CHEMICAL ENGINEERING TRANSACTIONS Volume 17, 2009
Editor Sauro Pierucci
 ISBN 978-88-95608-01-3 ISSN 1974-9791
DOI: 10.3303/CET0917035

Biological treatment of heavy metals contaminated waters

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The precipitation of metals with biologically produced H_2S by Sulphate Reducing Bacteria (SRB) in Permeable Reactive Barriers (PRB) has been proposed as a technology for the treatment of heavy metals contaminated waters. The aim of the work was the comparison between a selected reactive mixture containing organic matter for SRB and other electron donors, such as ethanol, glucose and polysaccharides. The different substrates were compared by testing their ability in sustaining SRB activities. Batch tests were conducted for the screening of solid mixtures for SRB growth. A continuously operating fixed-bed column was filled with the selected mixture (6% leaves, 9% compost, 3% zero valent iron, 30% silica sand, 30% perlite, 22% limestone) and inoculated by SRB. Column was regularly fed with a solution containing heavy metals and sulphates. At steady state 50±10% sulphate abatement was reached and metals were totally removed. Batch tests with ethanol showed the ability of SRB to grow on this substrate efficiently. Experimentation using ethanol was performed using two different column reactors filled with perlite, one inoculated by SRB and the other used as blank. Both columns were regularly fed with a solution containing sulphate and ethanol. Sulphate abatements of the inoculated column were 70±10% against 10±5% of the blank column. Preliminary batch tests with polysaccharides showed the ability of bacteria to grow on these substrates.

1. Introduction

Heavy metals are non biodegradable pollutants whose release in the environment is mainly related to industrial wastewaters discharged from industrial and mining activities. The use of bacterially mediated sulphate reduction in Permeable Reactive Barrier is an alternative technique for the remediation of heavy metals polluted streams.
SRB are known to grow using small organic molecules, essentially small molecular weight compounds, like acetate, lactate, propionate, butyrate, valerate, methanol, ethanol, glycerol, glucose (Postgate, 1979). However, pure substrates as carbon source may not be cost effective for this kind of treatment. Usually, organic mixtures are used in biological PRB construction as electron donor in the sulphate reduction: biodegradable materials are generally mixed with more recalcitrant ones to ensure long term SRB growth (Cocos et al., 2002; Gibert et al., 2004). Full scale applications of

Please cite this article as: Cruz Viggi C., Pagnanelli F., Cibati A., Berteletti C. and Toro L., (2009), Biological treatment of heavy metals contaminated waters, Chemical Engineering Transactions, 17, 203-208 DOI: 10.3303/CET0917035

organic-carbon based sulphate reducing PRB are also characterised by the addition of gravel to improve barrier permeability and limestone to increase pH and stimulate SRB growth (Ludwig et al., 2002). Batch experiments were preliminarly performed to determine the optimal mixture for treating heavy metals in biological PRB. Selected mixture was then tested in continuous fixed column experiments to simulate permeable reactive barriers running. Ethanol was also tested as electron donor for the sulphate-reduction for several reasons, including a well-defined and "clean" composition, ease of availability, low cost and the possibility to use bioethanol. Finally, preliminary batch tests starch were conducted to test the ability of SRB to grow on this substrate.

2. Materials and methods

2.1 Batch tests with solid reactive mixtures

Eight reactive mixtures were prepared consisting of three main functional components: a mix of organic materials, a neutralizing agent (limestone), and a non reactive porous medium (silica sand or perlite). A sample (20 g) of each mixture was added in flasks and filled with 80 mL of liquid C Medium (Postgate, 1979). Flasks were sealed and 20 mL inoculum of bacteria cultivated in C Medium were added. Best performing reactive mixture (RM: 6% leaves, 9% compost, 3% Fe(0), 30% silica sand, 30% perlite, 22% limestone) was further tested in presence and in absence of bacteria and single organic components (compost and leaves) were also investigated for sulphate removal without inoculum. All experiments were conducted at 37°C under shacking condition. pH, Eh, SO_4^{2-} and S^{2-} production were monitored for 22 days. Each test was performed twice and average values were considered.

2.2 Column tests with solid reactive mixture M8

Column tests were performed in a fixed bed column (height 1 m; diameter 0.2 m; column volume, V=6.65*10-3 m^3) made of Plexiglas with 10 equally distant outputs along the axial length, numbered from the bottom to the top of the column. It was packed with perlite (an expanded clay) and silica sand on the bottom (10 cm length) followed by reactive mixture (RM) (80 cm) and topped with perlite and silica sand (10 cm) (pore volume V_0 = 1.5 L) .SRB were inoculated in the core of the column. Column was regularly fed with a solution containing heavy metals (Cd 0.1 mM, Cr(VI) 0.1 mM, Cu 0.1 mM, Zn 0.1 mM and As(V) 27μM) and sulphate (31 mM). Samples from three different outputs (1, 5 and 9) were analysed for pH, Eh and the residual amounts of sulphates and metals.

2.3 Batch tests with ethanol

Glass reaction flasks (120 mL), containing a sampling port, were used for all the experiments. 80 mL of modified C Medium (ethanol 6 g/L instead of lactate) was added in flasks. Therefore the flasks were sealed and 20 mL inoculum of bacteria cultivated in C Medium were added by a sterile syringe through the sampling port. All experiments were conducted at 37°C under shacking condition. pH, Eh, SO_4^{2-} and S^{2-} production were monitored for 30 days. Each test was performed twice and average values were considered.

2.4 Column tests with ethanol

Column tests were performed in two fixed bed column like that used in column tests with mixture RM. Columns were filled with perlite (pore volume V_0 = 3.5 L), one inoculated by SRB and the other used as blank. Both columns were regularly fed with a solution containing sulphate (31 mM) and ethanol (65 mM). Samples from three different outputs (1, 5 and 9) were analysed for pH, Eh and the residual amounts of sulphates.

2.5 Batch tests with starch

Glass reaction flasks (120 mL), as those previously described, were used for the experiments with starch. 80 mL of C Medium (without carbon sources) and starch (100 g/L) were adder in flask. Therefore the flasks were sealed and 20 mL inoculum of bacteria cultivated in C Medium were added by a sterile syringe through the sampling port. All experiments were conducted at 37°C under shacking condition. pH, Eh, SO_4^{2-} and S^{2-} production were monitored for 100 days. Each test was performed twice and average values were considered.

3. Results and discussions

3.1 SRB growth using solid reactive mixtures

Sulphide formation was observed for all reactive mixtures by means of lead acetate paper, confirming that sulphate abatement was related to SRB metabolism. Considering together sulphate abatement, pH and Eh, RM showed to be the optimal mixtures in terms of both SRB performances (sulphate abatement: 83% on 22^{nd} day) and optimal growing conditions of pH and Eh (RM: pH = 7.8±0.1 and Eh -410±5 mV). The selected mixture was further tested in presence and in absence of inoculated microbial consortium, in order to isolate contributions due to bioreduction and biosorption. Sulphate abatement in mixture with bacteria was 83% and in mixture without bacteria was 58%. Difference between these values (25%) represents SO_4^{2-} removal due to sulphate reduction.

The efficiency of treatment was monitored during time for pH, Eh and the residual amounts of sulphates and metals in three different outputs of the column, the low and the upper zones filled with inorganic components and a reactive central core filled with mixture RM. Figure 1 shows sulphate and metals abatement versus pore volume on the upper output (9) of the column filled with solid reactive mixture (SS).

At steady state, sulphate abatement in the upper zone (output 9) of column was 50±10% caused both by SRB activity and sorption onto inorganic and organic components of the mixture. Heavy metals were totally removed, confirming the ability of the selected mixture in sustaining SRB activity and removing metals both by bioprecipitation and biosorption.

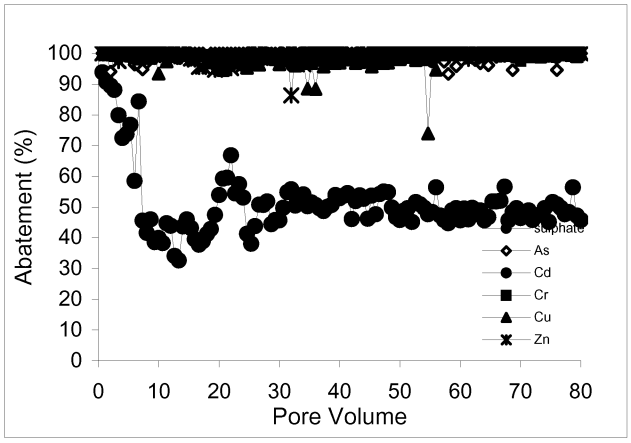

Figure 1: Sulphate and heavy metals abatement versus pore volume for column SS

Moreover, pH and Eh values can be considered optimal for growth and maintenance of the SRB inoculum (Table 1). Column apparatus, already working since 24 months, is still operated in order to determine specific kinetics of sulphate and heavy metals necessary for the project of a full-scale permeable reactive barrier.

Table 1. Average values of sulphate removal (%), pH and Eh of the different column systems (SS, Solid Substrate; LS, Liquid Substrate; BLS, Blank Liquid Substrate).

Column system	Output	SO_4^{2-} Abatement (%)	pH	E_h (mV)
SS	1	10±5	6.9±0.5	210±50
	5	40±5	8.3±0.4	-330±50
	9	50±5	8.7±0.3	320±40
LS	1	10±5	6.1±0.5	-220±20
	5	50±10	6.5±0.3	-270±30
	9	70±10	6.5±0.3	-280±20
BLS	1	5±2	6.7±0.6	-140±30
	5	10±5	6.8±0.4	-110±20
	9	10±5	6.9±0.4	-100±30

3.2 SRB growth using ethanol

Batch tests showed the ability to cultivate and maintain SRB growing on ethanol efficiently. The sulphate kinetic abatement showed a first phase of bacteria acclimatization in which the consumption of sulphate is almost absent (about 15 days). Following a second phase of rapid consumption associated with ethanol depletion. Batch tests with ethanol showed good performances in terms of both sulphate abatement (60%±5 on 30^{th} day) and operative conditions of pH and Eh (pH = 7.8±0.1 and E_h -425±5 mV).

Regarding column experiments, as for column filled with mixture M8, the performances of treatment was monitored during time for pH, Eh and the residual amounts of sulphates in three different outputs of both columns. At steady state, sulphate abatements in the upper zone (output 9) of the inoculated column were 70±10% against 10±5% of the blank column. In the inoculated column sulphate abatement was caused both by SRB activity and sorption onto perlite; moreover, pH and Eh values can be considered good for growth and maintenance of the SRB inoculum (Table1). In the blank column sulphate abatement was only due to sorption onto perlite, and pH and Eh values were different from those observed in the inoculated one (Table 1).
Figure 2 shows sulphate versus pore volume on the upper output (9) of the column inoculated by SRB (LS) and the other used as blank (BLS).

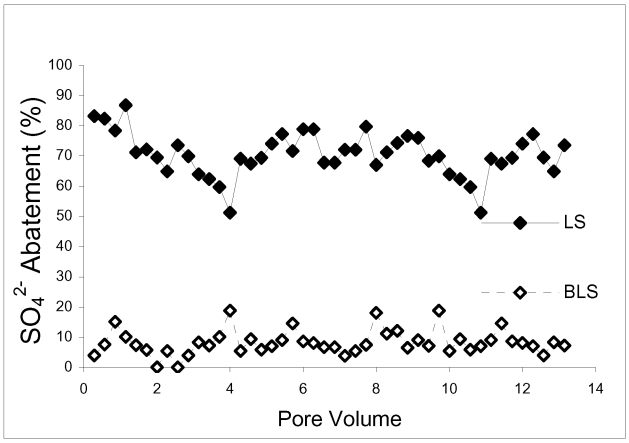

Figure 2: Sulphate abatement versus treated volume for the two columns fed by ethanol

Column apparatus, already working since 12 months, is still operated in order to determine specific kinetics of sulphate necessary for the project of a full-scale permeable reactive barrier.

3.3 SRB growth using starch

Biomass activity was evaluated by H_2S release and sulphate diminution during time. Batch tests showed the ability to cultivate and maintain SRB growing on starch efficiently. In Figure 3 sulphate abatement by SRB inoculum was reported for batch tests with starch. Experimental data from batch growth on sturch showed 85 ± 1 % abatement of sulphates in 100 days with an almost constant decrease rate. Batch tests with sturch showed good performances in terms of both sulphate abatement and operative conditions of pH and Eh (pH = 6.1±0.1 and E_h -305±5 mV at 100^{th} day).

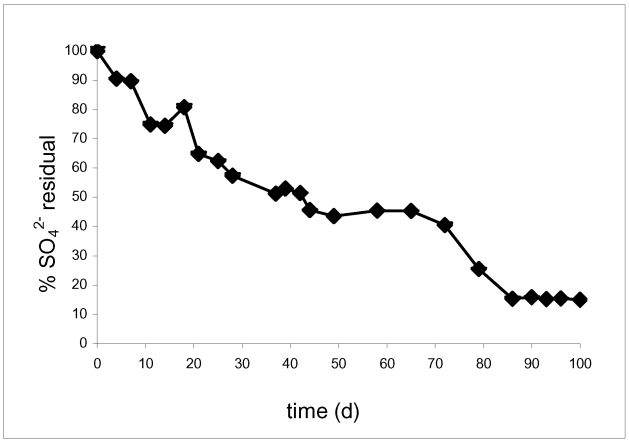

Figure 3: Sulphate abatement during time in batch tests with starch.

4. Conclusions

In this study a comparison between a selected reactive mixture containing organic matter for SRB and other electron donors, such as ethanol, glucose and polysaccharides was performed. The optimal selected mixture through batch tests allowed a sulphate abatement of 83% and showed also optimal pH and E_h conditions for SRB growth. A continuously operating fixed-bed column was filled with the selected mixture and inoculated by SRB. Column was regularly fed with a solution containing heavy metals and sulphate. At steady state sulphate abatement was 50±10% and heavy metals were totally removed. Batch tests with ethanol showed good performances in terms of both sulphate abatement and operative conditions for SRB growth. Column tests with ethanol showed that, at steady state, sulphate abatements of the inoculated column were 70±10% against 10±5% of the blank column. Preliminary batch tests with starch showed good performances in terms of both sulphate abatement and operative conditions for SRB growth.

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