A NEW THIOSALT DEPURATION BIOPROCESS FOR WATER- RECYCLING IN METALLIC SULPHIDE MINERAL PROCESSING.

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Abstract

Economic and environmental imperatives force the metallic sulphide processing industry to re-use liquors that normally contain thiosalts; this processing entails difficulties in operation control and losses in metal-extraction performance. Bio-oxidation presents a promising option for thiosalt removal. Hitherto, this method has been tested to treat synthetic liquors containing only one thiosalt, either tetrathionate or thiosulphate, usually at low concentration (< 1 g/L). In this article, bio-oxidation is studied of synthetic and real liquors containing mixtures of these thiosalts at concentrations close to 4.5 g/L. Coexistence of thiosulphate and tetrathionate in an acidic medium leads to strong and

irreversible inhibition phenomena (performance losses close to 80%). In order to solve this problem, a previous treatment is proposed consisting of the 8-hour aeration of inlet solution after adding up to 20 mg/L and pH 1.5 of Cu^{2+} and sulphuric acid, respectively, resulting in the complete removal of thiosulphate and sulphite. Pre-treated real liquors were fed into a 1L discontinuous stirred tank bioreactor and a 1L continuous flooded packed-bed bioreactor, which were successfully started up and operated, thereby obtaining bio-oxidation rates close to the highest rates found in the literature with synthetic liquors.

1. Introduction

Mineral processing by milling and flotation requires a high consumption of water. Cost effectiveness and impact on the conservation of freshwater resources, according to sustainability policies and goals, force this industry to re-use process liquors, thereby decreasing the demand for primary water supply.

Recycled liquors mainly come from classification ponds, tailings dams, and solid/liquid separation units containing dissolved and suspended substances (residual flotation reagents and ions and colloids coming from the ore). Water recycling leads to modifications of feed-water quality which hinders the operation control and even induces loss in flotation performance (Rao and Finch, 1989; Liu et al, 2013; Bicak, et al, 2018; Guerrero-Flores et al, 2018; Muzinda and Schreithofer, 2018).

Thiosalts are usually present in recycled liquors of metallic sulphide ore processing. Thiosulphate ($S_2O_3^{2-}$) and polythionates, mainly tetrathionate ($S_4O_6^{2-}$), are generated in milling and flotation circuits, usually in alkalinity conditions, by the partial oxidation of sulphides (Rolia and Chakrabarti, 1982; Silver, 1985). These anions can interact with added reagents, thereby affecting flotation selectivity (Rao and Finch, 1989; Kirjavainen et al, 2002; Petrus et al, 2012; Shengo et al, 2014; Ozturk et al, 2018). Thiosalts are metastable ions which consume O_2 and finally generate H_2SO_4 through spontaneous oxidation and disproportionation reactions (Silver and Dinardo, 1981; Suzuki, 1999; Druschel et al, 2003). These reactions are catalysed by metal ions and microorganisms of the *Acidithiobacillus* genus, usually present in mining environments. Discharging effluents containing thiosalts exert an indirect environmental impact in receptor aquatic bodies undergoing delayed dissolved O_2 depletion and progressive acidification (Silver and Dinardo, 1981; Chanda et al, 1984; Silver, 1985; Kuyucak and Yaschyshyn, 2007; Ghosh and Dam, 2009; Dopson and Johnson, 2012).

The complete removal of thiosalts in process liquors can become considered a critical target in the design and operation of flotation plants. Lagooning is the current, more extensively applied procedure. This passive depuration method is simple and inexpensive because it is based on the natural oxidation of thiosalts. Due to its low efficiency, this process requires huge ponds, which produce a visual impact and poor management of the territory. Facing the impossibility of temperature control, its applicability is limited in cold climates due to reduced bacterial and chemical activity (Kuyucak and Yaschyshyn, 2007; Montes-Rosúa et al, 2018). Active treatments based on the controlled addition of oxidants, such as H_2O_2, H_2SO_5 (Caro´s acid), O_3, or ClO^- (hypochlorite), have been tested. These methods are expensive and involve the handling, storage, and transport of hazardous substances. Adsorption using several adsorbents, such as activated carbon, specialty resins, and biomass ash/char, has also been studied. However the low adsorption capacities achieved make this technological option impractical on an industrial scale (Silver, 1985, Dinardo and Sally, 1998; Lu et al, 2010; Kuyucak and Yaschyshyn, 2007; Range and Hawboldt, 2018; Ozturk et al, 2018).

The majority of the aforementioned methods were proposed in the environmental context, and targeted effluent depuration with a low concentration of thiosalts (<1 g/L). The implementation of new purification technologies for water reuse, which are integrated in metallic sulphide mineral processing plants, requires procedures of a greater versatility regarding the thiosalt content that are also safer and more economical, with high oxidation rates and low residence times.

Bio-oxidation of thiosalts presents a promising option and almost always leads to a plunge in the level of pH. Bio-oxidation of $S_4O_6^{2-}$ generates SO_4^{2-} and H^+ as final products (r1):

$$S_4O_6^{2-} + 3\ H_2O + 7/2\ O_2 \rightarrow 4SO_4^{2-} + 6\ H^+ \qquad (r1)$$

Bio-oxidation of $S_2O_3^{2-}$ takes place in two steps: in the first step, $S_2O_3^{2-}$ is oxidised, thereby generating $S_4O_6^{2-}$ as an intermediary metabolite and increasing pH (r2).

$$2S_2O_3^{2-} + 2\,H^+ + ½O_2 \rightarrow S_4O_6^{2-} + H_2O \qquad (r2)$$

When $S_2O_3^{2-}$ concentration is depleted, $S_4O_6^{2-}$ is oxidised, which produces SO_4^{2-} and H^+ (r1) (Beard et al, 2011).

From among the very few studies into the bio-oxidation of thiosalts, only Iglesias et al. 2016 report the continuous purification of liquors. The majority of these studies are based on small-volume discontinuous tests using synthetic media with only one thiosalt, either $S_4O_6^{2-}$ or $S_2O_3^{2-}$ (Eccleston and Kelly, 1978; Silver and Dinardo, 1981; Pronk et al, 1990, Kupka et al, 2009; Shiers et al, 2011).

Fixed-bed bioreactors packed with plastic (MBBR^{TM}) and activated carbon as biofilm carrier materials have been studied (Sääf et al, 2009; Liljevqvist et al, 2011). These bioreactors (2L in volume) were fed at the top with synthetic solutions which then flowed through the bed by gravity. $S_2O_3^{2-}$ concentrations in feed were lower than 0.5 g/L and pH was maintained in the range from 3 to 4.5. The reactors were sparged with CO_2-enriched air. The microbial population consisted of *Acidithiobacillus ferrivorans* previously adapted from Fe^{2+} to thiosalts as substrate. During the continuous operation, a steady state was not achieved and an external supply of microorganisms was necessary. The maximum bio-oxidation rate of 0.32 kg/h·m^3 and a retention time of 0.9 h were observed when activated carbon was used as the microbial carrier.

Continuous bio-oxidation of $S_4O_6^{2-}$ was carried out in a flooded packed-bed bioreactor containing a microbial consortium attached to siliceous sand (Iglesias et al, 2016). Microorganisms were also previously adapted from Fe^{2+} to thiosalts, but in this case the adaptation process was performed inside the bioreactor. Air and $S_4O_6^{2-}$ solutions were fed

into the bottom of the bioreactor and flowed upwards through the bed thereby flooding it. The $S_4O_6^{2-}$ concentration in feed was 3.5 g/L and pH 2.8. Stable operation was feasible for 33 days without an external supply of biomass. In steady state, the maximum bio-oxidation rate achieved was 0.83 kg/h·m^3. In view of these results, flooded packed-bed bioreactor can be postulated as the most effective design for thiosalt bio-oxidation. However, the bio-oxidation of mixtures of thiosulphate and polythionates should be tested since this is the most representative scenario in metallic sulphide mineral processing.

In this article, the bio-oxidation of solutions containing $S_2O_3^{2-}$ and $S_4O_6^{2-}$ tetrathionate (synthetic and real liquors) is studied. Batch assays are carried out in 250 mL Erlenmeyer flasks and in a 1L stirred tank reactor. Continuous tests are run in fixed-bed reactors with cells attached to siliceous sand particles. The main goal of this work is to test the versatility of bio-oxidation with respect to the feed composition, and to introduce improvements in the design while ensuring the removal of thiosalts in the current scenario of metallic sulphide mineral processing.

2. Materials and methods.

2.1. Real liquor.

In this work, a real liquor from a polymetallic sulphide flotation plant placed in the Iberian Pyritic Belt (north of Huelva in Spain) was bio-oxidised. It mainly consisted of 5.66 g/L of $S_4O_6^{2-}$, 0.61 g/L of $S_2O_3^{2-}$, 0.15 g/L of SO_3^{2-}, 2.2. g/L of SO_4^{2-}, 1.41 g/L of Ca, 20 mg/L of Zn, 50 mg/L of Fe, 0.5 mg/L of Cu, and 0.2 mg/L of Co at pH 2.8.

2.2. Culture.

The culture used as inoculum in this work was originally isolated from drainage waters from the Rio Tinto mines whose dominant microorganisms are *Acidithiobacillus*

ferrooxidans and *Acidithiobacillus thiooxidans*. This culture is routinely maintained on an enriched medium containing 3.5 g/L of $S_4O_6^{2-}$, 3 g/L of $(NH_4)_2SO_4$, 3 g/L of K_2HPO_4, 0.5 g/L of $MgSO_4 \cdot 7H_2O$, and 0.2 g/L of $CaCl_2$.

2.3. Batch bio-oxidation of synthetic solutions in Erlenmeyer flasks.

Batch thiosalt oxidation assays in sterilized 250 mL Erlenmeyer flasks stirred in an orbital shaker at 180 rpm at 30 ºC were performed both with 1g/L of $S_2O_3^{2-}$ and without this synthetic medium. The pHs studied were 1.5 and 2.0. One hundred mL of culture medium was inoculated with 20 mL of grown culture. The evolution of cultures was monitored by the measurement of pH over time, and of concentrations of tetrathionate, thiosulphate, and sulphite. Moreover, abiotic control tests in analogous conditions were carried out.

2.4. Thiosulphate and sulphite removal.

Batch tests in sterilized 250 mL Erlenmeyer flasks stirred in an orbital shaker at 180 rpm at 30 ºC were carried out in order to study thiosulphate and SO_3^{2-} removal in an acidic medium in the presence of Cu^{2+}. Real liquor and synthetic solutions containing either only 1 g/L of $S_2O_3^{2-}$ or 4 g/L of $S_4O_6^{2-}$ and 1g/L of $S_2O_3^{2-}$ were used. The working volume was 150 mL. The pHs studied were 2, 1.5, and 1.25. The Cu^{2+} concentrations tested were 0, 10, 15, and 20 mg/L. $S_4O_6^{2-}$, $S_2O_3^{2-}$, and SO_3^{2-} concentrations were measured against time.

2.5. Batch bio-oxidation of real liquor in stirred tank bioreactor (STB).

A batch bio-oxidation test of real liquor was carried out in a 1L STB provided with 4 baffles and a Rushton turbine. The working volume was 800 mL. The stirring rate and aeration flow rate were 450 rpm and 1L/min, respectively. $S_2O_3^{2-}$ and SO_3^{2} were previously removed by aeration for one day after adding sulphuric acid and copper sulphate to reach pH 2 and Cu^{2+} 20 mg/L. Bio-oxidation was started by adding inoculum

(20 % of working volume). $S_4O_6^{2-}$, $S_2O_3^{2-}$, and SO_3^{2-} concentrations were measured over time.

2.6. Continuous bio-oxidation of real liquor in packed-bed bioreactor.

A flooded packed-bed bioreactor of 15 cm in height and 8.4 cm in diameter (Mazuelos et al, 1999; Iglesias et al, 2016) with immobilized cells was employed to carry out continuous bio-oxidation tests. At the bottom of the reactors there was a hollow chamber, 5 cm in height, with nozzles for air and liquid medium inlets. Above this chamber, a bed of 10 cm in height was placed supported in a plastic grille; randomly placed siliceous stone particles, 6-8 mm in size, formed the bed with a porosity of 0.45. At the top of the bioreactor there was a hollow chamber with a nozzle for liquid outlet by overflow. The liquid volume inside the bioreactor was 750 mL.

This bioreactor was fed with pre-treated real liquor without $S_2O_3^{2-}$ or SO_3^{2-}, which had previously been removed by aeration and H_2SO_4 and $CuSO_4$ were added to reach pH 2 and Cu^{2+} 20 mg/L.

Initially, inoculum and pre-treated real liquor were placed and aerated inside the bioreactor. When the thiosalts were exhausted, the bioreactor was connected with a 2.5L deposit where pre-treated real liquor was continuously recirculated. Once the thiosalts were exhausted, the continuous operation started. The liquid flow rate was 150 mL/h. Two air flow rates were tested: 750 and 1500 mL/min.

2.7 Analysis

$S_2O_3^{2-}$ and SO_3^{2-} were analysed using iodometry (Kurtenacker and Bittner, 1924; KoH, 1990) whereby, in order to discriminate between these ions, SO_3^{2-} was masked with CH_2O (formaldehyde). Acidimetric method with $HgCl_2$ was performed for the measurement of the total concentration of thiosalts (Makhija and Hitchen, 1978). The $S_4O_6^{2-}$ concentration

was calculated by subtracting SO_3^{2-} and $S_2O_3^{2-}$ concentrations from the total concentration of thiosalts. Traces of $S_3O_6^{2-}$ (trithionate) and $S_5O_6^{2-}$ (pentathionate) were occasionally detected via ion chromatography.

3. Results and discussion

3.1 Batch bio-oxidation of synthetic solutions in Erlenmeyer flasks.

As mentioned above, to the best of our knowledge, no studies concerning bio-oxidation of solutions containing mixtures of different thiosalts can be found in the literature. This issue was approached by performing four bio-oxidation tests in stirred Erlenmeyer flasks, two of which held liquid medium containing only $S_4O_6^{2-}$ (initial concentration 3.5 g/L), while the other flasks contained $S_2O_3^{2-}$ and $S_4O_6^{2-}$ (initial concentrations 1 g/L and 3.5 g/L, respectively). Initial pHs tested were 2 and 1.5; these values are in the typical pH range for waters whose thiosalts were completely oxidised (r1, r2) and consequently contain SO_4^{2-} and H^+ as final products, that is, they give rise to a SO_4^{2-}/HSO_4^- bisulphate buffer. In analogous operating conditions, abiotic tests were also carried out.

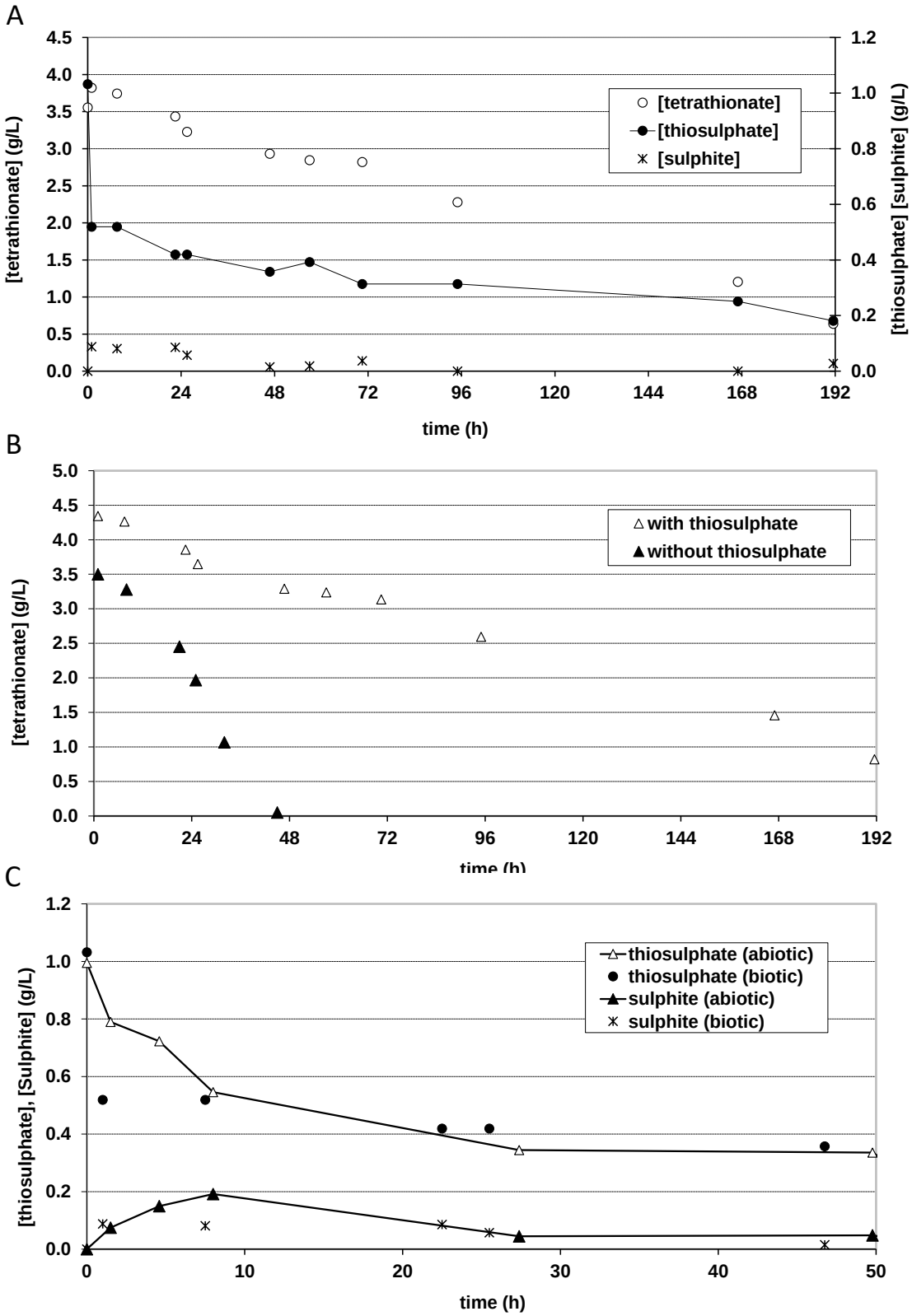
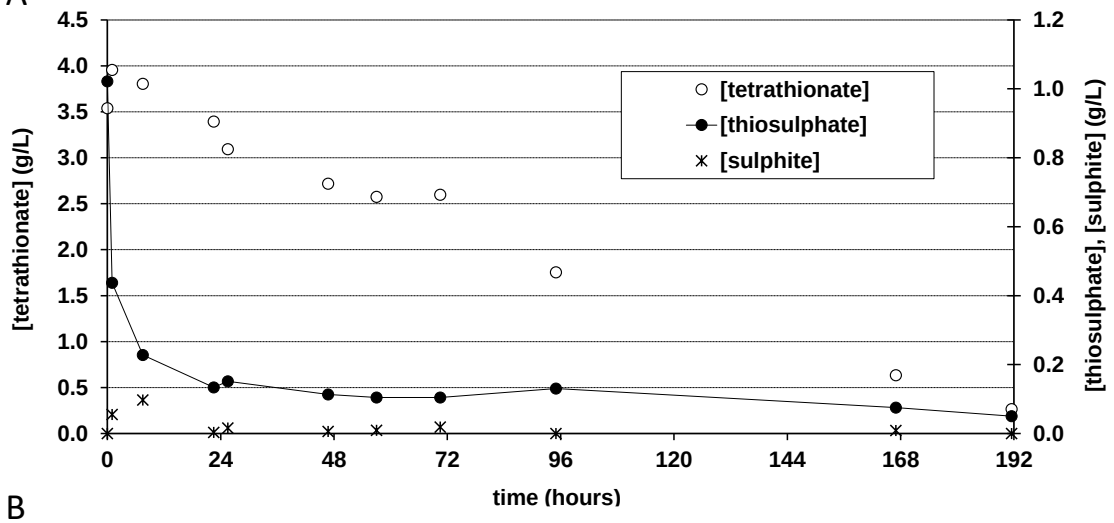


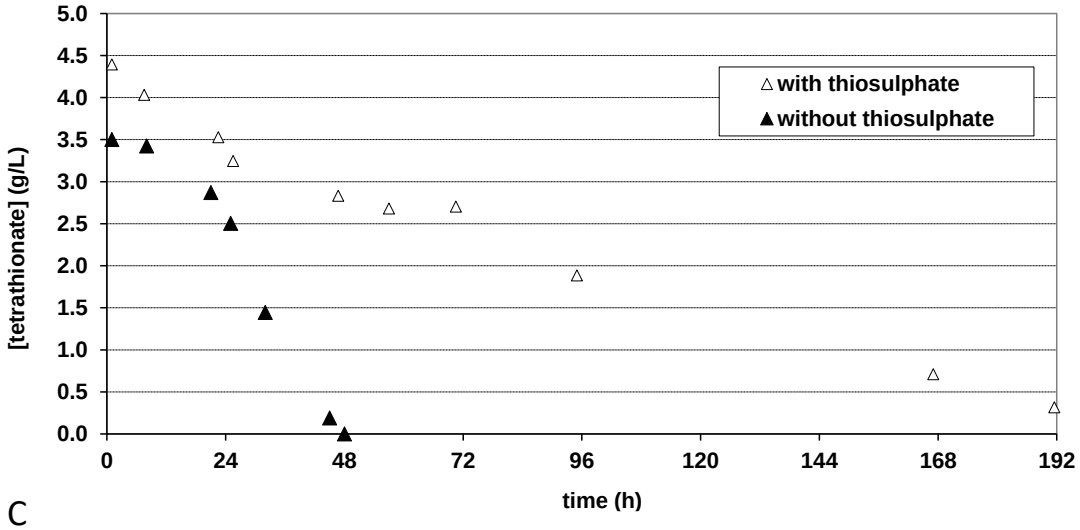
Figure 1: Batch bio-oxidation test of synthetic solutions in 250 mL Erlenmeyer flasks. pH 2, 30ºC, 180 rpm, initial concentration of tetrathionate 3.5 g/L, and initial concentration

of thiosulphate 1 g/L. A) Tetrathionate, thiosulphate, and sulphite concentrations vs. time. B) Comparison with a bio-oxidation test performed in analogous conditions in the absence of thiosulphate. C) Comparison with a test carried out under analogous conditions without microorganisms.

A


[tetrathionate] (g/L)
[thiosulphate], [sulphite] (g/L)
[tetrathionate]
[thiosulphate]
[sulphite]
time (hours)

B


[tetrathionate] (g/L)
with thiosulphate
without thiosulphate
time (h)

C

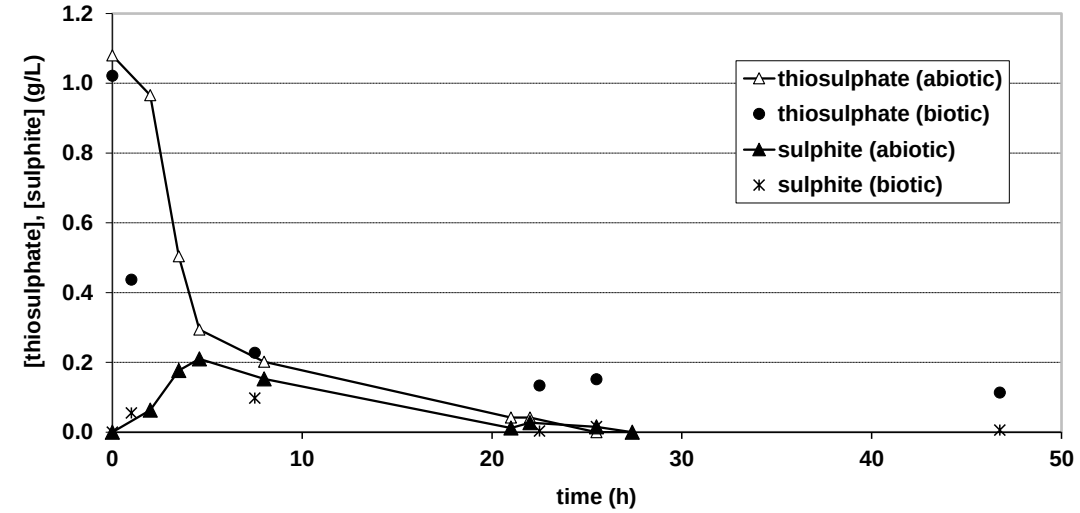

[thiosulphate], [sulphite] (g/L)
thiosulphate (abiotic)
thiosulphate (biotic)
sulphite (abiotic)
sulphite (biotic)
time (h)

Figure 2: Batch bio-oxidation test of synthetic solutions in 250 mL Erlenmeyer flasks. pH 1.5, 30ºC, 180 rpm, initial concentration of tetrathionate 3.5 g/L, and initial concentration of thiosulphate 1 g/L. A) Tetrathionate, thiosulphate, and sulphite concentrations vs. time. B) Comparison with a bio-oxidation test performed in analogous conditions in the absence of thiosulphate. C) Comparison with a test carried out under analogous conditions without microorganism.

Results obtained at pH 2.0 and pH 1.5 are shown in Figures 1 and 2, respectively. Figures 1A and 2A show that neither $S_2O_3^{2-}$ nor $S_4O_6^{2-}$ was completely consumed in 192 hours when mixtures of both thiosalts were fed, whereas $S_4O_6^{2-}$ was depleted in only 48 hours in the absence of $S_2O_3^{2-}$ (Figures 1B, 2B).

Without $S_2O_3^{2-}$, the $S_4O_6^{2-}$ concentration decreased exponentially, which clearly shows microbiological activity (Figures 1B, 2B). Neither $S_2O_3^{2-}$ nor SO_3^{2-} was detected. In the absence of bacteria, the $S_4O_6^{2-}$ concentration remained constant (result not shown) since this anion is stable in the mesophilic temperature range (Druschel et al, 2003).

When $S_2O_3^{2-}$ is fed, its concentration dramatically dropped early and SO_3^{2-} was detected (Figures 1A, 2A). This suggests that chemical disproportionation of $S_2O_3^{2-}$ (r3) took place (Johnston and McAmish, 1973):

$$S_2O_3^{2-} + 2\,H^+ \rightarrow S + H_2SO_3 \qquad \text{(r3)}$$

This assumption is reinforced when results obtained from biotic and abiotic tests are compared (Figures 1C, 2C), since $S_2O_3^{2-}$ and SO_3^{2-} concentrations show similar values. Concurrently, the $S_4O_6^{2-}$ concentration slightly increased (Figures 1A, 2A) due to bio-oxidation of $S_2O_3^{2-}$ to $S_4O_6^{2-}$ (r2) (Sorokin and Tourova, 2005). Subsequently, thiosalts were slowly oxidised, which indicated very limited biological activity. Deceleration of

$S_4O_6^{2-}$ bio-oxidation when $S_2O_3^{2-}$ is present can be attributed to the generated cytotoxicity of SO_3^{2-} (Tuovinen and Kelly, 1974; Masau et al, 2001; Sääf, 2009). Therefore, efficient bio-oxidation of mixtures of thiosalts at low pH (inherent to real liquors) would require the prevention of the presence of both SO_3^{2-} and $S_2O_3^{2-}$ inside the bioreactor.

3.2. Pre-treatment for thiosulphate and sulphite removal.

A set of experiments was carried out in order to study the depletion of $S_2O_3^{2-}$ and SO_3^{2-}in solutions containing mixtures of thiosalts. A simple method was proposed that consisted of adding H_2SO_4 and traces of Cu^{2+} in aerated reactors. Under these conditions, H_2SO_4 promotes chemical disproportionation of $S_2O_3^{2-}$ (r3) by generating SO_3^{2-} which is catalytically oxidised by O_2 in the presence of Cu^{2+} (r4).

$SO_3^{2-} + ½\ O_2 \rightarrow SO_4^{2-}$ (r4)

Three types of solutions were assayed:

A. Synthetic, initially containing 1 g/L of $S_2O_3^{2-}$.
B. Synthetic, initially containing 4 g/L of $S_4O_6^{2-}$ and 1 g/L of $S_2O_3^{2-}$.
C. Real liquor, initially containing 5.66 g/L of $S_4O_6^{2-}$, 0.61 g/L of $S_2O_3^{2-}$, and 0.15 g/L of SO_3^{2-}.

Table 1 summarises the results obtained in these experiences where $[Cu^{2+}]$ is the concentration of cupric ion, $t_{100\%}$ is the time taken to oxidise 100% of $S_2O_3^{2-}$ and SO_3^{2-}, $X(t)_8$ is the $S_2O_3^{2-}$ conversion at 8 hours, and $[SO_3^{2-}]_8$ is the concentration of sulphite at 8 hours.

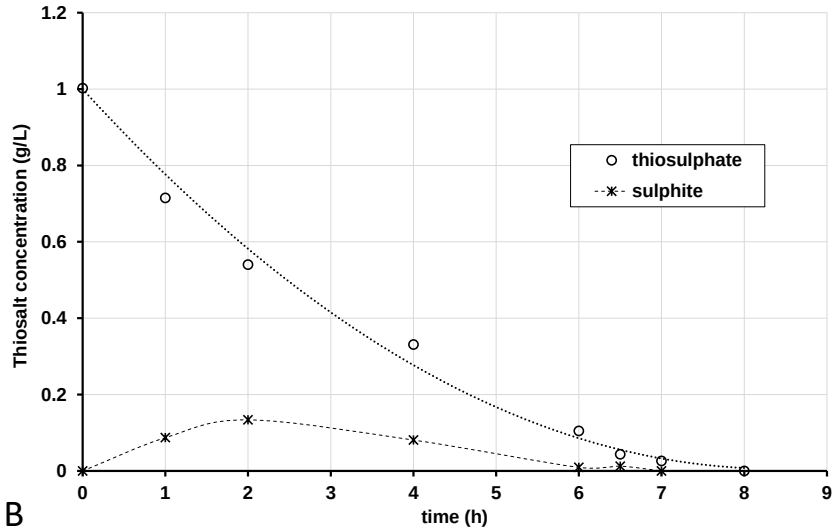
Table 1: Thiosulphate and sulphite removal by acidification, and oxidation catalysed by Cu^{2+} in 250 mL in Erlenmeyer flasks at 180 rpm and 30 ºC. $[Cu^{2+}]$ is the concentration of cupric ion, $t_{100\%}$ is the time taken to oxidise 100% of $S_2O_3^{2-}$ and SO_3^{2-}, $X(t)_8$ is the $S_2O_3^{2-}$ conversion at 8 hours, and $[SO_3^{2-}]_8$ is the concentration of sulphite at 8 hours.

Solution nature	Initial solution composition	Initial pH	$[Cu^{2+}]$ (mg/L)	$t_{100\%}$ (h)	$X(t)_8$ (%)	$[SO_3^{2-}]_8$ (mg/L)
A (synthetic)	1 g/L $S_2O_3^{2-}$	2	20	18	42	30
		2	15	>48	38	40
		2	10	>48	35	70
		2	0	>72	25	80
		1.5	20	7	100	0
		1.5	15	8	100	0
		1.5	10	12	91	10
		1.5	0	>24	74	100
		1.25	20	6	100	0
		1.25	15	7	100	0
		1.25	10	9	97	41
		1.25	0	10	93	60
B (synthetic)	4 g/L $S_4O_6^{2-}$ 1 g/L $S_2O_3^{2-}$	2	20	24	57	60
		2	15	>24	50	90
		2	10	>24	47	100
		2	0	>48	38	180
		1.5	20	8	100	0
		1.5	15	14	82	40
		1.5	10	14	81	60
		1.5	0	26	80	150
		1.25	20	5	100	0
		1.25	15	12	100	20
		1.25	10	14	100	30
		1.25	0	25	83	112
C (Real liquor)	5.66 g/L $S_4O_6^{2-}$ 0.61 g/L $S_2O_3^{2-}$ 0.15 g/L SO_3^{2-}	2	20	21	42	30
		2	15	>24	38	40
		2	10	>48	35	70
		2	0	>48	25	80
		1.5	20	7	100	0
		1.5	15	8	100	0
		1.5	10	9	91	10
		1.5	0	>24	74	102
		1.25	20	6	100	0
		1.25	15	8	100	0
		1.25	10	10	97	41
		1.25	0	18	93	60

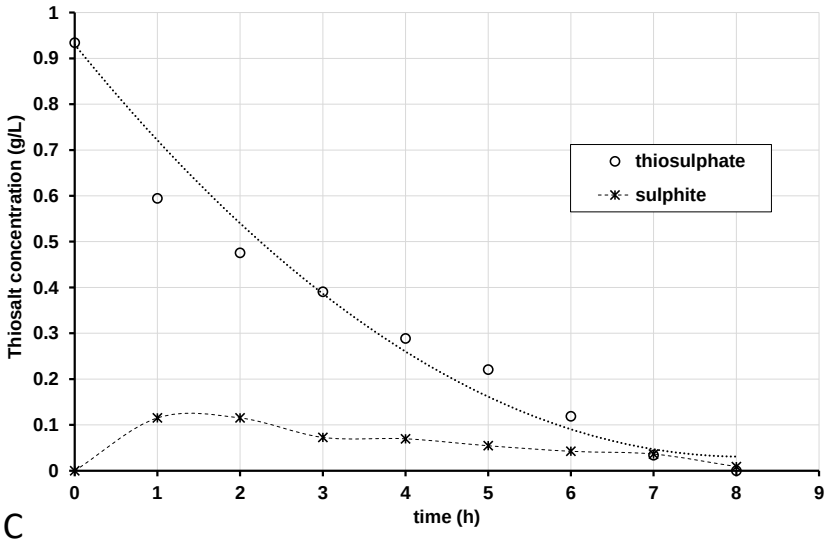
In Table 1, it can be observed the proposed pre-treatment is effective for $S_2O_3^{2-}$ and SO_3^{2-} removal with reasonable retention times. pH significantly influences $S_2O_3^{2-}$ and SO_3^{2-} removal rates which are faster as pH is decreased. However, it must be take into account that subsequent bio-oxidation of thiosalts implies an additional decrease of this parameter, and therefore if the final acidity in the bioreactor is very high, then there is a risk of triggering inhibitory effects by excessive acidity. Accordingly, pH must be chosen, depending on the thiosalt concentration in the feed and considering the following two technical criteria: a low retention time or a suitable pH for the direct bio-oxidation of liquors. The first criterion implies work at low pH, between 1.25 and 1.50, and the necessity for pH adjustment close to 2 before bio-oxidation, while in the second criterion of pH 2, longer retention times of around 24 hours are required.

In Table 1, the Cu^{2+} role as an effective catalyst to SO_3^{2-} removal can be observed. As representative examples, thiosalt concentration vs. time curves are shown in Figure 3 that correspond to the treatment of solutions A, B, and C at pH = 1.5 and $[Cu^{2+}]$ = 20 mg/L. For the three types of solutions, similar curves were obtained. It is interesting to note that the SO_3^{2-} concentration is always low (< 200 mg/L); SO_3^{2-} concentration initially increases up to values close to 0.15 g/L, and it then decreases until it disappears. These results suggest that kinetics for both disproportionation r2 and oxidation r4 reactions depend on $S_2O_3^{2-}$ and on SO_3^{2-} concentrations, respectively. Under these conditions, the $S_4O_6^{2-}$ content remained unaltered (results not shown).

A


Thiosalt concentration (g/L)
time (h)
thiosulphate
sulphite

B


Thiosalt concentration (g/L)
time (h)
thiosulphate
sulphite

C

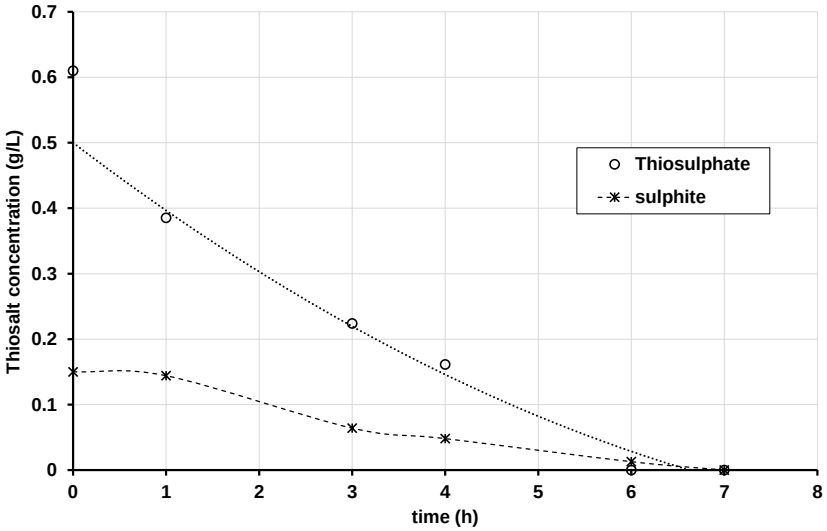

Thiosalt concentration (g/L)
time (h)
Thiosulphate
sulphite

Figure 3: Thiosulphate and sulphite removal for A, B, and C solutions by acidification and oxidation catalysed by Cu^{2+}. A: synthetic, initially containing 1 g/L of $S_2O_3^{2-}$; B: synthetic, initially containing 4 g/L of $S_4O_6^{2-}$ and 1 g/L of $S_2O_3^{2-}$; C: real liquor, initially containing 5.66 g/L of $S_4O_6^{2-}$, 0.61 g/L of $S_2O_3^{2-}$, and 0.15 g/L of SO_3^{2-}. 250 mL Erlenmeyer flasks, pH 1.5, $[Cu^{2+}]$ = 20 mg/L, 180 rpm, 30 ºC.

3.3. Bio-oxidation after thiosulphate and sulphite removal in STR.

A STR was operated in order to carry out the bio-oxidation of real liquors: a batch stirred tank, with planktonic microbial population, and a continuous flooded packed bed, where microorganisms were attached on siliceous sand particles.

This bioreactor was fed with the real liquor as received, i.e containing $S_2O_3^{2-}$ and SO_3^{2-}. After several attempts, the start-up operations were not success, in accordance with the results obtained with synthetic liquors. On the contrary, start-up was feasible when the inlet liquor was previously treated for $S_2O_3^{2-}$ and SO_3^{2-} removal. Results are shown in figure 4, where the straight line between 8 to 34 hours (black points) evidences exponential growth of biomass. A Specific bio-oxidation rate of 0.11 h^{-1} was calculated. This value is in the range found in the literature for this parameter when $S_4O_6^{2-}$ as only substrate was bio-oxidised in similar conditions; between 0.06 and 0.08 h^{-1} in Erlenmeyer flasks and 0.12 h^{-1} in aerated tanks (Eccleston and Kelly 1978, Montes-Rosúa et al, 2018). Therefore, bio-oxidation of pre-treated liquor must not have been affected by any meaningful inhibition phenomena; neither $S_2O_3^{2-}$ nor SO_3^{2-} was detected.

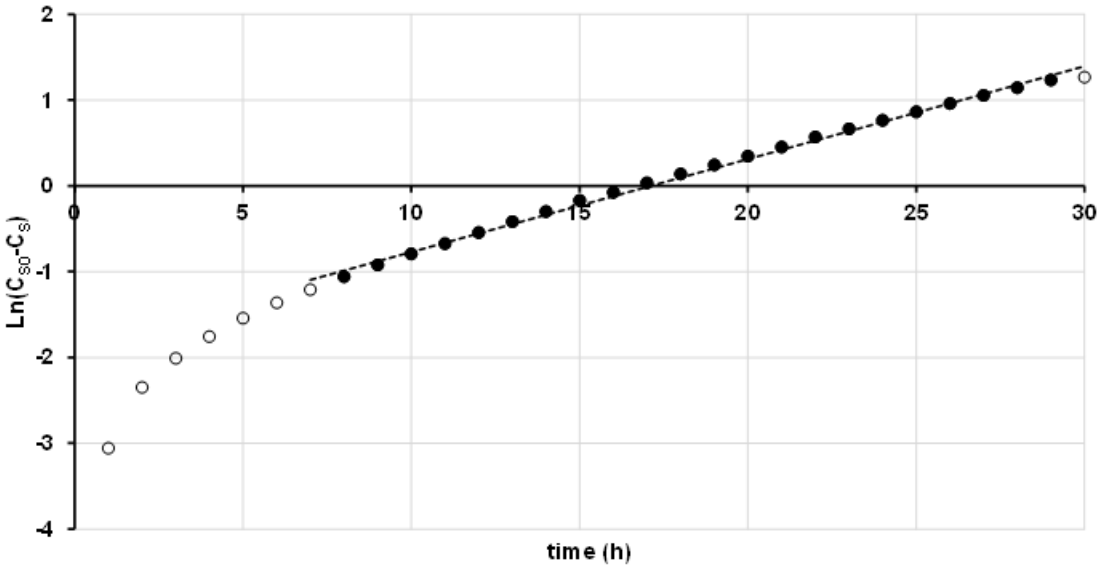


Figure 4: Batch bio-oxidation test of pre-treated real liquor at pH 2 and [Cu^{2+}] 20 mg/L in a stirred tank bioreactor. Initial pH 2, 450 rpm, 30ºC, air flow rate 1L/min.

3.4The continuous bio-oxidation of real liquors.

Once a methodology for the avoidance of detrimental effects associated to the presence of $S_2O_3^{2-}$ and SO_3^{2-} was established, the continuous bio-oxidation of real liquors was performed using a flooded packed-bed bioreactor with microbial population attached to siliceous sand particles.

Again, the start-up operation was only feasible when the liquors were previously treated for the removal of $S_2O_3^{2-}$ and SO_3^{2-}.

Figure 5 shows results obtained from the continuous bio-oxidation test performed in a flooded packed-bed bioreactor; the vertical lines define four areas corresponding to the four campaigns carried out:

- Campaign 1 (from 0 to 123 hours): feed consisted of pre-treated real liquor without $S_2O_3^{2-}$ nor SO_3^{2-}. A steady state was reached and the bio-oxidation rate

was the highest achieved in this work. Again, the effectiveness of the pre-treatment proposed for the prevention of the presence of $S_2O_3^{2-}$ and SO_3^{2-} was demonstrated.

- Campaign 2 (from 123 to 171 hours): $S_2O_3^{2-}$ was added in an inlet solution to a maximum concentration of 100 mg/L. The bio-oxidation rate dropped dramatically by approximately 50% in only 48 hours. A clear negative effect associated to the addition of $S_2O_3^{2-}$ was observed. SO_3^{2-} was detected in the outlet stream.
- Campaign 3 (from 171 to 292 hours): $S_2O_3^{2-}$ was suppressed in feed and the inlet liquor composition was the same as in Campaign 1. Despite the lack of $S_2O_3^{2-}$, the bio-oxidation rate continued to fall to values lower than 80% of those achieved in Campaign 1. Subsequently, the bio-oxidation rate remained almost constant for 76 hours. The loss of efficiency caused by adding $S_2O_3^{2-}$ in Campaign 2 was not reversible.
- Campaign 4 (from 292 to 400 hours): the air flow rate was doubled and no meaningful changes were observed; the irreversibility of the detrimental effect associated to the addition of $S_2O_3^{2-}$ was confirmed despite improvements in the aeration conditions.

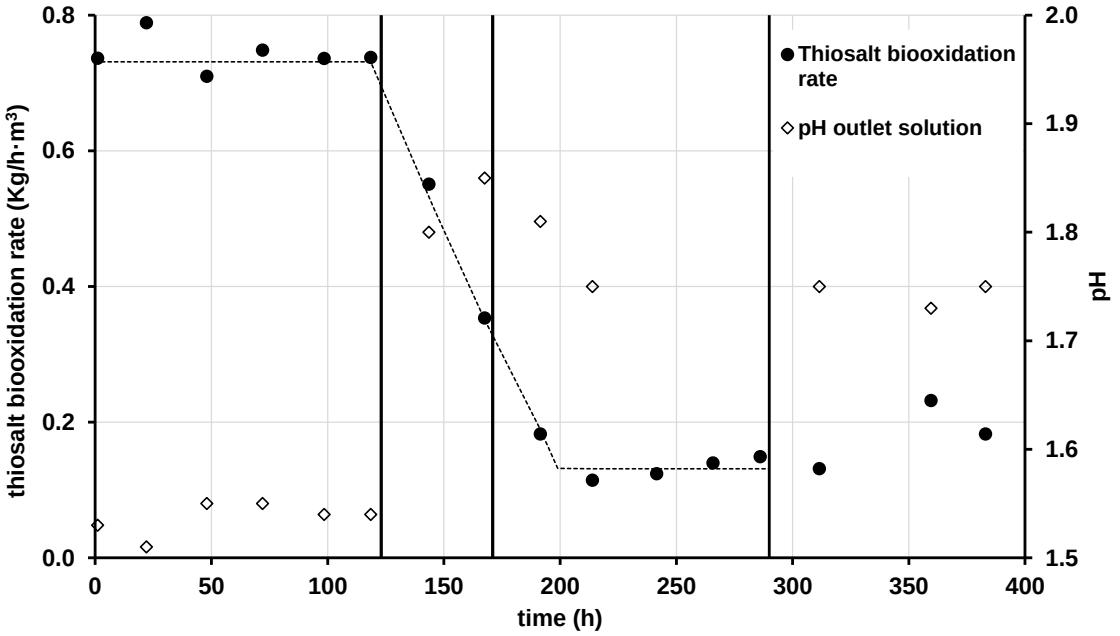


Figure 5: Continuous bio-oxidation test of pre-treated real liquor at pH 2 and [Cu^{2+}] 20 mg/L in a flooded packed bed. Initial pH 2, 30ºC, liquid flow rate 150 mL/h. Air flow rate 750 mL/min; before 292 hours, air flow rate 1500 mL/min.

The sudden decrease in the bio-oxidation rate when $S_2O_3^{2-}$ was added (Campaign 2) and the irreversibility of the resulting detrimental effect (Campaigns 3 and 4) provide evidence that a biological inhibition phenomenon took place, as happened in the discontinuous tests. Thiosalt bio-oxidation implies a noticeable fall in pH; in Figure 5, subsequent to the addition of $S_2O_3^{2-}$, an outlet pH level near to 1.5 was observed. Inside the bioreactor, the pH had to be similar since the flow in this kind of bioreactor fits the perfect mix model (Mazuelos et al, 2019). At this pH, $S_2O_3^{2-}$ disproportionation was promoted (r2) and, in consequence, SO_3^{2-} was generated.

This severe and irreversible decline of the bioreactor performance with partial loss of biomass activity brings to light that both pH and $S_2O_3^{2-}$ and SO_3^{2-} concentrations must be considered as critical control parameters for bio-oxidation of thiosalts in bioreactors.

4. Conclusions.

Bio-oxidation of solutions consisting of mixtures of thiosalts is incompatible with the presence of $S_2O_3^{2-}$ and SO_3^{2-} (inherent to real liquors). If $S_2O_3^{2-}$ and SO_3^{2-} are previously eliminated, then efficient bio-oxidation becomes feasible.

$S_2O_3^{2-}$ and SO_3^{2-} can be completely removed within reasonable retention times when liquors are aerated after acidifying with H_2SO_4 and adding traces of Cu^{2+}.

Flooded packed beds with attached sulfooxidant microorganisms are postulated as an effective design of bioreactor for the continuous removal of thiosalts.

In summary, the removal of thiosalts can be successfully carried out by a two-stage process: first, a previous chemical stage that consists of disproportionation and oxidation; and a second biotic stage consisting of bio-oxidation. Based on these statements, a new depuration technology to be used for water recycling in metallic sulphide mineral processing facilities has been proposed.

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