Influence of temperature in sulphate-reducing anaerobic bacteria (SRB) development and metal removal efficiency

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Abstract Treatment processes to remove metals and sulphate, neutralizing the acidity of acidic mining drainage (AMD) involve phenomena as sulphate-reducing anaerobic bacteria (SRB) development and biosorption. Processes have found widely varying efficiencies ranging from 40 and 90 %. We carry out a test plan to determine the effect of temperature: 6 °C, 13°C and 25 °C. At 25 °Cthe black precipitate formation begins during the first hours reaching a metal removal efficiency of 90.44%, while at6 °Cin general there was no evidence of SRB development, even at 30 days of incubation, without significant changes in pH and Eh.

Keywords temperature, bacteria, sulphate, biosorption, efficiency

Introduction

Passive systems to mitigate acid mine drainage (AMD) depends essentially of sulphate-reducing anaerobic bacteria (SRB) development to neutralize the acid drainage, producing SH_2. which reacts with metals producing metals sulphides. Other mechanisms that immobilize metals in treatment systems such as biosorption and co-precipitation may simultaneously take place in such systems.

As any other microorganisms sulphate-reducing bacteria are living beings with a high specific surface and sensibility to the physic-chemical conditions of the micro-environment around their membrane cell. So, they have a very high capacity to give a response (positive or negative) to each external stimulus. The main mechanism of biosorption is ionic-interchange which is also affected by factors as pH.

Systems to mitigate AMD at pilot or full scale have found widely varying efficiencies (Karathanasis *et al.* 2010). Several variables impact the efficiency including $SO_4^=$ concentration, temperature, AMD particular composition, residence time, pH, redox potential (Eh) and carbon source.

Laboratory tests plans have been carried out to obtain quantified answers to the prior questions (Paños *et al.* 2012). Only the complete knowledge of the phenomena involved in AMD treatment systems and the conditions affecting them, could allow predicting, designing and optimizing such systems. A test plan was carried out to analyze the influence of temperature at two levels of $SO_4^=$ and organic nutrients availability, in SRB development and the other mechanisms of iron removal.

Methods

Synthetic media were prepared by mixing the three following solutions:

Solution I Only tap water corresponding to tests indicated in Table 1 as $SO_4^=$: 0 or tap water with 3 g of $MgSO_4.7H_2O$ per litter and is indicated in Table 1 as added $SO_4^=$: 3.

Solution II Organic nutrients compounds coming from dry leaves of the blackberry tree (high availability in our region). To liberate tha maximum contained labile compounds, dry leaves were mixed with water in a relation 7.5:100 (W:V) and kept at 20–25 °C during 48 hours before to separate the solution from solids. Solution Ii was added in quantities of 1 or 5 mL each 100 mL of Solution I.

Solution III 5 g of $FeSO_4.7H_2O$ in 100 mL of SO_4H_2 acid 0.5 % (V:V). Iron was used as a marker to analyze SRB development. 1 mL of Solution III each 100 mL of Solution I was added to all the tests.

Once the media of each test was prepared with the corresponding composition, pH was adjusted to the indicated values around 7 in Table 1 (pHin) also measuring Eh (Ehin). Flasks were filled, closed and film-sealed to ensure an anaerobic medium. Tests were prepared by triplicate incubating one of them at 6 °C in a refrigerator, other at ambient temperature with a media of 13 °C and the other at 25 °C.

Observations were made every day to determine the time (days) required for the begining of SRB development given by the begining of the black precipitate formation. At 35 days all the flasks were open. At the moment of open each flask the final pH and Eh were measured (pHout and Ehout) and a sample was filtered to analyze iron and determine iron removal efficiency. In tests with possitive SRB development, the pHout measured at the moment of flasks opening is in the order or more acidic than pHin due to the dissolved SH_2. Nevertheless, when the SH_2 excess come off (some hours latter the flasks opening) pHout is stable and higher than pHin.

Results

Table 1 shows the results of tests prepared with two sulphate concentrations and two organic compounds concentrations, incubing each group at three temperatures: 6, 13 and 25 °C.

Even in tests with negative SRB development a yellow-brown precipitate was formed and the solution lost the typical brown colour given by the organic compounds coming from dry leaves. The iron removal efficiency may be as high as 78.06 % indicating other mechanisms as biosortion and co-precipitation are responsable of the iron remosion. It may be see in Fig. 1 showing tests incubated at 6 °C. In tests IV and X, prepared with higher organic compounds concentration, any very slow SRB growth is observed, pHout decreased by the dissolved SH_2 and the organic matter remain suspended probably due to the gas disolved.

Fig. 2 is a picture of the tests incubated at 25 °C. In tests III SRB development was negative due to the very low sulphate and organic matter availability. In the others SRB development was possitive and the time required for the begining of the black precipitate formation notably decreased with higher sulphate and organic matter availability, as it is shown in the results of Table 1.

Fig. 1 *Tests incubated at 6 °C, I and VII with negative SRB development in 35 days, IV and X with a very slowly growth starting from 30 and 25 respectively.*

Fig. 2 *Tests incubated at 25 °C, III with negative SRB development in 35 days and XII with a fast SRB development in less than 1 day, depending on sulphate and organic nutrients availability.*

Discussion

In tests with negative SRB development, pHin respect to pHout is kept constant or in the same order and Ehin didn't decrease to reach the required for the development of SRB showing that there was not any significant bacterial development. In tests with positive SRB development; pHout was increased respect to pHin and Eh was decreased to the order or below the order required for SRB development (-180 mV). To reach such Eh order others bacterial populations must have developed before. Obviously, their development is also temperature dependent.

By comparing the black precipitate formation time of tests X, XI and XII: 25 days, 5 days and less than 1 day respectively, with the same medium composition but incubated at 6 °C, 13 °C and 25 °C respectively, it is put in evidence the very high influence of temperature, either in SRB development velocity, as in the growth velocity of the prior necessary microbial populations.

Furthermore, sulphate concentration and organic compounds availability are important factors. At a constant temperature, for example 25 °C, the results comparison between tests III and IX (both with 1 mL of organic solution each 100 mL of Solution I) shows whereas SRB development was absolutely negative even at 35 days of incubation in test III, in test IX there

Test N°	Added[(a)] SO_4^{--}	Organic Sol. [(b)]	pH_{in}	Eh_{in}	pH_{out}	Eh_{out}	Time SRB Growth [(c)] (days)	Efficiency (%)	Observations
Incubation temperature: 6 °C									
I	0	1	6.994	-120.8	6.774	-46.5	(-)	78.06	Without SRB growth
IV	0	5	6.996	-110.2	6.855	-179.3	30	67.44	Any SRB low growth
VII	3	1	7.085	-121.2	6.797	-107.9	(-)	-	Without SRB growth
X	3	5	7.075	-11.04	6.774	-190	25	70.21	Any SRB low growth
Incubation Temperature: 13 °C									
II	0	1	6.994	-120.8	6.758	-137.6	(-)	-	Without SRB growth
V	0	5	6.996	-110.2	8.382	-191.2	6	72.00	Positive SRB growth
VIII	3	1	7.085	-121.2	6.868	-161.9	(-)	-	Without SRB growth
XI	3	5	7.075	-114.0	8.237	-213.0	5	79.73	Positive SRB growth
Incubation temperature: 25 °C									
III	0	1	6.994	-120.8	6.715	-126.3	(-)	-	Without SRB growth
VI	0	5	6.996	-110.2	7.803	-202.1	2	89.30	Positive SRB growth
IX	3	1	7.085	-121.2	7.700	-171.1	19	76.04	Low nutrients conc.
XII	3	5	7.075	-114.0	8.120	-216.7	< 1	90.44	Fast SRB growth

[(a)] : $MgSO_4.7H_2O$ (g/L) added to Solution I

[(b)] : Organic solution (solution II) added each 100 mL of Solution I

[(c)] : Times (days) required for the begining of the black precipitate formation

(-): Negative SRB development even at 35 days of incubation

- : Data non obtained

Table 1 *Results of tests prepared with two sulphate concentrations and two organic compounds concentrations, incubing each group at three temperatures: 6, 13 and 25 °C.*

was evidence of growth in 19 days. By comparing tests VI and XII (both with 5 mL of organic solution each 100 mL of Solution I) the addition of sulphate in test XII (moreover the added as $FeSO_4.7H_2O$ in Solution III), allows the bacterial growth in a time less than a day, while in test III without the extra-addition, 2 days are required. Equivalent analysis could be done for tests incubated at other temperatures. The influence of organic compounds availability is shown by comparing the results of tests III and VI as well as IX and XII.

Even in tests with negative SRB development during 35 days, the efficiency (% of removed iron) was relatively high, over 67 – 70 % due to iron biosorption by bio-molecules and micro-particles coming from dry leaves. As it is shown in Fig. 2, the organic material sorbing iron is deposited in the flask button turning translucent the medium solution.

Comparing tests I and X incubated at 6 °C, (see Table 1 and Fig. 2), in test X at 25 days of incubation SRB development began, while in test I, there was not any evidence of bacterial growth at 35 days. Nevertheless, the efficiency reached in test X (70.21 %) is lower than test I efficiency (78.06 %). It is suggesting SRB development and the associated production of SH_2 and CO_2 are making difficult or less efficient the mechanism of biosorption, mainly an ionic-interchange mechanism, and co-precipitation. An equivalent analysis may be done by comparing the results of test I and IX, taking furthermore into account test IX was incubated at 25 °C and a clear SRB development was obtained.

Conclusions

Temperature is a factor of very high influence in SRB development. In cold climes or during winter season, it should not be expected SRB development in passive processes for acidic mining drainage (AMD) treatment, unless steps were taken in the system design.

Biosorption is a mechanism that can be efficient in metals removal near neutral pH when SRB development is negative. Nevertheless, when SRB development is positive, it generate conditions that difficult in some extension the mechanism of biosorption.

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