence of the genera *Desulfovibrio*, *Desulfomona* and *Desulfomaculum* in the reactor.

Key Words sulfate removal, anaerobic treatment, sulfate-reducing bacteria.

Introduction

Acid rock (ARD) or mining drainage (AMD) account for water pollution with acidity, toxic metals as well as sulfate. While acidity neutralization and toxic metal removal have been extensively studied and technological solutions developed, less attention has been focused on the treatment of dissolved sulfate. Furthermore, sulfate containing compounds such as sulfuric acid are applied in a series of industries such as metal production (hydrometallurgy), food processing and paper mills. Therefore, sulfate is always present in effluents of these industries. Although this species is considered a low risk substance as compared to dissolved metals and acidity, regulatory agencies are becoming increasingly concerned over high sulfate levels on effluents and stricter standards are being imposed or expected in the near future (INAP, 2003).

The technologies to treat sulfate containing wastewaters comprise both chemical and biological routes. Biological treatment uses sulfate reducing bacteria (SRB), which are present in many anaerobic wastewater treatment systems and have been extensively studied because sulfate and transition metal concentrations can be reduced to very low levels, unlike most chemical treatments.

The upflow anaerobic sludge blanket reactor (UASB) is one of the most traditional anaerobic reactors applied to the treatment of domestic wastewater. It has some advantages as compared to other anaerobic technologies such as low investment and energy costs as well as short hydraulic retention time. This reactor has been investigated for sulfate reduction and parameters such as substrate type, COD/sulfate ratio, pH, sulfide concentration and temperature have been shown to affect sulfate reduction (Cao *et al.,* 2009; Lens *et al.,* 2003). In addition, much effort has also been directed to understanding the factors related to SRB competition with both fermentative and methane-producing microorganisms (MPM) (Cao *et al.,* 2009).

Many studies have confirmed that SRB compete with acidogenic, acetogenic and methanogenic microorganisms for substrate consumption. As lactate is the best substrate for SRB growth, it enables a deeper understanding of the different phases occurring in anaerobic reactors. Notwithstading, most works studied other carbon sources such as sucrose, ethanol, molasses and sewage sludge, most likely due to their lower costs as compared to that of lactate. Therefore, this work is a comprehensive study on the performance of a UASB reactor treating high sulfate loadings.

Materials and Methods

The total volume of lab-scale UASB reactor was 3.0L (94mm diameter, 380mm height). It was operated for 580 days at 24h of hydraulic retention time (HRT), placed inside a fume hood in a temperature controlled room where temperature was maintained at 24±1 °C. The microorganisms used in this study were harvested from a granular sludge collected from an UASB reactor treating domestic wastewater and enriched in modified postgate C medium so that a 5 liters sample was produced, in 300 days. The enrichment medium contained: 0.5g/L KH_2PO_4; 1.0g/L NH_4Cl; 0.06g/L $MgSO_4.7H_2O$; 0.1g/L $FeSO_4.7H_2O$; 0.25g/L yeast extract; 2.96g/L Na_2SO_4; and 3.76g/L lactate. Afterwards, the UASB reactor was fed with synthetic effluent (same growth medium utilized during enrichment) and lactate was applied as carbon and electron source. The organic loaded varied according to the sulfidogenic performance shown by the reactor, starting at a COD/Sulfate ratio of 2.0 ± 0.2. Phases I (3.48 ± 0.33 kg/m³.d) and II (4.87 ± 0.30 kg/m³.d) represented the SRB enrichment period, whereas phases III to V (3.55 ± 0.25 to 5.89 ± 0.48 kg/m³.d) were run at increasing organic loading for a constant sulfate concentration (2.0g/L). Phase VI (5.04 ± 0.33 kg/m³.d) is characterized by effluent recirculation (rate = 93), at an organic loading of 5 kgCOD/m³.d (COD/sulfate = 2.5). The reactor effluent was analysed twice a week for total and filtered chemical oxygen demand (COD), sulfate, alkalinity, volatile fatty acids (VFA), volatile suspended solids (VSS), pH, redox potential (Eh) and temperature. Once a week, a sample from inside the reactor was withdrawn for measuring VSS, alkalinity, pH and redox potential, whereas viable cell were determined monthly.

Sulfate concentration was determined by ionic chromatograph (Metrohm) whereas VFA (acetic, propionic, valeric, butyric, lactic) were determined by high performance liquid chromatography, (HPLC, Shimadzu); bicarbonate alkalinity was assayed by titration with 0.1M sulfuric acid solution to pH 4.5; VSS, gravimetrically, and COD, by the closed reflux method, according to the Standard Methods for Water and Wastewater (APHA, 2005). Before COD determination, the sulfide present in effluent samples was removed by adding a drop of HCl (35%) and flushing the sample during 10 min with N_2. Solution pH (Hanna HI931400) and redox potential (Digimed) (vs an Ag/AgCl electrode) were also recorded.

Microorganisms were enumerated by a three - tube most probable number (MPN) procedure using 10-fold serial dilutions in selective media. The SRB were enumerated in a specific medium for SRB (Postgate C) (Postgate, 1963). Prior to the experiments, culture tubes were degassed with pure N_2, sealed and autoclaved (120°C, 1.5atm, 20min). Afterwards, culture and control tubes were incubated for 30 days, at 35°C.

Results and discussion

Reactor start-up and biomass

The performance of sulfate reduction was investigated by the amount of sulfate and COD removal as well as volatile fatty acids (VFA) production in the reactors. Total biomass concentration and SRB population were followed, respectively by the VSS concentration and the MPN technique. The SRB population, depicted in figure 1 was measured at the end of each phase and show a 1000 times increase from phase I (5.3×10^6 cell/mL) to phase III (9.5×10^9 cell/mL). This linear increase suggests that up to phase III, the SRB population had not reached its maximum value. This is consistent with other works in which a longer lag period was required to stabilize the SRB population in a reactor without carrier material (Beaulieu *et al.,* 2000; Omil *et al.,* 1998). After phase III, the VSS values stabilized in the range 15.78 to 19.44 gVSS, without large variations.

The SRB population present in the UASB reactor was dominated by incomplete oxidizers, i.e, the *Desulfomonas*, *Desulfovibrio*, *Desulfolobus*, *Desulfobulbus* and *Desulfotomaculum* genera. It must be pointed out that although methanogens were detected in phase I, their growth is inhibited by the presence of sulfide (especially H_2S; O'Flaherty *et al.*, 1998) and their population was expected to decrease as the SRB predominated and sulfate reduction increased.

Reactor performance

The performance of the UASB reactor was monitored by pH, redox potential (Ag/AgCl) as well as VFA concentration and alkalinity. The optimum pH for SRB growth is around 7 and lower values (pH < 5) affect bacterial growth, thereby VFA accumulation and alkalinity production, both resulting from organic matter degradation, will define the effluent pH. As shown in figure 2, the pH inside the reactor remained fairly constant up to phase IV in the range 6.5—7.0, reducing to values between 6.0 and 6.5, at phase V due to the increase in the organic loading and the larger VFA production (data not shown). The pH increased again to values above 7, when recirculation was started (phase VI) and higher sulfate reduction resulted in higher alkalinity (equation 1) as compared to the previous phases. Accordingly, recirculation can be an alternative to alkalinity addition to maintain pH conditions suitable for SRB development.

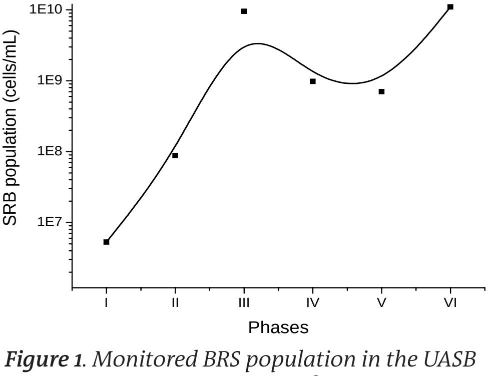

***Figure 1**. Monitored BRS population in the UASB reactor. (I) OLR = 3.48 kg/m³.d; (II) OLR = 4.87 kg/m³.d; (III) OLR = 3.55 kg/m³.d; (IV) OLR = 4.65 kg/m³.d; (V) OLR = 5.89 kg/m³.d; (VI) OLR = 5.04 kg/m³.d.*

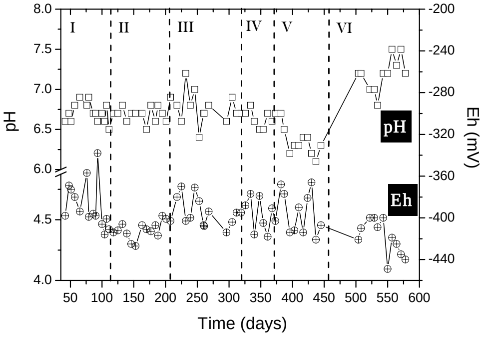

Figure 2*. Variation on pH and Eh during the UASB reactor performance. Experimental conditions: (I) OLR = 3.48 kg/m³.d; (II) OLR = 4.87 kg/m³.d; (III) OLR = 3.55 kg/m³.d; (IV) OLR = 4.65 kg/m³.d; (V) OLR = 5.89 kg/m³.d; (VI) OLR = 5.04 kg/m³.d.*

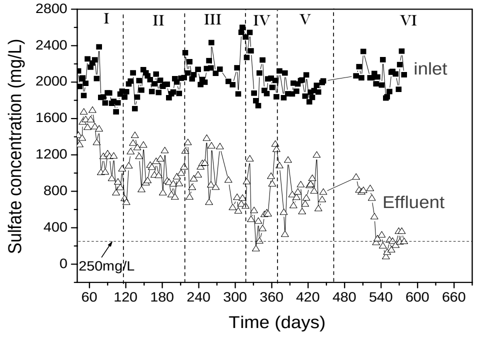

Figure 3*. Sulfate concentration during the sulfate reduction in a UASB reactor. Experimental conditions: (I) OLR = 3.48 kg/m³.d; (II) OLR = 4.87 kg/m³.d; (III) OLR = 3.55 kg/m³.d; (IV) OLR = 4.65 kg/m³.d; (V) OLR = 5.89 kg/m³.d; (VI) OLR = 5.04 kg/m³.d.*

$$2 \text{ lactate} + SO_4^{2-} \rightarrow 2 \text{ acetate} + 2\, HCO_3^- + HS^- + H^+ \quad (1)$$

Due to the reducing conditions, effluent redox potential fluctuated between -360mV and -420mV (Ag/AgCl) up to phase V and as recirculation improved reactor hydrodynamics, it decreased further to the range -400mV to -440mV (Ag/AgCl). It must be stressed that no reducing agent such as Na_2S was required for start-up as the potential steadily decrease up to the 150th day of operation.

Previous work has shown that with this inoculum, a COD/sulfate ratio of 2.0 was optimum for bacterial growth in batch conditions, resulting in 98% sulfate reduction (Barbosa *et al.,* 2009). Therefore, this value was chosen for phase I (start up) and in phases III to V, the organic load was changed and the sulfate reduction was followed (figure 3).

During phase I (3.48 kgCOD/m³.d) and II (4.87 kgCOD/m³.d), the average organic matter consumptions were 25% and 22% respectively. In addition, for an SRB population of 5.3×10^6 cells/mL, it was observe that 51% of electron donors were utilized for 36% sulfate reduction (phase I). Similarly, during phase II, a population of 8.8×10^7 SRB-cells/mL utilized 58% of electron donors, which is equivalent to 49% sulfate reduction. At phase III, the SRB population increased considerably (9.5×10^9 cells/mL), thus 40% COD and 60% sulfate were removed, implying 60% of electron flow was transferred to sulfate. Sulfate reduction improved up to phase IV (88%) but an increase on the organic loading to 4.65 kgCOD/m³.d (COD/sulfate ratio of 2.39 ± 0.33), resulted in lower sulfate reduction with a minimum at 32%, which is consistent with the work of Ren *et al.* (2007). At an even higher organic loading of 5.89 kgCOD/m³.d (phase V), no improvement on the reactor performance was observed and the COD consumption decreased to 23%, for a sulfate reduction efficiency that varied between 39% and 72%.

It was observed that up to phase VI, an increase on substrate concentration did not improved sulfate reduction, as the residual sulfate concentration on the reactor effluent was fairly high (780mg/L, on average) as shown in figure 3. Taking the incomplete lactate oxidation (equation 1), this latter species would be limiting at COD/sulfate ratios lower than 1.85 (theoretical), thereby COD/sulfate ratios lower than 1.67 ± 0.18 (phase III) were not tested as they would limit sulfate reduction. It was therefore decided to apply effluent recirculation and this is represented by phase VI in this work. For 5.0 kgCOD/m³.d, the improvement on reactor performance was clear since sulfate reduction increased from 60% (phase V) to 89% (235mg/L residual sulfate concentration) for a COD consumption of 41%. This value is consistent with the work performed by Kaksonen *et al.* (2003) in similar conditions.

In addition to acetate, propionate (an indication of lactate fermentation) was detected in the UASB reactor effluent, suggesting concurrent sulfidogenesis and acidogenesis. This result is consistent with the works of Zhao *et al.* (2008) and Lopes *et al.* (2010), which also observed sulfate reduction in acidogenic conditions. These parameters alongside the microbial characterization indicate two metabolic pathways for lactate degradation: (i) lactate is oxidized to piruvate followed by acetate and carbon dioxide formation by incomplete-oxidizer SRB; in which *Desulfovibrio* sp. plays a key role (reaction 2); (ii) lactate fermentation by propionate CoA-transferase enzyme by fermenting bacteria such as Propionibacterium (Barton, 1995; García, 1982). These observations are supported by acetate and propionate accumulation in the reactor.

$$3 \text{ lactate} \rightarrow \text{acetate} + 2 \text{ propionate} + HCO_3^- + H^+ \quad (2)$$

As shown in figure 3, effluent sulfate concentrations lower than 250mg/L are only achieved during phase VI, i.e. when recirculation was applied to the UASB reactor. This is an important parameter since some countries define 250mg/L as the maximum allowable sulfate concentration in mine drainages and industrial effluents. The application of produced sulfide (up to 250mg/L) for metal precipitation is under investigation.

Conclusions

This work showed that a COD/sulfate ratio of 1.7—2.5g-COD/g-sulfate resulted in the highest sulfate reduction yield in a lab-scale UASB reactor, containing a SRB population formed by incomplete oxidizers. Lower values produced lower sulfate reduction due to limiting carbon sources, whereas at COD/sulfate ratios higher than 2.5g-COD/g-sulfate, fermentation becomes predominant. In the best conditions, an average sulfate reduction value of 66% was observed without recirculation in the reactor, treating 2.0g/L sulfate and in the presence of 3.55COD kg/m^3.d. Sulfate reduction increased to 89% (0.087gSO_4^{2-}/gSSV.d) when recirculation was applied at an organic loading value of 5.04 kg/m^3.d. The residual COD is high and requires downstream treatment but it is easily degradable due to the presence of only acetate and propionate when lactate was the single carbon source.

Acknowledgements

The financial support from the funding agencies FINEP, FAPEMIG, CNPq, CAPES as well as Vale is gratefully appreciated.

References

APHA, 2005. Standard Methods for the examination of water and wastewater, 20th ed, Washington, DC.

Barbosa, L.P., Bertolino, S.M., Freitas, P.C., Oliveira, V.A., Pina, P.D., Leão, V.A., Teixeira, M.C., 2009. Effects of different COD/Sulfate ratios on the Growth of Metal Tolerant Sulfate Reducing Bacteria (SRB), Biohydrometallurgy: A meeting point beteween microbial ecology, metal recovery process and evironmental remediation., Proceddings of Biohydrometallurgy, Bariloche, pp. 569—572.

Barton, L.L., 1995. Sulfate-reducing bacteria. Plenum Press, New York.

Beaulieu, S., Zagury, G.J., Deschênes, L., Samson, R., 2000. Bioactivation and bioaugmentation of a passive reactor for acid mine drainage treatment., in: R.K. Singhal, a.A.K.M. (Ed.), Environmental Issues and Management of Waste in Energy and Mineral Production. the Netherlands, Rotterdam, pp. 533—537.

Cao, J., Zhang, G., Mao, Z., Fang, Z., Yang, C., 2009. Precipitation of valuable metals from bioleaching solution by biogenic sulfides. Minerals Engineering 22, 289—295.

García, J.L., 1982. Relations between acidogenesis and the utilization of lactate, sulfate and nitrate during anaerobic digestion, Simposio Panamericano de Combustibles y Productos Químicos Vía Fermentación "Avances en Digestión Anaeróbica", Mexico City, Mexico, pp. 20–30.

INAP, 2003. Treatment of sulphate in mine effluentes. International network for acid prevention, p. 129.

Kaksonen, A., Franzmann, P., Puhakka, J., 2003. Performance and Ethanol Oxidation Kinetics of a Sulfate-Reducing Fluidized-Bed Reactor Treating Acidic Metal-Containing Wastewater. Biodegradation 14, 207—217.

Lens, P.N.L., Gastesi, R., Lettinga, G., 2003. Use of Sulfate Reducing Cell Suspension Bioreactors for the Treatment of SO2 Rich Flue Gases. Biodegradation 14, 229—240.

O'Flaherty, V., Mahony, T., Kennedy, O., Colleran, E., 1998. Effect of pH on growth kinetics and sulphide toxicity thresholds of a range of methanogenic, syntrophic and sulphate-reducing bacteria. Process Biochemistry 33 (5), 555—569.

Omil, F., Lens, P., Visser, A., Hulshoff Pol, L.W., Lettinga, G., 1998. Long-term competition between sulfate reducing and methanogenic bacteria in UASB reactors treating volatile fatty acids. Biotechnology and Bioengineering 57, 676—685.

Postgate, J.R., 1963. Versatile Medium for the Enumetation of Sulfate-Reducing Bacteria. Appl. Microbiology. 11, 265—267.

Ren, N.-Q., Chua, H., Chan, S.-Y., Tsang, Y.-F., Sin, N., 2007. Effect of COD/SO_4^{2-} ratios on an acidogenic sulfate-reducing reactor. Industrial & Engineering Chemistry Research 46, 406—411.

Zhao, Y., Ren, N., Wang, A., 2008. Contributions of fermentative acidogenic bacteria and sulfate-reducing bacteria to lactate degradation and sulfate reduction. Chemosphere 72, 233—242.